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## Complexity of molecular alterations impacts pancreatic cancer prognosis

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### Abstract

Individualized cancer treatment (e.g. targeted therapy) based on molecular alterations has emerged as an important strategy to improve the current standard-of-care chemotherapy. A large number of studies have demonstrated the importance of biomarkers not only in predicting prognosis but more importantly in predicting the response towards therapies. For example, amplification or mutation status of the two biomarkers HER2 (human epidermal growth factor 2) and BRCA (breast cancer) can be used to decide on a specific targeted therapy in breast cancer. However, no biomarkers with a similar clinical impact have been identified in pancreatic ductal adenocarcinoma. Although many genome-wide and proteome-based high-throughput studies have identified candidate genes or proteins as promising biomarkers, none of them were eventually transferred into the clinical setting. Notably, the most reliable markers for predicting prognosis are still the tumor stage and grade and biomarkers for therapy response remain undefined. One reason lies in the lack of systemic approaches to analyze the complexity of dominating cancer pathways and the impact of such signal complexity on prognosis and therapy response.

### INVITED COMMENTARY ON HOT ARTICLES

In a recent seminal study, Breitkreutz *et al*<sup>[1]</sup> compared the complexity of core signaling pathways in a variety of tumor entities including pancreatic ductal adenocarcinoma (PDAC). Specifically, 14 different pathways specific for one type of cancer were extracted from the Kyoto Encyclopedia of Genes and Genomes (KEGG)<sup>[1-3]</sup>. In order to analyze the influence of such a pathway complexity on 5-year survival rates, a metrics for network complexity (node degree entropy) has been used to perform correlation analyses. Prostate cancer was excluded from this analysis due to its highly differentiated phenotype and slow growth. The remaining 13 types of cancer show a high correlation between the 5-year survival rate and the node degree entropy of the corresponding network ( $R^2 = 0.7$ ), e.g. pancreatic cancer with the shortest 5-year survival rate (5.5%) has a high node degree entropy ( $H = 2.05$ ) whereas thyroid cancer showing the highest 5-year survival rate (97.2%) has a low entropy ( $H = 1.48$ ). The authors concluded that complex structured networks generally point to a worse survival rate than simple structured networks. Moreover, they suggest intensifying research on network metrics in the context of survival probabilities and other clinical observations. Indeed, pancreatic cancer is an aggressive cancer entity with a very

complicated cancer signaling network. Although previous genome-wide sequencing efforts have identified a complex network of 12 core signaling pathways influencing the aggressive behavior of pancreatic cancer, it is not known how these 12 core pathways are coordinated or whether there are central players by which the pathways can be interconnected<sup>[4]</sup>. Assuming that the central players serve as connective 'linkers' within complex signaling networks, application of existing knowledge from protein-protein interaction analysis would reduce the complexity of networks, and would therefore help to uncover central players. To this end, Breitzkreutz *et al.*<sup>[11]</sup> analyzed protein-protein interaction networks of the individual specific cancer pathways extracted from KEGG. As many biological networks are scale-free, network analysis would focus on nodes with a high impact. Because node impact is not just given by its network degree, but by its property to connect different nodes or sub-networks, the authors use the betweenness centrality measure for further analysis. The betweenness centrality of a node is the proportion of the shortest paths in the network that include the node. Accordingly, nodes with a high betweenness centrality can be considered as potential therapeutic targets. For each network, the three nodes with the highest betweenness centrality were identified. This analysis yielded three candidate genes for pancreatic cancer consisting of *KRAS*, *JAK1* and *RALBP1*.

The network analysis suggests that *KRAS*, *JAK1* and *RALBP1* play an important role in mediating signal cross talks between different pathways in PDAC. Indeed, nearly all PDAC harbor oncogenic *KRAS* mutations, and *KRAS* mutations can also be detected in chronic pancreatitis and various early cancer lesions, such as pancreatic intraepithelial neoplasia, acinar-ductal metaplasia or cystic lesions<sup>[5,6]</sup>. Therefore, it is not surprising that *KRAS* has been identified by such analysis. However, *KRAS* mutations are neither a reliable prognostic marker nor a predictive biomarker for therapy, in as much as clinical trials targeting the *KRAS* signaling pathway do not show encouraging results<sup>[7]</sup>. Nevertheless, patients without *KRAS* mutations show a favorable response to combination treatment with gemcitabine and erlotinib<sup>[8]</sup>.

Mouse models of pancreatic cancer suggest that oncogenic *Kras* mutation, pancreas-specifically (starting during embryogenesis) expressed from its endogenous locus, initiates alone the development of invasive PDAC albeit at a low efficiency. A 'second hit' such as loss of a tumor suppressor or the initiation of inflammation is required to increase the rate of/accelerate malignant transformation<sup>[9,10]</sup>. These observations underscore the necessity of an interaction between the *RAS* pathway and other signaling pathways in driving the formation of malignant pancreatic tumors. In addition, they also imply that *KRAS* effectors are widely 'connected' and have a broad biological effect on tumor behavior. A downstream target of the *Ras* GTPase is *RALBP1*, the second protein identified by the protein-protein network analysis. The protein is involved in the cellular stress response and is

over expressed in several cancers in which it protects transformed cells from apoptosis and mediates resistance to various drugs<sup>[11,12]</sup>. Indeed, *RALBP1* has been considered as a prognostic biomarker in colorectal cancer and high expression of *RALBP1* is associated with shortened overall survival and early relapse<sup>[13]</sup>. *In vitro* studies of *RALBP1* inhibition demonstrate reduced tumor cell proliferation and enhanced apoptosis in non-small cell lung cancer cells<sup>[14]</sup>. Furthermore, *RALBP1* was identified as a possible mediator of metastatic invasion in PDAC<sup>[15]</sup>. Whether *RALBP1* may constitute a potential drug target or a prognostic biomarker in PDAC is unclear.

The third candidate gene is *JAK1*, which has previously been shown to have pro-tumorigenic effects. *JAK1* plays an important role in transmitting inflammatory signals through nuclear factor- $\kappa$ B signaling into epithelial cells. In general, inflammation signaling extensively interacts with oncogenic *KRAS* signaling and promotes the development of PDAC<sup>[16,17]</sup>. However, the exact role of *JAK1* in this context remains unknown. A clinical trial of a *JAK1* inhibitor demonstrated that *JAK1* may be a target for myelofibrosis because treatment reduced the level of inflammatory cytokines and improved systemic symptoms<sup>[18]</sup>. Hence, this data suggest that *JAK1* inhibition affects inflammatory processes. Additionally, *in vitro* studies revealed decreased tumor cell proliferation and activated apoptosis of glioblastoma cells and multiple myeloma cells following *JAK1* inhibition<sup>[19,20]</sup>. However, further investigation is necessary to uncover the potential link between *KRAS* and *JAK1* as well as the potential of *JAK1* as a prognostic marker or a drug able target in PDAC.

In conclusion, the study by Breitzkreutz *et al.*<sup>[11]</sup> reveals that *KRAS*, *RALBP1* and *JAK1* may constitute a biochemical network which coordinates the malignant behavior of cancer cells. Further analysis of this network may yield novel cancer biomarkers and therapy targets.

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## Clinical importance and surgical decision-making regarding proximal resection margin for gastric cancer

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### Abstract

Because of the intramural spread of gastric cancer, a sufficient length of a resection margin has to be attained to ensure complete excision of the tumor. There has been debate on an adequate length of proximal resection margin (PRM) and its related issues. Thus, the objective of this article is to review several studies on PRM and to summarize the current evidence on the subject. Although there is some discrepancy in the recommended values for PRM between authors, a PRM of more than 2-3 cm for early gastric cancer and 5-6 cm for advanced gastric cancer is thought to be acceptable. Once the margin is confirmed to be clear, however, the length of PRM measured in post-operative pathologic examination does not affect the patient's survival, even when it is shorter than the recommended values. Hence, the recommendations for PRM length should be applied only to intraoperative decision-making to prevent positive margins on the final pathology. Given that a negative resection margin is the ultimate goal of determining an adequate PRM,

development and improvement of reliable methods to confirm a negative resection margin intraoperatively would minimize the extent of surgery and offer a better quality of life to more patients. In the same context, special attention has to be paid to patients who have advanced stage or diffuse-type gastric cancer, because they are more likely to have a positive margin. Therefore, a wider excision with intraoperative frozen section (IFS) examination of the resection margin is necessary. Despite all the attempts to avoid positive margins, there is still a certain rate of positive-margin cases. Since the negative impact of a positive margin on prognosis is mostly obvious in low N stage patients, aggressive further management, such as extensive re-operation, is required for these patients. In conclusion, every possible preoperative and intraoperative evaluation should be thoroughly carried out to identify in advance the patients with a high risk of having positive margins; these patients need careful management with a wider excision or an IFS examination to confirm a negative margin during surgery.

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**Key words:** Resection margin; Proximal resection margin; Negative resection margin; Positive resection margin; Gastrectomy; Gastric cancer

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### INTRODUCTION

Although there have been great improvements in the diagnosis and treatment of gastric cancer, it remains a major health problem as the fourth most common



cancer and the second leading cause of cancer death worldwide<sup>[1,2]</sup>. Complete resection with negative surgical margins along with lymph node dissection has been accepted as the only possibly curative treatment for gastric cancer. The gross resection margin should be somewhat far from the edge of the mass to avoid the possibility of cancer involvement at the line of resection, because tumor cells spread intramurally beyond the macroscopically detectable boundaries of the lesion. Surgeons are also concerned about the possibility of recurrences with a short distance between tumor mass and resection margins. Hence, they try to remove the tumor completely with a wide range of normal stomach.

The adequate length for the required resection margin to be obtained during gastrectomy has been debated, but it is important because it determines the extent of the operation. Although there have been several studies on the sufficient length of margins that guarantees tumor-free resection and prevents local recurrences<sup>[3-9]</sup>, a definite consensus has not yet been reached, especially about the proximal resection margin (PRM). Furthermore, it is hard to secure the recommended length of PRM in some patients, and the appropriate management for these patients is still controversial.

The purpose of this article is to review issues and controversies about the importance of safe margins, an adequate length of PRM, and how to deal with the patients with insufficient PRM.

## SIGNIFICANCE OF NEGATIVE RESECTION MARGIN

According to the criteria of the International Union against Cancer/American Joint Committee on Cancer, curative (R0) surgery is defined as *en bloc* resection of primary tumor and complete lymphadenectomy without microscopic or macroscopic residual disease<sup>[10]</sup>. Thus, a microscopically negative resection margin is a prerequisite for R0 resection. To clearly discuss margin status in this article, we defined the 'positive resection margins' as the presence of viable tumor cells at the line of resection on the postoperative pathologic examination even in gastrectomy with curative intent with or without an intraoperative frozen section examination. Furthermore, the term, 'unintended positive resection margin', was used in the case of tumor involvement at the resection margin on the final pathologic examination, although it was thought to be negative by an intraoperative frozen section examination (false negative). We also defined the 'negative resection margins' as the absence of both macroscopic and microscopic tumor involvement at the resection line confirmed by the final pathologic examination.

Not surprisingly, most studies have demonstrated that a positive margin is an independent unfavorable factor for patients who have had a gastrectomy<sup>[11-21]</sup>. In this section, the negative impact of a positive margin on tumor recurrence and patient survival was analyzed in detail.

## Impact of positive resection margins on recurrences

Recurrence following curative surgery is a critical problem for patients with gastric cancer, because most patients die within the first year after diagnosis of recurrence and the mean survival time has been reported to be only 8.7 mo<sup>[22]</sup>. Since any residual tumor cells at the resection lines may contribute to a recurrence, it is not unexpected that patients with positive margins have more recurrences than those with negative margins<sup>[3,18,19]</sup>. In detailed analysis, however, there are several interesting issues.

First, recurrences do not always develop in all patients whose resection margins have remaining cancer cells on microscopic examination. This phenomenon can be partly explained by the successful eradication of these cells by postoperative adjuvant therapies which are performed in some patients with positive margins<sup>[5]</sup>. Furthermore, a few residual cancer cells could be eliminated by the patient's own immune system or poor blood supply at the resection margin<sup>[17,20]</sup>. Another possibility is that tumor cells are involved only in diagnostic resection margins but not in the true surgical margins. A discrepancy between diagnostic margins and true surgical margins due to the removal of the stapled resection lines before histological examination might lead to misinterpretation of margin status<sup>[20]</sup>.

Second, locoregional recurrence is not always the most common type of relapse in positive-margin patients. Clinically, recurrences are classified as locoregional, peritoneal, or distant. A locoregional recurrence is defined as any cancer recurrence in the gastric bed, upper abdominal retroperitoneal lymph nodes or at the local anastomotic sites<sup>[18]</sup>. Of the three patterns of recurrence, the locoregional type seems to be the most affected by positive margins, in that residual tumor cells can grow and lead to a recurrence at that location. However, Wang *et al.*<sup>[21]</sup> demonstrated that distant metastasis constituted the most common site of recurrence in positive-margin patients, whereas the rate of locoregional recurrence was the lowest. Considering that locoregional is reported to be the most common recurrence pattern in negative-margin patients<sup>[23,24]</sup>, these results are very interesting, because negative margins resulted in more locoregional recurrences but positive margins resulted in more distant recurrences. Since positive-margin patients are likely to suffer from more aggressive cancer which frequently results in distant or peritoneal recurrences, they might have more distant or peritoneal recurrences than locoregional. Consequently, the aggressiveness of the cancer rather than margin status is what really affects the recurrence patterns in positive-margin patients. Nonetheless, another group showed somewhat different results, which suggested the possible contribution of residual tumor cells at a resection line to locoregional recurrence<sup>[18]</sup>. Hence, the relationship between positive margins and locoregional recurrence remains in dispute and further studies are needed.

Third, in one study that compared the recurrence patterns by pT, pN, and tumor-node-metastasis (TNM)

**Table 1** Effects of positive margins on survival

Ref.	Inclusion	n	Effect of positive margins on survival		
			All patients P value	Subgroup analysis Nodal status	P value
Kim <i>et al</i> <sup>[11]</sup>	GC	619	< 0.0001	≤ 5 LNI	0.0001
				> 5 LNI	NS
Cascinu <i>et al</i> <sup>[19]</sup>	AGC	259	Significant	Node negative	0.001
				Node positive	NS
Cho <i>et al</i> <sup>[16]</sup>	AGC	2740	0.0028	Node negative	0.0001
				Node positive	0.259
Sun <i>et al</i> <sup>[16]</sup>	GC	2728	< 0.001	N0	< 0.001
				N1	0.007
				N3, N4	NS
Morgagni <i>et al</i> <sup>[17]</sup>	GC	89	< 0.0001	N0	0.001
				N1	0.003
				N2	0.009
				N4	NS

GC: Gastric cancer; AGC: Advanced gastric cancer; NS: Not significant; LNI: Lymph node involvement.

stage, higher recurrence rates for positive-margin patients were seen only in pT1-2, pN0-1, and stage I - II cancer<sup>[18]</sup>. In other words, the margin status did not affect recurrence rates in the patients with T3-4, N2-3, and stage III-IV cancer. The effects of a positive margin seem to be masked by the aggressiveness of the cancer, which is thought to have a strong influence on recurrences in advanced-stage patients. Thus, these patients might not benefit from negative resection margins, although every effort to make margins clean should still be made for curative surgery<sup>[25]</sup>.

### Impact of positive resection margins on survival

In the registry study by the American College of Surgeons, the 5-year survival rate of the patients with microscopically clear margins was 35% and 13% in those with positive margins<sup>[26]</sup>. Poorer survival of positive-margin patients was also reported by others<sup>[27-29]</sup>. As summarized in Table 1, many studies have demonstrated the negative predictive value of positive margins on survival. P-values for this association were always significant for the entire population. In subgroup analysis, however, positive margins were associated with poor survival only in the patients with low N stage gastric cancer<sup>[11,16-18]</sup>. The association was not significant for those who had many tumor-involved lymph nodes, possibly because the adverse effects of positive margins might be overwhelmed by the more detrimental impact of nodal metastasis on survival.

A similar tendency was seen after stratifying T stage. The negative impact of positive margins was limited to T1-2 stage patients<sup>[18]</sup>. Accordingly, this seems to impact patients with either low N stage or low T stage<sup>[30]</sup>. On the contrary, some authors reported discordant results with regard to T1 or early gastric cancer (EGC)<sup>[5,17,31]</sup>. Since EGC patients with positive margins had a good survival rate in their studies, they argued that a positive margin was not a significant adverse factor for EGC

patients. Their good survival was explained by limitation of laterally spreading T1 cancer along the resection line which lacked a good blood supply<sup>[17]</sup>. Putting all this together, the negative predictive value of positive margins is prominent in lower T stage disease, while it is still controversial in EGC (T1) patients.

Furthermore, when it comes to overall TNM stage, the predictive value of positive margins is less clear. This is because some have concluded that the margin involvement leads to poorer survival only in patients with overall stage I and II cancer<sup>[18,32]</sup>, whereas others have described different results<sup>[17,21]</sup>.

In conclusion, although margin status is an important prognostic factor for survival after gastrectomy, subgroup analyses revealed that this effect was restricted to early stage patients, especially those with minimal or no nodal involvement. Therefore, it is reasonable to consider the stage of cancer in predicting survival of patients with positive margins and plan further management for them. For example, positive margins in N0-N1 patients should be regarded as a more serious condition which needs aggressive retreatment. Furthermore, if N0-N1 stage is suspected before or during surgery, it would be better to avoid positive margins at all costs, including using a wide excision and an intraoperative frozen section (IFS) examination of margins.

### Predictors of positive resection margins

Many predictors of positive margins after curative resection of gastric cancer have been elucidated. Larger tumor size, higher T stage, higher N stage, higher overall stage, Borrmann type 4, diffuse histologic type, positive lymphatic vessel invasion, and upper tumor location were found to be associated with a higher probability of resection line infiltration by tumor cells<sup>[11,16-19,21]</sup>. On multivariate analysis, higher T stage, higher N stage, larger tumor size, and diffuse histologic type were significant independent predictors for a positive margin<sup>[16-18,21]</sup>. Surgeons should be more cautious about margin involvement when treating patients with these characteristics. Thus, thorough preoperative evaluations by an endoscopist, radiologist and pathologist are needed to determine the properties of the cancer and predict the risk of the patient having positive margins. Additionally, an IFS examination is also recommended for these high risk patients to prevent positive margins; this will be discussed later in this article. These preoperative and postoperative efforts are important as they have reduced the rates of positive margins in Japan<sup>[33]</sup>.

## ISSUES REGARDING PROXIMAL RESECTION MARGIN

### Why is the proximal resection margin a problem?

Surgeons try to remove gastric cancer completely with negative resection margins to reduce the risk of recurrences, which can result from even a few residual cells. For a gastrectomy to be curative, a sufficient distance

**Table 2** Studies on an adequate length of proximal resection margin in gastric cancer

Ref.	Characteristics	RLPRM	Brief results of the study
Bozzetti <i>et al</i> <sup>[4]</sup>	without SI with SI	≥ 3 cm ≥ 6 cm	No positive margin if gross PRM ≥ 3 cm No positive margin if gross PRM ≥ 6 cm (0% if PRM ≥ 6 cm vs 7% if PRM < 6 cm)
Ito <i>et al</i> <sup>[7]</sup>	Cardia T1, T2 T3, T4	≥ 4 cm ≥ 6 cm	No positive margin if gross PRM ≥ 4 cm No positive margin if gross PRM ≥ 6 cm
Papachristou <i>et al</i> <sup>[3]</sup>	Gastric cancer	≥ 6.5 cm	Median length of gross PRM in patients with or without recurrences: 6.5 cm vs 3.5 cm, respectively
Kim <i>et al</i> <sup>[36]</sup>	Upper third	≥ 2 cm	Recurrences: 8.2% (PRM > 2 cm) vs 14.5% (1-2 cm) and 30% (< 1 cm), <i>P</i> = 0.024
Ha <i>et al</i> <sup>[6]</sup>	EGC AGC	- ≥ 3 cm	PRM did not affect survival if margins were negative Recurrences: 32.9% (PRM ≥ 3 cm) vs 37.6% (< 3 cm), NS; 5-yr survival: 57% (PRM ≥ 3 cm) vs 46% (< 3 cm), <i>P</i> = 0.02

RLPRM: Recommended length of proximal resection margin; PRM: Proximal resection margin; SI: Serosa infiltration; NS: Not significant; EGC: Early gastric cancer; AGC: Advanced gastric cancer.

from the gross lesion to any surgical margin is necessary because of the following reasons. First, a grossly normal resection margin, determined by intraoperative inspection or palpation, is often insufficient to ensure pathologic clearance due to intramural spread of gastric cancer. Second, surgeons are concerned about the high probability of recurrence if the distance between the tumor and resection margin is short. Hence, complete resection of the tumor mass with a wide margin of normal stomach is required. There have been a number of studies and recommendations on the sufficient length of proximal and distal resection margins (PRM and DRM, respectively), which aimed to guarantee negative margins on final pathologic examination and to prevent recurrences after gastrectomy<sup>[3-7,34,35]</sup>. These references can help surgeons to decide the extent of surgery in the operative field by making them confident of negative margins whenever following the recommendations.

DRM has been generally determined as at least 2 to 4 cm distal to the pylorus<sup>[5,8,9]</sup>. More debate on an adequate length of DRM is meaningless, because it must be proximal to the orifice of the common bile duct and pancreatic duct no matter how long a DRM we want to secure. If a tumor requires a longer DRM that includes the orifice, it is likely to be metastatic disease and the surgical option needed is no longer gastrectomy alone.

On the other hand, the adequate length of PRM is more variable and there is still inconsistency in specific recommended values between authors<sup>[3-7]</sup>. To what extent the grossly normal stomach tissue needs to be excised proximally is important, because this is critical in deciding the type of resection. For example, for a tumor located in the middle part of the stomach, the length of PRM that surgeons try to achieve determines whether a total gastrectomy (TG) or distal gastrectomy (DG) should be performed. In addition, the recommendations regarding the way to manage the patient who has a shorter PRM postoperatively than was originally intended during surgery is another important issue. In this section, we will introduce several studies about PRM and discuss the related problems.

### Studies on the adequate length of proximal resection margin

Table 2 is a summary of various studies that have suggested an adequate length of PRM for a gastrectomy. Authors recommended such values either to ensure negative margins on final pathologic exam or to prevent recurrences which were thought to be a result of insufficient distance between gross resection margin and the lesion. Bozzetti *et al*<sup>[4]</sup> and Ito *et al*<sup>[7]</sup> documented that there were no positive-margin cases if gross PRM was longer than certain figures, and they recommended them as adequate lengths of PRM for negative margins. Other authors have compared the rate of recurrences and survival according to the length of PRM and suggested proper cut-off values that provided a significantly low rate of poor outcomes<sup>[3,6,36]</sup>.

Recent guidelines from the Japanese Gastric Cancer Association<sup>[35]</sup> recommended 2 cm or more gross PRM for T1 gastric cancer, 3 cm or more gross PRM for T2 or deeper tumors with an expansive growth pattern, and 5 cm or more gross PRM for T2 or deeper tumors with an infiltrative pattern. When these rules cannot be observed, the guidelines advised to examine the PRM by IFS.

Although the values for each situation were somewhat different between the studies, similarities were also found. First, a longer PRM was needed for more advanced or aggressive cancer. Three to six centimeters of gross PRM for advanced or aggressive gastric cancer was generally recommended, while 2-3 cm of PRM was adequate for EGC. Second, recommended lengths differed by the characteristics of the tumor, such as T stage, histologic type, and location. An infiltrative type of gastric cancer requires a longer PRM.

### Determining an adequate length of proximal resection margin: How long is safe?

One of the most important reasons for the efforts to determine an adequate length of PRM and to try to achieve it during surgery is to obtain negative resection margins in all cases. An adequate length of PRM, however, is emphasized not only to ensure negative

margins. Many surgeons are anxious about a short PRM, even when it is confirmed to be negative in the final pathologic exam. This is because a short PRM has been associated with more recurrences and poorer survival. The median length of gross PRM in patients with recurrences was found to be 3.5 cm *vs* 6.5 cm in patients who did not develop recurrences<sup>[3]</sup>. Kim *et al*<sup>[36]</sup> documented that a PRM shorter than 2 cm resulted in a higher rate of recurrences in patients with upper gastric cancer. Furthermore, the survival rate of AGC patients was lower if PRM was less than 3 cm in the final pathological examination<sup>[6]</sup>. According to these studies, a short PRM itself seemed to negatively affect the patient's outcome. Nevertheless, we have to be careful about the interpretation of these findings for two reasons. First, the PRM tended to be short in patients with advanced stage cancers in which poor prognosis was expected. This means the aggressiveness of the cancer in cases with a short PRM could be the real cause of a poor outcome, and thereby could serve as a confounding factor when assessing the correlation between a short PRM and adverse outcome. Second, the group of patients who had inadequate PRM included positive-margin cases. Poorer outcomes seen in that group might be partially attributed to these positive-margin patients. Therefore, it has been hard to know the pure effect of the length of PRM on prognosis.

Recently, some authors have found that the length of PRM measured by final pathology did not affect the 5-year survival rates if a negative margin was obtained<sup>[37,38]</sup>. These results could, to some degree, answer the question about the true impact of PRM length on prognosis. Based on these studies, the belief that short PRM would result in more recurrences and poorer survival has to be reconsidered. It also seems that the length of PRM is irrelevant if resection lines are clear on final pathology, and the concept of an adequate length of PRM should be applied to the intraoperative determination of a gross resection margin but not to the postoperative pathologic assessment.

The fundamental problem here is that no reliable method has been available thus far to ensure negative margins in the operating room, except resecting the tumor mass with a wide range of normal stomach. Therefore, current recommendations for gross PRM are still significant and any intraoperative decision about the extent of surgery has to be made in accordance with them. In some cases in which the recommended length of PRM cannot be attained during surgery, IFS examination is helpful to ensure negative margins.

### **IFS examination of PRM**

IFS examination of resection lines is commonly used to assess margin status. The accuracy of this procedure has been reported to be about 98%<sup>[39]</sup> and both sensitivity and specificity are seen to be high<sup>[40]</sup>. Some authors have encouraged the routine use of IFS examination<sup>[30,34]</sup>. Nonetheless, it is more practical to selectively perform this procedure in patients who may benefit from it, since

it is costly, time-consuming, and not always available<sup>[41]</sup>.

The most suitable candidates for IFS examination are those who have a high possibility of having positive margins, including patients with T3-T4 stage, poorly differentiated, Bormann type IV or signet ring cell type gastric cancers<sup>[42-44]</sup>. When the gross margin status is still suspicious despite acquisition of the recommended length of PRM, IFS examination will help to avoid positive margins. This technique is also used to determine the extent of surgery, providing negative margins when it is impossible to attain the recommended length of PRM. Even in this case, however, all attempts must firstly be made to achieve the recommended length of PRM, because IFS exam may give false-negative results<sup>[27,45]</sup>. An unintended positive margin, defined herein as a false-negative result of IFS exam, has been more frequently associated with signet ring cell or poorly differentiated type gastric cancers due to their extension under the submucosal layer of the gastric wall<sup>[46]</sup>. Of course, patients with unintended positive margins are also included in the positive-margin cases and need to be treated as such. Fortunately, the numbers are expected to decrease by virtue of several improvements in this procedure, such as cytokeratin immunohistochemistry<sup>[47]</sup>.

### **Inadequate proximal resection margin and re-operation**

Although surgeons have done their best to perform tumor-free resection based on the present recommendations, the prevalence of positive margins has been reported to be 0.8%-20.0%<sup>[11,19,26,29,42,48]</sup>. Also, the distance to resection margins measured intraoperatively sometimes differs from values measured in the final pathologic exams. For these reasons, the way to manage the patients with an insufficient length of PRM or a positive margin is an important issue. Do we have to re-operate these patients to provide adequate margins?

Studies have shown that if PRM is confirmed to be negative for malignancy but shorter than the recommended length, further resection to acquire a longer PRM is unnecessary, since better survival cannot be expected<sup>[37,38]</sup>.

Regarding the positive-margin cases, the necessity of re-operation depends on whether the patients will benefit from it or not. The benefits of reoperation always have to be balanced with the risks of this technically demanding procedure. In the previously mentioned studies, a negative margin improved the survival of patients with early stage cancer<sup>[16,19,30]</sup>. Hence, an extended re-operation appears to have the most obvious survival advantage in low-stage patients, especially when few nodes are involved (N0 or N1). In contrast, as advanced N stage patients with positive margins might not benefit from an extended re-excision, the decision has to be made with much deliberation. In fact, multidisciplinary options including chemotherapy and irradiation are more appropriate treatments for positive-margin patients<sup>[16,49]</sup>. Even with all options, however, the most important objective should be to prevent positive margins beforehand, by



evaluating the cancer status before and during surgery to determine the patients with a high risk of having positive margins and treating them more carefully.

### Optimal type of gastrectomy and the length of proximal resection margin

Different types of gastrectomy have been recommended for gastric cancers located in each part of the stomach. For proximally located gastric cancers, TG has been recommended as a first choice, excluding limited cases in which some authors have suggested proximal gastrectomy as an alternative<sup>[7,36,50]</sup>. DG is generally performed for gastric cancers of the lower third of the stomach, since DG showed a similar long-term prognosis, improved quality of life and lower morbidity for distal-third cancer in randomized prospective studies<sup>[51,52]</sup>.

When it comes to middle-third gastric cancers, the most appropriate procedure is controversial because of the ambiguity of their location. The issues surrounding adequate PRM greatly matter for these, because the choice between TG and DG depends on the length of PRM required. Generally, a longer PRM can be achieved by TG, whereas DG is associated with a better quality of life and similar or lower morbidity<sup>[51-55]</sup>. Of the two options, TG has been adopted as the standard treatment for middle-third gastric cancer by many surgeons who are concerned about the possibility of recurrences with a short PRM after DG. As explained above, however, the length of PRM does not impact prognosis if the lines of resection are free of tumor<sup>[37,38]</sup>. In these studies, the authors suggested DG should be the first surgical option for intermediately located gastric cancer if negative margins could be guaranteed. Furthermore, when the surgery has to be converted from DG to TG to gain a few more centimeters of PRM to obtain the recommended values, DG with IFS examination, which can provide better quality of life, is a better choice if negative margins are confirmed by the frozen exam. When doing this, there is a practical problem that the residual part of the stomach can become necrotic owing to the poor blood supply. Therefore, surgeons try to preserve a short gastric artery technically as much as possible to make it successful.

In addition, we expect that less extensive surgery can be performed more commonly in gastric cancer patients who are not eligible for DG based on the current recommendations on PRM length, if unintended positive margins can be prevented by improvement of IFS exam or if other reliable methods to confirm negative margins intraoperatively are developed.

## CONCLUSION

Since tumor infiltration at resection lines has been accepted as an adverse prognostic factor, negative resection margins are crucial components of curative surgery, which is the only currently available method offering a cure for gastric cancer. To ensure negative margins in the

final pathologic exam, a sufficient length of gross PRM is always required. Whenever surgeons try to attain 2-3 cm of gross PRM in EGC and 3-5 cm of gross PRM in AGC during the operation, positive margins should be avoided. If the final PRM examination is clear but shorter than that originally intended intraoperatively, a short PRM itself seems not to affect a patient's prognosis. Along with this principle, IFS examination of resection lines is also used to confirm margin status in various situations. If despite all attempts, however, there are still positive margins, then re-operation is reasonable, especially in those who have low N stage diseases. In conclusion, achieving a negative resection margin is the ultimate goal when determining the adequate length for PRM and debating related issues. Every possible pre-operative and intraoperative evaluation should be thoroughly carried out to find the patients with a high risk of having positive margins in advance, and subsequent careful management of these patients with a wider excision or an IFS examination to confirm a negative margin during surgery is necessary.

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In press

- 3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

- 4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

- 5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

- 6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

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Issue with no volume

- 8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; **(401)**: 230-238 [PMID: 12151900 DOI:10.1097/0000-3086-200208000-00026]

No volume or issue

- 9 Outreach: Bringing HIV-positive individuals into care. *HRS A Careaction* 2002; 1-6 [PMID: 12154804]

## Books

Personal author(s)

- 10 **Sherlock S**, Dooley J. Diseases of the liver and biliary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

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Conference proceedings

- 13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

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**Electronic journal** (list all authors)

- 15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: <http://www.cdc.gov/ncidod/eid/index.htm>

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- 16 Pagedas AC, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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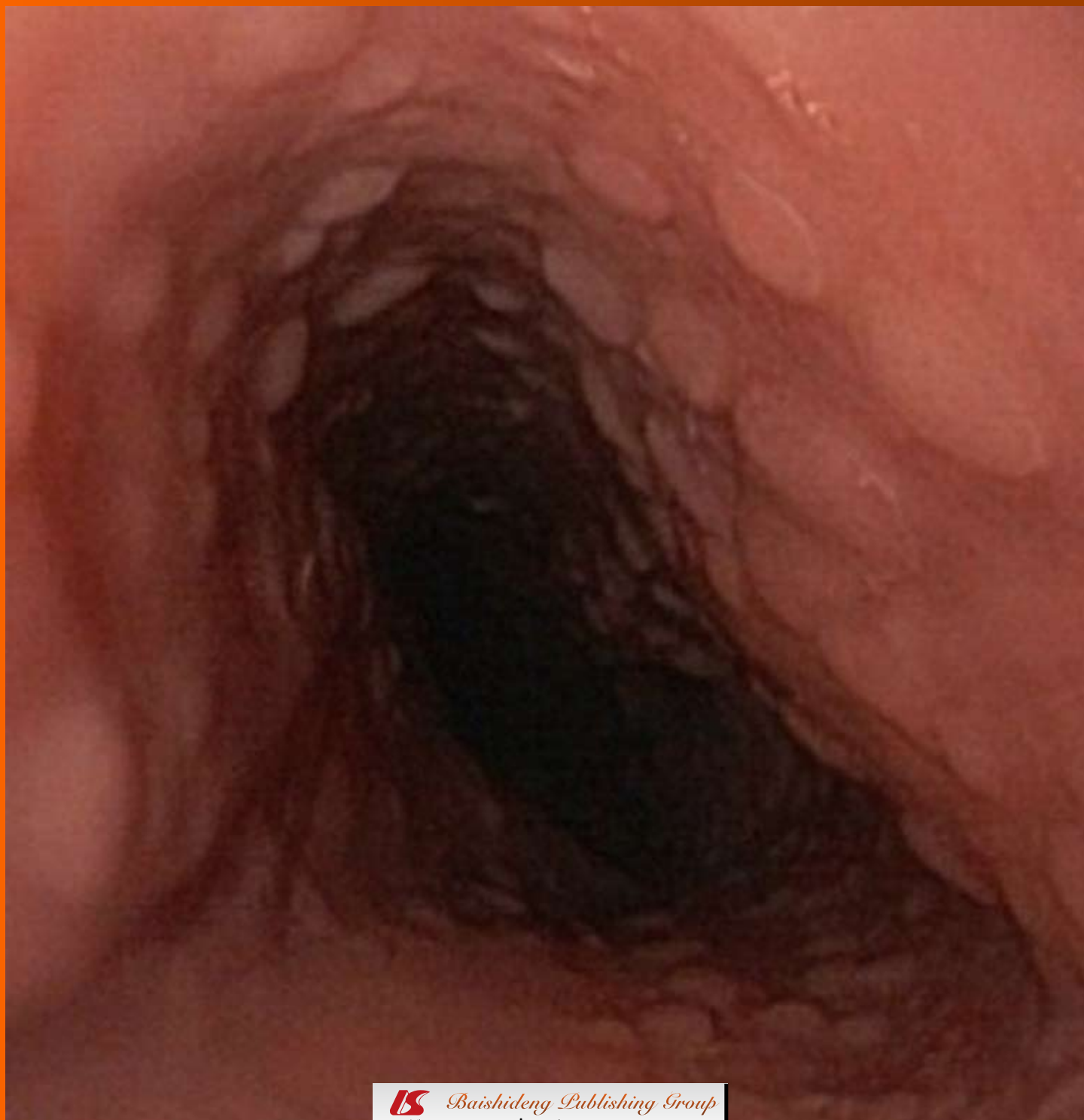
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## Era of universal testing of microsatellite instability in colorectal cancer

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are evidences that universal testing for MSI starting with either IHC or PCR-based MSI testing is cost effective, sensitive, specific and is getting widely accepted.

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**Key words:** Colorectal cancer; Lynch syndrome; Universal testing; DNA mismatch repair; Microsatellite instability

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### Abstract

Colorectal cancer (CRC) incidence and mortality are constantly decreasing, but CRC still remains the third most prevalent cancer and the third most common cause of cancer death in both males and females in the United States. Recent rapid declines in CRC incidence rates have largely been attributed to increases in screening that can detect and remove precancerous polyps, and the decrease in death rates for CRC largely reflects improvements in early detection, treatment and the understanding of molecular/genetic basis of CRC. One of the important molecular/genetic findings is the presence of microsatellite instability (MSI) in CRCs. Many studies have shown the importance of MSI testing in diagnosing Lynch syndrome and predicting prognosis and response to chemotherapeutic agents in CRCs. Increased emphasis has been placed on the importance of MSI testing for all newly diagnosed individuals with CRCs. Both immunohistochemical staining (IHC) and polymerase chain reaction (PCR)-based MSI testing show high sensitivity and specificity in detecting MSI. The current clinical guidelines and histopathology features are indicative of, but not reliable in diagnosing Lynch syndrome and CRCs with MSI. Currently, there

### INTRODUCTION

Colorectal cancer (CRC) is the third most prevalent cancer and the third most common cause of cancer death in both males and females in the United States<sup>[1]</sup>. However, widespread screening for CRC and progress in its treatment have both contributed to a recent decline in the incidence of mortality of the disease. In parallel, much progress has been made in the understanding of the molecular and genetic basis of CRC<sup>[2-4]</sup>. Chromosomal instability (CIN) and microsatellite instability (MSI) constitute the predominant tumorigenic pathways in CRC (Figure 1)<sup>[5-8]</sup>. CIN is associated with high mutation rates in genes tightly linked to the development of CRC, such as *APC*, *KRAS*, *SMAD4*, *PIKCA*, *SOX9*, *ARID1A*, *FAM123B* and *TP53*, which lead to the development of CIN tumors<sup>[5]</sup>. MSI is a form of genetic instability caused by alterations in the DNA mismatch repair (MMR) system. Although the majority of CRCs develop through the CIN pathway, approximately 15% of CRCs display MSI due to germline mutations, epigenetic silencing of *MMR* gene or a combination of these factors<sup>[9]</sup>.

Germline mutations in *MMR* genes cause a cancer susceptibility syndrome called Lynch syndrome, previ-



ously referred to as hereditary nonpolyposis CRC. These individuals are predisposed to CRC and multiple other cancers including endometrial, gastric, ovarian, urothelial, hepatobiliary tract, brain, small intestine, pancreatic, and skin (specifically sebaceous adenomas or carcinomas and benign keratoacanthomas) cancers<sup>[10-12]</sup>. Approximately 90% of CRCs occurring in Lynch syndrome patients exhibit MSI. The exact prevalence of Lynch syndrome among CRCs is unclear. A prospective, multicenter, nationwide study (the EPICOLON study), consisting of patients newly diagnosed with CRC in 20 community hospitals in Spain, showed the prevalence was only 0.9% compared with 2.9%-3.5% in other studies<sup>[13,14]</sup>. A most recent study showed that the prevalence of Lynch syndrome is 3.1% in all CRCs<sup>[15]</sup>. Many studies have found that some CRCs occurred in non-Lynch syndrome patients also showed MSI (sporadic CRCs with MSI) and CRCs with MSI showed different clinical-pathological features, prognosis and response to chemotherapeutic agents comparing to microsatellite-stable CRCs<sup>[9,16]</sup>. Increased emphasis has been placed on the importance of MSI testing for all newly diagnosed individuals with CRCs.

## MOLECULAR BASIS OF THE MMR SYSTEM

The human genome is dynamic. It is estimated that each cell undergoes > 20 000 DNA damaging events and > 10 000 replication errors per cell per day<sup>[17]</sup>. One of the mechanisms to repair replication errors is the MMR system. The MMR system, a DNA repair pathway which is conserved from bacteria to humans, targets base-base mismatches and insertion-deletion mismatches that arise as a result of replication errors<sup>[18]</sup>. A proficient MMR system enhances replication accuracy 1000-10 000-fold<sup>[16]</sup>. A hallmark of MMR-deficient cells is instability (replication errors) at microsatellite regions. Microsatellites are mono-, di-nucleotide or higher-order nucleotide repeats such as (A)<sub>n</sub> or (CA)<sub>n</sub> that are distributed throughout the entire genome, and due to their repetitive pattern, they are prone to errors during DNA replication. The terminology used for the MMR system in eukaryotes is based on the analogous system in prokaryotes, best characterized in *Escherichia coli* (*E. coli*)<sup>[16]</sup>. The major *E. coli* MMR proteins include MutS and MutL<sup>[19]</sup>. Eukaryotic *MutS* homologs include *MSH2*, *MSH3* and *MSH6*, and are primarily responsible for recognizing mismatches and recruiting MutL to the mismatch location. *MutL* homologs include *MLH1*, *PMS1* and *PMS2*. Eukaryotic cells possess two MutS activities that function as heterodimers and share MSH2 as a common subunit: MutS $\alpha$  (MSH2-MSH6 heterodimer) and MutS $\beta$  (MSH2-MSH3 heterodimer). Eukaryotic MutL activities also function as heterodimeric complexes with MLH1 serving as a common subunit including MutL $\alpha$  (MLH1-PMS2 heterodimer), MutL $\beta$  (MLH1-PMS1 heterodimer) and MutL $\gamma$  (MLH1-MLH3 complex)<sup>[20]</sup>. Specifically, when a mismatch exists, MSH2 will form a MutS $\alpha$  or MutS $\beta$  complex. Both MutS $\alpha$  and MutS $\beta$  can then recruit either

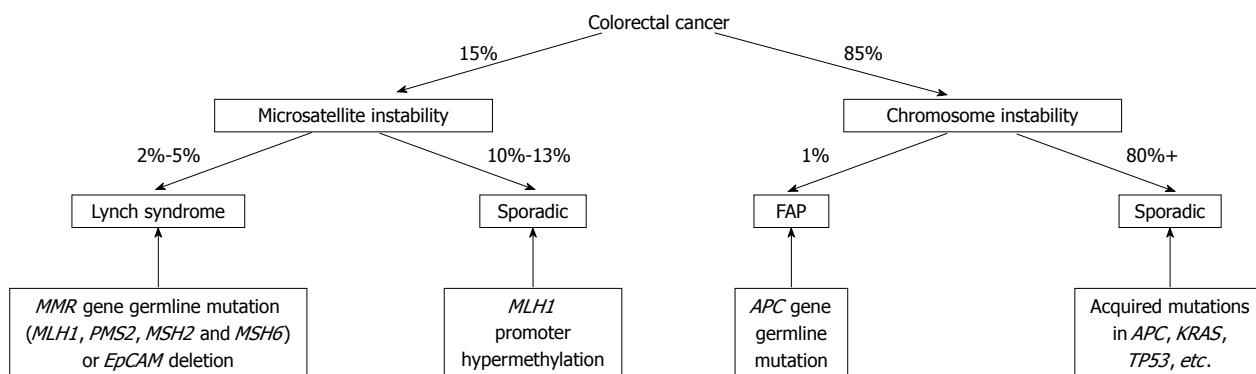
MutL $\alpha$ , MutL $\beta$ , or the MutL $\gamma$  complex, which in turn will mediate the processes of mismatch recognition and enzymatic repair<sup>[9,16,19,20]</sup>. Mutations in the MMR system lead to the accumulation of errors in DNA, which results in MSI.

## GENETIC BASIS FOR LYNCH SYNDROME AND SPORADIC CRCs WITH MSI

Lynch syndrome is a genetically heterogeneous disorder which is caused by autosomal dominant germline mutations in *MMR* genes. The overall risk of CRCs in individuals with this syndrome is 75% by the age of 70 years and cancers occur predominantly in the right side of the colon. The mean age at diagnosis of CRC in individuals with Lynch syndrome is younger (approximately 42-61 years) than that in the general population (approximately 65 years). Mutations in *MLH1*, *MSH2*, *MSH6* and *PMS2* are found in 32%, 38%, 14% and 15% of Lynch syndrome cases, respectively<sup>[11,21]</sup>. Individuals with mutations in the *MSH6* and *PMS2* genes have a somewhat lower risk of CRC and a later age of onset of CRC compared with individuals with mutations in the *MLH1* and *MSH2*<sup>[10,22,23]</sup>. Endometrial carcinoma is the most common extra-colonic carcinoma in Lynch syndrome and occurs in 28%-60% of women with an average age at diagnosis of 47-55 years, compared to a sporadic rate of 2%-3% in women with an average age at diagnosis in the mid 60s in the general population<sup>[10]</sup>. Endometrial cancers are more frequently associated with mutations in the *MSH2* and *MSH6* genes than the *MLH1* or *PMS2* genes.

Recently, deletions of the epithelial cell adhesion molecule (*EpcAM*) gene (previously known as *TACSTD1*, tumor-associated calcium signal transducer 1), which is located upstream of *MSH2*, have been described in a subset of families with Lynch syndrome<sup>[24]</sup>. Deletions affecting the 3' exons of the *EpcAM* gene lead to a transcriptional read-through and mediate epigenetic silencing of the *MSH2* allele in a mosaic pattern. Therefore CRCs in individuals with heterozygous germline *EpcAM* deletions will be *MSH2*-negative MSI cancers<sup>[25]</sup>. Though frequency of *EpcAM* deletions have been reported in different populations<sup>[24-26]</sup>, further research is needed to confirm the prevalence and clinical phenotype of *EpcAM* deletions.

MSI CRCs that are not associated with germline mutations in the MMR system and Lynch syndrome are commonly referred to as "sporadic CRCs with MSI". Sporadic CRCs with MSI account for about 10%-13% of CRCs with MSI. The most frequent cause of sporadic MSI is acquired promoter hypermethylation of *MLH1*. Hypermethylation of CpG islands in the promoter regions of both copies of *MLH1* gene leads to inactivation of the gene and loss of expression of the *MLH1* gene product in a manner analogous to the germline mutations of DNA MMR genes seen in Lynch syndrome. These sporadic CRCs with MSI are similar histologically to Lynch syndrome CRCs and, like Lynch syndrome, CRCs are more likely to be located in the right colon and



**Figure 1 Molecular classification of colorectal cancer.** EpCAM: Epithelial cell adhesion molecule; MMR: DNA mismatch repair; FAP: Familial adenomatous polyposis; APC: Adenomatous polyposis coli.

they tend to have a better overall prognosis. In contrast to Lynch syndrome, however, these MSI CRCs do not present with a strong hereditary background nor occur at a young age, but tend to be more common in older population. Testing for mutations in the gene for the B-type Raf kinase (*BRAF*) can help distinguish sporadic CRCs with MSI from Lynch syndrome-associated CRCs. *BRAF*, a serine/threonine protein kinase, is an immediate downstream effector of *KRAS* in the MAP kinase signaling pathway. An activating mutation of *BRAF* is often present when the promoter region of the *MLH1* gene is methylated. About 90% of the mutations in the *BRAF* gene in CRCs are transversion (1799 T>A), identified as V600E. Recently, reviewing the *BRAF* V600E mutation in 4562 tumors from 35 studies and *MLH1* promoter methylation in 2975 tumors from 43 studies, Parsons *et al.*<sup>[27]</sup> demonstrated that the *BRAF* V600E mutation occurred in 63.5% of CRCs displaying *MLH1* promoter hypermethylation or *MLH1/PMS2* protein loss. The frequency of *BRAF* V600E mutation in MSS CRCs was only 5.0%. More importantly, *BRAF* mutations are virtually absent in Lynch syndrome-associated tumors, and this is a very useful feature for distinguishing Lynch syndrome from sporadic CRCs with MSI. Evidence of *MLH1* promoter hypermethylation or a *BRAF* V600E mutation is highly predictive of a sporadic CRC with MSI. Individuals with unmethylated *MLH1* promoter and wild type *BRAF* should undergo further testing for Lynch syndrome. However, there are rare case reports of hypermethylation of the *MLH1* promoter as the “second-hit” in a patient with a germline mutation<sup>[22,23]</sup>.

## DETECTION OF MSI

Currently, MSI is detected indirectly by demonstrating absence of expression of MMR proteins by immunohistochemical staining (IHC), or more directly by polymerase chain reaction (PCR)-based amplification of specific microsatellite repeats.

### IHC of MMR proteins

The principle of using IHC of MMR proteins to indirectly indicate the presence of MSI is that the absence

of one or more of the MMR proteins can cause MSI. Antibodies against MMR proteins such as MLH1, PMS2, MSH2 and MSH6 are commercially available and can be used to provide information of functionality of the MMR system. Loss of expression and the pattern of loss of expression of one or more of these proteins suggest deficient MMR, and indicate which gene harbors a germline mutation or has been inactivated by hypermethylation. As mentioned earlier, eukaryotic MMR proteins form functional heterodimers. MSH2 dimerizes with either MSH6 or MSH3, and then recruits heterodimers of MLH1 and PMS2 or MLH1 and PMS1 to excise the mismatched nucleotides. MSH2 and MLH1 proteins are the common subunits of their respective heterodimeric complexes, and when mutated, a loss of both the common subunits and their associated partner proteins by IHC is typically observed. However, the opposite is generally not true, since other proteins, such as MSH3, MLH3 and PMS1, may bind to the common subunits to stabilize them. Loss of staining of MSH6 or PMS2 alone is typically observed with germline mutations in each of these respective genes but with retained positive staining of corresponding MSH2 or MLH1. Understanding the expression patterns of MMR proteins and genetic basis of Lynch syndrome and sporadic CRCs with MSI are crucial to the interpretation of the IHC results and for guiding the further molecular analysis. For example, a CRC that fails to stain for both MLH1 and PMS2, but retains expression of MSH2 and MSH6, is due to an alteration in the *MLH1* gene. However, determining whether the deficiency of MLH1 is due to a germline mutation or promoter hypermethylation requires further investigation (*MLH1* hypermethylation test and/or *BRAF* mutation test). A CRC that shows loss of expression of both MSH2 and MSH6 is most often consistent with defective MMR through *MSH2* germline mutations (Lynch syndrome), and this finding should be followed by genetic testing of *MSH2*. As mentioned earlier, a subset of Lynch syndrome is due to deletion of *EpCAM*, a gene upstream of *MSH2*. The deletion of *EpCAM* will lead to somatic hypermethylation of *MSH2* and finally loss of expression of *MSH2*. A recent study showed that a lack of EpCAM immunostaining in *MSH2*-negative CRCs is

indicative of *EpCAM* gene alterations with a 100% specificity<sup>[28]</sup>, and also *EpCAM* negative immunostaining can be detected even at a precancerous stage<sup>[29]</sup>. Therefore, performance of *EpCAM* IHC before molecular analysis is suggested to be included in the algorithm approach to Lynch syndrome identification in *MSH2*-negative CRC cases.

### PCR-based MSI testing

The principle of using PCR-based MSI testing is to detect the presence of different lengths of specific microsatellite repeats in tumor cells comparing to normal tissues caused by mismatches due to the absence of one or more of the MMR proteins. In 1997, National Cancer Institute (NCI) workshop established a reference panel of microsatellites for clinical and research testing, and also defined the criteria for diagnosing MSI. The core panel consists of two mononucleotide repeats (*BAT25*, *BAT26*) and three dinucleotide repeats (*D5S346*, *D2S123*, *D17S250*). Nineteen “alternative loci” are also suggested. Three categories of MSI have been established based on the following criteria: MSI-high (MSI-H), indicating instability at two or more loci (or > 30% of loci if a larger panel of markers is used); MSI-low (MSI-L), indicating instability at one locus (or in 10%-30% of loci in larger panels); and MSS, indicating no loci with instability (or < 10% of loci in larger panels)<sup>[30]</sup>. MSI-L CRCs do not appear to differ clinically or pathologically from MSS CRCs, and generally MSI-L CRCs are categorized as group of MSS CRCs<sup>[31]</sup>. MSI-L cases usually only show instability for dinucleotide markers, so the assessment of dinucleotides alone could lead to the misclassification of MSI-L as MSI-H. By contrast, mononucleotides *BAT25* and *BAT26* are nearly monomorphic. In 2002, NCI workshop (the revised Bethesda guidelines) added new guidelines with recommendations of testing additional mononucleotide markers in tumors with instability at only dinucleotide loci, as mononucleotide markers are more reliable in the identification of MSI-H tumors<sup>[31]</sup>. Recent years, the uses of panels containing more mononucleotide markers and the availability of commercial kits including predominant mononucleotide markers have been improving the sensitivity and specificity<sup>[32-34]</sup>.

### Comparison of IHC and PCR-based MSI testing

The results of MMR IHC and PCR-based MSI testing have been shown to be largely concordant (97.80% concordance, exact 95%CI: 96.27-98.82)<sup>[35]</sup>. Studies have shown that IHC for the MMR proteins *MLH1*, *PMS2*, *MSH2* and *MHS6* provides a rapid, cost-effective, sensitive, and highly specific technique for screening CRC for MSI. Reviewing the IHC results of 16 series representing 3494 cases, Rigau *et al.*<sup>[36]</sup> demonstrated that the following performances of IHC in assessing MSI: sensitivity, 92.4%; specificity, 99.6%; positive predictive value, 98.5%; and negative predictive value, 97.8%, which are comparable to PCR-based molecular MSI testing. In one previous large study, IHC in CRCs for *MLH1* and *MSH2* provided a rapid, cost-effective, sen-

sitive (92.3%), and extremely specific (100%) method for screening for DNA MMR defects. The predictive value of normal IHC for an MSS/MSI-L phenotype was 96.7%, and the predictive value of abnormal IHC was 100% for an MSI-H phenotype<sup>[37]</sup>. The major advantage of IHC is that it is widely available in general pathology laboratories. Another advantage of IHC is that tumors with *MSH6* germline mutations sometimes lack MSI in PCR-based testing owing to a functional redundancy in the MMR system, but demonstrate loss of *MSH6* staining by IHC<sup>[16]</sup>. Furthermore, a key advantage to the use of IHC is its ability to guide and direct genetic testing. However, rare missense mutations, which are reported usually in *MLH1* and *MSH6* genes, affect protein function other than protein translation and antigenicity. IHC will still show positive staining despite MSI<sup>[16,22]</sup>. In these cases, PCR-based MSI testing can help to determine whether there are true functional MMR proteins through these mutations.

## ERA OF UNIVERSAL MSI TESTING

The diagnosis of Lynch syndrome and recognition of sporadic CRCs with MSI have important implications regarding cancer prevention, surveillance and management. Studies have shown that MSI-H CRCs carry a better prognosis compared to those with MSS CRCs<sup>[38]</sup>. In addition, stage II MSI-H CRCs achieved similar progression free survival and overall survival with or without 5-fluorouracil (5-FU)-based neoadjuvant chemotherapy<sup>[39]</sup>. Therefore, patients with stage II MSI-H CRC are not recommended to receive 5-FU based adjuvant chemotherapy. As mentioned earlier, individuals with Lynch syndrome have significantly higher risks of developing extra-colonic malignancies besides early onset of CRC. Intensive cancer surveillance has shown to substantially reduce cancer-related death in this group of patients<sup>[40]</sup>. Most recently, it also has shown that aspirin can be used as a chemopreventive agent in carriers of Lynch syndrome to prevent the development of CRCs and extra-colonic carcinomas<sup>[41]</sup>.

Historically, diagnosis of Lynch syndrome relied on clinical characteristics of personal and family history of cancer. The Amsterdam criteria<sup>[42]</sup>, later revised to Amsterdam II criteria<sup>[43]</sup> are now well-recognized to be too stringent and insufficiently sensitive because of small family sizes, unfamiliarity with Lynch syndrome by clinicians, lack of documentation of tumors in the family, and/or reduced penetrance of the tumors in the family. With the availability of molecular diagnostic testing, the Bethesda guidelines<sup>[44]</sup>, and then the revised Bethesda guidelines<sup>[31]</sup>, were developed to select patients who should undergo MSI analysis. These guidelines incorporated tumor histopathology features into their criteria, including the presence of tumor infiltrating lymphocytes, Crohn's-like lymphocytic reaction, mucinous/signet-ring differentiation, and/or a medullary growth pattern. However, data suggests that the clinical guidelines and histopathology features are neither sensitive nor specific



in determining the presence or absence of MSI. For example, up to 50% of mutation carriers do not meet the Amsterdam criteria and 40%-45% of families who fulfill the Amsterdam criteria do not demonstrate MSI on tumor testing or *MMR* gene germline mutations<sup>[16,23]</sup>. In an effort to improve the detection rate of Lynch syndrome individuals and sporadic CRCs with MSI, it has been suggested that all CRCs (universal testing) should be tested for MSI using either a PCR-based or an IHC approach<sup>[45]</sup>. Julié *et al*<sup>[46]</sup> compared the performance of the revised Bethesda guidelines with universal molecular testing in 214 newly diagnosed CRC patients. The revised Bethesda guidelines identified 42.1% of patients for MSI testing. Of these 4.2% were MSI positive and 6 were *MMR* mutation-positive. However, using a universal MSI testing strategy in these patients, 9.8% were found to be MSI positive and 5.1% of the MSI positive patients were *MMR* mutation-positive. Thus, the authors concluded that the revised Bethesda guidelines does not adequately identify mutation carriers and CRCs with MSI<sup>[46]</sup>. Morrison *et al*<sup>[47]</sup> compared the MSI detection rate in 445 primary CRCs resected between November 2006 and March 2009, when MSI testing was based on histopathology features and age, with the rate in 145 CRCs resected between July 2009 and July 2010 when a universal testing paradigm was used. The overall Lynch syndrome screening rate between November 2006 and March 2009 was 34.8%, and the extrapolated MSI-H rate was 8.5% (38/445). Strict adherence to the revised Bethesda guidelines, that is, without testing CRC diagnosed in patients over 60 years, would have missed 26 (68.4%) MSI CRCs. The overall Lynch syndrome screening rate between July 2009 and July 2010 was 76.3% and the MSI rate was 20.6% (30/145). These data indicated that the revised Bethesda guidelines is inadequate for Lynch syndrome screening when personal and family cancer history is not available to the pathologist, a universal screening paradigm greatly increased the rate of MSI testing and MSI CRC detection<sup>[47]</sup>. Most recently, Pérez-Carbonell *et al*<sup>[48]</sup> investigated 2093 patients with CRC from the EPICOLON I and II cohorts and found the revised Bethesda guidelines strategy failed to detect 14.3% cases with Lynch syndrome and 57.1% cases with probable non-sporadic MSI-H tumors. The authors concluded that routine screening of patients with CRC for Lynch syndrome using immunohistochemistry or PCR-based MSI testing has better sensitivity for detecting mutation carriers than the Bethesda guidelines alone<sup>[48]</sup>. Many studies have identified other histopathologic features, which are included in the revised Bethesda guidelines, such as right-sided location, lack of “dirty necrosis”, a circumscribed/expansile growth pattern, histologic heterogeneity, lack of intratumoral budding, and carcinoma associated with sessile serrated adenoma/polyp (serrated pathway) are all suggestive of MSI-H<sup>[49-52]</sup>. However, our experience and that of others have shown that around 3%-6% of CRCs with feature of “dirty necrosis” and a portion of left-sided tumors do show MSI-H, especially with MSH6 loss<sup>[22]</sup>.

Recent data have shown that testing for *MMR* expression can be performed on the diagnostic CRC biopsy samples prior to definitive surgery<sup>[53]</sup>, with results comparable to those obtained on the surgical resection specimens<sup>[54,55]</sup>. Using this approach the diagnosis of Lynch syndrome can be made preoperatively, and this information can help the surgeon in planning the operative approach (extended colectomy, subtotal colectomy, or total colectomy) and in recommending screening for cancers in other organs. Another argument for early testing for *MMR* expression is the fact that neoadjuvant chemotherapy and radiation can cause aberrant or loss of immunoreexpression of *MMR* proteins. Diminished *MMR* staining in treated tumors should prompt IHC evaluation of pretreatment biopsy samples before genetic testing is pursued for Lynch syndrome<sup>[56]</sup>.

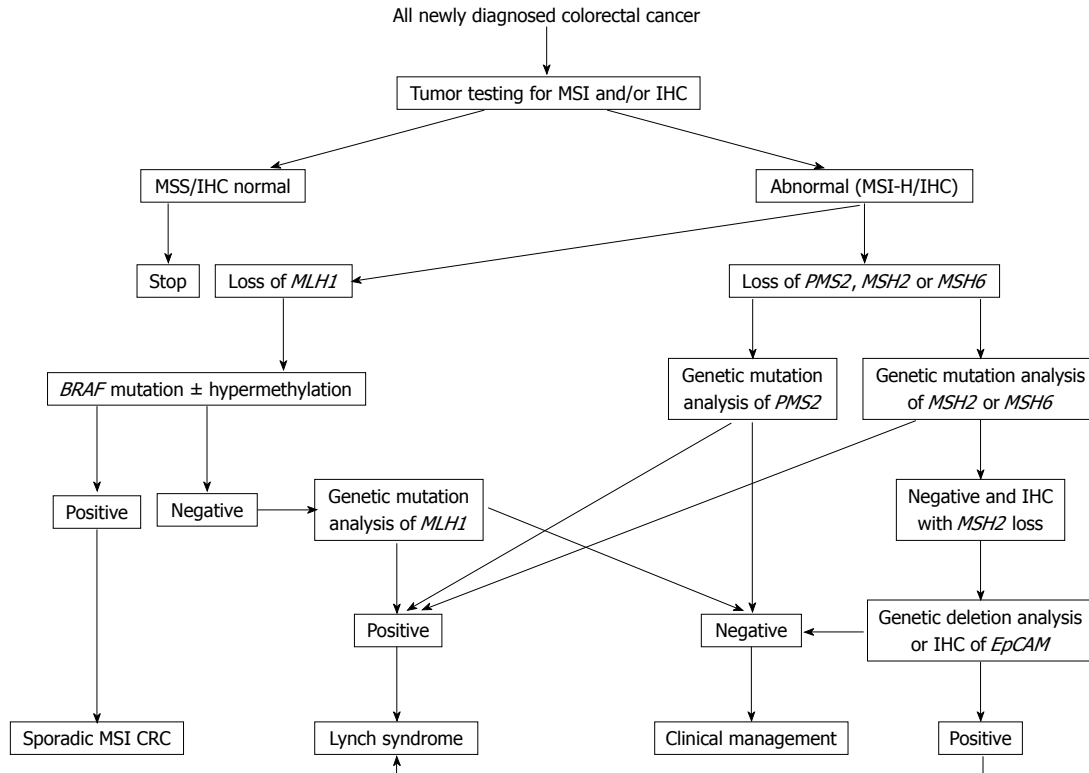
## ALGORITHM FOR MSI TESTING

Even though the increased emphasis has been placed on the importance of MSI testing and recommendations have been proposed to identify individuals at risk for Lynch syndrome<sup>[10]</sup>, and both PCR-based and IHC MSI detection are highly sensitive methods for the identification of individuals with defective *MMR*<sup>[21]</sup>, the approach of universal MSI testing for all newly diagnosed CRCs has not been widely accepted and understood. A recent survey of Canadian hospitals demonstrated that up to 21.2%, 42.1% and 38.2% of respondents either do not have access or are uncertain whether they have access to *MMR*-IHC, PCR-based MSI testing, and genetic counseling services respectively<sup>[57]</sup>. It has been demonstrated that the highest detection rate of Lynch syndrome in CRC is achieved through integrated efforts of pathologists, clinicians (surgeons, gastroenterologists, and family doctors) and genetic counselors<sup>[58]</sup>. However, only 13.1% of respondents have an integrated multidisciplinary approach to Lynch syndrome detection. A recent survey of United States hospitals reported that routine tumor testing with IHC, PCR-based MSI testing, or both is currently performed at 71% of NCI comprehensive cancer centers, 36% of American College of Surgeons-accredited community hospital comprehensive cancer programs, but only 15% of community hospital cancer programs<sup>[59]</sup>. Awareness of the importance of MSI testing and an appropriate algorithmic approach (Figure 2), starting with PCR-based MSI testing or IHC analysis on all newly diagnosed CRC specimens (universal testing) will help recognize Lynch syndrome and distinguish sporadic CRCs with MSI and Lynch syndrome effectively.

## CONCLUSION

Many studies have shown the importance of MSI testing in diagnosing Lynch syndrome and predicting prognosis and response to chemotherapeutic agents. Increased emphasis has been placed on the importance of MSI testing for all newly diagnosed individuals with CRCs. Both IHC and PCR-based MSI testing show close concordance and





**Figure 2 Testing algorithm for Lynch syndrome and sporadic microsatellite instability colorectal cancer.** MSI: Microsatellite instability; IHC: Immunohistochemical staining; CRC: Colorectal cancer; *EpCAM*: Epithelial cell adhesion molecule.

high sensitivity and specificity in detecting MSI. The current clinical guidelines and histopathology features are indicative of, but not sensitive and specific in diagnosing Lynch syndrome and CRCs with MSI. Currently, there are evidences that universal testing for MSI starting with either IHC or PCR-based MSI testing is cost effective, sensitive, specific and is getting widely accepted.

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## Current developments, problems and solutions in the non-surgical treatment of pancreatic cancer

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### Abstract

Pancreatic cancer is a common malignant neoplasm of the pancreas with an increasing incidence, a low early diagnostic rate and a fairly poor prognosis. To date, the only curative therapy for pancreatic cancer is surgical resection, but only about 20% patients have this option at the time of diagnosis and the mean 5-year survival rate after resection is only 10%-25%. Therefore, developing new treatments to improve the survival rate has practical significance for patients with this disease. This review deals with a current unmet need in medical oncology: the improvement of the treatment outcome of patients with pancreatic cancer. We summarize and discuss the latest systemic chemotherapy treatments (including adjuvant, neoadjuvant and targeted agents), radiotherapy, interventional therapy and immunotherapy. Besides discussing the current developments, we outline some of the main problems, solutions and prospects in this field.

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**Key words:** Treatment; Pancreatic cancer; Survival rate; Systemic chemotherapy; Radiotherapy; Interventional

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### INTRODUCTION

Pancreatic cancer is a malignant neoplasm of the pancreas whose prognosis is fairly poor. The incidence rate has risen in recent years and it comprises 1%-2% of common tumors. Each year about 185 000 individuals globally are diagnosed with this condition. As its symptoms are usually non-specific, pancreatic cancer is often not diagnosed until an advanced stage. The only potentially curative therapy for pancreatic cancer is surgical resection. Unfortunately, only 20% patients are resectable at the time of diagnosis. Even among those patients who undergo resection for pancreatic cancer and have tumor-free margins, the 5-year survival rate is only 10%-25%<sup>[1]</sup>. Therefore, developing new treatments to improve the survival rate has practical significance for patients with pancreatic cancer.

### SYSTEMIC CHEMOTHERAPY

#### Recent developments

The purpose of systemic chemotherapy is to relieve symptoms, improve the quality of life and prolong survival.

#### Chemotherapy

Compared with no chemotherapy or best supportive care, the combination of 5-fluorouracil (5-FU) with other drugs shows survival benefit in patients with pancreatic cancer. However, a retrospective study involving 5365



patients with pancreatic cancer showed no difference in survival between 5-FU combination therapy and 5-FU monotherapy<sup>[2]</sup>.

Gemcitabine (GEM) is a metabolic anti-tumor drug and has been approved by the United States Food and Drug Administration as the standard treatment for pancreatic cancer. The use of gemcitabine-cisplatin (GC) or capecitabine shows superiority over GEM monotherapy, while studies comparing GEM plus irinotecan or fluorouracil with GEM monotherapy show conflicting results. In studies of GC therapy, partial response (PR) was 10%-30%, time to tumor progression (TTP) was 2.8-7 mo, and median survival time (MST) was 5.6-8.1 mo<sup>[3]</sup>. In studies of GEM in combination with capecitabine therapy, PR was 8.9%, stable disease (SD) was 42%, TTP was 6.5 mo, overall survival (OS) was 8 mo, one-year survival rate was 34.8%, 53% of the patients experienced less pain, 44% of the patients reduced the dosage of analgesic, and 36% of the patients gained weight<sup>[4]</sup>.

Capecitabine is an orally-administered prodrug that is enzymatically converted to 5-FU. When used as first-line drug in patients with pancreatic cancer, its response rate (RR) is 24%. Therefore, it is recommended as the second-line drug for pancreatic cancer patients who failed GEM. Capecitabine monotherapy as second-line treatment for pancreatic cancer has only been studied in phase II trials, which showed that RR was 37%, TTP was 2.2 mo, and MST was 7.5 mo<sup>[5]</sup>. In studies of capecitabine plus oxaliplatin plus capecitabine as second-line treatment for advanced pancreatic cancer, RR was 28.2%, TTP was 9.9 wk, MST was 23 wk. The main side effect was fatigue and there were no severe hematological or nervous system side effects<sup>[6]</sup>. Capecitabine in combination with docetaxel showed a RR of 50%-83%, but showed no survival benefit because of frequent side effects such as grade 3-4 neutropenia, gastrointestinal reaction, and hand-foot syndrome<sup>[7]</sup>. Phase II clinical trials of capecitabine in combination with celecoxib as second-line treatment for pancreatic and bile duct cancer showed RR was 30% and MST was 16 wk<sup>[8]</sup>.

The addition of cetuximab to adjuvant gemcitabine was investigated in an open label, multi-center, phase II trial reported by Fensterer *et al.*<sup>[9]</sup>. Patients underwent R0 or R1 resection for pancreatic cancer, and were then treated with adjuvant chemotherapy consisting of 6 cycles of gemcitabine with weekly cetuximab for 24 wk. Of 76 patients enrolled, 73 patients received at least one dose of cetuximab. Median disease free survival (DFS) was 11.9 mo, and the DFS rate at 18 mo was 33.5%, failing to exceed the 35% level hypothesized by the authors. Median OS was 21.5 mo (95%CI: 16.9-28.2). Grade 3 or 4 toxicities were neutropenia in 11% of patients, thrombocytopenia in 8.2%, dermatological reaction in 6.9%, and allergic reaction in 6.9%. The authors concluded that the addition of cetuximab to gemcitabine in the adjuvant treatment of pancreatic cancer does not improve DFS compared with the use of gemcitabine alone.

S-1 and tegafur are also orally-administered 5-FU

prodrugs. Studies of tegafur as first-line monotherapy or combination therapy for advanced pancreatic cancer are ongoing. S-1 is a new orally-administered chemotherapy drug that combines tegafur with 5-chloro-2,4-dihydroxypyridine and oteracil at the ratio 1:0.4:1. Currently, its main use is in treating progressive stomach cancer. GEM in combination with S-1 was well tolerated and highly effective in patients with advanced pancreatic cancer in a phase I study. PR was 44%, SD was 48%, OS was 10.1 mo, and one-year survival rate was 33%. The side effects were acceptable and neutropenia was the most common, with an incidence rate of 80%<sup>[10]</sup>.

Currently, the use of camptothecins is limited in patients with pancreatic cancer. In studies of irinotecan monotherapy as second-line treatment for pancreatic cancer, RR was 48%, MST was 6.6 mo. Severe nausea occurred in 64% of the patients, and diarrhea occurred in 36%<sup>[11]</sup>. When used as second-line drug, camptothecins showed no survival benefit and demonstrated severe side effects. Rubitecan, an orally-administered camptothecin analog, failed to show positive effects. In an open-label phase II trial, RR of rubitecan monotherapy was only 7%, and MST was 3 mo<sup>[12]</sup>. In studies of paclitaxel monotherapy, RR was 6% and MST was 17.5 wk. It was well tolerated, with mild gastrointestinal reaction and hematological side effects.

In studies of pemetrexed monotherapy and raltitrexed monotherapy as second-line treatment for patients who failed GEM, RR was very low (0%-3.8%), MST was 18-20 wk. When used in combination with oxaliplatin or irinotecan, MST was 21-26 wk and showed more grade III-IV side effects<sup>[13]</sup>.

### Adjuvant and neoadjuvant therapy

Early stage pancreatic cancer is generally asymptomatic. As a result, the disease is often locally advanced or metastatic at the time of diagnosis, meaning that surgical treatment can only be performed in a minority of the cases. Furthermore, recurrence may occur after resection. Therefore, adjuvant chemotherapy and radiotherapy are very important for the treatment of this disease. 5-FU or GEM in combination with radiotherapy are widely used and have been showed to significantly increase the quality of life and prolong survival<sup>[14]</sup>. Adjuvant chemotherapy has shown a trend towards improved OS. Comparison of use of gemcitabine *vs* 5-FU was explored in the ESPAC-3 trial, which demonstrated equivalent survival for both treatments, but a more favorable safety profile with gemcitabine. There was also a trend toward improved survival in the gemcitabine arm in patients with node positive disease or those with positive resection margins<sup>[15]</sup>.

Kwon *et al.*<sup>[16]</sup> conducted a phase II trial of adjuvant gemcitabine and cisplatin chemotherapy followed by chemoradiation with gemcitabine and 5040 cGy of radiation, then 4 cycles of maintenance gemcitabine. Of the patients enrolled, 57 completed chemotherapy followed by chemoradiation. One-year DFS rate was 62.1%, median DFS was 17.4 mo, and median OS was 33.6 mo.

The majority of recurrences (66.2%) were distant metastases. Later disease stage and involved lymph nodes were associated with reduced DFS ( $P < 0.001$  and  $P = 0.01$ , respectively). These findings suggest promising efficacy with acceptable toxicity for adjuvant multimodality therapy.

The aim of neoadjuvant therapy is to turn the tumor from unresectable to resectable by reducing the volume. However, studies of neoadjuvant therapy in patients with pancreatic cancer at different stages showed conflicting results.

Neoadjuvant 5-FU-based chemotherapy showed modest effects for resectable tumors. 5-FU plus platinum anticancer drugs showed significantly improved effects. Trials of GEM as neoadjuvant therapy showed improvement in MST. However, a recently published retrospective analysis showed conflicting conclusions. Some studies indicated that neoadjuvant therapy for resectable tumor helped to improve CR, reduce the recurrence rate, and improve survival rate, while others suggested that neoadjuvant therapy showed no survival benefit and increased postoperative complications. Neoadjuvant therapy for resectable pancreatic tumor is still at the experimental stage and is not recommended as standard treatment.

The current neoadjuvant therapy for advanced local tumors is concurrent chemoradiotherapy. Studies of this therapy have demonstrated significant variation in its curative effects. This may be owing to the difference in the definition of “unresectable”. Moreover, such retrospective studies may have sample selection bias<sup>[17]</sup>.

### **Molecular targeted therapies**

These therapies are based on molecular biological differences between tumor and normal cells. They can inhibit the proliferation of tumor cells and promote their apoptosis by blocking signal transduction and prevent tumor angiogenesis. They interfere with specific targeted molecules needed for carcinogenesis and tumor growth, so they are more effective than conventional chemotherapy and less harmful to normal cells.

### **Epidermal growth factor receptor-targeted drugs:**

Epidermal growth factor (EGF) and epidermal growth factor receptor (EGFR) are overexpressed in the cells of pancreatic tumors, and are indicators of high aggressiveness and poor prognosis. Therefore, EGFR-targeted therapy is a promising strategy for the treatment of pancreatic tumor.

Cetuximab (C-225) is a chimeric monoclonal antibody, which is an inhibitor of EGFR. It prevents the growth of tumor cells by binding to the extracellular domain of EGFR, inhibiting phosphorylation caused by receptor-ligand binding, and blocking the EGFR-mediated signaling pathway. At the same time, it inhibits tumor angiogenesis and metastasis by reducing essential factors such as vascular endothelial growth factor (VEGF). Cetuximab in combination with GEM showed additive effects in patients with advanced pancreatic cancer<sup>[18]</sup>.

Phase I trials showed that cetuximab was well tolerated when used either as monotherapy or in combination with other cytotoxic drugs or chemotherapy. Cetuximab in combination with 5-FU, GEM, carboplatin or cisplatin demonstrated no drug interaction<sup>[19]</sup>. Phase II trials indicated that cetuximab in combination with GEM was effective in advanced pancreatic cancer although further clinical trials are needed.

Erlotinib, an EGFR tyrosine kinase inhibitor, is a small molecule compound that targets EGFR tyrosine kinase by blocking autophosphorylation and the downstream signal transduction pathway. According to results published at the 2005 American Society of Clinical Oncology annual meeting, GEM in combination with erlotinib showed longer one-year survival than GEM monotherapy. Therefore, GEM in combination with erlotinib is the only Food and Drug Administration approved combination therapy for unresectable or metastatic pancreatic cancer<sup>[20]</sup>. Moreover, a study of erlotinib plus capecitabine in 30 patients who failed GEM-based therapy showed that the combination therapy was well tolerated and that the outcome was positive<sup>[21]</sup>. No significant positive effects were observed in clinical trials of gefitinib.

ErbB-2 is a member of the receptor tyrosine kinase family and is over-expressed in cells of pancreatic tumors. Herceptin is a monoclonal antibody that suppresses proliferation of tumor cells with ErbB-2 overexpression. A study of GEM plus Herceptin showed RR was 6%, MST was 7 mo, and one-year survival rate was 19%, which was similar to results from GEM monotherapy.

**VEGF receptor inhibitors:** VEGF stimulates endothelial cell proliferation and angiogenesis, inhibits endothelial cells apoptosis by activating HSP90 and Bcl-2 expression, increases intercellular gaps and vascular permeability by making endothelial cells produce nitric oxide. It thus promotes tumor migration, activates kinase activity by autophosphorylation, triggers signal transduction, and stimulates tumor angiogenesis.

Bevacizumab is a humanized monoclonal antibody that recognizes and blocks VEGF-A. It blocks the chemical signal that stimulates the growth of new blood vessels and inhibits tumor angiogenesis and tumor cell proliferation. A study of bevacizumab in combination with GEM showed PR was 21% (11 patients), SD was 46% (24 patients), six-month survival rate was 77%, MST was 8.8 mo, and side effects included increased blood pressure (19%), thrombosis (13%), perforation of abdominal viscera (8%) and hemorrhage (2%)<sup>[22]</sup>. A multicenter phase II trial of GEM in combination with bevacizumab in pancreatic cancer demonstrated encouraging results, giving rise to optimism for further research on bevacizumab in combination with chemotherapy.

AEE788 is a new molecular-targeted drug and kinase inhibitor with potent inhibitory activity against ErbB and the VEGF receptor family of tyrosine kinases. It inhibits EGFR overexpression and VEGF-mediated growth of vascular endothelial cells. In animal experiments, AEE788 in combination with GEM showed higher control rate

(95%), increased cell apoptosis, reduced angiogenesis, and extended survival in mice with transplanted pancreatic tumors. Relevant phase I trials are underway<sup>[23]</sup>.

**Matrix metalloproteinases inhibitors:** Matrix metalloproteinases (MMPs) promote tumor cell invasion and migration, and stimulate tumor angiogenesis by degrading extracellular matrix and basement membrane, thereby regulating cell adhesion. Marimastat is an orally-administered broad-spectrum MMP inhibitor. It was well tolerated and showed a similar survival rate (19%-20%) to GEM monotherapy in patients with advanced pancreatic cancer<sup>[24]</sup>. There was no that its therapeutic effect may improve when used in combination with other drugs.

**Prostaglandin synthase:** Cyclooxygenase-2 (COX-2) plays an important role in the development and progression of tumors. It activates epithelial cell proliferation, inhibits tumor cell apoptosis, stimulates tumor angiogenesis, improves tumor cell invasion, and induces immunosuppression and mutation, in which angiogenesis is closely associated with malignant tumor growth, invasion and migration. Celecoxib is a highly selective COX-2 inhibitor. In a clinical trial involving 42 patients with advanced pancreatic cancer, celecoxib in combination with GEM showed CBR of 54.7%, MST of 9.1 mo, and only mild side effects<sup>[25]</sup>. However, no improved therapeutic effect or survival benefit (MST was 5.8 mo) was observed in studies of celecoxib plus GEM and DDP.

**Farnesyl protein transferase inhibitors:** Farnesyl protein transferase (FPT) is a critical enzyme for Ras protein synthesis. Therefore, inhibiting FPT and the activity of *Ras* gene may be a means to treat pancreatic cancer. FPT inhibitors include lonafarnib (SCH66336) and tipifarnib, BMS-214662. However, phase I and phase II trials of tipifarnib monotherapy in patients with advanced pancreatic cancer showed disappointing results<sup>[26]</sup>.

### Problems

The anatomical structure of the pancreas is very complicated. The high interstitial tension and inadequate blood perfusion of solid tumors, especially pancreatic tumors, give them extreme resistance to most chemotherapy drugs. Consequently, conventional systemic intravenous chemotherapy often fail to reach effective concentration<sup>[27]</sup>. Large dosages may cause severe adverse reactions, thus impairing the immune system and therapeutic effect.

GEM has replaced 5-FU as the most widely used drug in advanced pancreatic cancer. GEM and GEM-based combination therapies are recommended as standard for advanced pancreatic cancer by National Comprehensive Cancer Network. Several combination therapies based on GEM and 5-FU have been developed, although their therapeutic effects are still unknown. So far, they have mainly demonstrated improvement in the control of tumor growth and it remains unclear whether or not they have survival benefits.

No randomized controlled prospective study of neo-adjuvant therapy for pancreatic cancer has been conducted and, therefore, can not be recommended as treatment for pancreatic cancer, other than in clinical trials.

As the molecular pathway of tumor cellular differentiation, migration, apoptosis and metabolism are not clear, targeted cancer therapies still lack specificity.

### Solutions and prospects

In order to minimize the side effects of combination therapy, more data from phase II trials of monotherapy and combination therapy should be collected.

More clinical trials of topical medication, such as regional perfusion chemotherapy should be conducted. The arterial blood supply of the pancreas is from the common hepatic artery (division of the celiac artery), splenic artery, and superior mesenteric artery. Anti-tumor drugs infused through celiac artery or superior mesenteric artery can reach the whole pancreas. Hepatic artery infusion is also effective in pancreatic cancer metastases in the liver. The commonly used drugs include 5-FU, cisplatin, epirubicin, mitomycin and GEM. Regional perfusion significantly increases drug concentration within the pancreas, prolongs the presence of the drug in the body, and causes fewer side effects on other important organs, indicating its effectiveness in pancreatic cancer. Infusion *via* cannula of embolic agents into arteries that supply blood to the pancreas prolongs the presence of the drug in the body, reduces blood supply to the tumor, increases the cytotoxicity of the drug, and leads to necrosis of tumor cells. Studies showed that local ischemia inhibited the synthesis of DNA and protein of tumor cells, thereby inhibiting the growth of transplanted pancreatic tumors in mice.

Intra-tumor injection of chemotherapy drugs can break the blood-pancreatic barrier, increase drug concentration within the tumor, and causes fewer side effects than systemic chemotherapy. This is a good option for patients with unresectable pancreatic tumors.

We need to identify the molecular pathway of pancreatic cancer and look for highly specific targets. For example, S100P may reduce the side effects of chemotherapy drugs, breast cancer type 2 susceptibility protein may enhance pancreatic cancer's sensitivity to mitomycin, and human equilibrative transporter 1 overexpression can improve the survival rate of patients received GEM therapy<sup>[28]</sup>. This may be helpful to the future treatment for pancreatic cancer.

Pancreatic cancer cells are resistant to conventional treatments because they carry mutations which inhibit the activation of apoptosis. Therefore, developing a molecular targeted drug that inhibits mutation may be a solution.

## RADIOTHERAPY

### Recent developments

In recent years, the development of radiotherapy techniques, knowledge about the localization of tumor and radiation dosage have provided new and effective treat-



ment for pancreatic cancer.

**X knife:** This is a linear accelerator delivering high-energy X-rays to the region of the patient's tumor. Only a few cases of pancreatic cancer treated with the X knife have been reported. The X knife is only good option for pancreatic cancer treatment in patients diagnosed with early stage of the disease<sup>[29]</sup>.

**Three-dimensional conformal radiotherapy:** The profile of each radiation beam is shaped to fit the profile of the target from a beam's eye view, using lead or a multileaf collimator and a variable number of beams. When the treatment volume conforms to the shape of the tumor, the relative toxicity of radiation to the surrounding normal tissues is reduced, allowing a higher dose of radiation to be delivered to the tumor than when using conventional techniques. This is the most widely used radiotherapy technique for pancreatic cancer<sup>[30]</sup>. Studies showed that it relieved jaundice in patients with carcinoma of the pancreatic head, and one-year and two-year survival rates were 60%-90% and 25%-70%, respectively. A recent study showed one-year and two-year survival rates of 55.6% and 27.8% respectively, significantly higher than the 33% and 9.4% of traditional radiotherapy. Therefore, 3-dimensional conformal radiotherapy for local advanced pancreatic cancer will be the focus of future research.

**Intensity modulated radiation therapy:** This technique allows high radiation doses to be focused on regions within the tumor while minimizing the dose to surrounding normal critical structures, especially the dose to the duodenum. Therefore, higher and more effective radiation doses can safely be delivered to tumors with fewer side effects compared with conventional radiotherapy techniques<sup>[31]</sup>. This may make it be a suitable radical treatment for early stage local pancreatic cancer. Further clinical researches on this therapy are of great significance.

**Precision radiation therapy:** This method delivers a single high-dose of precisely-targeted radiation using highly focused gamma-ray beams that converge on the specific area where the tumor or other abnormality resides. In advanced pancreatic cancer patients who are not suitable for surgery, stereotactic radiotherapy may help control the growth of tumor, reduce jaundice, relieve symptoms, improve appetite, and improve the quality of life. "Gamma knife" is abbreviation of "gamma knife stereotactic radiosurgery system", and is composed of a radioactive source, collimator and movable treatment couch. The treatment couch can move in three (x, y, z) directions. Radiation can be delivered to the tumor from any angle by rotating the gantry and moving the treatment couch<sup>[32]</sup>.

### Problems

Radiotherapy is a treatment option for pancreatic cancer patients who don't have heart, liver, or kidney dysfunc-

tions or distant metastasis and whose predicted survival is more than 3 mo. Of the pancreatic cancer patients that seek radiotherapy, most have locally advanced unresectable tumors which are large and of irregular shape. It is difficult to give proper radiation doses to such tumors.

Pancreatic tumors have low radiosensitivity and, in order to inhibit or kill tumor cells, large doses of radiation are needed. However, the pancreas is located behind the peritoneum and near vital organs and important blood vessels such as stomach, intestines, liver, kidney, spinal cord, *etc.* These tissues are very sensitive to radiation and damage to them may lead to serious consequences.

The application of radiotherapy is limited by the high cost and difficult operation of radiotherapy equipment. It is still unknown whether the benefits of this technique outweigh its high cost in patients with locally advanced pancreatic cancer.

### Prospects

In future, we should be able to take precise images of pancreatic tumors by nanotechnology and perform conformal radiotherapy using such images. It will also be advantageous to develop more selective radioactive elements, such as radioactive elements against tumor cells or tumor stem cells, and to determine more accurate radiation dosage using biological equivalent dose, hyperfractionation, accelerated hyperfractionation and hypofractionation so as to achieve greater benefit.

## INTERVENTIONAL THERAPY

### Actualities

**Transvascular therapy:** As well as regional perfusion of chemotherapy drugs, radiation sources are also used. They are implanted into the tumor to deliver beams of radiation. Studies showed that this method improved therapeutic effect with a total effective rate of 70% (CR + PR), and MST of more than 10 mo. Injection of colloidal<sup>[32]</sup> phosphorus (P) into solid tumors helped to kill tumor cells and reduced the blood flow to the tumor<sup>[33]</sup>.

### Percutaneous puncture (or non-puncture) therapy:

Injection of absolute ethanol into tumors is an adjuvant therapy that inhibits the progression of tumor. It is safe and convenient and has led to better prognosis in pancreatic cancer patients whose primary tumor is relatively small but can not tolerate major surgery<sup>[34]</sup>.

To puncture the pancreatic tumor under the guidance of computer tomography (CT) or B type ultrasound, and utilize multi-stage radio frequency or microwave coagulation to dissolve tumor itself was safe, effective and minimally invasive<sup>[35]</sup>.

Resecting or dissolving a tumor or injecting drugs into a tumor could also be performed under endoscopy.

### Problems

It is difficult to perform interventional therapy in pa-



tients with pancreatic cancer. Most pancreatic tumors have decreased blood flow. They are supplied by several small blood vessels. The embolic agents often can not reach the nidus. Collateral circulation may appear near the embolized vessel after embolization which makes it difficult to kill the tumor cells. If peripheral vascular embolization material is used, it may enter normal pancreatic tissues through a communicating branch and lead to a disastrous result. CT-guided injection is only suitable for a nidus that can be visualized by CT. It can not be used in a nidus that has the same density as normal tissue. Moreover, the relationship between the dosage of drug and the size of the tumor has not been standardized. Percutaneous puncture may cause damage to the normal organs and may lead to massive hemorrhage if the nidus is located on the edge of the organ or near main vessels. Perfusion chemotherapy is far less effective than arterial perfusion plus embolization.

Although images taken immediately after embolization show that tumor vessels are blocked and the tumor blood supply cut off, images taken later may show some of the vessels become unobstructed or new vessels emerge, indicating the tumor is growing or recurring. In most cases, arterial embolization needs to be performed for at least twice.

### Solutions

Biological therapies mainly include gene therapy, immunotherapy and therapies that induce tumor cell apoptosis or inhibit tumor angiogenesis. Gene therapy inserts normal tumor suppressor genes into the patient's tumor cells and replaces deleterious mutant alleles to treat cancer. It is a new treatment option for patients besides surgery, chemotherapy, and radiotherapy. With the use of endosonography, gene therapy or cell-targeted therapy can be performed<sup>[35]</sup>.

With the help of a robot, rather than physician alone, puncture is performed more quickly and accurately, which causes less damage to the surrounding tissues.

Performing interventional therapy under the guidance of magnetic resonance imaging may avoid the influence of radioactive rays on patients and healthcare workers and minimize the CT scan error on tissues with the same density.

Micro catheter with a laser or catheter ablation system helps to avoid damage caused by percutaneous puncture.

Photodynamic therapy is a medical treatment that administers a photosensitizing drug to the patient and the tissue to be treated is exposed to light suitable for exciting the photosensitizer. The result is an activated oxygen molecule that can destroy nearby cells. It can damage endothelial cells of the tumor vessel, and lead to vascular thrombosis, microcirculatory disturbances, ischemia and necrosis of the tumor<sup>[36]</sup>.

Nanopolymers can be used to wrap chemotherapy drugs, radioactive particles, or biological agents into microspheres, which can be administered into the pancreatic tumor by percutaneous puncture under the guidance

of CT or B type ultrasound. Nanoparticles are slowly released and reach a high concentration in the tumor, killing tumor cells and minimizing the damage to the normal tissues.

## IMMUNOTHERAPY

### Recent developments

**Monoclonal antibody therapies:** Therapies include pure antibody therapy and conjugated antibody therapy. The former is the use of monoclonal antibodies to bind specifically to tumor antigens, leading to antibody-dependent cell-mediated cytotoxicity and complement-dependent cytotoxicity. In conjugated antibody therapy biological engineering technology is used to link the monoclonal antibody with drugs, toxins, radionuclides or enzyme prodrugs to create an entity to kill tumor cells.

MAb 17-1A is an IgG2a antibody created by immunizing mice with the SW1038 colorectal cancer cell line. It binds to the tumor cell surface, activates T-cells and kills tumor cells, as proved in animal experiments. MAb BW-494 is an IgG1 antibody created by immunizing mice with the BALB/C colorectal cancer cell line. It can mediate human monocytes and induce antibody-dependent cellular cytotoxicity against <sup>51</sup>Cr labeled pancreatic cells. <sup>131</sup>I labeled MAb BW-494 can inhibit the growth of tumor cells in mice with transplanted human pancreatic tumors. MAb YPC3 is an IgG1 antibody created by cell hybridization. Either MAb YPC3 or YPC3-mediated LAK can inhibit the growth of tumors. MAb C017-1A or the C017-1A analog bind the GA 733 antigen expressed in pancreatic tumor cells and induce cytotoxic immune response by antigen-specific proliferation, T cells and delayed-type hypersensitivity. Culture of anti-nuclear antibody P and several pancreatic tumor cell lines together and the antibody has been found to significantly inhibit the proliferation of pancreatic tumor cells, promote their apoptosis and reduce the tumor size. 425(scFv)-pseudomonas exotoxin A (ETA), a recombinant immunotoxin generated by fusing the anti-EGFR single chain variable fragment 425(scFv) to a truncated mutant of ETA, can significantly reduce the risk of pancreatic cancer metastasis to the lungs in mice. Trials of MAb in combination with chemotherapy showed large doses of chimeric MAb or humanized MAb were well tolerated by patients.

**Cytokine immunotherapy:** In exogenous cytokine therapy an antitumor cytokine is inserted into the tumor. interleukin (IL)-12 is an important anti-tumor cytokine. Injection of adenovirus encoding IL-12 plus adenovirus encoding MIP3a into tumors induces the generation of cytotoxic T lymphocytes and causes damage to the tumor cells in several ways. Tumor cell apoptosis is induced *via* Fas-pShuttle, although the recurrence rate is very high. Giving IL-2 to patients with pancreatic cancer *via* subcutaneous injection before surgery showed improved two-year survival rate compared with the control

group<sup>[37]</sup>. The *IL-2* gene plus interferon- $\gamma$  can increase the total amount of CD4<sup>+</sup>, CD8<sup>+</sup> lymphocytes, and induce anti-tumor immune response.

In cytokine-directed therapy, cytokines are conjugated with a toxin, radionuclide, or chemotherapy drug and act on the tumor cells that express the relevant cytokine receptor. IL-13 cytotoxin, composed of IL-13 and ETA, demonstrated antitumor activity in studies of many kinds of tumors. However, IL-13 is differently expressed in various kinds of tumors and its effects is not consistent. Tumor cells that express type I IL-13R may be more sensitive to IL-13 cytotoxin.

In cytokine gene therapy a cytokine gene is inserted into tumor cells resulting in production of cytokine which combats the tumor. After ras17 peptide vaccine combined with granulocyte-macrophage colony-stimulating factor was administered to patients with pancreatic cancer *via* subcutaneous injection, specific CD8 cytotoxic T-lymphocytes that could kill pancreatic tumor cells were detected in peripheral blood mononuclear cells<sup>[38]</sup>. MALP-2 is a synthetic lipopeptide that can inhibit tumor cells by inducing the synthesis of cytokines and chemokines, as well as the maturation of dendritic cells by toll-like receptor 2 and toll-like receptor 6<sup>[39]</sup>.

### Problems

Because pancreatic tumor-specific antigens have not yet been discovered, antigen immunotherapy lacks of specificity. Besides of this, immune escape mechanisms of tumors add to the obstacles to successful immunotherapy. Possible changes in tumor antigens are as follows: defects in tumor antigen and antigen modulation, blocking or coverage of tumor antigens, disorders of tumor antigen processing and presentation, underexpression or missing of major histocompatibility complex (MHC)-1 molecules, dendritic cell dysfunction, abnormal expression of tumor cell costimulatory molecules, overexpression of FasL in tumor cells, induction of CD4<sup>+</sup>CD25<sup>+</sup> T cells and suppression of antitumor immune response. The effects of monoclonal antibodies and cytokines have not been fully confirmed and high doses of them may not be tolerated by patients.

### Solutions

**Adoptive cellular immunotherapy:** This kind of treatment is used to help the immune system fight against cancer by giving cancer-specific T cells to the patient. It is seldomly used in pancreatic cancer and its therapeutic effect is not confirmed. (1) Adoptive transfer of dendritic cells: In the presence of granulocyte-macrophage colony-stimulating factor, dendritic cells are separated from peripheral blood mononuclear cells of patients with metastatic pancreas cancer, pulsed with supernatant of tumor cells, and administered to the patient by subcutaneous injection. Antitumor T-cells are produced, indicating the significant inhibition of tumors by this therapy<sup>[40]</sup>. GEM can induce the differentiation of CD14<sup>+</sup> and CD11c<sup>+</sup> DC and improve the therapeutic ef-

fect of GEM in combination with other therapies<sup>[41]</sup>; and (2) Adoptive transfer of lymphocytes: Allogeneic mixed lymphocytes cultured *in vitro* are injected into pancreatic tumors under the guidance of endoscopic ultrasound. The therapy is found to be effective and has no significant toxicity although controlled studies that involve more samples are needed. Through *in vitro* modification and immunostimulation, lymphocytes may be used as antigen presenting cells to treat pancreatic tumor cells with *p21* and *p53* mutations.

**Active immunotherapy:** Tumor vaccination may activate or strengthen specific anti-tumor immune response, prevent the growth, spread and recurrence of tumor cells. Tumor vaccines include cell vaccines, peptide vaccines and DNA vaccines. (1) Tumor cell vaccine technology: These vaccines are produced from actual cancer cells that have been removed during surgery. The cells are treated in the lab, usually with radiation, or modified by albumin. They are then injected into the patient. The immune system recognizes antigens on these cells, then seeks out and attacks any other cells with these antigens that are still in the body. Overexpression of heat shock protein in pancreatic tumors can inhibit the apoptosis of tumor cells. Quercetin is a HSP70 inhibitor which inhibits HSP70 in pancreatic tumor cells but not in normal pancreatic cells. Isolated HSP can bind to MHC-I molecules and can be recognized by the immune system. Thus, it can be used as tumor cell vaccine<sup>[42]</sup>; (2) Molecular vaccine technology: Tumor antigen peptide is synthesized by genetic engineering techniques and combined with the MHC-1 molecule, making it recognizable by antigen presenting cells; and (3) Idiotypic antibodies: Primary antibodies, obtained by using tumor antigens to immunize other animals, are utilized to create secondary antibodies, which can be used to activate anti-tumor activity of the immune system.

**Suicide genes:** Suicide gene therapy is also called drug sensitivity gene therapy, or virus-directed enzyme prodrug therapy. Suicide genes are prodrug converting genes or cytotoxic factor receptor genes from prokaryotes or lower organisms. In animal experiments, suicide genes introduced into tumor cells killed these cells by converting non-toxic or low-toxic prodrugs into toxic metabolites.

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## Gallbladder carcinoma in a pregnant patient with Crohn's disease complicated with gallbladder involvement

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This is the only case that describes all three factors. Common features in CD of the GB include acute cholecystitis, ileal involvement, and presence independent of active intestinal disease. Common features in CD patients with GB malignancy include younger age of detection, a long history of CD, extensive colonic and ileal involvement of disease, the absence of cholelithiasis, and pre-existing gallbladder disease (primary sclerosing cholangitis and gallbladder polyps). Pregnancy is specific to this case. The role of CD in the development of GB malignancy is not well understood nor is the contribution of pregnancy to the spread of disease. Chronic inflammation and immunosuppression compounded by hormonal influence is implicated.

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**Key words:** Crohn's disease; Cholangiocarcinoma; Gallbladder; Gallbladder carcinoma; Inflammatory bowel disease

### Abstract

Primary gallbladder (GB) carcinoma and Crohn's disease (CD) of the GB are individually rare. We present a case of a pregnant woman with CD found to have GB involvement and primary GB carcinoma. A 34-year-old female at 6 wk gestation with a 21 year history of CD of uncertain extent presented with 3 mo of diarrhea, urgency and abdominal pain. During work-up, she was found to have elevated transaminases and an abnormal alkaline phosphatase. Imaging revealed two gallbladder polyps both greater than 1 cm in size. Resection and histological evaluation was consistent with Crohn's involvement of the GB, poorly differentiated adenocarcinoma of the GB with invasion through the muscularis propria and matted lymph nodes in the porta hepatis positive for metastatic carcinoma (stage pT2N1). Six cases of CD involving the GB, two cases of primary GB carcinoma in CD, and ten cases of cholangiocarcinoma in pregnancy have been published.

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### INTRODUCTION

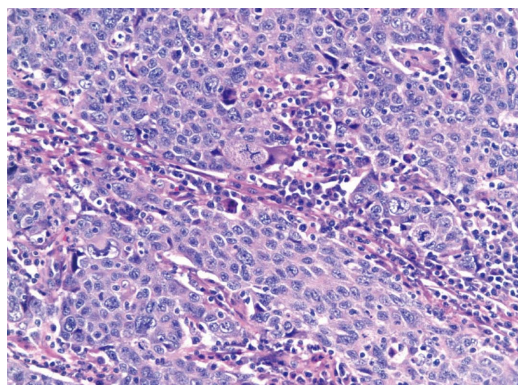
Primary gallbladder carcinoma (GC) is the second most common primary biliary malignancy and the fifth most common malignancy of the gastrointestinal tract. The prognosis of GC is dismal, with five-year survival rates of 0%-10% and median survival of less than 6 mo. Patients with inflammatory bowel disease (IBD) are at increased risk for cholangiocarcinoma. Several case reports<sup>[1-10]</sup>, population-based case control and cohort studies<sup>[11-18]</sup> report IBD as a risk factor for cholangiocarcinoma. The 10-year

cumulative risk of cholangiocarcinoma in IBD was found to be 0.07% in a national Danish cohort study<sup>[19]</sup> with a four-fold increase among IBD patients compared to the general population. However the absolute risk of cholangiocarcinoma and more specifically GC in patients with IBD remains unclear<sup>[19]</sup>. Furthermore, little is known about the impact of IBD on the development of GC. We herein report a case of a pregnant woman with Crohn's disease (CD) complicated with involvement of the gallbladder (GB) and primary GC. The purpose of this case is to illustrate the presentation of GB involvement in CD and primary GC and to discuss the putative risk factors that interplay to contribute to the development of these complications in the setting of pregnancy.

## CASE REPORT

A 34-year-old female at 6 wk gestation with a 21-year history of IBD of uncertain classification and extent presented with 3 mo of diarrhea, urgency and abdominal pain. Her disease had been indolent for the majority of her life. Her medications included an oral contraceptive (OC), which she had been on for the past 6 years, and mesalamine and mercaptopurine, which she took as needed at times of flare symptoms. Initial work up included a sigmoidoscopy that was consistent with mild patchy colitis of the sigmoid and descending colon, extending beyond the limit of the exam, with rectal sparing. Pathology showed patchy moderately active chronic colitis with crypt distortion and cryptitis without granuloma. The endoscopic distribution of disease was more consistent with CD, however she was unable to provide information on previous endoscopies. Her pregnancy limited further examination at that time. She was started on oral and topical mesalamine and symptomatically responded.

Initial labs were obtained including elevated alkaline phosphatase 515 U/L, aspartate aminotransferase 96 U/L, and alanine aminotransferase 181 U/L. Further labs included normal bilirubin, negative anti-mitochondrial antibody (AB), anti-smooth muscle AB, celiac and viral hepatitis serologies and anti-nuclear AB. An ultrasound revealed a large polypoid mass in the GB prompting further imaging. A non-contrast magnetic resonance cholangiopancreatography was obtained revealing two hypointense smooth margined masses with small stalks in the neck and fundus of the GB, 1.4 cm × 1.8 cm and 1.4 cm × 2.3 cm respectively. No GB wall thickening, cholelithiasis, ductal dilation, strictures, or liver parenchymal abnormalities were present. Laparoscopic cholecystectomy was performed at 18 wk gestation. Histology revealed a poorly differentiated adenocarcinoma of the GB (Figure 1) with invasion through the muscularis propria and an adjacent tubulovillous adenoma with highgrade dysplasia without nodal involvement (stage pT2N0). Additionally, there was widespread epithelial dysplasia in the GB with acute superficial inflammation, transmural chronic inflammation with numerous plasma



**Figure 1** Tumor cells are large with numerous mitotic figures consistent with poorly differentiated carcinoma of the gallbladder.

cells and one granuloma consistent with CD (Figure 2). Muscularis propria invasion prompted partial liver resection and portal lymphadenectomy, revealing matted lymph nodes in the porta hepatis positive for metastatic carcinoma (stage pT2N1). Liver tissue was negative for primary sclerosing cholangitis (PSC).

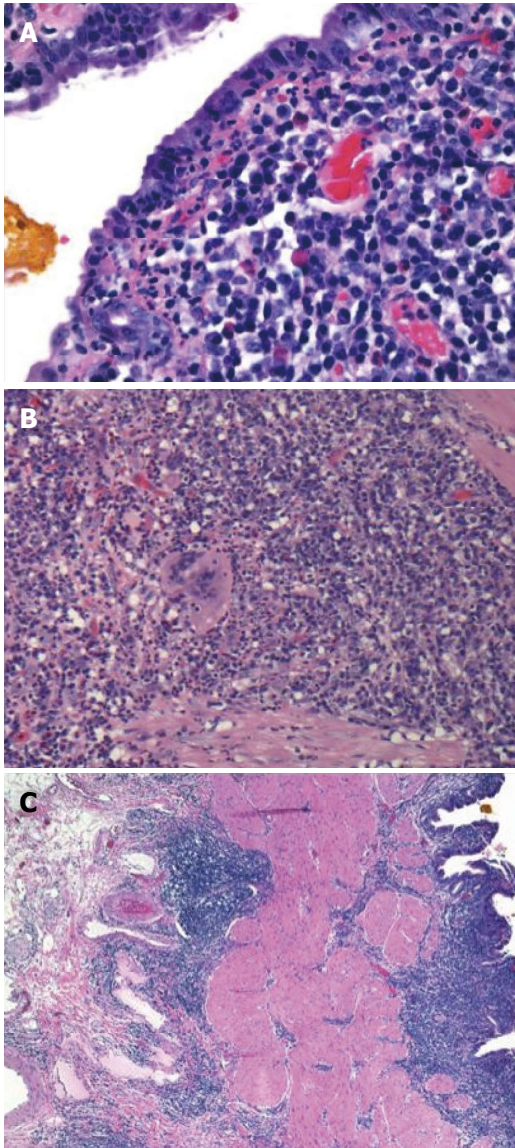
The immediate post-operative course was uncomplicated and the patient was discharged one week later. The patient was given the option to terminate the pregnancy and proceed with adjuvant radiation and chemotherapy or to carry out her pregnancy and delay further treatment until the post-partum period. She elected to defer radiation and chemotherapy until after she delivered. She has since had an uncomplicated vaginal delivery and is starting radiation and chemotherapy.

## DISCUSSION

Cholangiocarcinoma in ulcerative colitis is well established particularly with the presence of PSC<sup>[2,20-23]</sup>. In contrast cholangiocarcinoma in CD is less frequent. Primary GC in CD is even more infrequent with only 2 reported cases in the literature<sup>[24,25]</sup>. With inclusion of our case, common clinical features include detection at a young age (32 years, 34 years and 50 years), absence of gallstone formation (all cases), a long duration of disease (12 years, 13 years and 21 years), absence of biliary symptoms (all cases), and a history of ileal and pan-colonic disease (unknown extent in our case). Clinically active colitis was absent in two cases<sup>[24,25]</sup> and present in our case.

Primary GC in CD is rare enough that a true association can be questioned, despite consistent commonalities between cases. A study looking at 2645 CD patients and cancer incidence over a 17-year period from the Danish National Registry compared the rate of cancer in the CD population to the expected rate of cancer in the general Danish population<sup>[14]</sup>. In total, the authors found 143 malignant neoplasms in CD patients compared to 123 in the general population (95%CI: 0.97-1.36) of which only 2 were cholangiocarcinomas in CD (not location specified) compared to 1 in the general population. This agrees with the rare incidence of GB carcinoma





**Figure 2** Crohn's disease involving the gallbladder. A: Superficial acute inflammation and numerous plasma cells; B: Non necrotizing granuloma in the gallbladder wall; C: Transmural inflammation of the gallbladder wall.

in CD and the questionable contribution of IBD as an independent factor to the development of cholangiocarcinoma. Pre-existing GB diseases, include gallstones, chronic cholecystitis, polyps and premalignant lesions, adenomyomatosis, an anomalous junction of the pancreato-biliary duct, chronic infection, PSC and hormonal changes in women<sup>[26]</sup>, are known risk factors for the development of GC in the general population. These same factors may similarly be required for the development of GC in IBD however temporally accelerated in the setting of inflammation and immunosuppression. This is illustrated in the above cited cases with two of the three carcinomas originating from GB polyps<sup>[25]</sup> and one in the setting of concomitant PSC<sup>[24]</sup>.

Persistent inflammation is thought to promote carcinogenesis by causing DNA damage, activating tissue reparative proliferation, and by creating a local environ-

ment that is enriched with cytokines and other growth factors for autonomous proliferation and escape from apoptosis<sup>[27]</sup>. Population studies previously mentioned hint that IBD (*i.e.*, inflammation) alone does not account for these changes. A large survey investigating inflammatory patterns in post-cholecystectomy patients with IBD noted the absence of any specimens containing granulomatous disease or GC, including the 78 patients with CD. This lack of presence may be due to the low threshold to perform cholecystectomy in IBD patients preventing the progression of chronic inflammation, thus aborting the full development of biliary epithelial dysplasia and its associated malignancy as suggested by the authors<sup>[28]</sup>. Potentially, the presence of pre-existing immune modulating medications and the relative immunosuppressed state of IBD may act to down-regulate the immune response and in turn have a role in creation and progression of malignancy in the setting of long standing inflammation. Chronic GB inflammation as CD involvement was present in our case.

CD involvement of the GB itself is very rare with only six reported cases in the literature<sup>[29-34]</sup>. Common pathological features include transmural inflammation, granulomatous change, and lymphoid aggregation<sup>[29-34]</sup>. Similarly, our case demonstrated chronic transmural inflammation and granuloma formation. Common clinical characteristics include initial presentation with acute cholecystitis, as demonstrated in 4 cases<sup>[29-31,33]</sup> and ileal involvement occurred in 5 cases<sup>[29-32,34]</sup>. Of note, patients with ileal disease have a 10 fold increase in the incidence of cholelithiasis due to the disruption in bile salt metabolism<sup>[35]</sup>. Suggested mechanisms include disturbances in bile acid metabolism due to loss of absorptive function of the terminal ileum resulting in depletion of the bile acid pool and precipitation of cholesterol with subsequent stone formation<sup>[36,37]</sup>. However 2 of the 7 total cases, including ours, occurred in the absence of gallstone formation<sup>[31]</sup>. Alternatively, ileal disease may result in disturbance in the microbiome and colonization of the terminal ileum with anaerobic bacteria<sup>[38]</sup>. This results in deconjugation of bile acids to products that have an irritating effect on the mucosa of the GB resulting in inflammation<sup>[39]</sup>. The potential role of these mechanisms in its development is not clear.

And finally, the effect of pregnancy on the progression of our patient's disease is not clear. Ten cases of cholangiocarcinoma in pregnancy exist in the literature<sup>[40-48]</sup>. Pregnancy is associated with high estrogen levels and theoretically can aggravate a preexisting malignant lesion. The relative immunosuppressed state that exists during pregnancy may also play a role in enhancing aggressiveness of the malignancy. Human intrahepatic cholangiocarcinomas express the receptors for both estrogens and insulin-like growth factor-1 (IGF-1)<sup>[49]</sup> indicating that estrogens and IGF-1 coordinately regulate cholangiocarcinoma growth and apoptosis<sup>[49]</sup>. Additionally, GC is more common in women. Secreted mutagenic toxins persist in the GB in women due to stasis

which results from impaired contractility associated with progesterone<sup>[50]</sup>. This protracted exposure allows environmental carcinogens to then cause malignant transformation<sup>[43]</sup>. Additionally, the use of hormone based contraception remains a controversial issue. In some OC studies, no difference between the incidence of cholangiocarcinoma and healthy controls was found<sup>[51,52]</sup> but in other studies a positive association between OC use and extrahepatic bile cancer was found<sup>[53,54]</sup>. Our patient was on an OC agent for about six years.

GC carries the worst prognosis of any gastrointestinal or hepatobiliary neoplasm. The prognosis is equally grave independent of the presence of IBD. It is essential to identify IBD patients at high risks for developing GC. At this time, annual ultrasound examination of the GB is recommended in patients with PSC. Cholecystectomy is recommended in all PSC patients with mass lesions of any size due to high malignant potential<sup>[55]</sup>. The same recommendations should be extended to patients with chronic immunosuppressive states and chronic inflammation, such as IBD.

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## Diffuse intestinal ganglioneuromatosis an uncommon manifestation of Cowden syndrome

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### Abstract

Diffuse intestinal ganglioneuromatosis is a hamartomatous polyposis characterized by a disseminated, intramural or transmural proliferation of neural elements involving the enteric plexuses. It has been associated with MEN II, neurofibromatosis type 1 and hamartomatous polyposis associated with phosphatase and tensin homolog mutation. We report the case of a female patient with a history of a breast and endometrial tumor who presented in a colonoscopy performed for rectal bleeding diffuse ganglioneuromatosis, which oriented the search for other characteristic findings of Cowden syndrome given the personal history of the patient. The presence of an esophagogastric polyposis was also noted. Cowden syndrome is characterized by skin lesions, but it is rarely diagnosed by these lesions, because they are usually overlooked. Intestinal polyposis is not a major diagnostic criterion but it is very useful for early diagnosis. The combination of colonic polyposis and glucogenic acanthosis should orient the diagnosis to Cowden syndrome.

### INTRODUCTION

Intestinal ganglioneuromatosis is a hamartomatous polyposis usually reported in children and uncommon in adults consisting of hyperplasia of the myenteric plexus and the enteric nerve fibers<sup>[1]</sup>. The most common symptoms caused are change in bowel habit and gastrointestinal bleeding. Diagnosis is always microscopic although the digitiform morphology of this type of polyps may be suggestive. It may be a single, multiple or diffuse polyposis, characterized by a disseminated, intramural or transmural proliferation of neural elements involving the enteric plexuses. The diffuse form has been related to systemic diseases such as MEN II, neurofibromatosis type 1 and hamartomatous polyposis associated with phosphatase and tensin homolog (PTEN) mutation, including Cowden syndrome, although ganglioneuromatosis has not been associated with any specific gene mutation<sup>[2]</sup>. Cowden syndrome is an autosomal dominant disease characterized by the presence of multiple hamartomas of ectodermal, mesodermal and endodermal origin, and an increased risk of development of malignant disease. The most typical finding is mucocutaneous lesions present in

almost 100% of the cases<sup>[3]</sup>. It is also commonly associated with gastrointestinal polyposis, the most common histology being hamartomas, although fibromatous, lipomatous or hyperplastic polyps, adenomas and sometimes ganglioneuromas have also been reported. Many patients present several histological types simultaneously<sup>[4]</sup>. We report a case of intestinal ganglioneuromatosis that oriented the diagnosis to Cowden syndrome.

## CASE REPORT

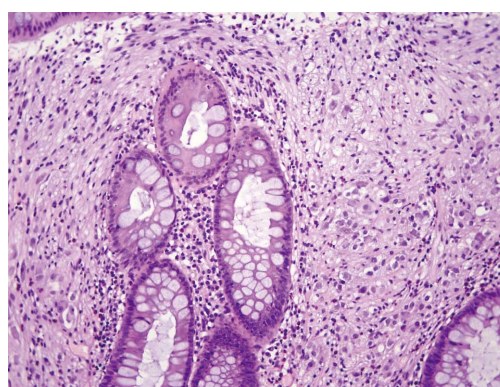
We report the case of a 40-year-old female patient who presented to the gastrointestinal clinic in 2005 for rectal bleeding associated with a change in bowel habit. She had as personal history at that time of hemithyroidectomy due to hyperfunctional thyroid nodules, a bilateral mastectomy for multicenter intraductal carcinoma and 4 abortions. Laboratory tests were performed with normal complete blood count, glucose, urea, creatinine, ions, liver profile, lipid profile, and hormone study. A colonoscopy was requested revealing multiple polyposis in all colon segments, with polypectomy of over 50 polyps being performed. Findings in the pathological study of the excised tissue were: hyperplastic, adenomatous polyps and ganglion cells in lamina propria of some excised polyps. In 2008, she required hysterectomy and right adnexectomy due to endometrial squamous metaplasia and an eroded ovarian cyst. That same year a colonoscopy was performed for postpolypectomy control in which multiple polypectomy was repeated. On this occasion, the pathological study revealed diffuse intestinal ganglioneuromatosis in the material provided (Figure 1). Based on these findings and given its possible relationship, it was decided to perform screening for MEN II, which was negative. Suspecting possible Cowden syndrome, a targeted skin examination was requested, in which multiple papular facial lesions were identified, some of them of papillomatous appearance, which were biopsied, with several showing pathological features of trichilemmomas. A gastroscopy was subsequently performed showing esophagogastric polyposis (Figure 2), with esophageal polyps consistent with glucogenic acanthosis. The patient met clinical diagnostic criteria for Cowden syndrome (Table 1), currently pending genetic study (*PTEN* gene). Associated pathology at the cerebellar level was discarded by magnetic resonance imaging. The recommended preventive follow-up was performed, requiring in 2010 thyroid resection of thyroid remnants due to papillary microcarcinoma and new endoscopic colonic polypectomies (Pathology report: ganglioneuromas with intestinal pneumatosis).

## DISCUSSION

Cowden syndrome is considered an uncommon syndrome of hamartomatous polyposis caused by germinal changes in the *PTEN* tumor suppressor gene localized on chromosome 10 (10q23)<sup>[5,6]</sup>, which could be involved as a regulator of multiple processes of cell proliferation,

**Table 1 2008 National comprehensive cancer network diagnostic criteria for Cowden syndrome**

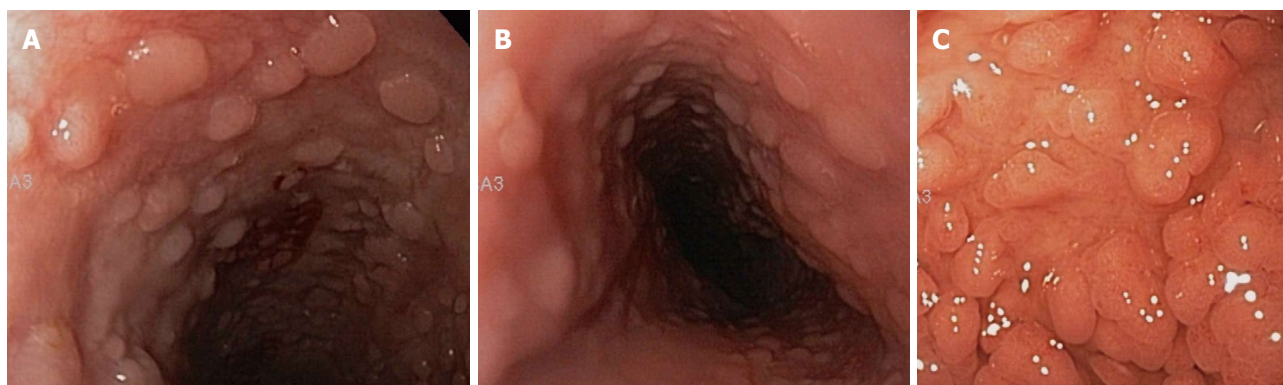
Pathognomonic criteria
Lhermitte-duclos disease-adult
Mucocutaneous lesions
Trichilemmomas, facial
Acral keratoses
Papillomatous lesions
Major criteria
Breast cancer
Thyroid cancer (papillary or follicular)
Macrocephaly ( $\geq 97\%$ ile)
Endometrial cancer
Minor criteria
Other structural thyroid lesions ( <i>e.g.</i> , adenoma, multinodular goiter)
Mental retardation ( <i>i.e.</i> , intelligence quotient $\leq 75$ )
Gastrointestinal hamartomas
Fibrocystic disease of the breast
Lipomas
Fibromas
Genitourinary tumours ( <i>e.g.</i> , uterine fibroids, renal cell carcinoma) or genitourinary structural malformations
Uterine fibroids
Operational diagnosis in an individual (any of the following)
Mucocutaneous lesions alone if:
There are six or more facial papules, of which three or more must be trichilemmoma; or
Cutaneous facial papules and oral mucosal papillomatosis; or
Oral mucosal papillomatosis and acral keratoses; or
Palmoplantar keratoses, six or more
Two or more major criteria, but one must include macrocephaly or Lhermitte-duclos disease
One major and three minor criteria; or
Four minor criteria
Operational diagnosis in a family where one individual is diagnostic for Cowden
One pathognomonic criterion
Any one major criterion with or without minor criteria
Two minor criteria
History of Bannayan-Riley-Ruvalcaba syndrome



**Figure 1** Pathological study revealed diffuse intestinal ganglioneuromatosis.

migration and apoptosis, all of which are important processes for adequate cell growth. Other syndromes that have been associated with a mutation of this gene are the Bannayan-Riley-Rubalcaba syndrome, proteus and proteus-like syndrome and adult Lhermitte-Duclos disease, as well as autism syndromes associated with macrocephalia<sup>[6]</sup>.





**Figure 2** A gastroscopy was subsequently performed showing esophagic (A, B) and gastric polyposis (C).

The prevalence of Cowden syndrome has been estimated at 1/200 000-250 000 inhabitants in a German series published in 1999<sup>[7]</sup>, but it is thought that its prevalence is underestimated as it is a difficult disease to diagnose because of the variability of its expression and since many of its manifestations may go unnoticed<sup>[5]</sup>.

Diagnosis is based on clinical criteria, the most recent criteria from 2008 have been previously described. Our patient had 2 pathognomonic criteria (papillomatous papules, trichilemmomas), 3 major criteria (breast cancer, thyroid cancer, endometrial cancer) and 2 minor criteria (gastrointestinal hamartomas and benign thyroid disease). These criteria lead us to the diagnosis but do not provide an early diagnosis of syndrome, since the skin lesions, pathognomonic of this disease, usually go unnoticed and are diagnosed by a targeted examination when one starts to suspect this condition, as occurred also in our case. Gastrointestinal polyposis is considered a minor criterion due to the lack of systematic studies to determine its true frequency and histology<sup>[4]</sup>. It is actually a very common finding, with an estimated prevalence of up to 80% in patients with Cowden syndrome. In a series of 127 patients with *PTEN* gene mutation, the presence of gastrointestinal polyposis was seen in 50% of the total and in 93% of patients who underwent an endoscopy, thus indicating an underestimated frequency of this manifestation since an endoscopic study is performed in only a percentage of patients, generally those who are symptomatic<sup>[4]</sup>. The histopathology of the polyps found in colon is similar to that found in duodenum and stomach but not in the esophagus, where it is usually a diffuse glucogenic acanthosis, as in our case, and less commonly consists of pseudo polyps of inflammatory appearance. It has been suggested that the association of benign gastrointestinal polyposis and esophageal glucogenic acanthosis should be considered as a pathognomonic criterion for Cowden syndrome<sup>[3,8]</sup>. The implementation of surveillance programs in patients with hereditary diseases with an increased risk of malignancy is necessary but in the case of Cowden syndrome it is a controversial subject since the association with increased breast, thyroid and endometrial cancer is clear, but the association to other cancers including melanoma, renal

cell or colon cancer has not been established due to the lack of sufficient data<sup>[6]</sup>. In the previously mentioned series of 127 patients<sup>[4]</sup>, colorectal cancer was detected in 7.1% of patients, all under 50 years of age. Based on these data and the earlier published cases referring to a possible association between Cowden syndrome and colon carcinoma, numerous recommendations for colon cancer screening were made in this type of patients. From the laxest which recommend monitoring as in the general population starting after 50 years<sup>[9]</sup> to the strictest which recommend starting at 15 years and monitoring every 1-2 years<sup>[10]</sup>, through an intermediate and more reasonable recommendation starting at 35 years, or earlier if there are symptoms, with a variable monitoring according to macroscopic and histopathological findings<sup>[4]</sup>.

In conclusion, Cowden syndrome is a disease with increased risk of malignancy, whose clinical manifestations are highly variable making diagnosis difficult. Gastrointestinal polyposis is a common manifestation but systematic studies are required to reach a criterion of greater weight in diagnosis of this pathology. The histology if gastrointestinal polyps is varied, and the same patient may present 2 or more histological types, including ganglioneuromas, which when they are of the diffuse type orient the diagnosis more quickly to Cowden syndrome. Additional studies are needed to assess the malignization risk of this polyposis and unify the recommendations for colon cancer screening in this type of population.

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## Complete remission of advanced hepatocellular carcinoma by sorafenib: A case report

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### Abstract

Hepatocellular carcinoma (HCC) is the fifth most common malignant disease worldwide, and curative treatment remains difficult because the majority of cases are diagnosed in the advanced stage. Sorafenib is the only known effective systemic treatment, but patients rarely achieve complete remission (CR). A 66-year-old man with a history of alcoholic liver cirrhosis with a diagnosis of advanced HCC, was initially treated with transarterial chemoembolization on four occasions. However, the disease progressed with portal vein thrombosis. Therefore, sorafenib was started, and 4 mo later, the patient achieved CR. The treatment was continued for 12 mo, and CR was maintained up to 4 mo after sorafenib discontinuation.

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**Key words:** Hepatocellular carcinoma; Sorafenib; Portal

vein thrombosis; Complete remission

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### INTRODUCTION

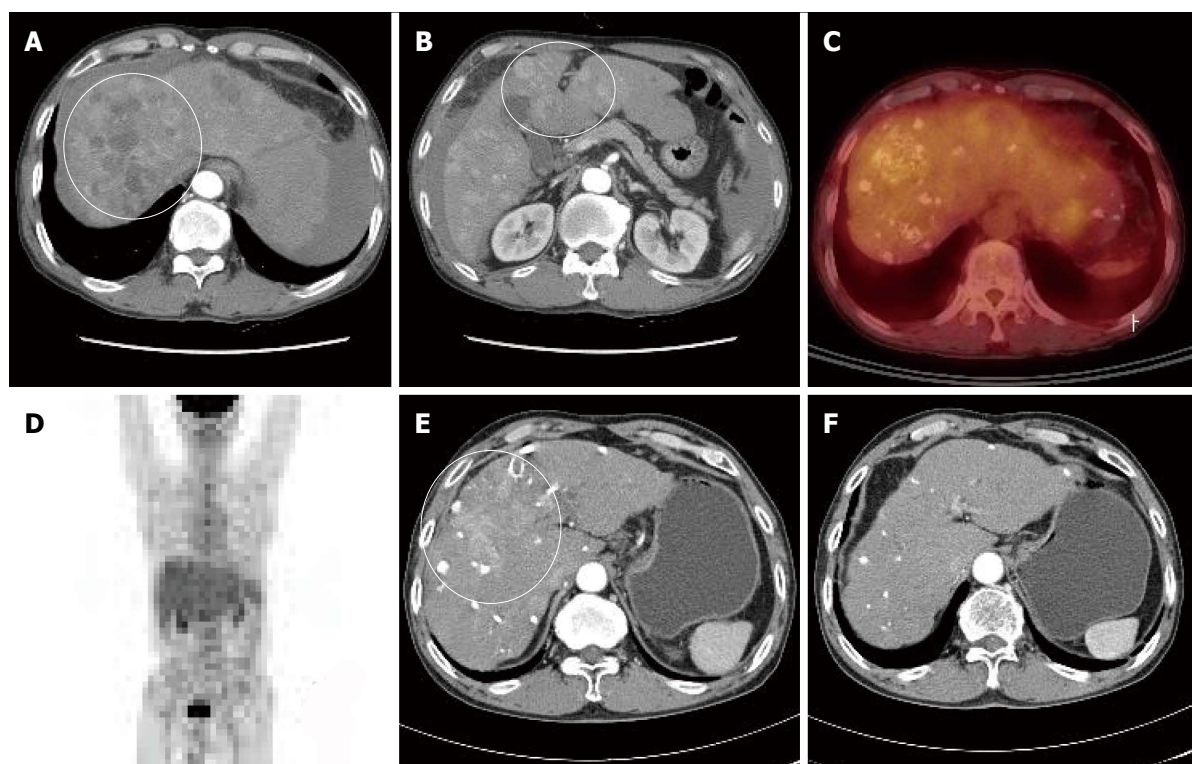
Hepatocellular carcinoma (HCC) is the fifth most common malignant tumor and ranks third on causes of death related to cancer<sup>[1]</sup>. However, only 30%-40% of HCC patients can expect to undergo curative treatment, as the majority of the patients are diagnosed in the advanced stage<sup>[2]</sup>. Therefore, only palliative treatment, such as, transarterial chemoembolization (TACE) or systemic treatment, is available for patients with advanced HCC<sup>[2]</sup>.

At the molecular level, sorafenib inhibits several types of tyrosine protein kinases, such as, vascular endothelial growth factor (VEGF) receptors 1, 2, and 3, platelet-derived growth factor (PDGF) receptor, and Raf kinase<sup>[3]</sup>. The SHARP trial reported that sorafenib has significant survival benefit in advanced HCC, and that is the only effective systemic agent<sup>[4]</sup>. Treatment response to sorafenib in HCC manifests in different ways, but only a handful of reports of complete remission (CR) on sorafenib have been issued<sup>[5-8]</sup>.

Here, we present a case of advanced HCC patient who have achieved CR on sorafenib despite disease progression after four sessions of TACE for multinodular intrahepatic HCC.

### CASE REPORT

A 66-year-old male visited our hospital with abdominal pain. He had a 40-year history of 120 g/d of ethanol consumption, and had been diagnosed with alcoholic liver cirrhosis two years ago, for which he had not received



**Figure 1** Computed tomography of the patient. A, B: Computed tomography (CT) scan taken at the time of hepatocellular carcinoma (HCC) diagnosis showing intrahepatic multinodular HCCs (white circles); C, D: Positron emission tomography CT scan showing multiple lipiodolized masses and abnormal fluorine-18 2-fluoro-2-deoxy-D-glucose uptake on liver (no distant metastasis was found); E, F: After repetitive transarterial chemoembolization, a viable hypervascular mass remained (E, white circle), but after 6 mo of oral sorafenib, this was not detected by liver dynamic CT (F, arterial phase).

any treatment. Laboratory test results at time of admission were as follows: aspartate aminotransferase 180 IU/L, alanine transaminase 75 IU/L, alpha-fetoprotein (AFP) 274 ng/mL, protein induced vitamin K absence > 2000 mAu/mL, total bilirubin 1.3 mg/dL, albumin 3.2 mg/dL, and prothrombin time international normalized ratio 1.25. He showed good reserve liver function with Child-Turcotte-Pugh class A, and did not have any ascites or findings of encephalopathy. Dynamic liver computed tomography (CT) findings depicted intrahepatic multinodular HCCs (Figure 1A and B). Tumor stage based on the Barcelona Clinical Liver Cancer (BCLC) staging system was BCLC stage B. Positron emission tomography (PET)-CT also showed increased multiple fluorine-18 2-fluoro-2-deoxy-D-glucose (PDG) uptake in liver without metastasis to any other organs (Figure 1C and D).

Because the tumor was not indicative for curative treatment, repeated TACE was performed with one or two month intervals. However, despite three sessions of TACE, follow-up dynamic liver CT revealed disease progression with viable intrahepatic HCCs (Figure 1E) and multiple paraaortic lymphadenopathy. At this time, serum AFP levels had increased to 2795 ng/mL, and thus, sorafenib was recommended. However, the patient refused for financial reasons, and TACE was performed once more. Followed-up CT scan taken after the fourth TACE session showed disease progression (Figure 2A) and portal vein tumor thrombosis (PVTT) that had not

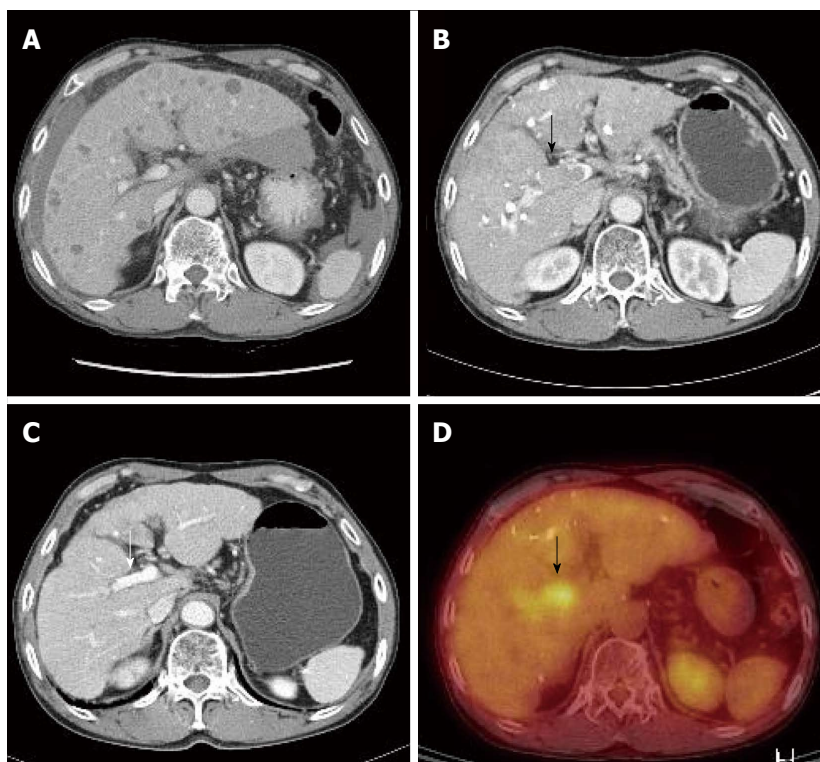
been previously identified was shown (Figure 2B and D).

Due to the PVTT, transarterial chemoinfusion (TACI) was performed, and oral sorafenib (400 mg *b.i.d.*) was initiated 4 d later. After 3 mo of sorafenib administration, serum AFP level returned to normal range (5.3 ng/mL), and dynamic liver CT visualized no remaining hypervascular intrahepatic mass, though it did show multiple lipiodol uptake. Furthermore, the PVTT and paraaortic lymphadenopathy had partially improved (Figure 2C). After 20 d of sorafenib administration, the patient developed a grade 2 hand-foot skin reaction and the dosage was reduced to 400 mg daily. After 6 mo of sorafenib treatment, serum AFP remained in normal range and no viable hypervascular mass was observed by dynamic liver CT (Figure 1F). The PVTT and the metastatic paraaortic lymph node had completely disappeared on dynamic liver CT, and no PDG uptake was observed by follow-up PET-CT (Figure 3). Therefore, he achieved clinical CR.

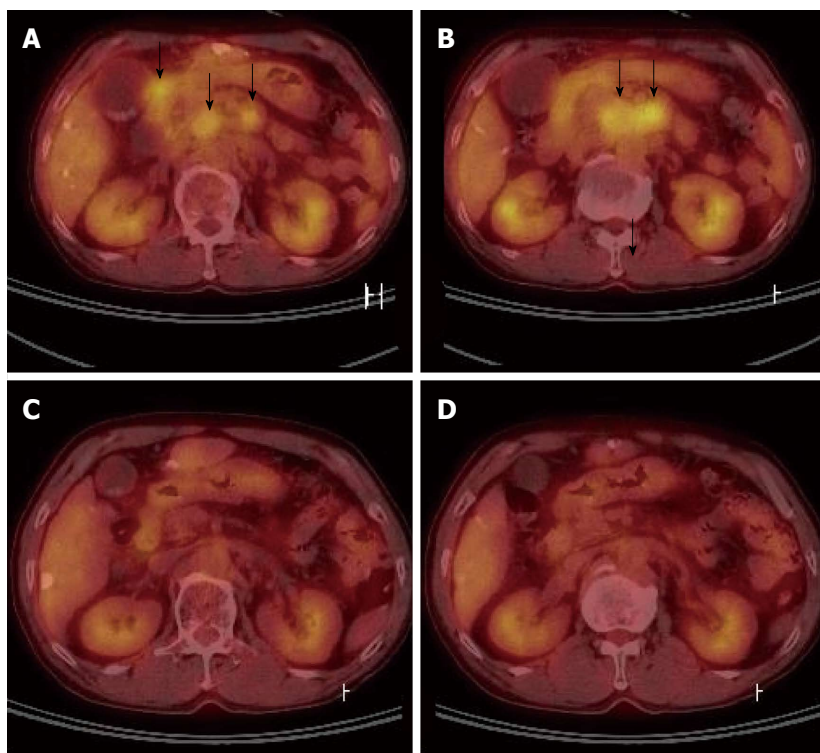
Sorafenib was maintained at 400 mg daily (200 mg, *b.i.d.*) for 6 mo after the achievement of CR. However, sorafenib was discontinued after 12 mo of administration for financial reasons. The patient was lost to follow up at 2 mo after sorafenib discontinuation, at which time, he had maintained a CR status for 8 mo.

## DISCUSSION

The majority of patients diagnosed with HCC have advanced stage disease, and for most, palliative treat-



**Figure 2 The diagnosis of portal vein tumor.** A, B, D: Portal vein tumor (A), which was not evident at diagnosis, was detected by liver dynamic computed tomography (CT) (B, black arrow) and positron emission tomography-CT (D, black arrow) after four sessions of transarterial chemoembolization; C: Portal vein tumor improved after 3 mo of sorafenib (white arrow).



**Figure 3 Positron emission tomography computed tomography.** A, B: After four sessions of transarterial chemoembolization, significant multiple abdominal lymphadenopathy was detected by positron emission tomography (PET)-computed tomography (CT) (black arrows); C, D: Fluorine-18 2-fluoro-2-deoxy-D-glucose uptakes disappeared on follow up PET-CT scans taken after 6 mo on sorafenib.

ments, such as, TACE or systemic treatment, are the only available therapeutic options. The SHARP trial

report concluded that sorafenib was the only treatment regimen capable of providing a survival benefit for ad-



vanced HCC<sup>[3]</sup>, and although several targeting agents and systemic cytotoxic agents have been developed, none has shown survival benefit in advanced HCC to date.

Sorafenib is a small molecule inhibitor of tyrosine protein kinases (*e.g.*, VEGFR and PDGFR) and of Raf kinases. Sorafenib blocks VEGF and PDGF signaling, suppresses tumor angiogenesis, and interrupts Raf kinase signaling by suppressing tumor cell proliferation and inducing apoptosis. These actions of sorafenib are attributed to the inhibition of the serine/threonine kinases Raf-1 and B-Raf and to its inhibition of the receptor tyrosine kinase activities of VEGFRs 1, 2, and 3 and PDGFR- $\beta$ . Furthermore, cellular signaling mediated by the Raf-1 and VEGF pathways has been implicated in the molecular pathogenesis of HCC, and this provides a rationale for the effect of sorafenib in HCC<sup>[9,10]</sup>.

Two large phase III clinical trials, the SHARP trial and the Asia-Pacific trial, established consensus that sorafenib has survival benefit in advanced HCC. In the SHARP trial, 602 patients with advanced HCC and cirrhosis were enrolled. Of the patients treated with sorafenib, only seven (2%) achieved partial response and no complete response was recorded<sup>[3]</sup>. In the Asia-Pacific trial, no complete response to sorafenib was observed among the 226 patients enrolled, despite a partial response rate of 3.3%. Furthermore, median overall survival was 6.5 mo in the sorafenib group, and 4.2 mo in the placebo group<sup>[4]</sup>. Based on the findings of these studies, sorafenib is now used in HCC patients with BCLC stage C, but, increased survival can only be expected when it is used in indicative patients. Furthermore, the achievement of CR by sorafenib is rare and only a handful of cases have been reported<sup>[5-8]</sup>. In our case, PVT and intraabdominal lymphadenopathy were also improved by administering sorafenib.

The treatment of advanced HCC with PVTT is much less effective than the treatment of HCC without PVTT, and median survival time of the former without treatment has been reported to be only 2.7-4 mo<sup>[11,12]</sup>. A variety of treatment modalities, such as, TACE, chemotherapy (5-fluorouracil, cisplatin, *etc.*), and radiotherapy have been attempted in cases with PVTT, but all have been found to be ineffective<sup>[13]</sup>. Interestingly, in the present case, PVTT improved after sorafenib treatment. PVTT is caused by portal vein invasion by cancer cells, and vascular specific growth factors are important for this process<sup>[14]</sup>. Sorafenib reacts by blocking VEGF and PDGF receptors, and is believed to promote PVT revascularization. Although PVTT revascularization after administering sorafenib has been reported in some cases, no case of complete response has been previously reported<sup>[15,16]</sup>. Furthermore, in the present case, sorafenib appeared to improve lymphadenopathy.

As in this case, the treatment for advanced HCC that has extrahepatic metastasis such as PVTT and abdominal lymphadenopathy is restricted<sup>[13]</sup>. The previous studies have reported the combined radiotherapy and TACE in advanced HCC with PVTT<sup>[17-19]</sup>, and there are

cases that report the effectiveness of radiation therapy in HCC that accompanies abdominal lymph node metastasis<sup>[20]</sup>. However, the standard treatment is not yet established. In this case, we started treatment with sorafenib according to BCLC guideline, and PVTT and abdominal lymphadenopathy responded to sorafenib.

The most common adverse effects of sorafenib are diarrhea (43%), rash (40%), fatigue (37%), hand-foot skin reaction (HFSR) (30%), and alopecia (27%); other adverse effects include nausea and pruritus, anorexia, hypertension<sup>[21]</sup>. HFSR is a common cause of dosage reduction, and affects quality of life, and usually occurs during the first 2-4 wk of administration. In our patient, grade 2 HFSR occurred after 20 d of administration and the dosage was halved to 400 mg daily, which was tolerable. Many patients treated with sorafenib complained about HFSR, and thus, require dosage adjustment or discontinuance. However, the dosage-response effects of sorafenib after dosage reduction have not been established. Well-designed studies are required to solve this issue in the future.

The pathogenesis of HFSR has not been elucidated. It has been reported that multi-targeting kinase inhibitors enter eccrine glands and directly affect toxicity to the skin<sup>[22]</sup>. However, HFSR is considered an indirect effect of sorafenib. Epidermal keratinocytes synthesize PDGF- $\alpha$  and PDGF- $\beta$ , which activate dermal capillaries, fibroblasts, and PDGFR in eccrine glands<sup>[22]</sup>. Furthermore, eccrine glands present c-KIT and PDGFR, which are targeted by sorafenib. Therefore, because sorafenib suppresses VEGFR and PDGFR, HFSR is believed to be an indirect effect of the suppression of the angiogenic pathway<sup>[22]</sup>.

We report a patient with advanced HCC patient with PVTT and abdominal lymphadenopathy, who achieved CR by sorafenib administration. Sorafenib is an important treatment option that has been shown to increase survival in HCC and to improve prognosis in selected cases. Therefore, we recommend active use of sorafenib to be considered in HCC patients capable of tolerating sorafenib but not indicative for curative treatment or TACE. Furthermore, our experience of this case recommends the use of sorafenib in HCC patients with PVTT, and suggests that sorafenib should be administered as aggressively as possible to such patients. In addition, given that many patients complain of HFSR during sorafenib administration, studies on the prevention and treatment of HFSR are required. Finally, studies are also required on the dosage/response characteristics of sorafenib with respect to ethnicity, age, gender, and disease status.

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# World Journal of *Gastrointestinal Oncology*

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## Epigenetic field defects in progression to cancer

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### Abstract

A field defect is a field of pre-malignant tissue in which a new cancer is likely to arise. Field defects often appear to be histologically normal under the microscope. Recent research indicates that cells within a field defect characteristically have an increased frequency of epigenetic alterations and these may be fundamentally important as underlying factors in progression to cancer. However, understanding of epigenetic field defects is at an early stage, and the work of Katsurano *et al* published this year, is a key contribution to this field. One question examined by Katsurano *et al* was how early could the formation of an epigenetic field defect be detected in a mouse colitis model of tumorigenesis. They highlighted a number of measurable epigenetic alterations, detected very early in normal appearing tissue undergoing histologically invisible tumorigenesis. They also documented the increasing presence of the epigenetic alterations at successive times during progression to cancer. In this commentary, we offer a perspective on the changes they observed within a broader sequence of epigenetic events that occur in progression

to cancer. In particular, we highlight the likely central role of epigenetic deficiencies in DNA repair gene expression that arise during progression to cancer.

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**Key words:** Field defect; Epigenetics; Tumorigenesis; Carcinogenesis; DNA damage; DNA repair; Colon cancer; Mouse; Human

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### COMMENTARY ON HOT TOPICS

We read with great interest the recent article by Katsurano *et al*<sup>[1]</sup> describing a mouse colitis model leading to tumor formation. They report that an epigenetic field defect forms early after treatment with dextran sulfate sodium (DSS) and that the epigenetic alterations continue to increase even with diminishing stimulation. Their study was unique in showing that particular epigenetic alterations, involving DNA methylation, increased even while inflammation was diminishing.

The term “field cancerization” was first used in 1953 to describe an area or “field” of epithelium that has been preconditioned by (at that time) largely unknown processes so as to predispose it towards development of cancer<sup>[2]</sup>. Since then, the terms “field cancerization” and “field defect” have been used to describe pre-malignant tissue in which new cancers are likely to arise.

Field defects are of crucial importance in progression to cancer, though they have not received a great deal of attention thus far. As pointed out by Rubin<sup>[3]</sup>, “The vast majority of studies in cancer research has been done on well-defined tumors *in vivo*, or on discrete neoplastic foci *in vitro*. Yet there is evidence that more than 80% of the somatic mutations found in mutator phenotype human

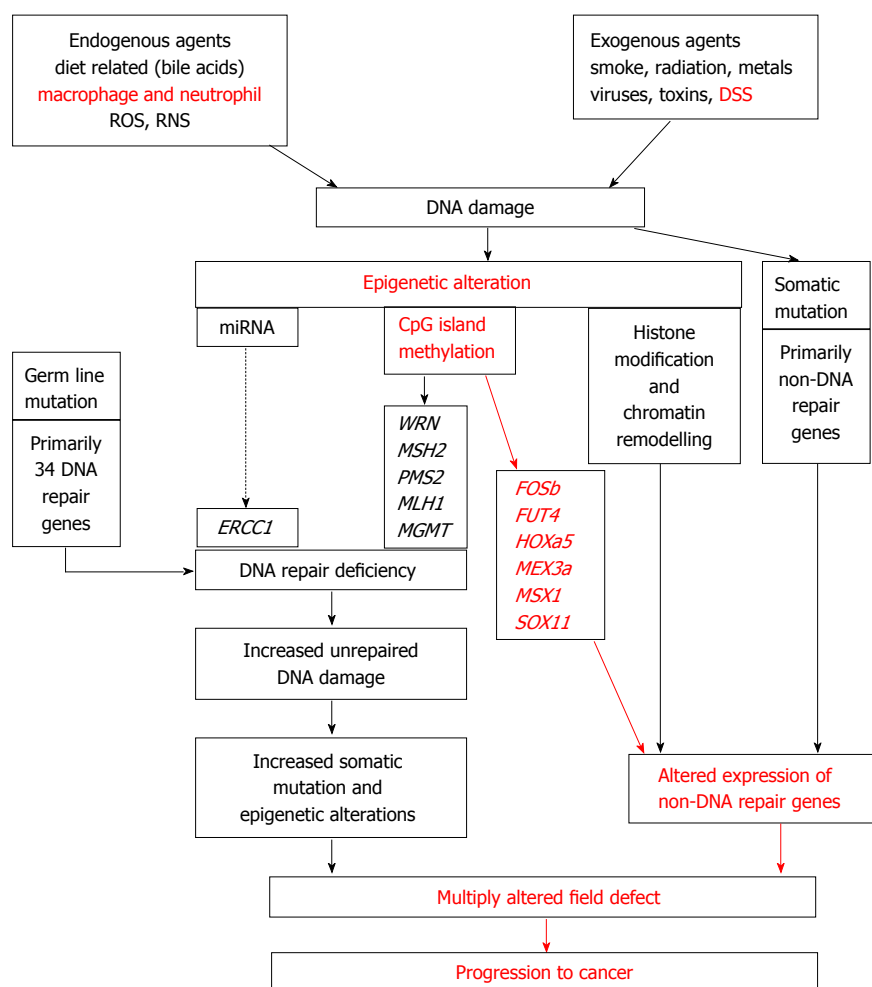


Figure 1 Roles of DNA damage, reduced DNA repair and epigenetic alterations in a field defect in progression to cancer.

colorectal tumors occur before the onset of terminal clonal expansion<sup>[4]</sup>." Field defects with mutations are precursors of cancers with those same mutations. Likewise, epigenetic alterations in field defects are precursors of cancers with those same epigenetic alterations.

Colon cancers contain a median of 76 non-silent sequence mutations, of which about 15 are "driver mutations" (the rest are "passenger mutations")<sup>[5]</sup>, as well as about 55 aneuploidy events<sup>[6]</sup>. By comparison, some frequent epigenetic alterations in colon cancers affect hundreds of genes. For example, CpG island methylation of the DNA sequence encoding microRNA miR-137 reduces its expression, and this is a frequent early epigenetic event in colorectal carcinogenesis, occurring in 81% of colon cancers and in 14% of the normal appearing colonic mucosa of the field defects associated with these cancers<sup>[7]</sup>. Silencing of miR-137 can affect expression of 491 genes, the targets of this miRNA<sup>[7]</sup>. Changes in the level of miR-137 expression result in changed mRNA expression of the target genes by 2 to 20-fold and corresponding, though often smaller, changes in expression of the protein products of the genes. Other microRNAs, with likely comparable numbers of target genes, are even more frequently epigenetically altered in colonic field

defects and in the colon cancers that arise from them. These include miR-124a, miR-34b/c and miR-342 which are silenced by CpG island methylation of their encoding DNA sequences in primary tumors at rates of 99%, 93% and 86%, respectively, and in the adjacent normal appearing mucosa at rates of 59%, 26% and 56%, respectively<sup>[8,9]</sup>. In addition to epigenetic alteration of expression of miRNAs, other common types of epigenetic alterations in cancers that change gene expression levels include direct hypermethylation or hypomethylation of CpG islands of protein-encoding genes and alterations in histones and chromosomal architecture that influence gene expression<sup>[10,11]</sup>.

The specific epigenetic alterations studied by Katsurano *et al*<sup>[1]</sup>, as well as epigenetic field defects in general, can be placed in a broad explanatory framework starting with the occurrence of DNA damage, a major primary event in progression to cancer as shown in Figure 1. In Figure 1 italicized capitalized abbreviations are symbols of epigenetically altered genes. *ERCC1*, *WRN*, *MSH2*, *PMS2*, *MLH1* and *MGMT* are symbols for specific DNA repair genes. *FOSb*, *FUT4*, *HOXA5*, *MEX3a*, *MSX1*, *SOX11* are symbols for epigenetically altered genes described by Katsurano *et al*<sup>[1]</sup>. These symbols for the genes (in the

order listed above) are: *FBJ* osteosarcoma oncogene B, fucosyltransferase 4, homeobox A5, mex3 homolog A, homeobox msh-like 1, and SRY-box-containing gene 1, respectively.

### DNA damage as a primary cause of cancer

Exogenous and endogenous agents that induce DNA damage have been identified as major causes of many common cancers (Figure 1). These include cancers of the lung (tobacco smoke<sup>[12]</sup>), colorectum (exposure to bile acids that cause increased reactive oxygen species (ROS) and reactive nitrogen species, and are produced in response to a high fat diet<sup>[13]</sup>), esophagus (exposure to stomach acids plus bile acids due to gastroesophageal reflux<sup>[14]</sup>), stomach (reactive oxygen species caused by *Helicobacter pylori* infection<sup>[15]</sup>), liver (*Aspergillus* metabolite aflatoxin B<sub>1</sub><sup>[16]</sup>), cervix/uterus (human papillomavirus plus increased nitric oxide from tobacco smoke or other infection<sup>[17]</sup>) and melanoma (UV light from solar radiation<sup>[18]</sup>). Inherited germ line mutations in DNA repair genes similarly cause an increase in DNA damages due to a deficiency in repair capability, and these also cause increases in cancer risk. At least 34 inherited human DNA repair gene mutations increase cancer risk, including, for example, germ line mutations in the *BRCA1*, *XPC* and *MLH1* genes<sup>[19]</sup>. From a study of 44 788 pairs of twins, it is estimated that overall, about 30% of cancers are familial (largely due to inherited germ line mutations or genetic polymorphisms) and 70% are sporadic<sup>[20]</sup>.

### DNA damages cause epigenetic changes and mutations

ROS, produced during inflammation and other types of cellular stress, cause a variety of types of DNA damage<sup>[21]</sup>, some of which lead to double strand breaks<sup>[22]</sup>. During repair of double strand breaks and other types of oxidative DNA damages, methylation of promoter CpG islands in DNA and/or modification of histones can occur, causing gene silencing (Figure 1)<sup>[23,24]</sup>. These epigenetic alterations are sometimes not reversed after repair is completed<sup>[23,24]</sup>. While it has long been known that oxidative damage can cause mutation<sup>[21]</sup>, it has only recently become clear that oxidative damage can also give rise to epigenetic changes (epimutation)<sup>[23,24]</sup>.

Other types of DNA damage can also give rise to epimutation during DNA repair. The DNA repair enzyme Parp1 [poly(ADP)-ribose polymerase-1] acts at sites of DNA damage, especially single strand breaks, where it adds poly(ADP)-ribose to specific proteins as part of the overall DNA repair process<sup>[25]</sup>. This, in turn, directs recruitment and activation of the chromatin remodeling protein ALC1 to cause nucleosome remodeling<sup>[26]</sup>. Nucleosome remodeling has been found to cause, for instance, epigenetic silencing of DNA repair gene *MLH1*<sup>[27]</sup>. In addition, certain chemicals previously identified as DNA damaging agents, including benzene, hydroquinone, styrene, carbon tetrachloride and trichloroethylene, cause considerable hypomethylation of DNA, leading to epigenetic modifications, and some of this hy-

pomethylation occurs through the activation of oxidative stress pathways<sup>[28]</sup>.

### Epigenetic changes in DNA repair gene expression are a likely source of genomic instability

While germ line (familial) mutations in DNA repair genes cause a high risk of cancer, in sporadic (non-familial) cancers, by contrast, somatic mutations in DNA repair genes are rarely found<sup>[5]</sup>. However, deficient expression of DNA repair genes is frequently observed within sporadic cancers, and this is almost always due to epigenetic alteration (Figure 1). Epimutation leading to silencing of a gene necessary for DNA repair will allow unrepaired damages to increase. Such additional DNA damages, in turn, will cause increased mutations and epimutations, including carcinogenic driver mutations and epimutations.

Truninger *et al.*<sup>[29]</sup> compared the frequencies of germ line mutations, CpG island methylations and other unidentified alterations in the down-regulation of expression of DNA mismatch repair (MMR) gene *MLH1* in colon cancer. They evaluated 1 048 unselected consecutive colon cancers. They found that 103 of these cancers were deficient in protein expression of *MLH1*. Among the *MLH1* deficient cancers, 68 were sporadic and the remaining 35 were due to germ line mutations. Among the 68 sporadic *MLH1* protein-deficient colon cancers, 65 (96%) were deficient due to epigenetic methylation of the CpG island of the *MLH1* gene. Reduced protein expression of *MLH1* in the remaining 3 sporadic *MLH1* protein-deficient cancers may have been caused by over expression of the microRNA miR-155. This explanation is suggested by the finding that transfection of miR-155 into cells caused reduced expression of *MLH1*<sup>[30]</sup>. Furthermore, high expression of miR-155 was found in colon cancers in which protein expression of *MLH1* was reduced and the *MLH1* gene was neither mutated nor hypermethylated<sup>[30]</sup>.

Some of the epigenetic alterations in DNA repair genes found in colon cancers, as well as in their associated field defects, are summarized in Table 1<sup>[31-35]</sup>.

Deficiencies in DNA repair genes cause increased mutation rates in MMR defective cells<sup>[36,37]</sup> and in homologous recombinational repair (HRR) defective cells<sup>[38]</sup>. Chromosomal rearrangements and aneuploidy also increase in HRR defective cells<sup>[39]</sup>. Thus, deficiency in DNA repair causes genomic instability (a mutator phenotype), the likely main underlying cause of DNA sequence alterations leading to tumorigenesis. Genomic instability permits the acquisition of a sufficient number of mutations and epimutations in tumor suppressor genes and oncogenes to fuel carcinogenesis. Deficiencies in DNA repair appear to be central to the genomic and epigenomic instability characteristic of cancer.

Figure 1 illustrates the chain of consequences of exposure of cells to endogenous and exogenous DNA damaging agents that lead to cancer. The role of germ line defects in DNA repair genes in familial cancers are also indicated. The large role of DNA damage and con-



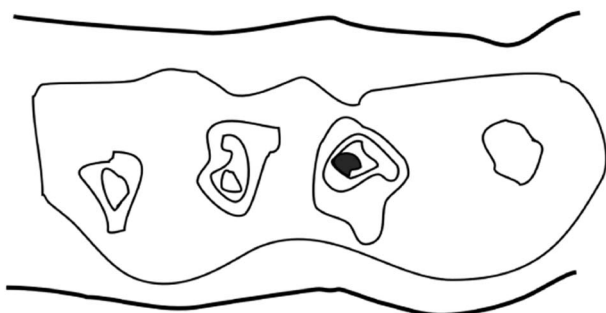


Figure 2 Schematic of a field defect in progression to cancer.

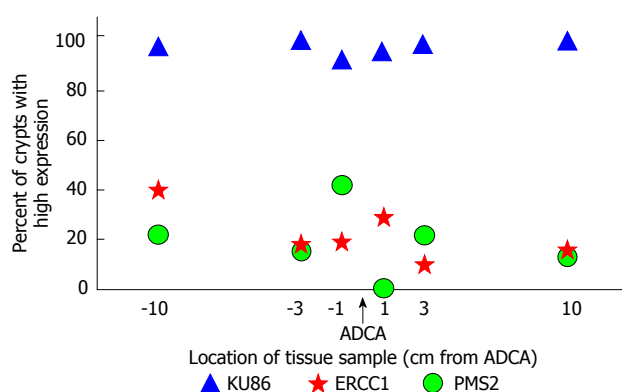


Figure 4 Reduced expression of DNA repair proteins ERCC1 and PMS2 and lack of reduction of DNA repair protein KU86 at distances up to 10 cm from an adenocarcinoma in a colon resection (graphed here from data reported on one of 8 similarly affected colon resections). ADCA: Adenocarcinoma.

sequent epigenetic DNA repair deficiencies leading to sporadic cancer are emphasized. The role of directly induced somatic mutation in sporadic cancer is indicated as well. The items shown in red lettering were demonstrated in the recent article of Katsurano *et al*<sup>[1]</sup>.

### Sequence of epimutation, mutation and natural selection leading to carcinogenesis

A field defect arises when an epimutation or mutation occurs in a stem cell that provides a reproductive advantage allowing clonal descendents of that stem cell to out-compete neighboring stem cells. These cells form a patch of somewhat more rapidly growing cells (an initial field defect). As the patch enlarges at the expense of neighboring cells, an additional epimutation or mutation may arise in one of the field defect stem cells so that this new stem cell with two advantageous epimutations and/or mutations generates daughter stem cells that can out-compete the surrounding field defect of cells that have just one advantageous epimutation or mutation. As illustrated in Figure 2, this process of expanding sub-patches within earlier patches can occur multiple times until a particular constellation of epimutations and mutations results in a cancer (represented by the small dark patch in Figure 2). The cancer, once formed, continues to evolve and to produce sub clones. A renal cancer, for example, sampled in 9 areas, had 40 ubiquitous mutations, 59 mutations

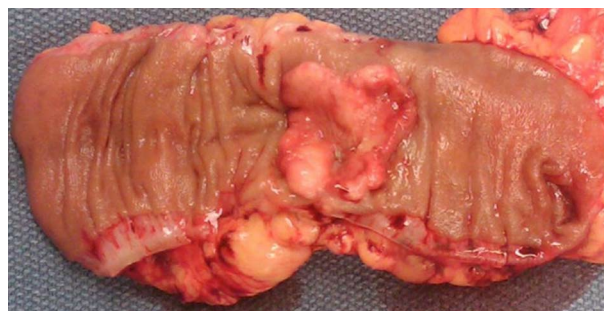


Figure 3 Human colon resection about 17 cm long, opened longitudinally to show inner epithelium, with cancer in center of inner surface.

shared by some, but not all regions, and 29 “private” mutations only present in one region<sup>[40]</sup>.

Figure 3 shows an opened resected segment of a human colon that has a colon cancer. There are about 100 colonic microscopic epithelial crypts *per* sq mm in the human colonic epithelium<sup>[41]</sup>. The colonic epithelium in the resection shown in Figure 3 has an area of about 6.5 cm by 17 cm, or 111 sq cm, or 11 100 sq mm. Thus this area has about 1.11 million crypts. There are 10-20 stem cells at the base of each colonic crypt<sup>[42,43]</sup>. Therefore there are likely 11 to 22 million stem cells in the grossly unremarkable colonic mucosal epithelium shown in Figure 3. Evidence reported by Facista *et al*<sup>[35]</sup>, listed in Table 1 and illustrated in Figure 4, indicates that in many such resections, most of the crypts, and thus the stem cells in such an area up to 10 cm distant (in each direction) from a colon cancer (such as in the grossly unremarkable area shown in Figure 3), and the majority of their differentiated daughter cells, are epigenetically deficient for protein expression of the DNA repair genes *ERCC1* and *PMS2*, although the epithelium is histologically normal.

The stem cells most distant from the cancer as well as those closer to the cancer in the resection defined by the data in Figure 4 appear to be deficient throughout the field defect for *ERCC1* and *PMS2*. The field defect of Figure 4, containing tens of millions of stem cells, presumably arose from an initial progenitor stem cell deficient in DNA repair (due to epigenetic silencing). Because of this repair deficit, the initial stem cell was genetically unstable, giving rise to an increased frequency of epimutations and mutations in its descendents. One daughter stem cell among its descendents presumably had a mutation or epimutation that, by chance, provided a replicative advantage. This descendent then underwent clonal expansion by natural selection because of its replicative advantage. Among the further descendents of the clone, new mutations and epimutations arose frequently, since these descendents had a mutator phenotype<sup>[44]</sup>, due to the repair deficiency passed down epigenetically from the original repair-defective stem cell. Among these new mutations and epimutations, some would provide further replicative advantages, giving rise to a succession of more aggressively growing sub clones (inner rings in Figure 2), and eventually to a cancer.

**Table 1** Examples of epigenetic alterations (epimutations) of DNA repair genes in colon cancers and in their field defects, with CpG island methylation indicated where known

Reference	Epimutations in genes found in colon cancer (mechanism)	Percentage of the sporadic cancers with that epimutation	Epimutations in genes in field defect (mechanism)	Percentage of the field defect with that epimutation
Agrelo <i>et al</i> <sup>[31]</sup>	WRN (CGI)	38%		
Shen <i>et al</i> <sup>[32]</sup>	MGMT (CGI)	46%	MGMT (CGI)	23%
Psofaki <i>et al</i> <sup>[33]</sup>	MGMT (CGI)	90%		
Psofaki <i>et al</i> <sup>[33]</sup>	MLH1 (CGI)	65%		
Truninger <i>et al</i> <sup>[29]</sup>	MLH1 (CGI)	96%		
Lee <i>et al</i> <sup>[34]</sup>	MLH1 (CGI)	2%		
Lee <i>et al</i> <sup>[34]</sup>	MSH2 (CGI)	13%	MSH2 (CGI)	5%
Lee <i>et al</i> <sup>[34]</sup>	MGMT (CGI)	47%	MGMT (CGI)	11%
Facista <i>et al</i> <sup>[35]</sup>	ERCC1	100%	ERCC1	60%
Facista <i>et al</i> <sup>[35]</sup>	PMS2	88%	PMS2	50%
Facista <i>et al</i> <sup>[35]</sup>	XPF	55%	XPF	40%

CGI: CpG island methylation.

The study by Katsurano *et al*<sup>[1]</sup> identified 14 genes that were epigenetically silenced or considerably reduced in expression due to CpG island methylation within at least 4 out of 5 of the cancers arising in their DSS induced mouse model of colon cancer. These appear to be “driver” epimutations. They then evaluated the non-neoplastic epithelial cells in the scraped off distal half of mouse colons undergoing DSS-induced tumorigenesis at 2 wk, 5 wk, 8 wk and 15 wk after transitory initial exposure of the mice to DSS. These epithelial cells constitute a field defect from which a mouse colon cancer is likely to arise, since 80%-100% of the mouse colons in their repeated experiments developed tumors 15 wk after exposure to DSS. By 5 to 8 wk after DSS exposure, and before any grossly visible tumors had formed, 6 of the possible “driver” epimutations present in the cancers were not only present, but were also increasing in extent with time, in the mouse colonic field defect.

Based on their own experiments and the literature, Katsurano *et al*<sup>[1]</sup> proposed that macrophages and neutrophils in the mouse colonic epithelium were the source of reactive oxygen species causing the DNA damage that initiated the tumorigenesis (Figure 1). However, even though these inflammatory cells were diminishing in frequency in the epithelium by 2 wk after their initial great increase upon DSS exposure, the level of CpG island methylation of the 6 possible “driver” genes *FOSb*, *FUT4*, *HOXA5*, *MEX3a*, *MSX1* and *SOX11* continued to increase in the isolated epithelial cells. This increase in the percentage of CpG island methylation of these 6 genes, as tumorigenesis progressed, may have been due to clonal expansion of epithelial cells that initially had these 6 methylated genes.

The work by Katsurano *et al*<sup>[1]</sup> constitutes the first mouse model of carcinogenesis in which the unique finding was made that DNA methylation frequency of some genes increased even as the initial inflammation causing DNA damage was decreasing. This work adds important experimental support for the idea that epimutation, natural selection and clonal expansion are key factors driving colon carcinogenesis.

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## CagA EPIYA polymorphisms in Colombian *Helicobacter pylori* strains and their influence on disease-associated cellular responses

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### Abstract

**AIM:** To investigate the influence of the CagA diversity in *Helicobacter pylori* (*H. pylori*) strains from Colombia on the host cell biology.

**METHODS:** Eighty-four *H. pylori*-cagA positive strains with different Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs patterns, isolated from patients with gastritis ( $n = 17$ ), atrophic gastritis ( $n = 17$ ), duodenal ulcer ( $n = 16$ ), intestinal metaplasia ( $n = 16$ ) and gastric cancer ( $n = 18$ ), were included. To determine the integrity of the cag pathogenicity island (cagPAI) we evaluated the presence of cagA, cagT, cagE, and cag10 genes by polymerase chain reaction. AGS gastric epithelial cells

were infected with each strain and assayed for translocation and tyrosine phosphorylation of CagA by western blot, secretion of interleukin-8 (IL-8) by enzyme-linked immuno sorbent assay after taking supernatants from cocultures and cell elongation induction. For cell elongation quantification, coculture photographs were taken and the proportion of "hummingbird" cells ( $> 15 \mu\text{m}$ ) was determined.

**RESULTS:** Overall 72% (60/84) of the strains were found to harbor a functional cagPAI. Levels of phosphorylated CagA were significantly higher for isolates from duodenal ulcer than the ones in strains from gastritis, atrophic gastritis, intestinal metaplasia and gastric cancer ( $49.1\% \pm 23.1\%$  vs  $21.1\% \pm 19.5\%$ ,  $P < 0.02$ ;  $49.1\% \pm 23.1\%$  vs  $26.2\% \pm 14.8\%$ ,  $P < 0.045$ ;  $49.1\% \pm 23.1\%$  vs  $21.5\% \pm 19.5\%$ ,  $P < 0.043$  and  $49.1\% \pm 23.1\%$  vs  $29.5\% \pm 27.1\%$ ,  $P < 0.047$  respectively). We observed variable IL-8 expression levels ranging from 0 to 810 pg/mL and from 8.8 to 1442 pg/mL at 6 h and 30 h post-infection, respectively. cagPAI-defective strains did not induce detectable levels of IL-8 at 6 h post-infection. At 30 h post-infection all strains induced IL-8 expression in AGS cells, although cagPAI-defective strains induced significantly lower levels of IL-8 than strains with a functional cagPAI ( $57.1 \pm 56.6$  pg/mL vs  $513.6 \pm 338.6$  pg/mL,  $P < 0.0001$ ). We did not observe differences in the extent of cell elongation induction between strains with a functional or a defective cagPAI in 6 h cocultures. At 24 h post infection strains with functional cagPAI showed high diversity in the extent of hummingbird phenotype induction ranging from 7% to 34%. cagPAI defective strains induced significantly lower levels of elongation than strains with functional cagPAI with one or more than one EPIYA-C motif ( $15.1\% \pm 5.2\%$  vs  $18.9\% \pm 4.7\%$ ,  $P < 0.03$ ; and  $15.1\% \pm 5.2\%$  vs  $20.0\% \pm 5.1\%$ ,  $P < 0.003$  respectively). No differences were observed in cellular elongation induction

or IL-8 expression among *H. pylori* strains bearing one and more than one EPIYA-C motifs, neither at 6 h nor at 24 h of coculture. There were no associations between the levels of induction of cell elongation or IL-8 expression and number of EPIYA motifs or pathology.

**CONCLUSION:** The present work describes a lack of association between *H. pylori* CagA protein EPIYA motifs variations from Colombian isolates and disease-associated cellular responses.

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**Key words:** *Helicobacter pylori*; cagA 3' region; CagA protein; Interleukin 8; Cell elongation; Glu-Pro-Ile-Tyr-Ala

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## INTRODUCTION

*Helicobacter pylori* (*H. pylori*) infects more than 50% of the world's population<sup>[1]</sup>. This pathogen has been associated with the development of chronic gastritis, duodenal ulcers and gastric cancer<sup>[2]</sup>, and was classified as a type I carcinogen by the International Agency for Research on Cancer<sup>[3]</sup>.

One of the most important virulence factors of *H. pylori* is the cag pathogenicity island (cagPAI), which encodes for a type IV secretion system (T4SS)<sup>[4]</sup>. Also encoded in the cagPAI is the CagA protein, which is translocated into gastric epithelial cells through the T4SS<sup>[5]</sup>, where it undergoes phosphorylation by members of the SRC and Abl families of kinases on tyrosine residues within the C-terminal Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs<sup>[6-8]</sup>. Phosphorylated CagA interacts with the cellular phosphatase SHP-2<sup>[9]</sup>, which in turn activates several signaling pathways involved, among others, in actin cytoskeletal rearrangements, leading to cell elongation (also known as the "hummingbird phenotype")<sup>[10,11]</sup>. Translocated CagA can also induce a proinflammatory response, resulting in the expression of interleukin-8 (IL-8) through the activation of nuclear factor  $\kappa$  B (NF- $\kappa$ B)<sup>[12-14]</sup>.

CagA varies in size, and this variation has been shown to be due to EPIYA motifs repeats within the C-terminal region of the protein<sup>[15,16]</sup>. Four types of EPIYA motifs have been described (A, B, C and D) based on the sequence flanking the motif<sup>[17]</sup>. Western *H. pylori* isolates have shown to harbor combinations of type A, B and C motifs, while East Asia isolates harbor combinations of type A, B and D motifs<sup>[17,18]</sup>.

A positive association between the number of EPIYA

motifs repeats and the phosphorylation of CagA protein has been reported<sup>[17,19]</sup>. Several studies have shown that strains with higher numbers of EPIYA-C motifs are more closely associated with gastric cancer<sup>[20-22]</sup>.

IL-8 expression in gastric tissue has been reported to correlate with the histopathological severity in *H. pylori*-positive patients<sup>[23,24]</sup>. Furthermore, it has been shown that strains with higher number of EPIYA-C motifs significantly increased IL-8 expression in gastric epithelial cells<sup>[25]</sup>. As with IL-8 expression, CagA proteins with higher number of EPIYA motifs, and especially EPIYA-C motifs, have shown to potentiate cell elongation in AGS cells<sup>[17,19,26,27]</sup>.

The aim of this study was to evaluate the possible association between CagA EPIYA motifs variations in *H. pylori* isolates from Colombia with the phosphorylation of CagA protein, the expression of IL-8 and cell elongation induction in gastric epithelial cells. Associations between disease severity and *H. pylori*-induced cellular responses *in vitro* were also evaluated.

## MATERIALS AND METHODS

### *H. pylori* strains

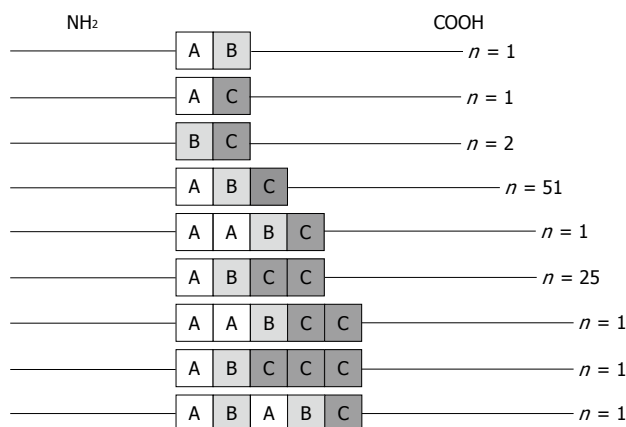
In total, 84 cagA-positive and 6 cagPAI-negative strains obtained from the stock collection at the Instituto Nacional de Cancerología, in Bogotá, Colombia were included in the study. CagA-positive strains were isolated from patients diagnosed with gastritis ( $n = 17$ ), atrophic gastritis ( $n = 17$ ), duodenal ulcer ( $n = 16$ ), intestinal metaplasia ( $n = 16$ ) and gastric cancer ( $n = 18$ ). Isolates' cagA genotyping was reported previously<sup>[28]</sup> and EPIYA motifs combinations used in this study are summarized in Figure 1. cagA-positive reference strain NCTC 11 637, with an ABCCC polymorphism, was used as a positive control.

### Detection of cagPAI genes

*H. pylori* genomic DNA was obtained from plate cultures of each isolate using DNAzol (Invitrogen) extraction method according to the manufacturer's instructions. The primers used in this study are listed in Table 1. To determine the integrity of cagPAI we evaluated the presence of cagA, cagT, cagE and cag10 genes. All PCR reactions were performed in a volume of 25  $\mu$ L containing 10 mmol/L Tris, 50 mmol/L KCl, 1.5 mmol/L MgCl<sub>2</sub>, 200  $\mu$ mol/L dNTPs, 25 pmol of the primers, 100 ng of *H. pylori* genomic DNA and 1U Taq polymerase. The polymerase chain reaction (PCR) conditions for each reaction were previously described<sup>[29,30]</sup>. Positive (strain 11637) and negative controls (strain 3062) for the cagPAI were included in each run. PCR products were analyzed by agarose gel electrophoresis with ethidium bromide staining.

### Culture of H. pylori strains

*H. pylori* strains were grown on blood agar plates, supplemented with 7% horse serum (Invitrogen), 1% Vitox (Oxoid), and Campylobacter selective supplement (Oxoid), at 37 °C in a 10% CO<sub>2</sub>-humidified atmosphere for



**Figure 1** *Helicobacter pylori* CagA Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs variations included in this study. Eighty-four cagA-positive strains isolated from Colombian patients were evaluated. All strains possessed Western EPIYA motifs (A, B and C)<sup>[17]</sup> ranging from 2 to 5 in number.

3 d. Grown plates were subcultured into brucella broth (DIFCO) containing 10% horse serum (Invitrogen) and Campylobacter selective supplement (Oxoid), and were incubated under microaerophilic and shaking conditions for 24 h. Overnight cultures were set to an optical density of 0.1 at 600 nm (approximately  $1.2 \times 10^8$  bacteria/mL) by dilution. Brucella broth was discarded after centrifugation of liquid cultures at 7000 rpm for 10 min and bacteria were resuspended in serum- and antibiotic-free RPMI medium (GIBCO) prior to infection.

### Co-culture assays

AGS epithelial cells were seeded into 6-well plates ( $4 \times 10^5$  cells/well) or 25 cm<sup>2</sup> flasks ( $5 \times 10^5$  cells) and grown in RPMI 1640 (GIBCO) supplemented with 10% fetal bovine serum (GIBCO), 100 U/mL penicillin (Invitrogen), 100 µg/mL streptomycin (Invitrogen) and 2.5 µg/mL amphotericin (GIBCO) at 37 °C in a 5% CO<sub>2</sub> atmosphere for 24 h. Eighty percent confluent cell cultures were then washed with phosphate buffered saline (PBS), and serum- and antibiotic-free RPMI was added to the wells. Sixteen hours serum-starved cell cultures were infected with *H. pylori* suspensions at a multiplicity of infection (MOI) of 100. Cocultures were incubated at 37 °C in a 5% CO<sub>2</sub>-humidified atmosphere.

### CagA phosphorylation assays

After 6 h of coculture the medium was removed and cells were washed with PBS containing 1.0 mmol/L CaCl<sub>2</sub> and 0.5 mmol/L MgCl<sub>2</sub> and scraped from the flasks into 3 mL PBS containing 1 mmol/L sodium vanadate, harvested by centrifugation at 1000 g by 10 min, resuspended in 100 µL of PBS-sodium vanadate and lysed with 4 × Laemli sample buffer [0.2 TRIS-HCl pH 6.8, 0.4 mmol/L dithiothreitol, 8% sodium dodecyl sulfate (SDS), 40% glycerol, 0.4% bromophenol blue]. Cell lysates were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis using a 6% resolving gel and a 4% stacking gel. Proteins were then transferred onto nitrocellulose

membranes by semidry transfer. For protein detection, membranes were probed with a 1:1000 dilution of anti-phospho-tyrosine monoclonal antibody (Santa Cruz Biotechnologies) followed by a 1:4000 dilution of HRP-conjugated goat anti-mouse (Zymax, Invitrogen). Blots were developed using the Amersham ECL detection reagents (GE Healthcare). Membranes were subsequently stripped (using a 62.5 mmol/L TRIS-HCl pH 6.8, 100 µmol/L β-2-Mercaptoethanol solution, 2% SDS at 50 °C for 30 min) and reprobed with 1:1000 polyclonal anti-CagA antibody (Santa Cruz Biotechnologies) followed by 1:60 000 HRP-conjugated goat anti-rabbit secondary antibody (Zymax, Invitrogen) and developed as described above. Densitometry was performed using a Gel Doc GS-670 (Biorad) and results were expressed as the ratio of phosphorylated CagA to total CagA multiplied by 100.

### IL-8 assay

Medium samples from 6 h and 30 h co-cultures were collected, centrifuged at 7000 rpm for 10 min to discard unattached bacteria or cells, and supernatants were stored at -80 °C until further use. IL-8 concentration was measured using an IL-8 Human ELISA kit (Invitrogen) according to the manufacturer's instructions. Uninfected AGS cells were used as a negative control.

### Cellular elongation assay

Six hours and twenty-four h cocultures were examined by differential interference contrast microscopy with a Leica DM IL phase contrast inverted microscope (Leica). For this, 3 randomly chosen 20 × fields were photographed with a MD800-CK camera for microscope (Amscope). Hummingbird cells were measured and counted with the software ImageJ v1.44c (developed by Wayne Rasband at the National Institutes of Health, Bethesda, MD, United States and available at <http://rsb.info.nih.gov/ij/>). Hummingbird cells are characterized by the formation of needle-like projections<sup>[11]</sup>. We defined hummingbird phenotype as cells with needle-like projections > 15 µm. Uninfected AGS cells were used as a negative control.

### Statistical analysis

Mann Whitney *U* test was used for statistical analysis. A *P* value < 0.05 was considered statistically significant. All data were analyzed with the software Graphpad Prism 5 (Graphpad Software, Inc.). All experiments were run in duplicates.

## RESULTS

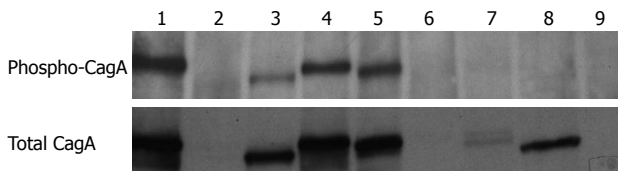
### cagPAI status, CagA expression and tyrosine phosphorylation

From the 84 cagA-positive strains, 74 (88.1%) tested positive for cagE, 72 (85.7%) for cagT and 68 (81%) for cag10. Overall, 67 (79.8%) tested positive for all four cagPAI genes by PCR, and were therefore predicted, on the basis of this limited testing, to have an intact cagPAI. The remaining 17 strains, which tested negative for one or more genes, were collectively predicted to have a partial (*i.e.*, in-



**Table 1** Primers used for the detection of *CagPAI* genes

Gene	Primer	Sequence 5'-3'	Product size (bp)	Reference
<i>cagE</i>	101	TTGAAAACCTTCAAGGATAGGATAGAGC	510	[16]
	102	GCCTAGCGTAATATCACCATTACCC		
<i>cagT</i>	<i>cagTF</i>	ATGAAAGTGAGAGCAAGTGT	823	[30]
	<i>cagTR</i>	TCACTTACCACTGAGCAAAC		
<i>cag10</i>	<i>cag10F</i>	ATGGAAGACTTTTGTATAA	2208	[30]
	<i>cag10R</i>	TCACAGITCGCTTGAACCCA		



**Figure 2** Phosphorylation of CagA protein in AGS cells after coculture with *Helicobacter pylori* CagA-positive strains. Cell lysates were evaluated by western blot using anti-phosphotyrosine or anti-CagA antibodies. A representative assay is shown. Lane 1: 11 637 control strain with a functional *cagPAI* and 5 Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs (ABCCC); Lanes 2 and 6: Isolates with a defective *cagPAI* lacking expression of CagA; Lanes 7 and 8: Isolates with a defective *cagPAI* expressing CagA, with absence of CagA phosphorylation; Lanes 3-5: Isolates with a functional CagPAI with three (ABC; lane 3) or four (ABCC; lanes 4 and 5) EPIYA motifs; Lane 9: Uninfected AGS cells.

complete) *cagPAI*. Bacterial lysates of the 84 strains were assessed by Western blot with anti-CagA antibodies, from which 75 (89.3%) expressed the CagA protein. The nine strains lacking CagA expression harbored a partial *cagPAI*.

Once CagA is expressed, it is delivered into host cells *via* the T4SS and becomes phosphorylated by host cell kinases<sup>[11]</sup>. Seventy-three out of the seventy-five CagA-expressing strains were evaluated for CagA phosphorylation in coculture with AGS cells (Figure 2). From these, in 58 strains (79.4%) CagA was phosphorylated during infection. In 15 strains CagA was not phosphorylated, including eight CagA-expressing strains bearing a partial *cagPAI*. The remaining seven strains lacking CagA phosphorylation were predicted to have an “intact” *cagPAI* according to *cagT*, *cagE*, and *cag10* PCR results. This last result indicates that PCR detection of selected *cagPAI* genes is not sufficient to predict the functionality of the *cagPAI*. In addition, and as described below, these seven strains failed to induce IL-8 secretion in AGS cells, which supports this conclusion. Based on this *in vitro* characterization of the strains, we grouped isolates bearing a partial *cagPAI* (*i.e.*, strains which tested negative for one or more *cagPAI* genes) and strains with a non-functional *cagPAI* (*i.e.*, strains showing no CagA phosphorylation nor induction of IL-8 secretion) as strains with a defective *cagPAI*. In summary, 24 out of the 84 strains (28.5%) were found to harbor a defective *cagPAI*: 17 strains with a partial *cagPAI* and 7 strains with a non-functional *cagPAI*.

We further evaluated the association degree between the levels of CagA phosphorylation and the histopathological diagnoses for strains with functional *cagPAI*. Interestingly, the mean of CagA phosphorylation in strains from duodenal ulcer was shown to be significantly higher

than the ones in strains from gastritis, atrophic gastritis, intestinal metaplasia and gastric cancer ( $P < 0.02$ , 0.045, 0.043 and 0.047 respectively; Figure 3A).

We also investigated the relationship between the number of EPIYA-C motifs and the levels of CagA phosphorylation. Isolates from this study ranged from one to three EPIYA-C motifs (Figure 1). CagA-expressing *H. pylori* strains bearing one and more than one EPIYA-C motifs were grouped together. Although higher levels of CagA phosphorylation were observed in strains with more than one EPIYA-C motif in comparison with strains with one EPIYA-C motif, this difference was not significant (Figure 3B).

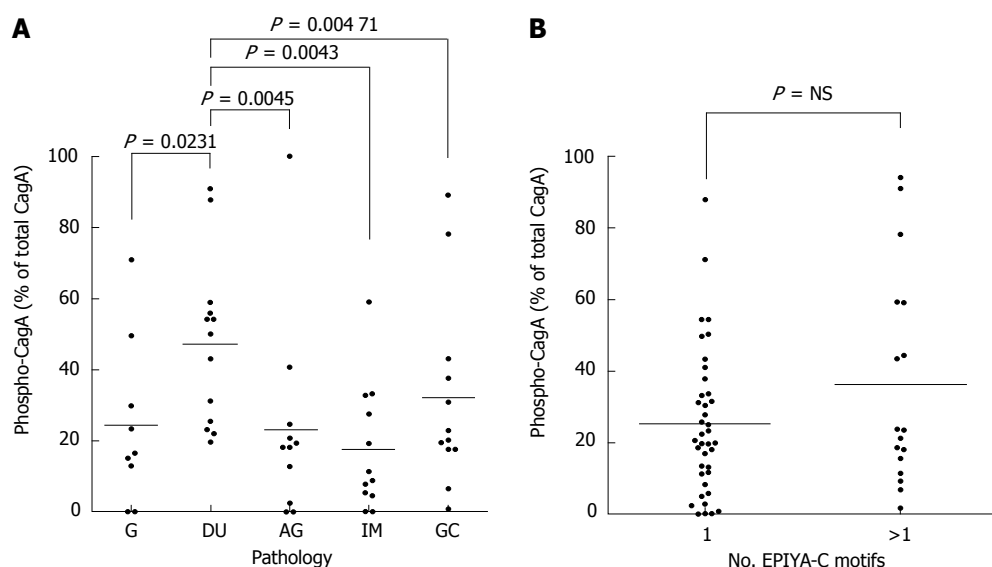
#### **Influence of *cagPAI* status and *cagA* polymorphisms on IL-8 expression in AGS cells**

The 84 *cagA*-positive and 6 *cagPAI*-negative strains isolated from Colombian patients were tested for IL-8 induction in AGS cells. *CagA*-positive strains induced variable expression levels of IL-8 ranging from 0 to 810 pg/mL and from 8.8 to 1442 pg/mL at 6 h and 30 h post-infection, respectively. Ten out of the 67 strains classified by genotyping to bear an intact *cagPAI* did not induce IL-8 expression after 6 h of coculture with AGS cells. Three of these strains showed CagA translocation and phosphorylation in contrast to the remaining seven strains, in which no phosphorylation was observed. These last seven strains were likely to bear defects in other *cagPAI* components not detected by the PCR of the selected *cagPAI* genes. This showed, as previously observed by Argent *et al.*<sup>[19]</sup>, that PCR prediction of *cagPAI* intactness is a poor test for the presence of a T4SS capable of inducing IL-8 expression in AGS. We therefore considered these strains to have a non-functional *cagPAI* and classified them, along with the partial-*cagPAI* strains, as *cagPAI*-defective isolates, as described above.

*CagPAI*-negative and *cagPAI*-defective strains did not induce detectable levels of IL-8 at 6 h post-infection (Figure 4A). At 30 h post-infection all strains induced IL-8 expression in AGS cells, although *cagPAI*-negative and *cagPAI*-defective strains induced significantly lower levels of IL-8 than strains with a functional *cagPAI* with one or more than one EPIYA-C motif ( $P < 0.001$ ; Figure 4B).

A previous report has suggested a positive association between the number of EPIYA-C motifs and IL-8 expression<sup>[25]</sup>. We therefore evaluated *H. pylori*-IL-8 induction according to the number of EPIYA-C motifs in each strain. There were no differences in IL-8 expression





**Figure 3** CagA-protein phosphorylation and its relationship to Glu-Pro-Ile-Tyr-Ala-C (EPIYA-C) motifs and disease severity. Sixty functional-cagPAI strains were cocultured with AGS cells for 6 h. Coculture lysates were assessed by Western blot and levels of CagA phosphorylation were determined by densitometry. A: Evaluation of CagA phosphorylation levels according to the pathology from which strains were isolated; B: Relationship between the number of EPIYA-C motifs and the levels of CagA phosphorylation. G: Gastritis; DU: Duodenal ulcer; AG: Atrophic gastritis; IM: Intestinal metaplasia; GC: Gastric cancer. NS: Not significant

among *H. pylori* strains bearing one and more than one EPIYA-C motifs, neither at 6 h nor at 30 h of coculture, suggesting a lack of association between CagA EPIYA-C motifs variations in *H. pylori* isolates from Colombia and IL-8 induction (Figure 4A and B).

#### Influence of cagPAI status and cagA polymorphisms on hummingbird phenotype induction

AGS cells were cocultured with the same 84 strains tested for IL-8 expression and evaluated for hummingbird phenotype formation. We did not observe differences in the extent of cell elongation induction between strains with a functional or a defective cagPAI in 6 h cocultures (Figure 4C). At 24 h post infection strains with functional cagPAI showed high diversity in the extent of hummingbird phenotype induction ranging from 7% to 34%. CagPAI-negative and cagPAI-defective strains induced significantly lower levels of elongation than strains with functional cagPAI with one or more than one EPIYA-C motif ( $P = 0.032$  and  $0.003$  respectively; Figure 4D).

Similarly to IL-8 expression, no differences were observed in cellular elongation induction among *H. pylori* strains bearing one and more than one EPIYA-C motifs, neither at 6 h nor at 24 h of coculture (Figure 4C and D). Unexpectedly, three cagPAI-defective strains induced elongation in more than 20% of the cells.

#### *H. pylori*-induced cellular responses and their association to disease severity

To assess the degree of association between the disease severity and IL-8 or cell elongation induction, strains with a functional cagPAI were grouped by the pathology from which they were isolated.

No differences were found in IL-8 expression among

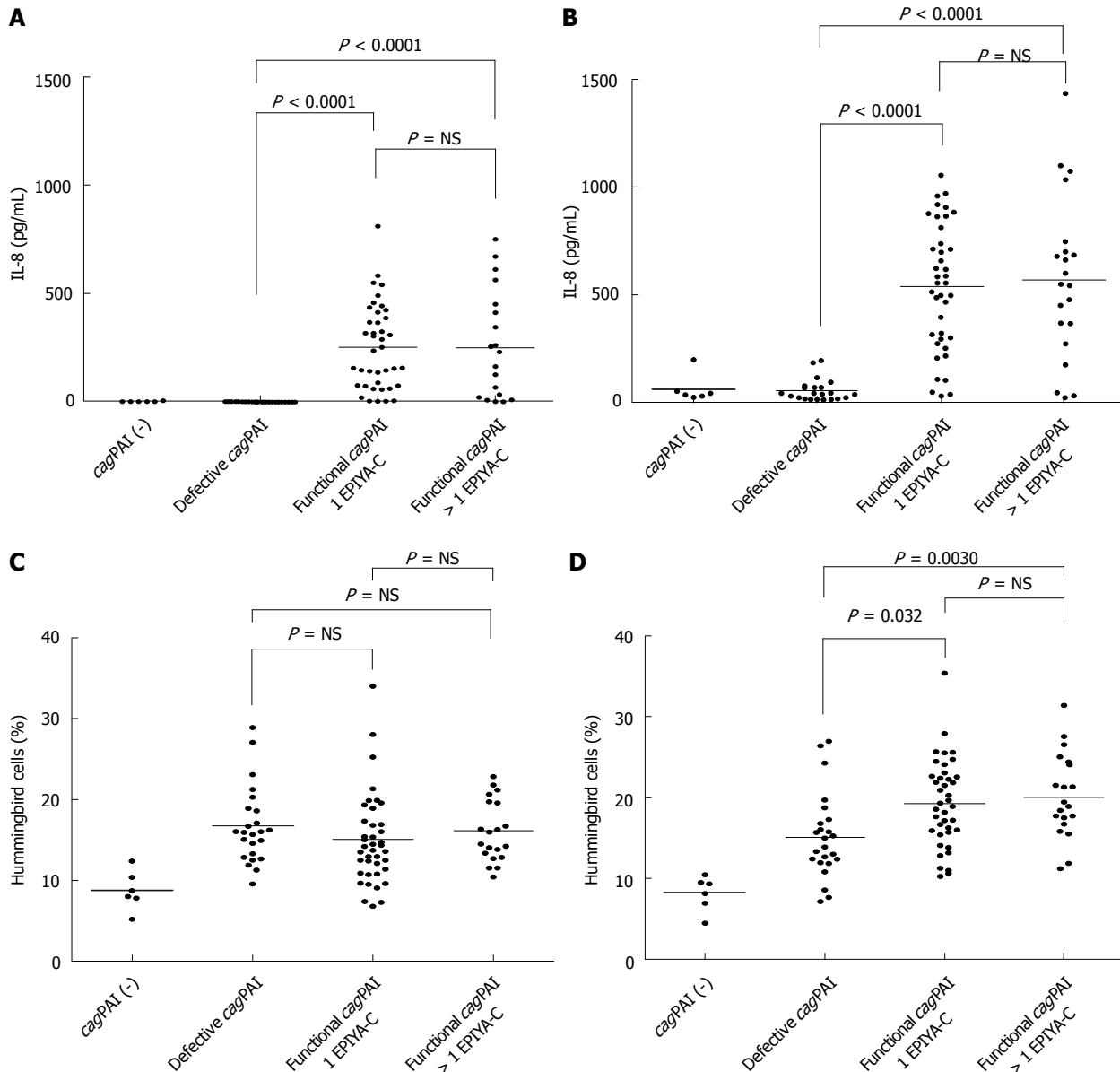
pathology groups, although small variations among IL-8 mean values were observed (Table 2). A slight increase in IL-8 mean values after 30 h of coculture in the direction Atrophic Gastritis, Intestinal Metaplasia, Gastric Cancer was observed. However, differences among groups were not significant. Interestingly, the two strains that showed the highest induction were isolated from patients diagnosed with gastric cancer.

Hummingbird phenotype induction had no significant association to disease severity either (Table 2). As in IL-8 induction, the two strains showing the highest cell elongation induction belonged to the gastric cancer group. However, these two strains were different from those inducing the highest IL-8 levels.

## DISCUSSION

In Colombia, a country with high incidence of gastric cancer, 72% to 90% of *H. pylori* isolates harbor the cagA gene<sup>[31,32]</sup>, a virulence factor associated with more severe disease<sup>[33]</sup>. It has been shown that the number of EPIYA-C motifs on CagA is associated with the levels of CagA tyrosine phosphorylation, SHP-2 binding activity and cytoskeletal alterations<sup>[17,26]</sup>. In this study we have evaluated the biological activities of cagA-positive Colombian strains on gastric epithelial cells according to the CagA polymorphisms, and their potential association with the severity of gastroduodenal diseases.

Although we included 84 cagA-positive strains in this study, the presence of this gene did not show strict concordance with the integrity of cagPAI, nor with the expression and delivery of CagA into epithelial AGS cells. About 28% of the strains were found to have a defective cagPAI, and 10%, in addition of being cagPAI-defective,



**Figure 4** Influence of *cagPAI* status and CagA Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs variations on interleukin-8 expression and cell elongation. *CagPAI*-negative strains ( $n = 6$ ), *cagPAI*-defective strains ( $n = 24$ ) and functional-*cagPAI* strains with either one ( $n = 40$ ) or more than one ( $n = 20$ ) EPIYA-C motifs were cocultured with AGS cells. (A) 6 h and (B) 30 h coculture supernatants were collected and assessed for interleukin-8 concentration by ELISA. Mean values are represented by horizontal lines within the scatterplots. Experiments for each strain were run in duplicates; (C) 6 h and (D) 24 h coculture photographs were taken and the percentage of hummingbird cells was determined. Mean values are represented by horizontal lines within the scatterplots. Experiments for each strain were run in duplicates. NS: Not significant.

**Table 2** Induction of interleukin-8 expression and cell elongation by *cagPAI*-functional *Helicobacter pylori* strains according to the histopathological diagnosis

Pathology	IL-8 (pg/mL)				Elongation			
	6 h		30 h		6 h		24 h	
	mean	95%CI	mean	95%CI	mean	95%CI	mean	95%CI
Gastritis	256.1	(145.1-367.1)	594.5	(419.5-769.6)	15.3%	(10.7-19.9)	18.98%	(15.7-22.3)
Atrophic gastritis	224.7	(58.7-390.6)	463.7	(244.9-682.5)	14.9%	(12.2-17.6)	19.84%	(16.9-22.7)
Intestinal metaplasia	192.7	(89.0-296.4)	532.1	(398.2-666.0)	15.32%	(12.2-18.4)	17.7%	(18.5-20.6)
Gastric cancer	265.5	(107.5-423.5)	589.5	(355.4-823.5)	16.3%	(12.8-19.9)	19.55%	(15.9-23.1)
Duodenal ulcer	278.9	(154.8-402.9)	572.2	(343.6-800.8)	14.72%	(13.6-15.8)	19.8%	(17.2-22.4)

IL-8: Interleukin-8.

did not express the CagA protein. It is likely, that the promoter region of the *cagA* gene was disrupted in these strains, as previously reported for isolates from different human populations<sup>[34]</sup>. These results reinforce previous reports indicating that the presence of the *cagA* gene alone is not an accurate marker for an intact *cagPAI*<sup>[19,34,36]</sup>.

Strains bearing an intact *cagPAI* showed high variability in CagA phosphorylation levels. *In vitro* experiments have shown that the number of EPIYA-C motifs is associated with the degree of CagA phosphorylation<sup>[26]</sup>, and some studies with clinical isolates have also reported this association<sup>[19,27]</sup>. In our study we observed higher levels of CagA phosphorylation in strains with more than one EPIYA-C motifs than in strains with one EPIYA-C motif, although the differences were not significant. Considering that it has been proposed that CagA EPIYA motifs polymorphisms influence the degree of virulence as well as the oncogenic potential of individual *cagA*-positive strains<sup>[26]</sup>, we evaluated the association between phosphorylation levels and histopathological diagnosis. We observed significant higher levels of CagA phosphorylation in strains from duodenal ulcer patients when compared to strains from the other pathologies, which is in agreement with a previous study reporting a similar behavior in strains isolated from duodenal ulcer patients<sup>[37]</sup>. It has been shown that *H. pylori*-induced inflammatory response is triggered upon CagA translocation into the host cell, where it activates NF- $\kappa$ B leading to IL-8 expression<sup>[12,13]</sup>. Furthermore, CagA-mediated IL-8 induction has been shown to be time- and strain-dependent. There is evidence demonstrating the importance of CagA for IL-8 expression in long incubation periods (24-48 h)<sup>[12,25]</sup>. However, the role of CagA in short incubation periods has been controversial. One study has found that isogenic *cagA*-mutant strains induce lower levels of IL-8 expression than their parental strains after 6 h and 9 h of co-culture<sup>[12]</sup>. In contrast, two studies, one involving isogenic *cagA* mutants and the second involving independent strains, found that IL-8 expression was not affected after 6 h of incubation<sup>[19,38]</sup>. Given these contradictory results, we tested IL-8 induction after 6 h and 30 h post-infection. We observed a clear CagA-dependent IL-8 expression pattern, as evidenced by the differences in IL-8 induction between *cagA*-negative/*cagPAI*-defective and *cagPAI*-functional strains. Strains bearing a functional *cagPAI* induced variable levels of IL-8 expression at 6 h of coculture, whereas *cagA*-negative and *cagPAI*-defective strains failed to induce IL-8 secretion. Furthermore, we confirmed the importance of CagA on IL-8 induction after long incubation periods, although we also detected low levels of IL-8 expression for *cagA*-negative and *cagPAI*-defective strains. These low levels of IL-8 induction at 30 h are probably the effect of other delayed responses like the CagA-independent IL-8 expression mechanism, in which *H. pylori* peptidoglycan translocated through bacterial membrane vesicles into epithelial cells activates, *via* Nod1, NF- $\kappa$ B resulting in IL-8 expression<sup>[39,40]</sup>. Furthermore, Crabtree *et al.*<sup>[41]</sup> also reported low levels of

secretion of IL-8 by *cagA*-negative strains after 24 h of infection. Taken together, our results support the concept of *H. pylori* time-dependent IL-8 induction, highlighting the importance of CagA for both, short and long, incubation periods.

We observed a lack of association between the number of EPIYA-C motifs and the level of IL-8 induction after coculture with AGS cells, even in prolonged incubation times. Neither an increasing number of EPIYA motifs nor an increasing number of EPIYA-C motifs had a boost effect on IL-8 expression. There are reports in the literature supporting our findings<sup>[42-44]</sup>. Reyes-Leon *et al.*<sup>[43]</sup> reported no differences in IL-8 induction between Mexican *H. pylori* strains bearing one EPIYA-C motif and those with two or more C motifs. Interestingly, Mexican and Colombian *H. pylori* populations share common predominant polymorphisms (ABC and ABCC)<sup>[28,43,45]</sup>. Moreover, Sgouras *et al.*<sup>[44]</sup> observed no differences in the levels of secreted IL-8 induced by individual isogenic subclones expressing CagA protein with different number of EPIYA-C motifs isolated from the same patient. It is worth noting the contrast of our results with those found by Argent *et al.*<sup>[25]</sup>, in which they observed a direct association between the number of EPIYA-C motifs and IL-8 expression in microevolved *H. pylori* strains from England. Discrepancies between studies may be explained by contrasting the geographical origin of the strains in each study. Our strains were isolated in Colombia and Argent strains were isolated in England, which are regions with high and mild gastric cancer risks, respectively<sup>[46]</sup>. It has recently been shown that *H. pylori* strains from different geographical and gastric cancer risk regions have distinct IL-8 induction behaviors in AGS cells<sup>[27]</sup>. Cellular inflammatory response in AGS cells was shown to be independent of the pathology from which strains were isolated, although the two strains showing the highest IL-8 expression levels were isolated from patients diagnosed with gastric cancer. These results are in agreement with previous studies showing that *H. pylori* strains isolated from different gastric pathologies varied in their ability to induce IL-8 expression in AGS cells, but did not associate to disease severity<sup>[19,47]</sup>. Moreover, Schneider *et al.*<sup>[27]</sup> reported in a recent study involving *cagA*-positive *H. pylori* strains isolated from Colombian patients, that IL-8 expression induced by isolates from precancerous lesions did not differ from that induced by isolates from nonatrophic gastritis.

The induction of hummingbird phenotype upon infection with *cagA*-positive *H. pylori* strains has long been proposed as one of the mechanisms contributing to CagA oncogenic transformation<sup>[8,17]</sup>. Strains carrying biologically more active CagA have been associated with an increased risk of developing gastric carcinoma<sup>[17,19,22]</sup>. *H. pylori*-mediated cell elongation is potentiated by CagA proteins with higher number of EPIYA motifs<sup>[19,27]</sup>. Furthermore, proteins harboring higher number of EPIYA-C repeats increase hummingbird cells in AGS cells<sup>[17,26]</sup>. Our results disagree with these statements, as neither of both CagA molecular variations groups affected hummingbird

phenotype formation, but are in agreement with previous studies suggesting a lack of association between the number and type of EPIYA motifs and cellular elongation in *H. pylori* clinical isolates<sup>[22,37,43,48]</sup>.

We found no differences in cell elongation induction when evaluating strains according to the pathology from which they were isolated. These results are in agreement with the results reported by Backert *et al.*<sup>[37]</sup>, in which strains isolated from German patients with different gastric pathologies showed no differences in cell elongation in AGS cells.

It is also important not to take *H. pylori* infection as the ultimate factor involved in gastric carcinogenesis, as there are many environmental and host factors associated with the disease. Polymorphisms in cytokine genes, such as *IL-8*, *IL-1β* and tumor necrosis factor- $\alpha$ , affect cytokine production upon *H. pylori* infection, increasing the risk of developing gastric diseases<sup>[2]</sup>. In addition to host genetic factors, environmental factors (*e.g.*, dietary and smoking habits) may also play an important role in *H. pylori* pathogenesis<sup>[49,50]</sup>. More interestingly, a recent study based on epidemiological and geographical data has proposed altitude as a surrogate for host, bacterial and environmental factors associated with gastric cancer risk<sup>[51]</sup>.

In conclusion, we have reported a lack of association between *H. pylori* CagA protein EPIYA motifs variations from Colombian isolates and disease-associated cellular effects. Taken together, these results suggest that other factors (*e.g.*, host or environmental) may play a more important role than *H. pylori* CagA protein EPIYA variations in gastric cancer development in Colombia.

## COMMENTS

### Background

*Helicobacter pylori* (*H. pylori*) infect more than 50% of the world's population. Around 10%-15% of the infected individuals develop gastroduodenal diseases such as chronic gastritis, duodenal ulcers and gastric cancer. Currently, the determinants of the variable clinical outcomes have not been fully elucidated. One of the most important virulence factors of *H. pylori* is the *cag* pathogenicity island (*cagPAI*), which encodes several proteins, including CagA.

### Research frontiers

CagA varies in size, and this variation has been shown to be due to Glu-Pro-Ile-Tyr-Ala (EPIYA) repeats within the C-terminal region of the protein. In Western *H. pylori* strains three types of EPIYA motifs have been described (A, B and C) based on the sequence flanking the motif. Strains with higher numbers of EPIYA-C motifs are more closely associated with gastric cancer and with an increased CagA *in vitro* activity, although this is controversial.

### Innovations and breakthroughs

In contrast with studies in other populations, this study reports a lack of association between CagA EPIYA motifs variations from *H. pylori* colombian isolates and disease-associated cellular responses or gastroduodenal disease severity.

### Applications

These results suggest that other factors (*e.g.*, host or environmental) may play a more important role than *H. pylori* CagA protein EPIYA variations in gastric cancer development in Colombia, a country with a high incidence of gastric cancer.

### Terminology

*H. pylori* CagA protein is a virulence factor encoded in the *cagPAI* of the bacterium which is translocated into gastric epithelial cells through a type IV secretion system. Once in the cell, CagA becomes phosphorylated on tyrosine residues within the EPIYA motifs, mediating in turn the activation of several sig-

naling pathways involved in the expression of pro-inflammatory cytokines such as interleukin-8 or in cell elongation, among others.

### Peer review

The authors investigate the role of *cagA H. pylori* gene polymorphisms in the various bacterial-related chronic conditions. The paper is well designed and the results represent novel aspects of the infection consequences.

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## Does in-house availability of multidisciplinary teams increase survival in upper gastrointestinal-cancer?

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### Abstract

**AIM:** To investigate the effect of the establishment of in-house multidisciplinary team (MDT) availability (iMDTa) on survival in upper gastrointestinal cancer (UGI) patients.

**METHODS:** In 2001, a cancer centre with irradiation and chemotherapy facilities was established in the Norwegian county of West Agder with a change of iMDTa (WA/MDT-Change). "iMDTa"-status was defined according to the availability of the necessary specialists within one institution on one campus, serving the population of one county. We compared survival rates during 2000-2008 for UGI patients living in counties with (MDT-Yes), without (MDT-No), with a mix (MDT-Mix) and WA/MDT-Change. Survival was calculated with Kaplan-Meier method. Cox model was used to uncover differences between counties with different MDT status when adjusted for age, sex and stage.

**RESULTS:** We analyzed 395 patients from WA/MDT-Change and compared their survival to 12 135 UGI

patients from four other Norwegian regions. Median overall survival for UGI patients in WA/MDT-Change increased from 129 to 300 d from 2000-2008,  $P = 0.001$ . The regions with the highest level of iMDTa achieved the largest decrease in risk of death for UGI cancers (compared to the county with MDT-Mix: MDT-Yes 11%,  $P < 0.05$  and WA/MDT-Change 15%,  $P < 0.05$ ). Analyzing the different tumour entities separately, patients living in the WA/MDT-Change county reached a statistically significant reduction in the risk of death [hazard ratios (HR)] compared to patients in the county with MDT-Mix for oesophageal and gastric, but not for pancreatic cancer. HR for the study period 2000-2004 are given first and then for the period 2005-2008: The HR for oesophageal cancers was reduced from [HR = 1.12; 95%CI: 0.75-1.68 to HR = 0.60, 95%CI: 0.38-0.95] and for gastric cancers from [HR = 0.87, 95%CI: 0.66-1.15 to HR = 0.63, 95%CI: 0.43-0.93], but not for pancreatic cancer [HR = 1.04-, 95%CI: 0.83-1.3 for 2000-2004 and HR = 1.01, 95%CI: 0.78-1.3 for 2005-2008]. UGI patients treated during the second study period in the county of WA/MDT-Change had a higher probability of receiving chemotherapy. In the first study period, only one out of 43 patients (2.4%, 95%CI: 0-6.9) received chemotherapy, compared to 18 of 42 patients diagnosed during 2005-2008 (42.9%, 95%CI: 28.0-57.8).

**CONCLUSION:** Introduction of iMDTa led to a two-fold increase of UGI patients, whereas no increase in survival was found in the MDT-No or MDT-Mix counties.

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**Key words:** Gastric cancer; Gastroesophageal cancer; Oesophageal cancer; Pancreatic cancer; Multidisciplinary treatment; Multidisciplinary team; Survival

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## INTRODUCTION

There is a lack of evidence that clinical decision making by a multidisciplinary team (MDT) leads to increased survival for oesophageal, gastric and pancreatic cancers<sup>[1-3]</sup>.

The rationale for introducing MDTs is that modern cancer management has become increasingly complex, necessitating the involvement of various key professional groups in clinical decision making<sup>[4,5]</sup>. In addition, MDTs serve to monitor adherence to clinical guidelines and promote effective use of resources<sup>[1-3]</sup>. Evaluations of the effectiveness of a MDT on survival are warranted, but complicated to perform due to difficulties regarding: (1) its definition; (2) availability of valid measurement of its performance and, most importantly; and (3) the ethical and organizational hurdles of conducting prospective randomized studies of MDTs. Therefore, in this study we do not focus on the actual practice of MDTs but aim to analyse the effect of their in-house availability (iMDTa).

The most common upper-gastrointestinal (UGI) cancers in Norway are oesophageal, gastric and pancreatic<sup>[6]</sup>. Although these cancers have a dismal prognosis, the timely involvement of different medical specialists is advocated, based on a few studies on patients treated with curative intention<sup>[5,7-9]</sup>. However, these findings do not necessarily apply to palliative patients<sup>[10]</sup>.

In 2001, a cancer centre with irradiation and chemotherapy facilities was established in the Norwegian county of West Agder with a change of iMDTa (WA/MDT-Change). Thus, iMDTa was established and the potential to work according to international MDT guidelines was created<sup>[4]</sup>.

Our hypothesis was that the county of WA/MDT-Change, with its increasing iMDTa during the study period (from 2000-2008), would reach UGI cancer survival levels similar to those of a comparable Norwegian county with iMDTa. In addition, we hypothesized that the county of WA/MDT-Change would have favourable UGI cancer survival figures compared to a county without iMDTa during said period.

The primary objective was to evaluate the effect of the establishment of iMDTa on overall survival in a cohort of UGI cancer patients living in the Norwegian county of WA/MDT-Change.

## MATERIALS AND METHODS

For detailed information about Norway's health care system, the Cause of Death Registry, The Cancer Registry, the establishment of MDT-availability and the actual performance of MDT in the county of WA/MDT-Change, as well as a description of the other counties included for comparison, see electronic supplement. A short overview is given below.

### Norway

Norway is a country with very little migration or socio-

economic disparity<sup>[11,12]</sup>, amongst its 5 million inhabitants (Table 1). Health care coverage in Norway is provided through a single-payer universal government funded system. All persons residing in Norway are assigned a unique 11-digit identification number, making it possible to link information from various national registries.

### Cause of Death Registry

Physicians are required by law to complete a death certificate for all deaths in Norway. The Cause of Death Registry<sup>[13]</sup> collects all death certificates for coding and registration of the cause of death.

### The Cancer Registry

The Cancer Registry of Norway<sup>[6]</sup> (CRN), has collected data on all cancers that have occurred in Norway since 1953. Medical doctors are required by law to report these diagnoses, ensuring high levels of completeness<sup>[14]</sup>. Cancer type, date of diagnosis, extent or stage of the disease at diagnosis, and initial treatment in broad terms, are recorded.

### Changes in WA/MDT-Change during 2000-2008

The Sørlandet Hospital Trust is the regional hospital in the county of WA/MDT-Change, which serves a stable population of approximately 170 000 inhabitants. In 2001, the Centre for Cancer Treatment was established at this hospital, thereby creating the potential for in-house MDTs. During the study period, the number of oncologists has increased from one consultant one day every fourth week to six full-time oncologists and two house officers. Prior to the establishment of the cancer centre, patients had to be referred for irradiation or complex chemotherapy to Oslo University Hospital, 300-500 km (a four to six hour drive) away.

In the ensuing years, increasing oncologic and palliative care expertise has developed and was practiced in conjunction with the already well-established pathological, radiological, gastrosurgical and gastroenterological specialties. Specifically, the following services were founded: A mobile palliative care team in 2002, an outpatient palliative care day centre in 2004 and an in-patient palliative care unit with ten beds in 2007. Prior to 2005, the management of cancer patients across specialties was discussed in informal and undocumented encounters between practitioners. From the summer of 2005 and onwards however, weekly MDT-meetings with a designated focus on gastrointestinal cancers have been held, with gastroenterologists, gastrointestinal surgeons, radiologists and oncologists present.

### Other analyzed regions

Throughout the study period the inhabitants of the analysed regions selected for comparison had the same life expectancies and very similar socioeconomic conditions. Further details can be found in the electronic supplement (Table 1).

The choice of the Norwegian counties used for comparison to WA/MDT-Change in this study, is based on



**Table 1** Cox proportional hazards model adjusted for age and the different regions

		MDT-No	MDT-Change	MDT-Yes	Rest of Norway
All UGI cancers	2000-2004	HR 0.96, (0.83-1.10)	HR 0.96, (0.82-1.13)	HR 0.90 <sup>1</sup> , (0.80-1.0)	HR 0.96, (0.83-1.11)
	2005-2008	HR 0.96, (0.82-1.13)	HR 0.81 <sup>1</sup> , (0.67-0.97)	HR 0.79 <sup>1</sup> , (0.70-0.89)	HR 0.85 <sup>1</sup> , (0.77-0.93)
Oesophagus	2000-2004	HR 0.75, (0.49-1.15)	HR 1.12, (0.75-1.68)	HR 0.86, (0.63-1.17)	HR 0.89, (0.73-1.1)
	2005-2008	HR 1.08, (0.72-1.61)	HR 0.60 <sup>1</sup> , (0.38-0.95)	HR 0.74 <sup>1</sup> , (0.53-1.02)	HR 0.84, (0.67-1.06)
Gastric	2000-2004	HR 0.94, (0.70-1.26)	HR 0.87 (0.66-1.15)	HR 0.99, (0.84-1.12)	HR 0.94, (0.70-1.25)
	2005-2008	HR 0.94, (0.70-1.23)	HR 0.63 <sup>1</sup> , (0.43-0.93)	HR 0.79 <sup>1</sup> , (0.65-0.97)	HR 0.82 <sup>1</sup> , (0.70-0.95)
Pancreas	2000-2004	HR 0.97, (0.79-1.2)	HR 1.04, (0.83-1.3)	HR 0.90, (0.77-1.06)	HR 1.02, (1.02-1.03)
	2005-2008	HR 0.92, (0.74-1.2)	HR 1.01, (0.78-1.3)	HR 0.84 <sup>1</sup> , (0.71-1.0)	HR 0.88, (0.78-1.0)

UGI: Upper gastrointestinal cancer; MDT: Multidisciplinary team. Hazard ratios (HR) are given with 95% confidence interval, and the county of Oslo with MDT-Mixed serves as reference. <sup>1</sup>Statistically significant with *P*-value < 0.05.

their stable status of iMDTa during the study period.

In this manuscript, we define “iMDTa” as a county’s theoretical possibility of MDT meetings within a single administrative institution with all departments on one campus (MDT-Yes). Thus, we measured the possibility of multidisciplinary in-house cooperation of necessary specialists, rather than the formal performance of MDTs. A county with stable iMDTa during the entire study period (MDT-Yes) was hypothesized to have the best survival figures for UGI patients. MDT-No describes a county with an absence of radiation units and medical oncologists within the hospital, where patients were referred to a tertiary university hospital for oncologic treatment during the entire study period. However, gastrointestinal surgeons, gastroenterologists, radiologists and, pathologists were available in such county. The population of the county of Oslo was treated partly in hospitals with all these services available and partly in hospitals without some of these services in the same institution. Therefore this region was defined as MDT-Mixed.

### Patients

For the county of WA/MDT-Change, we used the hospital’s electronic database and confirmed and supplemented it with data from CRN. Further, we identified patients diagnosed with oesophageal, gastric or pancreatic cancers during the study period. Only patients with adenocarcinomas and squamous cell carcinomas were included. The clinical course of the disease of each patient was reviewed. Data regarding oncological, surgical and endoscopic interventions were collected. If surgery had been performed, it was characterized as curative or palliative. Survival figures between different regions were compared using data from CRN.

### Ethics

The study was approved by the Regional Ethics Committee of Southern Norway. The anonymity of the patients included in the analysis was preserved according to the institutional guidelines of our hospital as well as those of the National Data Protection Commission of Norway.

### Statistical analysis

Complete follow-up data were available on all patients.

They were followed from the date of diagnosis to their death or the date of censoring (July 2011). Crude survival was calculated using the Kaplan-Meier method. Crude differences in survival were assessed with log-rank test. Further, to adjust for possible confounding multivariate Cox regression models were fitted. All models were adjusted for age, sex, stage and region and fitted separately for the two diagnostic periods. The results were presented as hazard ratios (HR) with 95% confidence intervals (CI). When assessing the regional differences, the county with MDT-Mix was used as a reference because its population was the largest (to ensure stability of the estimates). *P*-values of less than 0.05 were considered statistically significant. All analyses were performed with SPSS and Stata.

## RESULTS

### Patients

The annual incidences of oesophageal, gastric and pancreatic cancers in Norway from 2004 through 2008 were 4.1/100 000, 11.1/100 000, and 13.6/100 000, respectively. We analyzed 12 530 UGI patients living in five Norwegian regions, there of 395 patients in the county of WA/MDT-C. Median age at diagnosis was 74 years (17-98 years) and median follow-up was 5 mo (0-138 mo).

The baseline characteristics of the patients are listed in Table 2.

No clinically relevant differences in stage distribution of UGI cancers were revealed among the analyzed regions or between the two calendar periods. Furthermore, the stage distribution remained stable during the whole study period. Roughly 40% of all UGI cancer patients had distant metastases at the time of their diagnosis.

The changes in survival over time are illustrated with Kaplan-Meier curves in Figure 1.

During the study period, the largest increases in survival were seen in the county of WA/MDT-Change, see green curve in Figure 1. Here, median survival for oesophageal cancer patients increased from 5 mo (3-12 mo) to 11 mo (9-23 mo) and from 7 mo (4-12 mo) to 15 mo (4-35 mo) for gastric cancer patients. However, these increases were not statistically significant. This numerical survival gain could not be observed in the MDT-No county (red curve) or in the MDT-Mix county (blue

**Table 2 Patient characteristics *n*(%)**

	Diagnosed Jan 2000-Dec 2004					Diagnosed Jan 2005-Dec 2008				
	MDT-Mix	MDT-No	MDT-Change	MDT-Yes	Other regions	MDT-Mix	MDT-No	MDT-Change	MDT-Yes	Other regions
Tumor type										
Oesophagus	113 (15.4)	31 (11.8)	30 (14.6)	68 (10.0)	657 (12.9)	91 (15.2)	35 (16.2)	30 (19.6)	72 (12.8)	571 (14.1)
Gastric	258 (35.2)	110 (41.8)	77 (37.6)	318 (47.0)	2146 (42.2)	214 (35.8)	73 (33.8)	45 (29.4)	240 (42.6)	1534 (38.0)
Pancreas	362 (49.4)	122 (46.4)	98 (47.8)	291 (43.0)	2278 (44.8)	293 (49.0)	108 (50.0)	78 (51.0)	252 (44.7)	1935 (47.9)
Total	733 (100.0)	263 (100.0)	205 (100.0)	677 (100.0)	5081 (100.0)	598 (100.0)	216 (100.0)	153 (100.0)	564 (100.0)	4040 (100.0)
Stage										
No metastasis	109 (14.9)	38 (14.4)	28 (13.7)	81 (12.0)	694 (13.7)	59 (9.9)	30 (13.9)	28 (18.3)	71 (12.6)	543 (13.4)
Lymph node metastasis	155 (21.1)	62 (23.6)	49 (23.9)	182 (26.9)	1195 (23.5)	125 (20.9)	52 (24.1)	44 (28.8)	141 (25.0)	926 (22.9)
Distant metastasis	293 (40.0)	104 (39.5)	85 (41.5)	303 (44.8)	2083 (41.0)	237 (39.6)	97 (44.9)	57 (37.3)	237 (42.0)	1642 (40.6)
Unknown	176 (24.0)	59 (22.4)	43 (21.0)	111 (16.4)	1109 (21.8)	177 (29.6)	37 (17.1)	24 (15.7)	115 (20.4)	929 (23.0)
Total	733 (100.0)	263 (100.0)	205 (100.0)	677 (100.0)	5081 (100.0)	598 (100.0)	216 (100.0)	153 (100.0)	564 (100.0)	4040 (100.0)

MDT: Multidisciplinary team. No clinically relevant differences in stage distribution were revealed among the analyzed regions or between the two calendar periods. In addition, there were no clinically relevant differences in stage distribution among the studied counties.

curve), whereas a survival gain could be observed in the MDT-Yes county (yellow curve).

After analyzing crude survival, survival was adjusted for age, region, sex and stage (even though no differences regarding sex and stage were found among the analysed regions). Comparing the two calendar periods of 2000-2004 and 2005-2008, the regions with the highest level of iMDTa achieved the largest decrease in risk of death for all UGI cancers (Table 1, compared to the county with MDT-Mix: MDT-Yes 11% and WA/MDT-Change 15%).

Analyzing the different tumour entities separately, the WA/MDT-Change county reached a statistically significant reduction in the risk of death (HR) compared to the county with MDT-Mix for oesophageal and gastric, but not for pancreatic cancer. HR for the study period 2000-2004 are given first and then for the period 2005-2008: The HR for oesophageal cancers was reduced from [HR = 1.12, 95%CI: 0.75-1.68 to HR = 0.60, 95%CI: 0.38-0.95] and for gastric cancers from [HR = 0.87, 95%CI: 0.66-1.15 to HR = 0.63, 95%CI: 0.43-0.93], but not for pancreatic cancer [HR = 1.04-, 95%CI: 0.83-1.3 for 2000-2004 and HR = 1.01, 95%CI: 0.78-1.3 for 2005-2008].

### **Treatment and survival changes in the county of WA/MDT-Change**

Hospital records for the region with changing status of iMDTa were analyzed to confirm the UGI cancer incidence numbers from the national registries, as well as the gain in survival. Further, we searched for changes in use of potentially life-prolonging oncologic interventions for the county WA/MDT-Change.

A total of 395 patients with UGI cancers were identified in the hospital records of the WA/MDT-Change county. These data are in full accordance with the incidence figures estimated by The National Cancer Registry<sup>[6]</sup>.

The survival for all UGI cancer patients in the WA/MDT-Change county increased especially after 2004,

when MDT meetings became more formalized. Median overall survival for all MDT-Change UGI cancer patients increased significantly from 129 d in the year 2000 to 300 d in 2008,  $P = 0.001$ . Also these data were in accordance with figures from CRN<sup>[6]</sup>.

During the study period, several organizational changes were made at the Sørlandet Hospital Trust in the county of WA/MDT-Change. In line with the increased iMDTa, changes in the rates of curative surgery, oesophageal or bile duct stent placement, irradiation or chemotherapy were likely to have occurred and these were therefore analyzed.

UGI patients treated during the second study period had a higher probability of receiving chemotherapy. In the first study period, only one out of 43 patients in WA/MDT-Change (2.4%, 95%CI: 0-6.9) received chemotherapy, compared to 18 of 42 patients diagnosed during 2005-2008 (42.9%, 95%CI: 28.0-57.8).

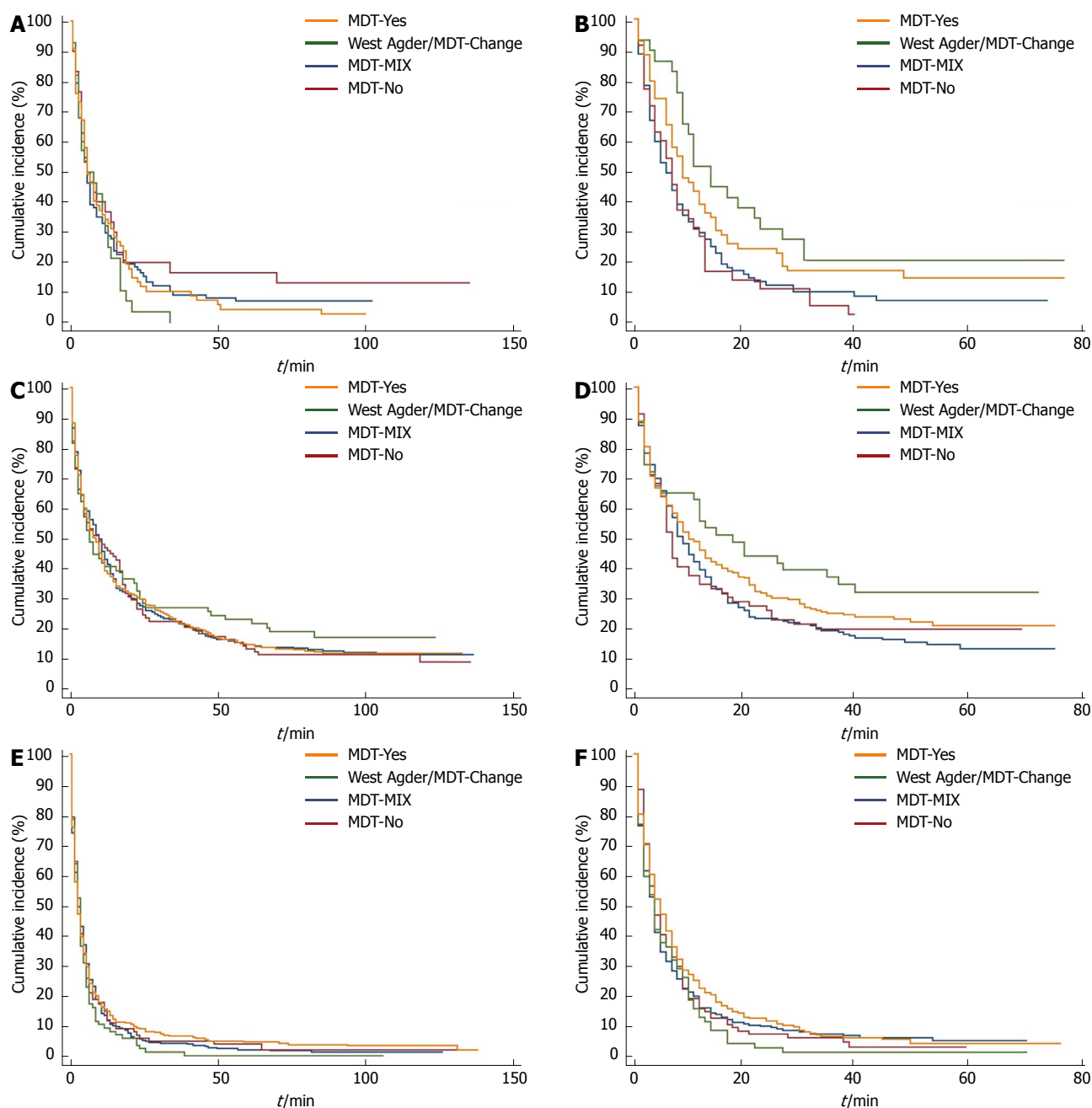
The number of irradiation series did not increase for the diagnoses in question (data not shown).

During the study period, no major changes in surgical practice took place and there was no statistically significant increase in the number of curative UGI cancer surgeries (data not shown). No statistically significant increase in the use of gastro oesophageal or bile duct stents was observed during the two calendar periods of 2000-2004 and 2005-2008 (data not shown).

## **DISCUSSION**

In this study, we found a more than two-fold increase in median survival for UGI cancer patients living in a Norwegian county during a time period in which in-house MDT has become available there. This increase in survival was not observed in counties without full iMDTa, but we saw a survival gain in both counties with iMDTa (MDT-Yes and MDT-Change).

The results of the described organizational changes are striking and clinically relevant, particularly in light of the limited advances in medical treatment of UGI cancer



**Figure 1** Increase in survival in counties with high in-house multidisciplinary team-availability during the second study period. A, B: Oesophagus; C, D: Stomach; E, F: Pancreas; A, C, E: 2000-2004; B, D, F: 2005-2008. MDT: Multidisciplinary team.

patients during the same time period<sup>[15]</sup>.

This study is one of very few, that report a survival benefit of MDTs in cancer care. MDT meetings require a considerable amount of time from core specialists. Therefore, the need to confirm MDTs' effectiveness on survival is of increasing importance, since there is an accelerating shortage of professional groups required for MDTs<sup>[16]</sup>.

A major strength of the present study is its unique setting. Typically, before-after series<sup>[1-3]</sup> are confounded by concurrent changes in other factors, such as better treatments or different stage mixes, over the studied time period. In Norway, relatively few and stable socioeconomic

differences are combined with an egalitarian public health service. In addition, high quality national cancer and death registries have been established decades ago. Furthermore, life expectancies were stable and similar in the analyzed regions throughout the study period. We were therefore able to analyze the un-confounded effect of changes in the organization of health care on survival of selected patient groups living in different regions.

Most importantly, survival outcomes can be attributed to patients' residence, even if a few of them were operated or irradiated in other regions, thus indicating the quality of health care provided for the population living in a defined region. In addition, we have compared patient

survival among regions between two time periods which were consecutive. Therefore, it is unlikely that significant changes in the possible confounding factors over a time period of 3-4 took place.

The precise role and composition of MDTs in cancer care vary throughout the world. Moreover, these variations exist even from hospital to hospital within the same region or country. Further hurdles in MDT research are the different interpretation of MDT-guidelines and the validity of documentation of the actual performance according to these guidelines<sup>[2]</sup>. Moreover, we are just starting to understand the individual factors of MDTs affecting the clinical outcome<sup>[17]</sup>. In the county of WA/MDT-Change, the MDTs were organized in line with international MDT guidelines<sup>[4]</sup>, and aimed to perform accordingly.

While using registry data for patient identification prevents bias associated with clinician selection of patients, registry retrieved data has limitations with respect to the variables available for analysis. In addition to the CRN, we had complete hospital records for the region with changing status of iMDTa and could therefore analyze the changes in use of potentially life-prolonging oncologic interventions for the MDT-Change county. One measurable factor potentially contributing to the increase in survival in WA/MDT-Change may be the increased use of chemotherapy. This increase is higher than expected for this time period. A 50% increase in the use of chemotherapy for every year of the study period may be a result of more patients getting therapy. In that respect, MDT seems to result in increased referral of UGI patients to the medical oncologist. Travel distance to hospitals has been shown by others to be a barrier to treatment among patients with most types of cancer, including UGI cancers<sup>[18-20]</sup>. Furthermore, the EURO CARE working group found striking differences in gastric cancer survival and the quality of management logistics has been proposed as an important variable for patient survival<sup>[21]</sup>. In line with this argument, gastric cancer patients at district hospitals more often received adjuvant chemotherapy, than patients treated in university hospitals in Norway<sup>[22]</sup>. Unfortunately, we have not been able to analyze to what extent patients in other regions had received chemotherapy. However, in light of the striking survival gains, it appears that the increasing use of chemotherapy is unlikely to be the only reason for the survival gain seen in WA/MDT-Change.

The number of irradiation series or the use of gastro oesophageal or bile duct stents did not increase during the study period and we do not consider that the changes of the palliative services had a major impact on the life expectancy of the study patients, since the in-patient service was established at the very end of the study period.

The role of surgery for survival of the whole study cohort in this setting is more complex, since the group of UGI cancers as a whole has a low rate of curative surgery. Pancreatic and esophageal cancers are operable in less than one of five cases<sup>[15]</sup>, and small changes in this ratio affect median overall survival to a limited extent.

Concerning gastric cancer, 43% of cases are operated in Norway<sup>[22]</sup>. This proportion was already higher before (data not shown), but stable throughout the study period, in our clinic. Thus, in light of a relatively high rate of surgery before iMDTa, the rate of surgery was not affected through the establishment of iMDTa in the WA/MDT-Change county. When looking at the results for gastric and esophageal cancers (Table 2), two findings are interesting: Both MDT-No and WA/MDT-Change centralized surgical treatment of esophageal, but not gastric cancer during the second interval of the study period to the MDT-Mix county of Oslo. In the WA/MDT-Change county, a survival benefit was seen for both entities, whereas the survival for these two diagnoses decreased in the MDT-No county (Table 2). These findings may support the theory that, in these particular geographic regions, the presence of oncologists in a hospital may have a greater impact than the place of curative surgery, at least on short term survival.

In this respect, the increased use of chemotherapy should be interpreted as an effect modifier for survival and the MDT members in WA/MDT-Change agree upon a during the study period gradually improved team spirit and more effective communication, although it seems easier to measure the results, rather than formally proof the process of such increased human interdependency.

A limitation of our study is the relatively low incidence of UGI cancers. We therefore analysed survival changes for all stages combined for each cancer type. As the main goal of our study was to assess changes in survival for the entire group of UGI cancer patients, we consider our results valid because the stage distribution for a given diagnosis in the different regions did not change during the course of the study period. In addition, the limited life expectancy for UGI cancer patients makes it possible to compare and assess results concerning improved patient outcome after treatment changes in a way that cannot be achieved in entities with long term survival. Seventy percent of UGI cancer patients live shorter than one year, making short term survival figures important both for the patients and health care administrators when considering organizational changes.

The explanation of the increased survival seen after the introduction of in-house MDT-availability is most likely multi-factorial. Future prospective studies should also analyze the communicative implications at play when a team is formed in-house over a period of several years and assessment tools for this purpose have been created<sup>[23]</sup>. Most importantly, it is not clear if the present findings for UGI cancer patients can be extrapolated to other cancer entities. This question should be addressed in future research.

In conclusion, we present one of the first studies showing a survival benefit for oesophageal and gastric cancer patients after the establishment of MDTs. We found a striking and more than two-fold increase in survival among patients with UGI cancers living in a Norwegian county with increasing iMDTa. During the analysed time period,



no increase in survival was found in counties without consistent MDT availability. The survival gain might be partly explained by increased use of chemotherapy.

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## COMMENTS

### Background

There is a lack of evidence that clinical decision making by a multidisciplinary team (MDT) leads to increased survival for oesophageal, gastric and pancreatic cancers

### Research frontiers

The rationale for introducing MDTs is that modern cancer management has become increasingly complex, necessitating the involvement of various key professional groups in clinical decision making. In addition, MDTs serve to monitor adherence to clinical guidelines and promote effective use of resources. Evaluations of the effectiveness of a MDT on survival are warranted, but complicated to perform due to difficulties regarding: (1) its definition; (2) availability of valid measurement of its performance and, most importantly; and (3) the ethical and organizational hurdles of conducting prospective randomized studies of MDTs.

### Innovations and breakthroughs

In this study, authors did not focus on the actual practice of MDTs but aim to analyse the effect of their in-house availability. This is the first study to document a survival benefit of upper gastrointestinal (UGI) cancers after the implementation of MDT.

### Applications

This study gives evidence to the wideheld belief of a survival benefit in UGI cancer patients, when treated in a setting of MDT.

### Terminology

Here, the term "in-house MDT" was introduced and defined as a county's theoretical possibility of MDT meetings within a single administrative institution with all departments on one campus (MDT-Yes).

### Peer review

The authors examined the survival benefit of UGI cancer patients after the introduction of in-house MDT and compared the survival to the geographic regions with and without in-house MDT.

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## Gastroesophageal cancer and retroperitoneal fibrosis: Two case reports and review of the literature

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### Abstract

Retroperitoneal fibrosis secondary to malignant disease is a rare condition associated with a dismal prognosis. We herein present the first ever reported case of retroperitoneal fibrosis related to esophageal adenocarcinoma in a 63-year-old patient who developed bilateral ureteral obstruction due to extensive retroperitoneal fibrosis 18 mo after having completed neoadjuvant chemoradiation followed by surgery for a pT3N0 adenocarcinoma of the distal esophagus. We also report the case of a previously healthy woman who presented with bilateral ureteral obstruction and diffuse narrowing of the common biliary duct and was found to have extensive retroperitoneal fibrosis as a consequence of metastatic gastric adenocarcinoma. Both patients had poor performance status and were unsuitable for palliative chemotherapy. This paper shows that urinary and biliary obstructive symptoms might represent retroperitoneal fibrosis as a consequence of gastroesophageal malignancy.

**Key words:** Gastric cancer; Esophageal cancer; Retroperitoneal fibrosis

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### INTRODUCTION

Retroperitoneal fibrosis is a rare clinical condition characterized by the presence of pathologic collagen plaque around the abdominal aorta and iliac vessels, as well as the inferior vena cava and the ureters. Approximately 70% of retroperitoneal fibrosis is idiopathic in nature, while the remaining 30% are believed to be related to certain drugs (ergot-derivatives, methysergide, bromocriptine, beta-blockers, methyldopa, analgesics, hydralazine), malignancy (carcinoid, lymphoma, sarcoma, carcinomas of the colon, prostate, breast, stomach), infections (tuberculosis, histoplasmosis, actinomycosis), radiotherapy (testicular seminoma, colon carcinoma, pancreatic carcinoma), surgery (lymphadenectomy, colectomy, hysterectomy, aortic aneurysmectomy), trauma, amyloidosis<sup>[1,2]</sup>. From our knowledge, there are only nine reported cases of retroperitoneal fibrosis associated with gastric cancer<sup>[3-11]</sup>, while there is no report associated with esophageal cancer.

### CASE REPORT

#### Case 1

A 63 year-old man with a past history of pT3N0 adenocarcinoma of the distal esophagus treated with neoadjuvant chemoradiation (5 wk of chemoradiation at dose 50.4 Gy with cisplatin 25 mg/m<sup>2</sup> days 1-3 and 5-fluorouracil 1000 mg/m<sup>2</sup> daily × 4, weeks 1 and 5) followed

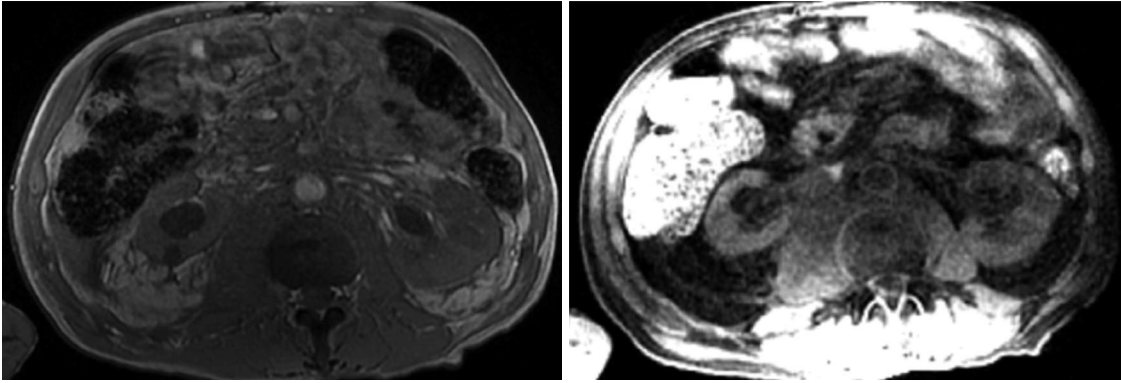


Figure 1 Magnetic resonance imaging of the abdomen revealing an ill-defined retroperitoneal infiltrate.

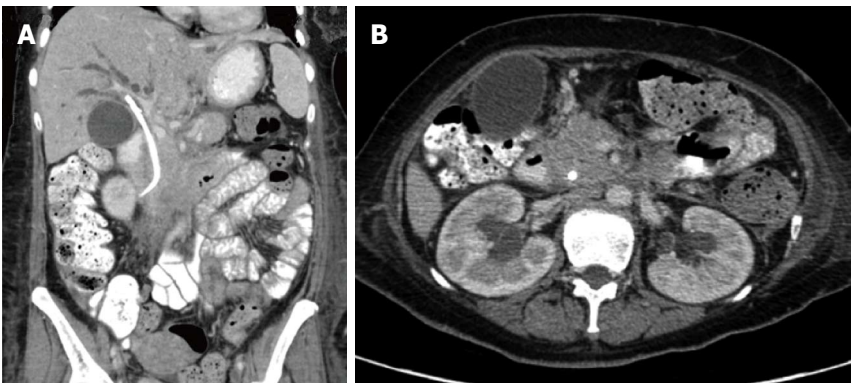


Figure 2 Computed tomography scan of the abdomen revealing an extensive retroperitoneal soft tissue mass. A: Sagittal view; B: Transverse view.

two-hole esophagectomy with gastric reconstruction presented 18 mo after the completion of treatment with acute kidney injury caused by bilateral ureteral obstruction. Bilateral nephrostomy tubes were placed. Magnetic resonance imaging of the abdomen revealed an ill-defined retroperitoneal infiltrate extending from the level of the renal vessels towards the presacral space distally demonstrating retroperitoneal fibrosis (Figure 1). The patient underwent a biopsy of the retroperitoneum which showed poorly differentiated adenocarcinoma consistent with metastasis from the previous esophageal cancer.

### Case 2

A previously healthy 54-year-old woman who presented to the emergency department with a 4-wk history of flank pain, persistent nausea with vomiting, anorexia and progressive oliguria as well as intermittent hematuria. The patient was found to have bilateral hydronephrosis due to ureteral obstruction with consequent renal insufficiency and underwent bilateral nephrostomy. Shortly after admission, she also became jaundiced. Subsequent work-up revealed diffuse narrowing of the common biliary duct and a biliary stent was inserted. A liver biopsy was consistent with cholestasis and immunoglobulin G4 level was normal. Computed tomography (CT) scan of the abdomen showed extensive retroperitoneal soft tissue mass, extending all the way up to the liver hilum as well as diffusely narrowed caliber of the inferior vena cava and portal vein (Figure 2). These findings were consistent

with retroperitoneal fibrosis. During an attempt to an endoscopic-ultrasound guided fine-needle aspiration of the retroperitoneum mass, she was found to have thickening of the gastric wall. Gastric biopsy revealed invasive adenocarcinoma in a scenario of linitis plastica while the ascetic fluid revealed malignant cells.

### DISCUSSION

Symptoms caused by this fibrotic process are usually secondary to compression and constriction of local anatomic structures. The most frequent presenting symptom is pain in the lower back, flank or abdomen, which tends to increase over time<sup>[12]</sup>. Other common symptoms include weight loss, anorexia, testicular pain, edema, and gross hematuria<sup>[2]</sup>. In late stages, patients may develop progressive ureteral obstruction with renal insufficiency due to encasement of both ureters by the retroperitoneal mass. More rarely, involvement of the biliary tree by the fibrotic tissue may cause obstructive jaundice<sup>[13]</sup>, as was the case of our second patient.

In most cases of retroperitoneal fibrosis secondary to malignant disease, abnormal collagen plaque in the retroperitoneum results from an exuberant desmoplastic response to retroperitoneal metastases<sup>[2]</sup>. It is believed to be an immune-mediated process, in which macrophages release cytokines that stimulate fibroblast proliferation with subsequent fibrosis. However, its etiology and pathobiology remain obscure. This mechanism is different in car-



conoid tumors, which may lead to retroperitoneal fibrosis without the presence of metastasis probably through a serotonin-mediated mechanism<sup>[14]</sup>. Another possible explanation for carcinoid-induced retroperitoneal fibrosis is the release of profibrogenic growth factors such as platelet-derived growth factor, insulin-like growth factors, epidermal growth factor, and the family of transforming growth factors  $\alpha$  and  $\beta$ <sup>[15]</sup>.

The diagnosis of retroperitoneal fibrosis is primarily made by imaging studies. Contrast-enhanced CT scan is the method of choice as it visualizes the extent of fibrosis and may assess the presence of metastatic tumor. Moreover, CT scan may also enable CT-guided biopsy<sup>[16]</sup>. Positron emission tomography (PET)-CT has recently been reported as a useful imaging modality in idiopathic retroperitoneal fibrosis, not only for diagnosis but also for treatment response evaluation<sup>[17]</sup>. Because retroperitoneal fibrosis is a metabolically active tissue, it will show increased radiotracer uptake, irrespective of a malignant or idiopathic cause. However, PET-CT scan may reveal an occult primary tumor as well as metastatic disease. Biopsy of the retroperitoneum is highly recommended if there is suspicion for an underlying malignancy.

Usually retroperitoneal fibrosis secondary to malignant disease is associated with a dismal prognosis. The nonspecific symptoms often make the diagnosis very difficult and during late stages patients may have organ dysfunction and poor performance status, being unsuitable for palliative chemotherapy. Both of our patients were not able to undergo chemotherapy. Unfortunately, there is no evidence in the literature that chemotherapy would help reducing malignancy-related retroperitoneal fibrosis. The decision to offer chemotherapy must be done from case to case, taking into consideration performance status and organ dysfunction. Although corticosteroids are the most used drugs for idiopathic retroperitoneal fibrosis, there is no evidence of effectiveness when retroperitoneal fibrosis is secondary to malignancy. The only exception is retroperitoneal fibrosis related to carcinoid tumors, which can achieve great response to corticosteroids<sup>[14]</sup>.

Despite the lack of effective systemic options for the management of retroperitoneal fibrosis associated with malignancy, these patients might draw benefit from palliative surgical approaches in order to relieve obstructive complications. Moreover, pain management is of great importance.

In summary, retroperitoneal fibrosis secondary to malignant disease is a rare condition associated with a dismal prognosis. Organ dysfunction and poor performance status usually preclude the use of systemic chemotherapy.

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## Current oncologic applications of radiofrequency ablation therapies

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### Abstract

Radiofrequency ablation (RFA) uses high frequency alternating current to heat a volume of tissue around a needle electrode to induce focal coagulative necrosis with minimal injury to surrounding tissues. RFA can be performed *via* an open, laparoscopic, or image guided percutaneous approach and be performed under general or local anesthesia. Advances in delivery mechanisms, electrode designs, and higher power generators have increased the maximum volume that can be ablated, while maximizing oncological outcomes. In general, RFA is used to control local tumor growth, prevent recurrence, palliate symptoms, and improve survival in a subset of patients that are not candidates for surgical resection. It's equivalence to surgical resection has yet to be proven in large randomized control trials. Currently, the use of RFA has been well described as a

primary or adjuvant treatment modality of limited but unresectable hepatocellular carcinoma, liver metastasis, especially colorectal cancer metastases, primary lung tumors, renal cell carcinoma, bone metastasis and osteoid osteomas. The role of RFA in the primary treatment of early stage breast cancer is still evolving. This review will discuss the general features of RFA and outline its role in commonly encountered solid tumors.

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**Key words:** Radiofrequency ablation; Hepatocellular carcinoma; Colorectal cancer liver metastasis; Lung cancer; Renal cell carcinoma

**Core tip:** We have described the technical aspects of radiofrequency ablation (RFA), advances in delivery mechanisms, indications for usage, and its equivalence or lack of equivalence to surgical resection. We emphasized studies that reported long term oncologic outcomes associated with RFA use for primary and metastatic liver and lung tumors, and described the evolving role of RFA for breast and solid renal tumors.

Shah DR, Green S, Elliot A, McGahan JP, Khatri VP. Current oncologic applications of radiofrequency ablation therapies. *World J Gastrointest Oncol* 2012; 5(4): 71-80 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v5/i4/71.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v5.i4.71>

### INTRODUCTION

Surgical resection of all malignant cells remains the gold standard for treatment of most solid tumors<sup>[1]</sup>. However, surgical resection is not always an option in patients with coexistent morbidities or poor functional status where

resection would be associated with a high morbidity and mortality. As a result, a variety of local ablative methods, including chemical (ethanol, acetic acid, hot saline) and thermal (radiofrequency ablation, microwave ablation, laser ablation, cryoablation), have been developed to destroy cancer cells *in situ*. Radiofrequency ablation (RFA) has risen to the forefront amongst these local ablative modalities due to refinements in technology that maximize effectiveness and simplicity of use while minimizing associated morbidity. RFA is now used in the treatment, both curative and palliative, for solid tumors throughout the body. This minimally invasive technique can serve both as treatment for patients who are not surgical candidates, as well as an adjunct to surgery, facilitating resection or in combination with surgery achieving total tumor burden control.

## TECHNICAL FEATURES OF RFA

RFA uses radiowaves, which are of low frequency (460-480 kHz) and long wavelength, to generate heat within a tumor mass causing thermal coagulative necrosis. RFA differs from other local methods in that the electrode itself does not supply the heat. Needle electrodes supply an alternating electric current, which travels from the electrode to a grounding pad (monopolar) or between two electrodes (bipolar). As the ions within the tissue attempt to follow the alternating path of the current, ionic agitation creates frictional heat. This friction heats the surrounding tissue to 50-100 °C, inducing instantaneous coagulative necrosis. Temperatures greater than 100 °C result in tissue desiccation and charring with loss of ions thus stopping current flow. This leads to a sudden rise of impedance<sup>[2]</sup>, thus limiting the volume of tissue that can be successfully ablated.

The energy from the electrode tip produces a temperature that is proportional to the square of the radiofrequency current, which in turn decreases as the square of the diameter from the electrode<sup>[2]</sup>. Larger tumors require overlapping spheres, which increases the risk of incomplete necrosis and, therefore, local recurrence. Over the past several years, advances in delivery mechanisms that can either increase the amount of energy deposited or the conduction of heat through the tissue have increased the sphere of tissue that can be ablated<sup>[3]</sup>. There are currently five companies that produce commercially available RFA systems, four of which are approved by the Food and Drug Administration and available in the United States<sup>[4]</sup>. The specifications of each system are presented in Table 1.

Multiprobe array electrodes, in which multiple tines apply current simultaneously, achieve coagulation zones of 3-5 cm. Internally cooled (or cool-tip) electrodes also allow for greater ablation volumes. While it seems paradoxical to cool the electrode with a continuous infusion of fluid within the lumen, this cooling results in no local charring around the uninsulated electrode tip, thus allow-

ing longer flow of current. Longer duration of current flow allows for a larger volume of local tissue coagulation, compared to non-internally cooled electrodes. Wet electrodes using saline (either isotonic or hypertonic) infused through the electrode into surrounding tissue, increase conductivity with greater amounts of infusion of ions in the tissue, increasing current flow and thus allowing longer duration of current flow and increasing volume of coagulation.

Several strategies have been developed to decrease tumor tolerance to heat and increase the effectiveness of thermal ablative techniques. The “heat-sink” effect created by proximity of tumors to large vessels that can dissipate heat is a primary mechanism by which the extent of thermal injury can be limited<sup>[5,6]</sup>. The Pringle maneuver, which involves occluding portal inflow during open RFA. This has been shown to improve volume of tissue (tumor) coagulation by increasing local heat deposition, rather than having heat being dissipated in the portal vein<sup>[6,7]</sup>. Tissue damage from chemotherapy and hypoxic injury to tumors cells from embolization have also been shown to increase tumor sensitivity to hyperthermia. A synergistic effect between neoadjuvant transarterial chemoembolization and RFA in the treatment of hepatocellular carcinoma has also been demonstrated<sup>[7]</sup>.

## RFA technique

RFA can be performed percutaneously, or during laparoscopic or open surgery. There are advantages and disadvantages to each, and the approach will depend on the condition of the patient, tumor characteristics such as location, size, number and growth pattern, and experience and preference of the provider<sup>[8]</sup>. There is insufficient evidence as of date indicating which delivery method is the preferred due to a lack of randomized control trials and varying patient and tumor characteristics between single technique studies. In a study comparing open, laparoscopic, and percutaneous approaches for liver tumors, there was no difference in mortality, major complications, or overall survival; but open compared to percutaneous approach resulted in improved disease free survival and decreased local tumor recurrence<sup>[9]</sup>.

The percutaneous approach has the advantage of being performed under conscious or deep sedation, providing an option for patients who are higher surgical risk. This can usually be done as an outpatient or with a very short hospital stay, and can be performed multiple times if needed. The percutaneous approach can also be performed under anesthesia. Other advantages of this technique are the use of sonographic, computed tomography (CT) or magnetic resonance imaging (MRI) to guide precise electrode placement. At the same setting, contrast enhanced sonography or contrast enhanced CT can be done during the procedure to check for adequacy of ablation. Disadvantages of the percutaneous technique are lack of visualization of small surface tumors or deeper tumors which can be better identified with the

**Table 1** Radiofrequency ablation systems commercially available in the United States<sup>[4,8]</sup>

RFA system	Electrodes	Generator power/ frequency	Control system	Algorithm used to maximize volumes
Boston scientific	14 gauge, 10-12 tines, umbrella shaped	200 W/460 kHz	Impedance controlled	Coaxial system
Valleylab (radionics)	17.5 gauge, single cooled needle or three cooled needles in triangular cluster	200 W/480 kHz	Impedance controlled	Cool-tip
RITA medical systems		250 W/460 kHz	Temperature controlled	
Starburst XL	14 gauge, 9 tines, Christmas tree shape max diameter 5 cm			Expandable
Starburst XLi	14 gauge, 9 tines, max diameter 7 cm			
Starburst Flex	13 gauge, flexible			Expandable, wet electrode
Berchtold	18-14 gauge	60 W/375 kHz	Impedance or temperature controlled	Wet electrode

Modified from<sup>[4,8]</sup>. Cool-tip: Cooled electrode achieved with chilled water flowing through electrode but not entering tissue; Wet-electrode: Saline infusion into tissue adjacent to electrode creates larger “virtual” electrode around metal electrode tip.

open technique. Percutaneous RFA has shown excellent results for small < 3 cm neoplasms in the liver, lung or kidney. However, higher local recurrence has been shown with the percutaneous approach for larger tumors<sup>[10]</sup> and tumors in close proximity to major vessels, such as the portal vein.

Open RFA allows for better visualization and the ability to manipulate adjacent structures. It has the advantage of being able to detect occult metastatic disease with use of intra-operative ultrasound (US) and allows for treatment within a greater anatomic range. With hepatic RFA, another advantage is the ability to occlude portal inflow (Pringle maneuver) which, as described above, reduces heat dissipation and, therefore, increases the volume of tissue ablated. This technique is particularly valuable when tumors are located in proximity to vascular structures.

Laparoscopic RFA combines many of the benefits of both the percutaneous and open approaches. It is minimally invasive with less morbidity of a large incision while still allowing better visualization of the tumor and of adjacent structures to optimize staging. Pneumoperitoneum may also work in a similar manner to the Pringle maneuver and decrease the heat sink effect in tumors in proximity to large vessels by decreasing portal flow<sup>[11]</sup>. It also allows resection or displacement of structures adjacent to tumors that cannot be performed with the percutaneous technique.

### Imaging

Imaging plays an important role in the diagnosis and localization of the tumor, in real-time monitoring of the ablation zone, in assessment of tissue response to RFA therapy, and finally in patient follow-up. The RF probe is usually placed under CT or US guidance, and the RFA procedure monitored with real-time US. Ablation zones are seen on US as hyperechogenic areas which represent microbubbles created from the vaporization of interstitial fluid from ablated tissue. However, these hyperechogenic areas do not completely parallel the ablated zone. To determine the extent of necrosis following RFA in countries outside of the United States, US con-

trast is used at the time of the procedure to check for complete ablation and whether re-treatment is needed at the setting<sup>[12]</sup>. In the United States, a follow-up contrast-enhanced CT or MR is typically used, with successfully ablated areas failing to enhance. A thin enhancing rim representing either inflammation or hemorrhagic granulation tissue may surround the ablated zone for several weeks following treatment<sup>[13]</sup>. Follow-up may be done with CT, MRI or positron emission tomography scan, depending on the type, size and location of tumor.

The goal of RFA is usually to ablate 1 cm margin of normal tissue surrounding the tumor on all sides<sup>[8,14,15]</sup>. This surgical margin is necessary because of the difficulty of accurately determining the extent of the coagulation zone, and because of the possibility of microscopic malignancy surrounding the gross tumor<sup>[8]</sup>. Exceptions to the 1 cm margin rule may include organs such as the kidney, in which preservation of normal renal parenchyma would be a priority, or when tumor debulking for palliation or relief of neuroendocrine symptoms is the goal of treatment or when surrounding vital structures limit the extent of ablation.

### Complications

RFA has been shown to be a relatively safe procedure, with mortality between 0.3% and 0.8% and morbidity 2% and 10%<sup>[16,17]</sup>. Complications include post-procedural pain, post-RFA syndrome with fever and flu-like symptoms that usually resolves within the first 24 h, skin burns from improperly placed grounding pads, thermal injury to adjacent structures, bleeding, secondary infection, and tumor seeding, which can be prevented by cauterization of the needle tract on withdrawal of the probe<sup>[8]</sup>.

## SOLID TUMOR ABLATIVE EXPERIENCE

### Liver

The most extensive body of literature on RFA for the treatment of solid tumors involves its use with hepatic malignancies, both primary and metastatic. Currently, RFA is considered a first line treatment modality for local control of hepatocellular carcinoma in patients with

**Table 2** Studies reporting survival after use of radiofrequency ablation for colorectal liver metastases

Ref.	Patients (tumors) <i>n</i>	Median tumor size (cm)	Extra-hepatic disease	Chemotherapy	Method	% complete ablation	Local recurrence	Overall survival		
								1 yr	3 yr	5 yr
Abdalla <i>et al</i> <sup>[26]</sup>	57 (110) for RFA  190 for HR  101 for RFA + HR	2.5	No	NR	0	NR	9% for RFA  5% for HR + RFA 2% for HR	NR	37% for RFA 43% for HR + RFA 73% for HR	NR
Siperstein <i>et al</i> <sup>[27]</sup>	234 (665)	3.9 (mean)	Yes	80% before RFA	L	NR	NR	NR	20% <sup>2</sup>	18% <sup>2</sup>
Park <i>et al</i> <sup>[28]</sup>	30 for RFA 59 for HR	2.0 for RFA 3.1 for HR	No	73% after RFA 81% after HR	P	NR	23% for RFA 2% for HR	NR	NR	19% <sup>2</sup> for RF1 48% <sup>2</sup> for HR
Abitabile <i>et al</i> <sup>[54]</sup>	47 (147)	2	Yes	After RFA	O, P	97%	9% for overall 0%-5% for < 3 cm	88% <sup>1</sup>	57% <sup>1</sup>	21% <sup>1</sup>
Gillams <i>et al</i> <sup>[55]</sup>	167 (167)	3.9 (mean)	Yes	80% before RFA	P	NR	14.00%	99% <sup>1</sup> 91% <sup>2</sup>	58% <sup>1</sup> 28% <sup>2</sup>	30% <sup>1</sup> 25% <sup>2</sup>
Jakobs <i>et al</i> <sup>[56]</sup>	68 (183)	2.28 (mean)	No	78% parallel or after	P	NR	18.00%	96% <sup>2</sup>	71% <sup>2</sup>	
Machi <i>et al</i> <sup>[57]</sup>	100 (507)	3.0 (mean)	NR		O, L, P		7%	90%	42%	31%
Schindera <i>et al</i> <sup>[58]</sup>	14 (20)	1.8	No	NR	P	89%	15%	72% <sup>2</sup>	60% <sup>2</sup>	NR
White <i>et al</i> <sup>[59]</sup>	30 (56)	3.0 (0.8-7)	No	36% before, 50% after	P	89%	17%	75% <sup>2</sup>	45% <sup>2</sup>	NR
Solbiati <i>et al</i> <sup>[60]</sup>	117 (179)	2.6	Yes	72% parallel	P	98%	39%	93% <sup>2</sup>	46% <sup>2</sup>	NR

<sup>1</sup>Calculated from time of diagnosis of liver metastases; <sup>2</sup>Calculated after radiofrequency ablation (RFA) treatment of liver metastases. P, L, O: Percutaneous, laparoscopic, open; NR: Not reported; HR: Hazard ratio.

Child-Pugh B or higher cirrhosis where resection would have a higher associated mortality. It is indicated in patients with 3 or fewer tumors that are 3 cm or smaller (Milan criteria)<sup>[18]</sup>. It has recently been shown to be superior to percutaneous ethanol injection with regards to survival and local recurrence<sup>[19]</sup>. Its equivalence to surgical resection in patients who satisfy the Milan criteria remains controversial. A prospective randomized trial and a large retrospective analysis comparing local ablative techniques with surgical resection for patients with small solitary tumors, stage T1, found no difference in overall survival between RFA and surgical resection<sup>[20-22]</sup>. Smaller observational studies have demonstrated similar results<sup>[11]</sup>. A meta-analysis comparing RFA to hepatic resection in all subsets of patients found improved 3 and 5 year overall and disease free survival and decreased local recurrence in patients who underwent hepatic resection<sup>[23]</sup>. However, in patients with tumors smaller than 3 cm, the overall survival was comparable. In patients with larger tumors (> 3 cm), the combination of chemoembolization with RFA has been demonstrated to be superior to RFA alone in improving survival<sup>[24,25]</sup>. This is based on the hypothesis that RFA results in a zone of inflammation that can then be strategically used for targeted delivery of chemotherapeutic agents *via* chemoembolization.

The majority of the literature regarding hepatic metastases comes from single arm, retrospective or prospective studies evaluating RFA for treatment of unresectable colorectal metastases. In such studies, hepatic resection is superior to both RFA alone or combination of RFA with hepatic resection in regards to local recurrence and overall survival<sup>[26]</sup>. However, during open resection, ad-

ditional tumors may be detected on the liver surface or deep metastases may be seen with intra-operative US. These additional lesions can be resected or treated with intra-operative RFA. Randomized control trials directly comparing RFA to hepatic resection for resectable disease have yet to be performed.

There is considerable overlapping variability in the 5 year survival and the local recurrence rates due to differences in definition of local recurrence, inclusion criteria for unresectability, extent of extrahepatic disease, and patient and tumor characteristics between the studies. Local recurrence rates varied between 9% and 40% and 5 year overall survival varied between 18% and 30% (Table 2). The best outcomes were in patients with solitary tumors less than 3 cm and slightly less in patients with 3 or fewer tumors less than 3 cm<sup>[27]</sup>. Local recurrence was significantly larger in patients with tumors between 3-5 cm<sup>[20]</sup>. Retrospective studies comparing hepatic resection to RFA for patients who were potentially resectable but poor candidates for surgery due to co-morbidities or refusal, demonstrated decreased local recurrence and improved overall survival with hepatic resection<sup>[28]</sup>. Therefore it is evident that surgical resection remains the gold standard; but for those who are not candidates for surgery, an alternative such as RFA is valuable.

### Lung

RFA is increasingly being applied to malignant lung nodules for local control as well as for palliation as its feasibility and efficacy is becoming more established in the literature. Surgical resection remains the gold standard for curative treatment of primary lung cancers and malignant metastasis. However, only about 30% of



**Table 3** Studies involving survival using radiofrequency ablation for primary lung tumors and metastases

Ref.	Patients (tumors) <i>n</i>	Mean tumor size (cm)	Tumor type	Median local progression free interval	Overall survival			Complications
					1 yr	2 yr	3 yr	
Ambroggi <i>et al</i> <sup>[1]</sup>	54 (64)	2.4	40 for NSCLC 24 for Mets	< 3 cm - 15.8 mo > 3 cm - 6.6 mo	72% for NSCLC <sup>1</sup> 88% for Met <sup>1</sup>	46% for NSCLC <sup>1</sup> 72% for Mets <sup>1</sup>	30% for NSCLC <sup>1</sup> NR for Mets <sup>1</sup>	6 for PTX 1 for chest wall hematoma
Kim <i>et al</i> <sup>[30]</sup>	8 for RFA 14 for SR	3.66 for RFA 3.99 for SR	All stage I NSCLC	NR	88% for RFA 93% for SR	50% for RFA 77% for SR	25% for RFA 67% for SR	1 for PTX 4 for hemoptysis
Simon <i>et al</i> <sup>[35]</sup>	153 (189)	2.7	75 for stage I NSCLC 57 for Mets	< 3 cm - 45 mo > 3 cm - 12 mo	78% for NSCLC 70% for Met	57% for NSCLC <sup>1</sup> 54% for Mets <sup>1</sup>	36% for NSCLC <sup>1</sup> 44% for Mets <sup>1</sup>	18 for PTX 5 for hemoptysis 4 for death
Chua <i>et al</i> <sup>[37]</sup>	148	4	108 for CRCM Other	11 mo	NR	NR	60%	66 for PTX 16 for pleural effusion
Lencioni <i>et al</i> <sup>[61]</sup>	106 (183)	3.5	40 for Mets 33 for NSCLC 73 for Mets	NR	70% for NSCLC 89% for CRCM 92% for Other	48% for NSCLC 66% for CRCM 64% for Other		1 for vbleeding 27 for PTX 4 for effusion
Yan <i>et al</i> <sup>[62]</sup>	55	2.1	All CRCM	NR	85%	64%	46%	16 for PTX/ 9 requiring drainage
Hiraki <i>et al</i> <sup>[63]</sup>	20	2.4	All stage I NSCLC	9 mo	90%	84%	74%	5 for hemoptysis 13 for PTX/ 1 requiring drainage

<sup>1</sup>Calculated based on Kaplan-Meier survival curves. NR: Not reported; NSCLC: Non-small cell lung cancer; CRCM: Colorectal cancer metastasis; Mets: Other tumor metastases; PTX: Pneumothorax; SR: Surgical resection; RFA: Radiofrequency ablation.

patients with primary lung cancer are eligible for surgery at the time of diagnosis due to poor functional status and chronic obstructive pulmonary disease<sup>[29]</sup>. In patients with pulmonary metastasis, multiple lesions and advanced stage usually precludes curative surgical resection.

Currently, there is insufficient evidence to prove that RFA is comparable to surgical resection. There are currently no prospective randomized controlled trials comparing RFA with standard surgical treatment options in patients with malignant lung nodules. Data is limited to case series with differences in number of primary and secondary lung lesions, criteria for unresectability, number of prior resections, history of prior radiation therapy, differences in follow-up protocols, and criteria for determining extent of response to RFA treatment.

However, a small matched case series of 22 patients comparing RFA to resection in patients with stage I non-small cell lung cancer (NSCLC) demonstrated comparable survival in RFA patients at 1, 2, and 5 years<sup>[30]</sup>. The RAPTURE study, a large prospective multicenter single arm trial, using RFA in patients with early stage NSCLC or lung metastases demonstrated 1 and 2 year overall survival rates of 70% and 48% respectively in patients with primary lung tumors, and 89% and 66% 1 and 2 year overall survival in patients with colorectal metastases. The cancer specific survival was higher in both groups; 92% and 73% at 1 and 2 years in the NSCLC cohort and 91% and 68% in the cohort with colorectal metastases.

The 1, 2, 3 year overall survival for patients with early stage primary lung cancer treated with RFA varies from 70% to 90%, 48% to 84%, 25% to 74%, respectively (Table 3). This is comparable to the 1, 3, 5 year overall sur-

vival of patients who undergo lobectomy or segmental resection for early stage lung cancer<sup>[31-34]</sup>. In most studies that compare outcomes based on size of tumor ablated, patients with tumors smaller than 3 cm had longer median progression free intervals and overall survival<sup>[35]</sup>.

The median procedure related morbidity and mortality are 37.5% and 0% respectively<sup>[36]</sup>. The majority of complications from thoracic RFA are minor with the most frequently encountered being pneumothorax and pleural effusions (4.5%-61%) and hemoptysis. Others include pain, fever and pneumonia. Despite the high incidence of pneumothorax, only a minority, 11%, require pleural drainage<sup>[36]</sup>. The incidence of pneumothorax increases as the number of lesions ablated<sup>[37]</sup>.

### Breast

The role of RFA in breast cancer is still emerging. There is a growing trend towards breast conservation techniques that minimize scarring, breast deformity, and improve overall post procedure cosmesis. Several small single institution studies have established the feasibility of RFA and outlined potential complications (Table 4). In majority of these studies, RFA was followed by lumpectomy or mastectomy, either immediately or in a delayed fashion. The procedure was done under local or general anesthesia depending on whether resection was delayed or followed immediately after RFA, respectively. Response was assessed by pre- and post-procedural MRI which correlated better with pathologic response than US<sup>[38]</sup>. HE staining, immunohistochemistry with CK 18/8, or nicotinamide adenine dinucleotide-diaphorase cell viability assay were used to assess histopathologic response. There

**Table 4** Studies involving survival using radiofrequency ablation for primary lung tumors and metastases

Ref.	Patients	Range tumor size (cm)	Mean tumor size (cm)	Complete coagulation necrosis <i>n</i> (%)	Resection	Assessment of cell viability	Complications
Burak <i>et al</i> <sup>[38]</sup>	10	0.8-1.6	1.2	9 (90)	Delayed	HE CK8/18	None
Singletary <i>et al</i> <sup>[40]</sup>	29	≤ 2.0	-	25 (86)	Immediate	HE NADH-diaphorase	1 skin burn
Oura <i>et al</i> <sup>[41]</sup>	52	0.5-2.0	1.3	52 (100)	Delayed	NR	1 skin burn
Khatri <i>et al</i> <sup>[64]</sup>	15	0.8-1.5	1.28	13 (93)	Immediate	HE NADH-diaphorase	2 skin puckering
Noguchi <i>et al</i> <sup>[65]</sup>	10	0.5-2.0	1.1	10 (100)	Immediate	HE NADH-diaphorase	None
Fornage <i>et al</i> <sup>[66]</sup>	20	0.6-2.0	1.2	21 (100)	Immediate	HE NADH-diaphorase	None
Hayashi <i>et al</i> <sup>[67]</sup>	22	0.5-2.6	0.9 (median)	19 (86)	Delayed	HE NADH-diaphorase	1 skin burn
Izzo <i>et al</i> <sup>[68]</sup>	26	0.7-3.0	1.8	25 (96)	Immediate	HE NADH-diaphorase	1 skin burn

NR: Not reported; HE: Hematoxylin and eosin stain; NADH: Nicotinamide adenine dinucleotide.

are several studies that have reported HE staining maybe inadequate to assess histopathologic response since it gives a broad spectrum of necrosis and that techniques that assess cell viability are better<sup>[38,39]</sup>. Complete coagulative necrosis was achieved in 80%-100% of the patients, with skin burn being the most common complication in a very small subset of patients.

Patient selection criteria were strict, including mostly patients with invasive tumors less than 2 cm in size; a few studies had a small portion of patient with non-invasive tumors. The presence of extensive intraductal component was also a relative contraindication to RFA. In addition, estrogen and progesterone receptor status, her 2 status, grade, histology, and need for chemotherapy had to be known prior to RFA since no residual tumor cells would be available post-procedure if 100% successful. Superficial tumors within 1 cm of the skin are a relative contraindication as well, due to increased risk for skin burns. Various strategies to minimize skin burns have been employed in the studies including cooling the breast with sterile ice packs and subcutaneous injection of sterile saline or a high resistance solution to displace the tumor away from the skin. In addition, preoperative chemotherapy is a contraindication since it can lead to an underestimation of tumor size and leave occult foci of residual carcinoma<sup>[40]</sup>.

There are currently no studies comparing RFA to surgical resection, and no long term studies depicting local recurrence rates or survival in patients who receive RFA instead of surgical resection. Very few studies have evaluated RFA as an alternative to surgical resection. Oura *et al*<sup>[41]</sup> reported their experience treating 52 patients, with a mean tumor size of 1.3 cm (range 0.5-2.0 cm), with RFA following sentinel node biopsy. There was no local-regional or distant recurrence after an average 15-mo follow-up (range 6-30 mo).

Patient response to RFA has been favorable. Oura *et al*<sup>[41]</sup> retrospectively evaluated cosmetic results, which were found to be excellent in 43 patients (83%), good in 6 patients (12%) and fair in 3 patients (6%). The authors found that a major factor leading to poor cosmesis was mass formation at the site of RFA, especially in women with small breasts. This can lead to increased patient anxiety as well.

Progress in the application of RFA for breast tumors is at present hampered by our ability to accurately judge the margin status which is a critical variable in local recurrence rate. Evolution in imaging technology will foster such advancements. Nonetheless, as more breast cancers are being diagnosed at a smaller size, a focused image-guided ablation can minimize destruction of normal breast tissue and thus may positively impact cosmesis.

### Kidney

As with other solid tumors, RFA is increasingly being applied for the therapy for renal tumors as less invasive and nephron-sparing techniques, including partial nephrectomy and laparoscopic nephrectomy, have proven to have comparable 5-year and disease-free survival<sup>[42]</sup>.

Currently, RFA as primary treatment for renal malignancy is limited in study to a select group of patients with early T1a disease or for whom surgical resection is not an option. These include patients with only one kidney, multifocal disease, Von Hippel Lindau, limited renal function, elderly patients or patients with comorbidities that are poor candidates for surgery<sup>[8,43-45]</sup>. Contraindications include a life expectancy less than one year, the presence of distant metastases, tumors > 5 cm, or tumors in the hilum or central collecting system. Studies have consistently shown 91%-97% complete first ablation success for small (< 3-4 cm), exophytic, peripherally located tumors (Table 5). This is due to the fact that peripherally located tumors are surrounded by peri-renal fat that provides insulation, allowing the high temperatures necessary for successful ablation to be achieved. Conversely, hilar blood flow creates a heat-sink effect making treatment of central tumors more challenging. The recurrence free survival varies from 79%-91% in biopsy proven renal cell cancers, while the 3 and 5 year cancer specific survival ranges from 95%-100% in the few long term studies.

### Bone tumors and metastatic bone lesions

RFA has been long proven efficacious for the treatment of osteoid osteomas. It is performed in patients with typical clinical and radiographic characteristics of an osteoid osteoma (radiolucent nidus surrounded by reactive sclerosis) for treatment of bone pain. It is successful initially in 73%-98% of patients with 92%-100% secondary

**Table 5** Studies involving survival after radiofrequency ablation for solid renal tumors

Ref.	Patients (tumors) <i>n</i>	Method	Mean tumor size (cm)	RCC	Complete first ablation	Recurrence free survival	Overall survival (yr)			Cancer specific survival (yr)		Complications
							1	3	5	3	5	
Tracy <i>et al</i> <sup>[69]</sup>	208 (243)	P, L, O	2.4	79%	97%	90% at 3 yr <sup>2</sup>	99% <sup>1</sup>	93% <sup>1</sup>	85%	95% for RCC	99% for RCC	NR
Levinson <i>et al</i> <sup>[70]</sup>	31 (34)	P, L	2.1	58%	91%	80% at 5 yr <sup>2</sup>	NR	NR	63% for all <sup>3</sup> 58% for RCC <sup>4</sup>	NR	100% for all 100% for RCC	4 for perinephric hematoma ; 1 for liver burn; 1 for death from pneumonia
Zagoria <i>et al</i> <sup>[71]</sup>	41 (48)	P	2.6	100%	NR	88% at 5 yr	NR	NR	66%	NR	NR	2 for pneumothorax no drainage; 2 for ureteral strictures
Stern <i>et al</i> <sup>[72]</sup>	40	P, L	2.4	81%	97%	91% at 3 yr <sup>2</sup>	NR	NR	NR	100% for RCC	NR	2 for minor; 3 for major

<sup>1</sup>Calculated based on Kaplan-Meier curve and life table; <sup>2</sup>In biopsy proven renal cell carcinoma (RCC); <sup>3</sup>80 mo overall survival; <sup>4</sup>57 mo overall survival. P, L, O: Percutaneous, laparoscopic, open; NR: Not reported.

success rates and majority of patients experiencing pain relief within the first 1-2 wk of treatment<sup>[46-49]</sup>. Complication rates are minimal with skin necrosis and burns being the most common. It has been demonstrated to be comparable to surgical resection with regards to recurrence<sup>[50]</sup>. RFA has also been described in case reports for the treatment of other benign bone tumors.

More recently, RFA has been applied as a palliative modality for the treatment of painful metastatic bone lesions. External beam radiation remains the gold standard for treatment of localized bone pain from a metastatic focus. However, 20%-30% of patients don't respond and are recalcitrant to pharmacotherapy<sup>[51,52]</sup>. In addition, patients previously irradiated at a recurrent site, may not be eligible for repeat radiation therapy. Ninety percent to ninety-five percent of patients treated with RFA experience a clinically significant reduction in pain that can be seen within the first week of treatment lasting up to 24 wk<sup>[52,53]</sup>. Complication rates are minimal and can vary from bleeding, pathologic fractures, skin and muscle burns and damage to adjacent neurovascular structures<sup>[46]</sup>.

## CONCLUSION

RFA has been demonstrated to be an effective local ablative technique in patients with a variety of solid tumors. More prospective randomized studies are needed before RFA will replace surgical resection for small, limited tumors involving the lung or liver. Long term studies establishing its oncological effectiveness in breast and solid renal tumors are still needed. The future of thermal ablative techniques may or may not involve radiofrequency waves as newer ablative techniques involving microwaves are currently being developed which offer the advantages of higher intratumoral temperatures, larger ablative volumes, and faster ablation times while minimizing energy dissipation. However, the safety and efficacy of microwave ablation is still under evaluation. Regardless of the ablative technique, proper patient selection remains a key

factor in determining who will most likely benefit.

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## Prognostic factors in resectable cholangiocarcinoma patients: Carcinoembryonic antigen, lymph node, surgical margin and chemotherapy

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### Abstract

**AIM:** To evaluate outcomes in resectable cholangiocarcinoma patients and to determine prognostic factors.

**METHODS:** A retrospective study was conducted among newly-diagnosed cholangiocarcinoma patients from January 2009 to December 2011 who underwent curative resection in Srinakarind Hospital (a 1000-bed university hospital). Two hundred and sixty-three cholangiocarcinoma patients with good performance were enrolled. These patients had pathological reports with clear margins or microscopic margins. Prognostic factors which included clinical factors, serum liver function test as well as serum tumor makers at presentation,

tumor data, and receiving adjuvant chemotherapy were determined by uni- and multivariate analysis.

**RESULTS:** The median overall survival time was 17 mo (95%CI: 13.2-20.7); and 1-, 2-, and 3- year survival rates were 65.5%, 45.2% and 35.4%. Serum albumin levels, serum carcinoembryonic antigen (CEA) levels, staging classifications by American Joint Committee on cancer, pathological tumor staging, lymph node metastases, tumor grading, surgical margin status, and if adjuvant chemotherapy was administered, were shown to be significant prognostic factors of resectable cholangiocarcinoma by univariate analysis. Multivariate analysis, however, established that only abnormal serum CEA [hazard ratio (HR) 1.68;  $P = 0.027$ ] and lymph node metastases (HR 2.27;  $P = 0.007$ ) were significantly associated with a decrease in overall survival, while adjuvant chemotherapy (HR 0.71;  $P = 0.067$ ) and surgical margin negative (HR 0.72;  $P = 0.094$ ) tended to improve survival time.

**CONCLUSION:** Serum CEA and lymph node metastases which were associated with advanced stage tumors become strong negative prognostic factors in cholangiocarcinoma.

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**Key words:** Cholangiocarcinoma; Prognosis; Carcinoembryonic antigen; Lymph nodes; Neoplasm metastasis; Surgical margin status; Hepatectomy; Chemotherapy; Adjuvant; Survival rate

**Core tip:** Cholangiocarcinoma has a high prevalence in the Asian countries, particularly Thailand. Cholangiocarcinoma patients usually have a high mortality rate and poor treatment outcomes. Curative surgery is the only treatment for early stages of this cancer. Cholan-

giocarcinoma has a high rate of recurrence. This study aimed to evaluate outcomes in resectable cholangiocarcinoma patients and to determine prognostic factors. The results demonstrated serum carcinoembryonic antigen and lymph node metastases which were associated with advanced stage tumors become strong negative prognostic factors in cholangiocarcinoma, while additional treatment including adjuvant chemotherapy and adequate surgical resection may improve survival time.

Wirasorn K, Ngamprasertchai T, Chindaprasirt J, Sookprasert A, Khantikaew N, Pakkhem A, Ungarereevittaya P. Prognostic factors in resectable cholangiocarcinoma patients: Carcinoembryonic antigen, lymph node, surgical margin and chemotherapy. *World J Gastrointest Oncol* 2012; 5(4): 81-87. Available from: URL: <http://www.wjgnet.com/1948-5204/full/v5/i4/81.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v5.i4.81>

## INTRODUCTION

Cholangiocarcinoma is a malignant tumor of intrahepatic and extrahepatic bile duct epithelium<sup>[1]</sup>. It is a second most common malignancy of primary liver tumors worldwide<sup>[2]</sup>. The highest incidence is in the Northeast region of Thailand, while it is a rare tumor in Europe and America<sup>[3,4]</sup>. *Opisthorchis viverrini* infestation is a major risk factor in Thai patients, while primary sclerosing cholangitis, obesity, viral hepatitis B and viral hepatitis C infection are the risk factors in Western countries<sup>[5,6]</sup>. Cholangiocarcinoma is commonly classified into 3 groups based on the location of the tumor: intrahepatic, perihilar, or distal types<sup>[1]</sup>.

Surgery with clear surgical margin is an important treatment for patients with local disease<sup>[7]</sup>. Standard surgery for cholangiocarcinoma depends on its location. Major hepatectomy is a surgical procedure for intrahepatic cholangiocarcinoma and perihilar cholangiocarcinoma, while pancreaticoduodenectomy is performed in distal cholangiocarcinoma<sup>[7,8]</sup>.

Although most patients receive surgical treatment, the five-year survival rate is extremely low<sup>[9]</sup>. High locoregional recurrence and metastases are common causes of death in resectable patients<sup>[10]</sup>. Benefits of adjuvant therapy in achieving long-term survival in resectable cholangiocarcinoma patients are controversial<sup>[11]</sup>. Previous studies attempted to identify prognostic factors in this group<sup>[12-15]</sup>. Surgical margin status and lymph node involvement are important prognostic factors<sup>[9,11,16]</sup>. Other risk factors may be differentiation of tumor cells, preoperative tumor markers like carbohydrate antigen 19-9 (CA 19-9) and carcinoembryonic antigen (CEA), and site of tumor<sup>[13,17,18]</sup>. Data about prognosis in resectable cancer patients, however, are still limited. Moreover, only a few participants were enrolled in former reports. Therefore, this study aimed to determine prognostic factors in cholangiocarcinoma patients who underwent curative resection.

## MATERIALS AND METHODS

### Patients

A retrospective study was conducted among newly-diagnosed, cholangiocarcinoma patients from January 2009 to December 2011, who underwent curative surgery in Srinakarind Hospital, Khon Kaen University (a 1000-bed university hospital), Khon Kaen, Thailand. The study was reviewed and approved by the institutional review board (HE 551183). Curative resection was defined as a total excision of the entire tumor, including the primary tumor and the associated lymph node drainage fields. Two hundred and sixty-three cholangiocarcinoma patients with good performance status were enrolled. All patients with curative resection had pathological reports with a negative surgical margin or microscopic surgical margin. Demographic data including sex, age, underlying disease especially type 2 diabetes mellitus, body weight, height, and clinical manifestations were collected. Body mass index (BMI) was calculated from weight in kilograms divided by the square of the height in meters ( $\text{kg}/\text{m}^2$ ). BMI cutoffs were classified according to the World Health Organization criteria for Asian and Pacific populations (underweight,  $< 18.5 \text{ kg}/\text{m}^2$ ; healthy,  $18.5\text{--}22.9 \text{ kg}/\text{m}^2$ ; at risk,  $23\text{--}24.9 \text{ kg}/\text{m}^2$ ; obese I,  $25\text{--}29.9 \text{ kg}/\text{m}^2$ ; and obese II,  $\geq 30 \text{ kg}/\text{m}^2$ )<sup>[19]</sup>. Preoperative liver function status including total bilirubin, cholesterol, alanine transaminase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), as well as serum tumor markers including CA 19-9 and CEA were evaluated.

Tumor data included tumor location, staging classification by the 7<sup>th</sup> edition of American Joint Committee on Cancer (AJCC), pathological tumor staging (pT), lymph node metastasis, tumor differentiation, and surgical margin status. All patients received the appropriate surgical procedure. Adjuvant chemotherapy was administered in patients who accepted the risk-benefit after a discussion with their physicians.

### Statistical analysis

The survival time was defined as date of diagnosis to date of death from any cause. Patients' characteristics and tumor data were summarized as mean and percentage. The cumulative survival rate is presented by the Kaplan-Meier curve. The following variable factors were analyzed: sex, age, diabetic status, hepatomegaly, BMI status, serum total bilirubin level, serum cholesterol level, serum albumin level, serum ALT level, serum AST level, serum ALP level, serum CEA level, serum CA 19-9 level, AJCC staging, tumor location, pT, lymph node status, tumor differentiation, surgical margin status and adjuvant chemotherapy. Differences in survival between subgroups were compared using the log-rank test. Univariate analysis was performed using the chi-squared testing. Multivariate analysis was performed with the Cox proportional hazard model. The statistical analyses were performed by using SPSS software version 20.0. A *P*-value of less than 0.05 was considered statistically significant. The database was closed for analysis in August 2012.



**Table 1** Baseline characteristics of 263 resectable cholangiocarcinoma patients *n* (%)

Age, yr	
mean $\pm$ SD	59.0 $\pm$ 8.9
Range	35-80
Male	181 (69.6)
DM	19 (6.5)
BMI (mean $\pm$ SD), kg/m <sup>2</sup>	
< 18.5	23 $\pm$ 8.7
18.5-22.9	127 $\pm$ 48.3
23-24.9	47 $\pm$ 17.9
25-29.9	48 $\pm$ 18.3
$\geq$ 30	13 $\pm$ 4.9
Not available	5 $\pm$ 1.9
Clinical manifestation	
Abdominal pain	164 (62.4)
Jaundice	54 (20.5)
Fever	6 (2.3)
Cholangitis	4 (1.5)
Weight loss	1 (0.4)
Asymptomatic	17 (6.5)
Hepatomegaly	153 (58.2)
Total bilirubin (mg/dL)	
< 10	213 (81.0)
$\geq$ 10	50 (19.0)
Cholesterol (mg/dL)	
< 200	168 (63.9)
$\geq$ 200	95 (36.1)
Albumin (g/dL)	
< 3	42 (16.0)
$\geq$ 3	220 (83.7)
ALT (U/L)	
< 30	46 (17.5)
$\geq$ 30	151 (82.5)
AST (U/L)	
< 30	25 (9.5)
$\geq$ 30	238 (90.5)
ALP (U/L)	
< 100	82 (31.2)
$\geq$ 100	180 (68.5)
CA 19-9 (U/mL)	
< 35	108 (41.1)
$\geq$ 35	148 (56.3)
CEA (ng/mL)	
< 2.5	65 (24.7)
$\geq$ 2.5	183 (69.6)
Receiving adjuvant chemotherapy	
Yes	138 (52.5)
No	125 (47.5)

CA 19-9: Carbohydrate antigen 19-9; CEA: Carcinoembryonic antigen; ALT: Alanine transaminase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; BMI: Body mass index; DM: Diabetes mellitus.

## RESULTS

The patients' characteristics and tumor data are presented in Tables 1 and 2. Abdominal pain was the most common clinical presentation. The majority of the patients had normal a BMI, level of serum total bilirubin below 10 mg/dL, level of serum albumin above 3 g/dL, elevation of serum liver enzymes as well as abnormal serum tumor markers, CA 19-9 and CEA. Intrahepatic cholangiocarcinoma was the most common site of tumor. Most patients were in an advanced stage, *i.e.*, stage III or IV. One hundred and thirty-three patients received

**Table 2** Tumor data of 263 resectable cholangiocarcinoma *n* (%)

Tumor location	
Intrahepatic	166 (63.1)
Perihilar	91 (34.6)
Distal	6 (2.3)
AJCC staging	
0	11 (4.2)
1	37 (14.1)
2	54 (20.5)
3	89 (33.8)
4	72 (27.4)
pT stage	
0	10 (3.8)
1	47 (17.9)
2	85 (32.3)
3	95 (36.1)
4	25 (9.5)
pN stage	
0	167 (63.5)
1	96 (36.5)
Tumor grading	
Well diff	198 (75.3)
Moderate diff	14 (5.3)
Not available	51 (19.4)
Margin surgical resection	
Free	134 (51.0)
Not free	129 (49.0)

AJCC: American Joint Committee on Cancer; pN: Pathologic node; pT: Pathologic tumor.

adjuvant chemotherapy of which the combination of fluorouracil and mitomycin C was the most administered regimen (60.9% of these patients). Other regimens included combination of gemcitabine and capecitabine, gemcitabine, fluorouracil, and capecitabine.

Median overall survival of the entire cohort was 17 mo (95%CI: 13.2-20.7) as shown in Figure 1. One, two, and three-year survival rates were 65.5%, 45.2%, and 35.4%. Serum albumin, serum CEA, AJCC staging, pT staging, lymph node metastases and whether or not having received adjuvant chemotherapy were significant prognostic factors in resectable cholangiocarcinoma by univariate analysis as shown in Table 3. Figure 2 revealed Kaplan-Meier survival curve regarding significant prognostic factors. Receiving adjuvant chemotherapy prolonged survival in resectable cholangiocarcinoma patients, however, the combination between fluorouracil and mitomycin C was not different other regimen to improve survival benefit [median survival time was 17.3 mo (95%CI: 12.8-21.7) *vs* 22.3 mo (95%CI: 20.3-24.3), respectively; *P* = 0.20]. Abnormal serum CEA and lymph node metastasis significantly impacted the overall survival in multivariate analysis (Table 4).

## DISCUSSION

This cohort study had several similar and different characteristics from the previous reports<sup>[3,20,21]</sup>. Most patients in this study had a BMI below 23; whereas, the majority of patients in the cited previous report were over-

**Table 3** Differences of survival time among significant variable factors when analyzed by univariate analysis

Variable	Median survival (mo)	95%CI	P value
Albumin (g/dL)			0.04
< 3	12.8	7.1-18.4	
≥ 3	19.1	14.6-23.5	
CEA (ng/mL)			0.02
< 2.5	27.7	14.1-41.3	
≥ 2.5	16.5	13.0-20.0	
AJCC staging			< 0.001
0	Not reached		
1	Not reached		
2	23.5	16.9-30.1	
3	12.8	10.6-15.1	
4	12.5	9.3-15.7	
Tumor grading			0.01
Well differentiated	17.9	12.6-23.2	
Moderate differentiated	7.7	0.0-21.7	
Margin in resection group			0.001
Negative	26.7	19.6-33.8	
Positive	14.1	11.9-16.4	
pT stage			< 0.001
0	Not reached		
1	28.6	23.1-34.1	
2	19.9	12.9-26.9	
3	12.8	9.4-16.3	
4	15.5	9.9-21.1	
pN stage			< 0.001
0	25.1	20.0-30.1	
1	10.0	6.7-13.3	
Receiving adjuvant chemotherapy			0.01
Yes	21.6	16.9-26.4	
No	13.4	10.7-16.2	

CEA: Carcinoembryonic antigen; AJCC: American Joint Committee on Cancer. pN: Pathologic node; pT: Pathologic tumor.

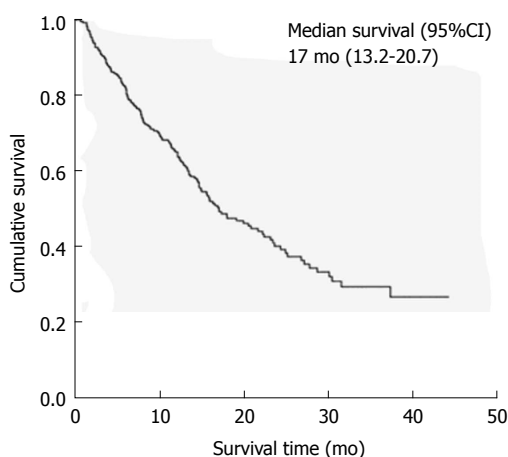
weight<sup>[20]</sup>. The tumor data showed that intrahepatic cholangiocarcinoma was the most common subtype, whereas, perihilar subtype was the most common location in other reports<sup>[3]</sup>. These findings were correlated with the first clinical presentation of abdominal pain and level of serum bilirubin below 10 mg/dL. Furthermore, this study found that asymptomatic presentation was more common in patients with intrahepatic cholangiocarcinoma than in other previous studies<sup>[21]</sup>. The authors' results demonstrated serum albumin was a significant prognostic factor by univariate analysis. Serum albumin is marker of nutritional status in cancer patients<sup>[22]</sup>. A low level of serum albumin is usually found in malnourished patients, and associated with poor treatment outcomes such as postoperative infection and impaired wound healing<sup>[23,24]</sup>. Advanced stages of cancers, including cholangiocarcinoma, also lead to a decrease in serum albumin level<sup>[25]</sup>. Additionally, previous studies reported that low serum albumin was associated with an increased postoperative mortality in cholangiocarcinoma patients<sup>[26]</sup>.

AJCC staging of cholangiocarcinoma, pT staging, and the differentiation of tumor cells were an associated prognostic factor, as well and were demonstrated in our results by univariate analysis. These results were similar

**Table 4** Significant prognostic factors by multivariate analysis

Variable	HR	95%CI	P value
Serum CEA (< 2.5 ng/mL vs ≥ 2.5 ng/mL)	1.68	1.05-2.66	0.027
Lymph node metastasis (yes vs no)	2.27	1.24-4.12	0.007
Receiving adjuvant chemotherapy (yes vs no)	0.71	0.49-1.02	0.067
Surgical margin (negative vs positive)	0.72	0.49-1.06	0.094

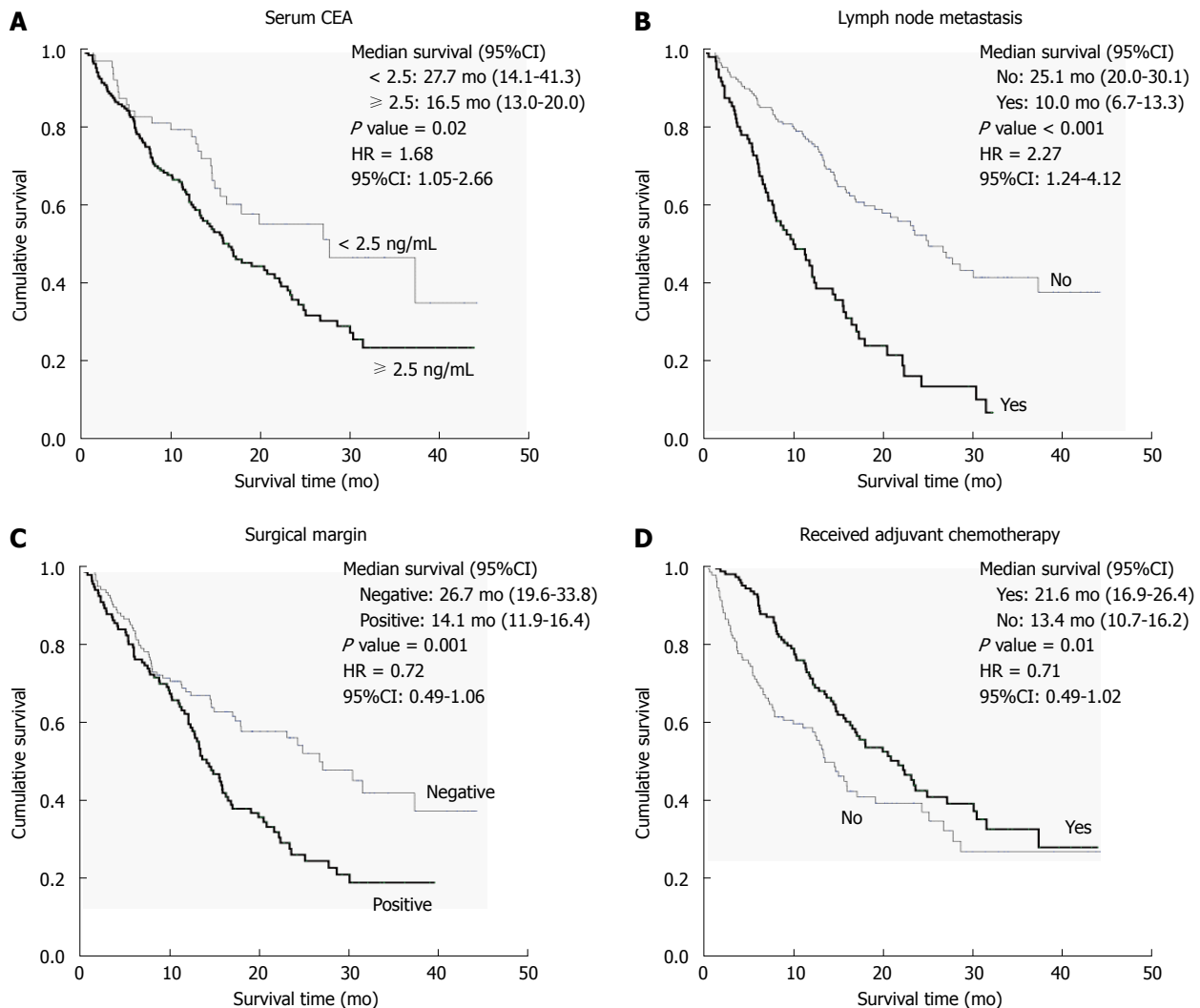
CEA: Carcinoembryonic antigen; HR: Hazard ratio.

**Figure 1** Kaplan-Meier survival curve used to analyze the overall survival time of 263 resectable cholangiocarcinoma.

with previous results<sup>[18,25]</sup>. A well-differentiated tumor histology was related to early staging and was a good prognostic factor from results of the previous studies<sup>[27,28]</sup>.

The results showed that the level of serum CEA above 2.5 ng/mL and lymph node metastases were significant independent poor prognostic factors by univariate and multivariate analysis. CEA was demonstrated in fetal gut tissue and in tumors from the gastrointestinal tract<sup>[29]</sup>. Serum CEA in cancer patients was significantly higher than in healthy controls and may be a prognostic factor in several gastrointestinal cancers, including cholangiocarcinoma<sup>[30,31]</sup>. A previous study demonstrated that cancer patients with a high level of serum CEA was associated with an advanced stage of cancer and may signal poor prognosis<sup>[32,33]</sup>. This study demonstrated that cholangiocarcinoma patients with high level of serum CEA were associated high risk of death (HR 1.68, 95%CI: 1.05-2.66), which is similar to previous studies<sup>[25]</sup>. The preoperative serum CEA level in cholangiocarcinoma patients was correlated with the stage of cancer and could help determine their prognosis<sup>[32,34]</sup>.

Lymphatic dissemination is a common metastatic pathway of cholangiocarcinoma. Previous studies demonstrated that up to 55% of cholangiocarcinoma patients who underwent operations had tumor cells in the regional lymph nodes<sup>[9]</sup>. Several studies showed that overall survival rate in cholangiocarcinoma patients with lymph node involvement was lower than other groups<sup>[35-37]</sup>. These findings were similar in both resectable and unresectable patients<sup>[25,26,38,39]</sup>. The findings of the present



**Figure 2** Kaplan-Meier survival curve showed significant difference in survival rate regarding prognostic factors. A: Serum carcinoembryonic antigen (CEA) level  $\geq 2.5$  ng/mL at presentation; B: Lymph node metastasis; C: Surgical margin; D: Receiving adjuvant chemotherapy. HR: Hazard ratio.

study also showed that lymph node metastases had an impact on survival.

Surgical margin status is a prognostic factor in several cancers, including cholangiocarcinoma. Previous studies showed overall survival rate in cholangiocarcinoma patients with positive surgical margin was lower than patients with negative surgical margin<sup>[15,28,40-42]</sup>. The present results demonstrated that a negative surgical margin was associated long-term survival time.

Adjuvant chemotherapy is a controversial issue in resectable cholangiocarcinoma. The present authors' results showed that patients with adjuvant chemotherapy may have longer overall survival time than patients without adjuvant chemotherapy. Previous retrospective studies showed benefits of adjuvant chemotherapy<sup>[12,15,43]</sup>. Randomized studies, however, did not demonstrate a definite advantage in cholangiocarcinoma<sup>[44]</sup>. Recently, a meta-analysis showed that chemotherapy as a part of adjuvant therapy which included radiotherapy and concurrent chemoradiotherapy may be beneficial in resect-

able cholangiocarcinoma patients with high risk features, such as lymph node metastases and positive surgical margins<sup>[45]</sup>. In our institute, combination of 5-fluorouracil and mitomycin C was the most administered regimen. However, the survival of this combination was not significantly different from the other regimens.

In conclusion, serum CEA and lymph node metastasis which are associated with advanced tumor stages become strong negative prognostic factors in cholangiocarcinoma, while additional treatment including adjuvant chemotherapy and adequate surgical resection may improve survival time.

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## COMMENTS

### Background

Cholangiocarcinoma has a high prevalence in the Asian countries, particularly Thailand. Cholangiocarcinoma patients usually have a high mortality rate and poor treatment outcomes. Curative surgery is the only treatment for early stages of this cancer. Cholangiocarcinoma has a high rate of recurrence. This study aimed to evaluate outcomes in resectable cholangiocarcinoma patients and to determine prognostic factors.

### Research frontiers

A retrospective study included newly-diagnosed 263 cholangiocarcinoma patients from January 2009 to December 2011 who underwent curative resection and had pathological reports with clear margins or microscopic margins in Srinakharind Hospital (a 1000-bed university hospital).

### Innovations and breakthroughs

The results demonstrated serum carcinoembryonic antigen and lymph node metastases which were associated with advanced stage tumors become strong negative prognostic factors in cholangiocarcinoma, while additional treatment including adjuvant chemotherapy and adequate surgical resection may improve survival time.

### Applications

Adjuvant chemotherapy and adequate surgical resection may improve survival time.

### Terminology

Curative resection was defined as a total excision of the entire tumor, including the primary tumor and the associated lymph node drainage fields.

### Peer review

This is an interesting study aimed to evaluate outcomes in resectable cholangiocarcinoma patients and to determine prognostic factors. The results are interesting and suggest that adjuvant chemotherapy which includes combination of fluorouracil and mitomycin C and other regimens may improve overall survival in resectable cholangiocarcinoma patients.

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# World Journal of *Gastrointestinal Oncology*

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**APPENDIX** I-V Instructions to authors

**ABOUT COVER** *World Journal of Gastrointestinal Oncology* Editorial Board, Yu-Tong He, Vice-director, Department of cancer epidemiology, Hebei Cancer Institute/Hospital, 12 Jiankang Road, Shijiazhuang 050011, China

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## Targeted treatments for metastatic esophageal squamous cell cancer

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tor; Vascular endothelial growth factor receptor; Mammalian target of rapamycin

**Core tip:** This paper discusses some of these targeted agents in more advanced development in metastatic esophageal squamous cell carcinomas, as well as some promising drugs with pre-clinical or initial clinical data in the disease.

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### Abstract

Squamous cell carcinoma, one of the two major subtypes of esophageal carcinomas, constitutes the great majority of tumors in the upper and middle third of the organ. Declining in incidence in western countries, it continues to be a significant public health problem in the far east. Targeted treatments are novel therapies introduced in the clinical therapeutic armamentarium of oncology in the last 10-15 years. They represent a rational way of treating various cancers based on their molecular lesions. Although no such agent has been approved so far for the treatment of esophageal squamous cell carcinomas (ESCC), several are in clinical trials and several others have displayed pre-clinical activity that would justify the efforts and risks of pursuing their clinical development in this disease. This paper discusses some of these targeted agents in more advanced development in metastatic ESCC, as well as some promising drugs with pre-clinical or initial clinical data in the disease.

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**Key words:** Esophageal carcinoma; Squamous; Targeted therapies; Clinical trials; Epidermal growth factor recep-

### INTRODUCTION

Two major histologic types of esophageal cancers exist and differ in their epidemiology and risk factors. Esophageal adenocarcinomas arise almost exclusively in the lower third of the esophagus and esophagogastric junction, have an increasing incidence in western populations and are associated with Barrett's metaplasia and chronic gastro-esophageal reflux<sup>[1]</sup>. In contrast, esophageal squamous cell carcinomas (ESCC) are mostly situated in the upper two thirds of the organ and are associated with smoking and alcohol<sup>[2]</sup>. Salty foods and nitrosamine compounds in foods have also been implicated<sup>[3]</sup>. Their incidence decreases in western countries but remains a significant public issue in Asian populations<sup>[4]</sup>.

Unfortunately, despite these differences that imply a distinct pathogenesis, the two types are mostly lumped together in clinical trials, a fact that would dilute a possible benefit in only one of the two histologies if the other histology would not benefit from a given treatment. Although this may be true even for chemotherapy as evidenced in the case of pulmonary adenocarcinomas and

squamous cell carcinomas where differences in response to various regimens have been revealed<sup>[5]</sup>, it is particularly important for targeted therapies that would work only if the intended target is expressed, functional and involved in the pathogenesis of a carcinoma. A recent change in the trend of trials in the field of esophageal cancers necessitated by the development of targeted therapies adjoins esophageal adenocarcinomas and adenocarcinomas of the gastro-esophageal junction with gastric adenocarcinomas with which they share a common histology and are at times difficult to discern at the margins of the two organs. For the development of trastuzumab therapy, adenocarcinomas of the gastro-esophageal junction have been included in the trials and those of lower esophagus are often treated with the drug if they display an increased expression or amplification of human epidermal growth factor receptor 2 (HER-2)/Neu<sup>[6]</sup>. With these facts in perspective, the current paper will discuss only data concerning targeted therapies in metastatic or locally advanced inoperable ESCC. For studies that have included both esophageal histologies, discussion will be restricted to patients with squamous histology. When there are significant data available specifically for ESCC on expression of a possible tumor target and preclinical anti-tumor activity of a corresponding therapy, they will be mentioned, as they might represent an opportunity for future clinical development.

## ANTI-EPIDERMAL GROWTH FACTOR RECEPTOR THERAPIES

Immunohistochemical (IHC) studies showed epidermal growth factor receptor (EGFR) protein over-expression in 50% of patients with ESCC and gene amplification was evident in 28% of over-expressors (or 14% of the total patients in the series)<sup>[7]</sup>. Over-expression was significantly correlated with the depth of tumor invasion<sup>[7]</sup>. Mutations in exons 19 and 21 of EGFR were not identified in any of the patients examined. Others have found amplification of EGFR in 15% in a series of 55 patients with ESCC<sup>[8]</sup> and rare EGFR mutations in ESCC specimens and a ESCC cell line<sup>[9,10]</sup>. Half of the patients displayed high levels of EGFR protein expression measured by a semi-quantitative IHC-based method. Protein expression correlated with gene amplification in this and in another series of 105 ESCC patients<sup>[11]</sup>. In this last series EGFR amplification or polysomy by fluorescence *in situ* hybridization (FISH) was seen in 31% of patients<sup>[11]</sup>.

Several studies have examined the efficacy of anti-EGFR therapies in ESCC. Two types of agents targeting the EGFR signaling pathway are available: the anti-EGFR monoclonal antibodies cetuximab and panitumumab and the small molecule tyrosine kinase inhibitors (TKIs) erlotinib and gefitinib.

In metastatic ESCC, a randomized phase II study compared cisplatin 100 mg/m<sup>2</sup>, day 1 and 5-fluorouracil (5-FU) 1000 mg/m<sup>2</sup>/d continuous infusion, days 1-5 every

4 wk with or without cetuximab 250 mg/m<sup>2</sup> weekly (after a loading dose of 400 mg/m<sup>2</sup>) in the first line setting<sup>[12]</sup>. A trend towards longer progression-free survival (PFS) (5.9 mo *vs* 3.6 mo) and overall survival (OS) (9.5 mo *vs* 5.5 mo) was noted in the cetuximab arm. Of interest cetuximab did not exacerbate grade 3 or 4 toxicities, except for rash and diarrhea. A randomized three arm phase II study (CALGB 80403/ECOG 1206) took a reverse approach and sought to determine what chemotherapy is best in combination with cetuximab in metastatic esophageal and gastroesophageal junction cancer<sup>[13]</sup>. Patients were randomized to epirubicin, cisplatin, 5-FU (ECF) or 5-FU, folinic acid, oxaliplatin (FOLFOX) or irinotecan/cisplatin. All three arms received concomitant cetuximab. Only a few (about 10%) of patients had ESCC. Results have been presented so far in an abstract form for the adenocarcinoma patients. The two first arms were more effective and the FOLFOX arm less toxic<sup>[13]</sup>. Conclusions regarding the clinical utility of adding cetuximab to first line chemotherapy are awaiting information from ongoing randomized phase III trials.

Regarding the role of cetuximab in the 2<sup>nd</sup> line chemotherapy setting there is a lack of published trials. A phase II study in the 2<sup>nd</sup> line setting adding cetuximab to cisplatin and irinotecan in patients with irinotecan and cisplatin-refractory metastatic esophageal cancer (NCT 00397904) has completed accrual. This study has included both squamous and adenocarcinomas and results are awaited.

The other clinically available anti-EGFR monoclonal antibody, panitumumab is investigated in combination with chemotherapy in a phase III study (NCT01627379) of non-resectable advanced or metastatic ESCC. Patients included have not been treated with chemotherapy previously (except in the neo-adjuvant setting). All patients receive cisplatin and 5-FU and are randomized to receive or not panitumumab.

A third investigational anti-EGFR antibody, nimotuzumab has been studied in patients with ESCC in the first line metastatic setting in combination with paclitaxel and cisplatin<sup>[14]</sup>. Results of 25 patients treated in a phase II study showed a 63.6% partial response (PR) rate and 31.8% stable disease (SD). The same investigators study nimotuzumab in the second line setting in combination with mFOLFIRI chemotherapy (Trial NCT01486992).

Both orally active TKIs gefitinib and erlotinib that are currently available in clinical practice have been tested in metastatic ESCC. These TKIs block the ATP binding site of the EGFR tyrosine kinase molecule. A phase II trial of gefitinib 500 mg daily in the 2<sup>nd</sup> line treatment of metastatic esophageal cancer showed a higher disease control rate (PR *plus* SD) in patients with SCCs compared with adenocarcinomas ( $P = 0.013$ ). Patients with high EGFR expression and lower levels of phosphorylated kinase adams kara taylor (Akt) had higher disease control rates ( $P = 0.002$  and  $0.009$  respectively)<sup>[15]</sup>. Nine patients with SCC were among the 36 patients enrolled in this study and five (55.5%) showed a PR or SD. Five of six patients tested had a strong expression of EGFR (more than 25%

of tumor cells stained strongly) by IHC. Among the five patients with a PR or SD, all four tested had high EGFR expression. Another phase II trial of gefitinib in recurrent or metastatic esophageal or gastroesophageal junction cancer included 58 patients but only 4 among them had squamous histology<sup>[16]</sup>. Authors state that both histologies derived a clinical benefit but obviously the small number of ESCC patients precludes any definitive conclusion from this study.

A phase III study (NCT01243398) randomizing patients with ESCC and adenocarcinoma to gefitinib versus placebo after one or two lines of chemotherapy is currently ongoing.

Recently a phase II study of erlotinib monotherapy in previously treated esophageal cancer was published<sup>[17]</sup>. Similarly to gefitinib, erlotinib shows activity in squamous cancer. Among the 30 patients included in this study, thirteen patients had squamous histology and twelve of them had some degree of EGFR positivity by IHC (defined as more than 10% of tumor cells staining for the receptor tyrosine kinase). Two patients obtained a response which lasted for 5.5 and 7 mo while seven additional patients had stable disease for a median of 5 mo. The median time to disease progression in all squamous histology patients in the study was 3.3 mo. No correlation of EGFR status and degree of expression with erlotinib efficacy could be established possibly due to the small number of patients.

Overall, interesting activity with acceptable toxicity of anti-EGFR agents is seen in these initial studies. More definitive results from larger trials are expected. Well-validated biomarkers will certainly help to define sub-sets of patients that will benefit most.

## ANTI-HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR 2 THERAPIES

The benefit of treatment with the humanized anti-HER-2 monoclonal antibody trastuzumab in cases of HER-2 protein over-expressing or gene amplified metastatic breast and gastric adenocarcinomas is well established<sup>[16,18]</sup>. About 15%-20% of squamous esophageal cancers show over-expression of HER-2 by IHC and about 1%-20% show gene amplification by FISH<sup>[19,22]</sup>. Few studies correlated HER-2 status with clinical outcomes in ESCC<sup>[20,21]</sup>. These retrospective studies have shown a worse survival in ESCC when HER-2 is over-expressed. Preclinical data show that trastuzumab, targeting the extracellular domain of the HER-2 protein, has anti-proliferative activity directly but also through antibody dependent cellular cytotoxicity in esophageal carcinoma cells<sup>[23-26]</sup>, providing a rational for clinical trials in ESCC with HER-2 over-expression/amplification. Nevertheless no such studies investigating trastuzumab treatment or treatment with the newer anti-HER-2 agents pertuzumab and trastuzumab emtansine in ESCC have been conducted so far. A phase I study of paclitaxel and trastuzumab with interleukin

12 (trying to take advantage of a natural killer cell mediated cytotoxicity) in HER-2 overexpressing carcinomas has included 4 patients with ESCC<sup>[27]</sup>. Two of them had a partial response lasting for 25 and 43 wk.

## DUAL ANTI-EGFR AND ANTI-HER-2 THERAPIES

Given that a percentage of ESCC overexpress both EGFR and HER-2<sup>[25]</sup>, there is a rational for use of drugs that inhibit both receptors. Lapatinib is a small TKI that inhibits both EGFR and HER-2. A phase II study of lapatinib in recurrent or metastatic ESCC has been initiated (NCT00239200) but has been terminated and there are no published data regarding the outcomes. Another oral TKI pan-HER inhibitor, PF-00299804 is studied in a Korean phase II trial in patients with recurrent and metastatic ESCC (trial NCT01608022). The dual EGFR and HER-2 inhibitor afatinib has been investigated in a phase I study in which one of 7 esophageal cancer patients participating had an unconfirmed partial response<sup>[28]</sup>. Phase II development is pursued only in esophago-gastric adenocarcinomas. Based on the accumulated evidence from other malignancies, it can be expected that the efficacy of anti-EGFR/anti-HER-2 agents would be restricted to tumors with high expression or specific mutations of these receptors. Further development of targeted EGFR/HER-2 drugs should be focused to these sub-sets of ESCC. Although this focusing would limit the pool of available patients and make clinical trials more cumbersome and slow to accrue, it will, on the other hand, increase the probability of obtaining positive results.

## ANTI-VASCULAR ENDOTHELIAL GROWTH FACTOR THERAPIES

Angiogenesis plays a crucial role in carcinogenesis and progression of malignancies. Vascular endothelial growth factor (VEGF) pathway is among the important signal transducers in this process<sup>[29]</sup>. Several ligands including VEGF-A, -B, -C and -D initiate signals by binding cell surface receptor tyrosine kinases VEGF-R1 (also known as flt1), VEGF-R2 (also known as flk1) and VEGF-R3 (also known as flt4) and triggering down-stream cascades of cell proliferation and survival. VEGF pathway alterations are involved in pathogenesis of esophageal cancer<sup>[30]</sup>. VEGF-A expression is present in most ESCC and ranges between 24%-93% in different studies<sup>[31]</sup>. Moreover the over-expression of VEGF isoforms has been significantly correlated with poorer prognosis of ESCC<sup>[32,33]</sup>.

Bevacizumab is a monoclonal antibody targeting VEGF. It is used clinically in colon, breast, ovarian and lung adenocarcinomas. Its development in gastric and gastro-esophageal junction adenocarcinomas has progressed through phase III trials<sup>[34]</sup> but in squamous

cancers there is a reluctance to pursue development given safety issues with hemorrhage in squamous non-small cell lung cancer<sup>[35]</sup>. Small molecule TKI of VEGF receptor (VEGFR) have been studied in early trials that have included ESCC patients. Pazopanib, a TKI targeting VEGFR, platelet-derived growth factor receptor and c-Kit is already approved for the treatment of clear cell renal carcinoma. A phase I study combining it with carboplatin and paclitaxel in patients with various advanced cancers found the maximal tolerated dose to be 200 mg daily<sup>[36]</sup>. Among the patients in this trial there were 4 with esophageal carcinoma (histology not specified) and two of them had a complete response. The TKI sunitinib is evaluated in phase II studies as a single agent or in combination with paclitaxel in patients with advanced esophagogastric cancer of both histologies<sup>[37,38]</sup>. Another anti-VEGF TKI, sorafenib is studied as monotherapy. Responses so far have been reported in adenocarcinomas but only 2 patients with ESCC were included in this preliminary report<sup>[39]</sup>. A fourth anti-VEGFR TKI, vandetanib is in phase I development in combination with oxaliplatin and docetaxel in advanced esophageal and gastroesophageal junction adenocarcinomas and squamous carcinomas (trial number NCT00732745). It is evident from these data that the field of anti-VEGF therapy development in esophageal cancer is dominated by adenocarcinoma histology and few results for ESCC are available. It remains to be seen if further development, optimally aided by predictive markers, will be pursued in ESCC.

## ANTI-MTOR THERAPIES

Mammalian target of rapamycin (mTOR) is an intracellular serine/threonine kinase that plays important roles in RNA translation, cell proliferation and angiogenesis. Inhibition of mTOR by everolimus has shown activity and is used clinically in renal cell carcinoma<sup>[40]</sup>, pancreatic neuroendocrine tumors<sup>[41]</sup> and in breast cancer where it has been found to reverse tumor resistance to hormonal treatments<sup>[42]</sup>. In ESCC mTOR is reported to be activated in 25% of cases and co-relates with a lower degree of differentiation<sup>[43]</sup>. Another group found activation of mTOR in 50% to 70% of ESCC and showed worse overall and cancer specific survival in cases with activated mTOR compared with non-activated cases<sup>[44,45]</sup>. Proliferation of ESCC cell lines with activated mTOR was inhibited by everolimus *in vitro* and *in vivo* in mouse xenograft models<sup>[44]</sup>.

Very few clinical data on mTOR inhibitors in ESCC are available. In a phase I trial of everolimus a single esophageal cancer patient included (histology not reported) treated with 10 mg/d showed a marked response in a metastatic supraclavicular lymphadenopathy before dying of tumor-related hemorrhage<sup>[46]</sup>. An additional strategy for mTOR inhibitor development in ESCC would be their combination with other targeted treatments. As mTOR may be activated by the phosphati-

dylinositol 3-kinase (PI3K)/Akt pathway down-stream of receptor tyrosine kinases, a combined inhibition with inhibitors of these kinases may be advisable. Combination with direct inhibitors of PI3K could be an alternative. PI3K inhibitors are in development<sup>[47,48]</sup>. The hedgehog pathway is also co-operating with the PI3K/Akt pathway<sup>[49]</sup> and is activated in a sub-set of ESCC<sup>[50,51]</sup>. Hedgehog signaling is important in foregut development, a fact that may underline its importance in carcinogenesis in both squamous and adenocarcinomas of the esophagus<sup>[51]</sup>. Hedgehog pathway inhibitor vismodegib is used for the treatment of basal cell cutaneous carcinomas<sup>[52]</sup>. Preclinical studies have shown synergy of vismodegib with everolimus in esophageal adenocarcinomas<sup>[53]</sup>. A phase II study of the addition of vismodegib to FOLFOX chemotherapy in gastro-esophageal adenocarcinomas is in progress<sup>[54]</sup>. Further development of mTOR inhibitors in ESCC enriched for activated mTOR with or without other targeted or chemotherapeutic treatments seems to be warranted.

## OTHER TARGETED THERAPIES

Hepatocyte growth factor/scatter factor (HGF) is the ligand for proto-oncogenic cell surface receptor c-Met. c-Met transduces proliferative and pro-angiogenic signals and has been related to prognosis of different malignancies<sup>[55,56]</sup>. A study in ESCC patients has shown that the serum level of HGF is higher than controls and correlates with levels of interleukin-8 and VEGF, both important mediators of angiogenesis<sup>[57]</sup>. HGF was also an independent prognostic factor for survival. Patients with higher than the median serum HGF had a median survival of 34 mo and a 2 year survival of 63% while those with serum HGF lower than the median had a median survival of only 15 mo and a 2 year survival of 37%<sup>[57]</sup>. Antibodies blocking this pathway could be a potential therapeutic strategy in ESCC. The small molecule kinase inhibitor against anaplastic lymphoma kinase crizotinib is concomitantly an inhibitor of c-Met and could be used to target this pathway.

Bryostatin-1 is an agent with antitumor activity *via* the inhibition of protein kinase C and has synergistic activity with chemotherapeutic agents such as paclitaxel<sup>[58]</sup>. In esophageal cancer a phase II trial of weekly paclitaxel and bryostatin-1 that included 22 patients demonstrated a response rate above 25% and up to 40% in the higher doses reached<sup>[59]</sup>. Nevertheless this trial has included only 2 patients with ESCC and had to close prematurely because of high rates of toxicities (myalgia) and two possibly treatment-related deaths. Bryostatin-1 analogs have been synthesized and could be alternatively developed if proved to have a better toxicity profile<sup>[60]</sup>.

Bortezomib is a proteasome inhibitor that is used in the treatment of myeloma and lymphoma. In pre-clinical studies in ESCC, bortezomib has shown activity by co-operating with both intrinsic and extrinsic pathways in



apoptosis induction<sup>[61,62]</sup>. A possible role of the drug in enhancing radiotherapy-induced cell death was suggested<sup>[62]</sup>. Despite the rational in targeting the proteasome as an anti-neoplastic treatment, in several solid tumors bortezomib has shown minimal activity. Thus an alternative approach with the identification and use of predictive biomarkers could be more effective if bortezomib (or other newer proteasome inhibitors such as carfilzomib<sup>[63]</sup>) were to be developed in ESCC.

The inducible form of the pathway enzyme cyclooxygenase (COX)-2 plays a role in the promotion of ESCC. Studies in preclinical models have shown that COX-2 inhibition has anti-tumor effects in ESCC cells<sup>[64]</sup>. These effects have been attributed to induction of apoptosis, inhibition of angiogenesis and suppression of invasion. In human ESCC, increased COX-2 expression correlates with reduced OS<sup>[65,66]</sup> and more aggressive tumor characteristics<sup>[67]</sup>. Furthermore, increased expression of EP2, the receptor for the COX-2 product prostaglandin PGE2, is associated with worse survival in patients with localized ESCC<sup>[68]</sup>. Dawson *et al.*<sup>[69]</sup> report a response rate of 54% in a phase I / II study of the COX-2 inhibitor celecoxib in combination with 5-FU/cisplatin/radiotherapy in 13 patients, with 3 of them having squamous histological type. This small number precludes any conclusions and no data are available in the metastatic setting. Moreover there are safety concerns with the coxib class of COX-2 inhibitors that limits their potential for further development. A recent study demonstrates that celecoxib antagonizes the cytotoxicity of cisplatin<sup>[70]</sup>, further complicating a putative development of coxibs in ESCC. Other non-steroidal anti-inflammatory drugs with a better safety record such as aspirin may be preferable, although many of them lack the selectivity of coxibs for COX-2 and inhibit concomitantly the constitutive form, COX-1.

Other opportunities for targeted treatments clearly exist based on detected abnormalities in ESCC cells. For example global histone H3 and H4 hypoacetylation was detected in tumors from patients with ESCC<sup>[71]</sup>. This may be the result of increased histone deacetylase 1 (HDAC1) expression in ESCC cells compared with adjacent normal tissues<sup>[72]</sup>. Inhibition of the expression of HDAC1 by RNAi resulted in enhanced radiosensitivity of ESCC cells *in vitro*. Histone deacetylase inhibitor vorinostat inhibited invasion of ESCC cells pretreated with tumor necrosis factor  $\alpha$  and transforming growth factor  $\beta$  in an *in vitro* assay<sup>[73]</sup>. The combination of vorinostat with the aforementioned proteasome inhibitor bortezomib further increased these invasion-inhibiting effects. Despite these pre-clinical encouraging data, no clinical data in ESCC are available for the time being regarding HDAC inhibitors.

Natural products contained in berries have been found to prevent ESCC in Fischer-344 rats treated with N-nitrosomethyl benzylamine (NMBA)<sup>[74]</sup>. This is a model of ESCC in which rats develop pre-neoplastic lesions passing from hyperplasia to dysplasia and finally to neo-

plasia (papillomas). In a clinical trial conducted in China, treatment with 60 mg daily of freeze-dried strawberries reversed mild to moderate dysplasia of esophagus<sup>[75]</sup>. Several carcinogenesis-involved molecules such as COX-2, nuclear factor  $\kappa$ B and targets of the mTOR pathway have been modified after this treatment. Whether the treatment could have beneficial effect in established carcinomas and what compound or compounds in the extracts provide the beneficial effect remains to be investigated.

## CONCLUSION

No targeted treatment agent has been introduced in the clinic for the treatment of ESCC until now. This relates to several factors that impede the clinical development of new drugs in ESCC. The rarity of these tumors not only makes the execution of trials with satisfactory numbers to extract conclusions more difficult but also obliges investigators to perform trials with both histologies in the organ or even including gastric cancer patients. As a result, effective treatments for only one of the histologies may be missed. The problem of patient recruitment will not be helped in the future as ESCC incidence (fortunately) decreases in western countries. Thus other solutions are needed including judicious “use” of the patient pool at hand. This implies that new agents to be entered in the clinical development face should have robust pre-clinical data supporting them and a molecular rational.

Another factor that may dilute possible positive results even within the same histology stems from the significant heterogeneity of cancer. This is probably of even greater importance for the development of targeted treatments than for chemotherapy agents. A promising strategy to overcome the heterogeneity barrier is the identification of prognostic markers which can help in the selection of patients. Such identification would lead in trials that will test a new targeted agent only in patients whose tumors over-express the target or express a mutated form of it. In some instances mere over-expression of the target is not enough or is not even present and demonstration of lesions in other proteins of the pathway(s) in which it participates is required for determination of sensitive sub-groups of a given tumor type. An illustrative example is anti-EGFR agents in colorectal cancer which are more effective in the sub-group of tumors with wild type Kras protein<sup>[76]</sup>. This protein is a down-stream effector of EGFR and when mutated blunts the activity of anti-EGFR agents because it is active even without receiving signals from the EGFR up-stream.

Related to the problem of tumor heterogeneity is the theory of tumor stem or tumor initiating cells. According to this theory only a generally small sub-set of neoplastic cells has the ability to propagate the tumor, while the bulk of the tumor derived from the sub-set of stem cells is less important because it lacks the capacity to proliferate indeterminably except if it acquires a stem cell phenotype<sup>[77]</sup>. In addition stem cells have been found to be drug

resistant and possess the ability to undergo epithelial to mesenchymal transition, a process endowing them with metastatic potential<sup>[78]</sup>. Thus a potential strategy for clinical development of targeted agents would be to determine the expression of their targets in stem cells and the dependence of those cells to these targets. By targeting tumor initiating cells one can argue that the anti-tumor effect would be more pronounced and durable.

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## RAS signaling pathways, mutations and their role in colorectal cancer

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oncogene operates physiologically. It also describes the mutations in these pathways that lead to colorectal cancer (CRC), as well as other mutations outside these cascades affecting RAS function and also leading to CRC. The prognostic value of each mutation is assessed and linked to response rates to available biological treatments. Monoclonal antibodies under development are also briefly discussed.

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### Abstract

Two of the main cellular pathways in which the RAS protein operates are the mitogen-activated protein kinases (MAPK) and phosphoinositide-3 kinase (PI3K) pathways. In a normal cell, these are important in controlling several functions, such as cell growth and survival. It becomes self-evident that these events will be disrupted in a malignant cell with a deregulated MAPK or PI3K pathway. Mutations in genes involved in these pathways and interacting with RAS, as well as RAS itself will be discussed. The second part of this review concentrates on how crucial RAS signaling is in colorectal cancer progression, with references to treatment response and prognosis when RAS or other related mutations are present.

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**Key words:** Genes; RAS; Colorectal neoplasms; Therapeutics; Mitogen-activated protein kinase signaling system

**Core tip:** This review outlines clearly the normal function of the mitogen-activated protein kinases and phosphoinositide-3 kinase cascades, in which the RAS proto-

### MITOGEN-ACTIVATED PROTEIN KINASES SIGNALING CASCADE

Two of the main cellular pathways in which the RAS protein operates are the mitogen-activated protein kinases (MAPK) and phosphoinositide-3 kinase (PI3K) pathways. In a normal cell, these are important in controlling several functions, such as cell growth and survival<sup>[1,2]</sup>. The first step towards activating this pathway occurs when a ligand binds to a receptor tyrosine kinase (RTK). For example, a well known ligand is epidermal growth factor (EGF), whose receptor is EGFR. Before being able to bind EGFR, EGF must first be released from the cell surface membrane where it resides. This is achieved by means of the TACE/ADAM-17 enzyme, which is particularly capable of cleaving transforming growth factor- $\alpha$  and amphiregulin, two of the ligands belonging to the EGF family<sup>[3]</sup>.

Following ligand binding, the receptor becomes dimerised and phosphorylated<sup>[4]</sup>. Next, a complex of proteins is established within the cell, with growth factor receptor-bound protein 2 (GRB2) becoming attached to the receptor, whilst being bound by son of sevenless

(SOS). Then the SOS protein, whilst still attached to GRB2, binds RAS as well. It should be noted here that there are several subtypes of the RAS protein, such as HRAS, NRAS and KRAS, but the most important one with regards to colorectal carcinogenesis is the latter, followed by NRAS<sup>[1]</sup>.

After the attachment of SOS to RAS, SOS shows guanine nucleotide exchange factor activity. This means that SOS is capable of displacing guanosine diphosphate (GDP) molecules from RAS and thus allowing guanosine triphosphate (GTP) molecules to bind and activate it. Active GTP-RAS is able to recruit the RAF proteins (A-RAF, B-RAF and C-RAF) to the cell surface. The RAF proteins are normally bound to, and therefore inhibited by, the 14-3-3 proteins in the cytosol. However, after binding to GTP-RAS, the RAF proteins are released from the 14-3-3 proteins and are therefore activated; they pair up amongst them and form heterodimers, which are then capable of binding and activating the KSR1 enzyme<sup>[1]</sup>.

The KSR1 enzyme is a relay hub connecting RAF heterodimers with the MEK protein. Hence, RAF proteins are able to phosphorylate and activate MEK, which in turn phosphorylates and activates ERK. ERK then enters the cell nucleus to activate a range of transcription factors, such as Jun and Fos<sup>[1]</sup>; these bind to the AP-1 DNA domain of the nucleus and transcribe genes involved in cell proliferation<sup>[5]</sup>.

The above process is entirely normal in a healthy cell, and is terminated by means of RAS-GTPase activating (GAP) proteins. As their name suggests, these proteins activate GTPase enzymes found within RAS, which hydrolyse GTP to GDP and therefore switch RAS off<sup>[1]</sup>.

## MUTATIONS RELATIVE TO THE MAPK PATHWAY

### RAS mutations

One of the most frequent ways in which the MAPK is set to overdrive is by a mutation in the RAS protein; mutations in the KRAS protein are found in about 40% of all colorectal cancer (CRC) cases, whereas NRAS mutations are less common, having a frequency of 5%. Both in KRAS and NRAS, the most typical mutations are found at codons 12, most of the times, 13 and 61 (the latter being rarely affected). These mutations are sometimes present in early adenomas and in cells with minimal potential to develop a malignancy. However, they are also thought to enhance the malignant behaviour of cells with advanced CRC; both *in vitro* and animal studies indicate that silencing these mutated codons leads to attenuation of the tumourigenic growth properties of the affected cells<sup>[6]</sup>.

In molecular terms, mutations in these three KRAS/NRAS codons may lead to conformational changes so that the RAS-GAP protein cannot activate the inherent GTPase enzyme anymore. As a result, the GTP molecules are not hydrolysed and instead they maintain RAS continuously in its active state, thus causing protumorigenic

effects by amplifying signaling in the MAPK pathway<sup>[7]</sup>.

### BRAF mutations

BRAF can also be mutated in the MAPK pathway, and this appears to happen in about 5%-10% of all colon cancer cases<sup>[6]</sup>. The commonest BRAF mutation amongst all cancers, including colorectal, is the V600E mutation. This occurs when adenine replaces thymine at nucleotide 1799. Consequently, glutamic acid (E) substitutes valine (V) at codon 600, hence the name of the mutation<sup>[8,9]</sup>.

Two basic models were proposed to explain how CRCs arise, and BRAF mutations occur in both of them. The first model proposed by Fearon and Vogelstein in 1990 suggested that CRC is a result of multiple adenomatous lesions progressing to carcinomas, following several somatic and inherited gene alterations<sup>[10]</sup>. Apparently, this is what happens in the majority of the cases<sup>[11]</sup>. In this model, the mutation of adenomatous polyposis coli (APC) leading to initial formation of the polyps is of paramount importance<sup>[12]</sup>.

The second model, true in approximately 15% of all CRC cases<sup>[6]</sup>, holds that CRC is caused by mutation of mismatch repair (*MMR*) genes, which normally fix errors in DNA replication. Hence the mutations result in replicative errors not being corrected and therefore microsatellites (short DNA repetitions) start accumulating or become abnormally short, leading to microsatellite instability (MSI)<sup>[13]</sup> and colorectal carcinogenesis. Inactivation of *MMR* genes can be observed in the hereditary non-polyposis CRC (HNPCC) syndrome, but it usually occurs epigenetically; epigenetic inactivation most often involves the hypermethylation of MutL homolog 1 (*MLH1*), one of the *MMR* genes, and falls under a category of colorectal tumours called CpG island methylator phenotype (CIMP). CIMP tumours have a specific histological appearance, termed sessile serrated adenomas (SSAs). BRAF mutations are very frequent in SSAs, but not so frequent in HNPCC. Overall, they are mostly found amongst sporadic, high in MSI (MSI-H) colorectal tumours<sup>[6]</sup>.

In any case, a BRAF mutation will lead to increased kinase activity and therefore increased downstream signaling in the MAPK cascade<sup>[6]</sup>.

### EGFR and other RTK mutations

EGFR (*HER-1*) gene amplifications or point mutations may cause an up-regulation of the receptor, thus increasing the probability of its activation by EGF binding and thus increasing signaling. However, such events are quite uncommon and appear in less than 5% of CRCs<sup>[14]</sup>. The human epidermal growth factor receptor 2 (*HER-2*)/neu receptor can also be overexpressed; though the evidence is inconsistent and ranges are anywhere between 0% and 83%, it is unlikely that this is a major determinant of colorectal tumorigenesis<sup>[15]</sup>.

## PI3K SIGNALING CASCADE

The other main pathway in which RAS is involved is the

PI3K pathway. This is a very complex pathway, therefore only some of its key elements will be mentioned here. Just like in the case of the MAPK pathway, various growth factors initially bind on receptor tyrosine kinases, leading to their dimerisation and autophosphorylation. The next stage involves PI3Ks. There are three different classes of PI3Ks, but the most important class in human cancer is 1A. The regulatory subunit of this class, p85, attaches to phosphotyrosine residues and/or other adaptors found on the RTKs. As a result, p110, the catalytic subunit of the PI3Ks is disinhibited and phosphorylates PIP2 to PIP3<sup>[2]</sup>. RAS can also activate the pathway physiologically by directly binding p110<sup>[16]</sup>. Conversely, the tumour suppressor protein PTEN dephosphorylates PIP3 back to PIP2, thus terminating signaling<sup>[2]</sup>.

Once PIP3 is formed, it recruits PDK1 and AKT kinases and brings them in close proximity. PDK1 phosphorylates AKT. Consequently, AKT becomes activated and generates several signals, the details of which are probably unrelated to this topic. These signals essentially contribute to cellular growth and evasion of apoptosis<sup>[2]</sup>.

## MUTATIONS RELATIVE TO THE PI3K PATHWAY

### PIK3CA mutation

*PIK3CA* is the gene encoding for P110 $\alpha$ . Mutation of RAS often coexists with mutations at exons 9 and 20 of *PIK3CA*<sup>[17]</sup>. It has been hypothesised that, when RAS is mutated, it can no longer bind the physiological form of P110 $\alpha$  efficiently. This necessitates the mutation of *PIK3CA*, which apparently will encode for a truncated version of P110 $\alpha$ , on which the mutant RAS will be able to bind effectively. The estimated frequency of *PIK3CA* mutations in CRC is 15%-25%, and these may lead to increased PI3K activity<sup>[6]</sup>.

### Phosphatase and tensin homolog mutation

Nonsense mutations and deletions in the phosphatase and tensin homolog (*PTEN*) gene makes the PTEN protein unable to convert PIP3 to PIP2, thus it can no longer act as an antagonist to PI3K signaling. This mutation is cardinal to the manifestation of Cowden syndrome, as it appears in 85% of all its cases. Cowden syndrome is an autosomal dominant disorder that predisposes to multiple cancers, including colorectal. Overall, it is estimated that *PTEN* is mutated in 10%-20% of all CRCs<sup>[6]</sup>.

## OTHER MUTATIONS AFFECTING RAS SIGNALING

### Neurofibromin 1 mutations

The neurofibromin 1 (*NF1*) gene is responsible for causing the genetic disease neurofibromatosis type 1. *NF1* acts as a negative regulator of RAS because it transcribes neurofibromin, a GAP; as mentioned above, these proteins hydrolyse RAS-bound GTP to GDP, therefore inactivating RAS. It has been suggested that NF1 may play a

role in colorectal carcinogenesis when mutated, because it can no longer inhibit RAS signaling effectively. Indeed some studies have found increased *NF1* mutations in malignant colorectal tissue<sup>[18,19]</sup>, and in concurrence with *KRAS* mutations as well. Having said that, *NF1* mutations may also occur with wild type *KRAS*. In addition, one study found that the majority of *NF1* mutations were actually concurrent with *BRAF* mutations, especially in MSI-H tumours<sup>[18]</sup>. Paradoxically, a more recent *in vitro* study observed that the mitogen-activated protein kinase (MAPK) pathway signaling was upregulated in malignant colorectal cells with wild type *BRAF* and a knocked out *NF1* gene<sup>[20]</sup>. Therefore, the role of NF1 in human colorectal carcinogenesis remains largely controversial.

The inverse relationship between CRC and neurofibromatosis type 1 is also evident; rarely, children with homozygous deficiency of the *MLH1* gene, which leads to HNPCC, exhibit features of neurofibromatosis type 1<sup>[21]</sup>.

### RASSF mutations

The RASSF is a family of ten genes (*RASSF1-10*), members of which seem to act as tumour suppressors. An emerging body of evidence indicates that they can stimulate growth arrest and proapoptotic signals mediated by RAS. The exact way they achieve this is still unclear; there are suggestions that there is a domain in RAS which *RASSF* can bind, and indeed this holds true to date for *RASSF1*, *RASSF2*, *RASSF4* and *RASSF5*. *RASSF1A* is one of the most well studied members of the family<sup>[22]</sup>, and it has been said that it either binds farnesylated *KRAS* directly<sup>[18,23]</sup> or it has to form a heterodimer with *RASSF5* before is able to bind RAS<sup>[18,24]</sup>.

Silencing of *RASSF1A* may occur when a specific sequence on the gene, the CpG island promoter region [*i.e.*, a region rich in cytosine (C) and guanine (G) nucleobases linked by phosphodiester (p) bonds] is methylated. This event is regarded to be a major contributor to early CRC development, ranging from 12% to 81% amongst different studies. Methylation can also affect *RASSF2* and *RASSF5* in the context of CRC. Nevertheless, exactly how they bring about malignancy is currently under investigation<sup>[22]</sup>.

## HOW IMPORTANT RAS SIGNALING IS FOR CRC

In order to evaluate the importance of RAS signaling, it is reasonable to examine how RAS and associated mutations behave in the clinical setting; whether they respond to current treatments, and how good the prognosis is when such mutations are evident.

### Issues with KRAS

Screening for *KRAS* mutation is the only widely used and accepted prognostic tool to decide eligibility for monoclonal antibody therapy<sup>[25,26]</sup>. This is largely because, the only molecular treatment currently licensed to be used in clinical practice is anti-EGFR therapy<sup>[26]</sup>. Hence, the



identification of a *KRAS* mutation is used as a means of exclusion from anti-EGFR therapy. The rationale for this is that since the MAPK pathway signaling is upregulated by a constitutively active *KRAS* protein, it is worthless to try and block the EGFR since MAPK signaling is no longer dependent on the activation of the receptor<sup>[25]</sup>.

However, the above notion, though probably true most of the times, is not universally accepted. Although mutant *KRAS*, especially the G12V mutation, is often associated with poor response to anti-EGFR agents<sup>[26]</sup>, some studies have not found the same results. Several clinical studies observed that patients with a p.G13D (codon 13) mutation in *KRAS* actually responded to treatment with cetuximab, as they had increased progression-free and overall survival compared to those on best supportive care or chemotherapy alone. This is a conclusion not to be ignored, because p.G13D positive patients are often refused administration of cetuximab (based on the rationale described above), albeit they could potentially benefit from it. Hence, further prospective clinical trials should be performed to confirm these data, since the value of the *KRAS* p.G13D mutation as a negative predictive biomarker is still contradictory<sup>[27]</sup>.

In addition, regarding metastatic CRC, there is also a question of whether the primary or the metastatic lesion should be analysed for genetic mutations. Some researchers postulate that there is no difference between the two, whereas others report significant variations. It is also argued that the genetic profile of the metastatic lesion is what matters most, because it is the metastasis that causes the bulk of the morbidity and mortality related to the disease. These hypotheses may again have ethical implications. In a hypothetical scenario, a patient has a genetic variation between his primary and metastatic lesions; the majority of his metastatic cells carry the wild type *KRAS* gene, but the primary lesion has mutant alleles. Based on the latter, he is wrongfully denied potentially beneficial anti-EGFR therapy, if indeed the metastasis is what's causing the major problem<sup>[26]</sup>.

Finally, there is uncertainty regarding how many and which *KRAS* codons should be screened, as well as issues with cost-effectiveness<sup>[26]</sup>.

### Clinical status of other mutations

There are a great number of cases with wild type *KRAS* tumours which fail to respond to anti-EGFR therapy. This of course might happen because there are other mutations disrupting the MAPK pathway. These may include mutations in other regions of the *KRAS* gene which are not commonly tested. Indeed, the majority of clinical trials regarding *KRAS* mutations in CRC involved screening codons 12 and 13 only, whereas there are reported mutations in exons 3 and 4 as well<sup>[26]</sup>. *NRAS* also becomes mutated occasionally.

*BRAF* mutation is associated with very poor prognosis as it does not respond to anti-EGFR therapy. In fact, in a study performed by Di Nicolantonio *et al.*<sup>[28]</sup>, it was observed that none of the patients with a V600E

*BRAF* mutation responded to either cetuximab or panitumumab. *BRAF* mutations are also mutually exclusive with *KRAS* mutations, *i.e.*, these two do not occur together<sup>[18,25-28]</sup>. This means that if mutant *KRAS* is identified in a patient, there is no point of screening for *BRAF* as well. It is rather more useful to screen for *KRAS* first, since some particular mutations, as already discussed, may validate the use of anti-EGFR therapy. This is not the case for *BRAF*, where there is no response to monoclonal antibodies whatsoever. Hence, as a prognostic biomarker, *BRAF* can only be used to indicate complete insensitivity to anti-EGFR agents.

Contrary to *BRAF*, *PIK3CA* and *PTEN* are not mutually exclusive to *KRAS*<sup>[29]</sup>. Loss of function of *PTEN* or mutation in *PIK3CA* is often associated with poorer response to cetuximab or panitumumab, as expected. However, there is no standard, reliable scoring system by which *PTEN* loss can be detected, thus making it an unsuitable prognostic biomarker. At the same time, the data regarding *PIK3CA* mutations is not uniform, as some studies report no overall difference in 5-year survival for patients with *PIK3CA* mutation, whilst others report positive response to cetuximab<sup>[29]</sup>.

### The future

Most patients responding well to current treatments are essentially those who only have EGFR upregulation. All the other mutations necessitate the discovery of agents that can block RAS signaling further down the pathway. Such a discovery will render any specific gene alteration irrelevant. Indeed, there has been a development of a *RAF* inhibitor, called PLX4032, which showed inhibition of *RAF* in melanoma, but had little success with CRC cells<sup>[30]</sup>. Similarly, AZD6244, a *MEK* inhibitor which recently entered phase 2 trials for CRC showed no significant advantage over chemotherapy<sup>[26]</sup>.

## CONCLUSION

It has recently been said that the MAPK and PI3K cascades are important to carcinogenesis and progression of CRC<sup>[25]</sup>. Apart from *KRAS* mutations, which are the main reason why anti-EGFR agents fail, several other genes related to RAS signaling also contribute to CRC manifestation and, some more than others, to anti-EGFR therapy insensitivity. If nothing else, *KRAS* and *BRAF* mutations can be used as negative biomarkers to identify patients who will not benefit from cetuximab and panitumumab. Nevertheless, as mentioned earlier, we do not know everything, as there are still several controversies in the data regarding the clinical status of some mutations, as in the case of p.G13D *KRAS*, *PIK3CA* and others. These indicate that there is a need to perform larger clinical trials that will minimise statistical error and will find out what exactly happens in these cases. In doing so, perhaps a deeper insight will be gained into the molecular mechanisms giving rise to CRC, thus allowing for pioneering pharmacological agents to be successfully developed.

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# World Journal of *Gastrointestinal Oncology*

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**REVIEW**

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*Rammohan A, Sathyanesan J, Rajendran K, Pitchaimuthu A, Perumal SK,  
Srinivasan UP, Ramasamy R, Palaniappan R, Govindan M*



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**APPENDIX** I-V Instructions to authors

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## A gist of gastrointestinal stromal tumors: A review

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enchymal tumors. Both traditional and minimally invasive surgery are used to remove these tumors with minimal morbidity and excellent perioperative outcomes. The revolutionary use of specific, molecularly-targeted therapies, such as imatinib mesylate, reduces the frequency of disease recurrence when used as an adjuvant following complete resection. Neoadjuvant treatment with these agents appears to stabilize disease in the majority of patients and may reduce the extent of surgical resection required for subsequent complete tumor removal. The important interplay between the molecular genetics of GIST and responses to targeted therapeutics serves as a model for the study of targeted therapies in other solid tumors. This review summarizes our current knowledge and recent advances regarding the histogenesis, pathology, molecular biology, the basis for the novel targeted cancer therapy and current evidence based management of these unique tumors.

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**Key words:** Gastrointestinal stromal tumors; c-KIT; Imatinib mesylate; Surgery; Review

### Abstract

Gastrointestinal stromal tumors (GISTs) have been recognized as a biologically distinctive tumor type, different from smooth muscle and neural tumors of the gastrointestinal tract (GIT). They constitute the majority of gastrointestinal mesenchymal tumors of the GIT and are known to be refractory to conventional chemotherapy or radiation. They are defined and diagnosed by the expression of a proto-oncogene protein detected by immunohistochemistry which serves as a crucial diagnostic and therapeutic target. The identification of these mutations has resulted in a better understanding of their oncogenic mechanisms. The remarkable antitumor effects of the molecular inhibitor imatinib have necessitated accurate diagnosis of GIST and their distinction from other gastrointestinal mes-

**Core tip:** Gastrointestinal stromal tumors have been recognized as a biologically distinctive tumor type, different from smooth muscle and neural tumors of the gastrointestinal tract. This review summarizes our current knowledge and recent advances regarding the histogenesis, pathology, molecular biology, the basis for the novel targeted cancer therapy and current evidence based management of these unique tumors.

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## INTRODUCTION

Gastrointestinal stromal tumors (GISTs) are uncommon mesenchymal tumors that arise predominantly in the gastrointestinal tract (GIT). In the past, there has been considerable debate regarding its nomenclature, cellular origin, diagnosis and prognosis<sup>[1-3]</sup>. Due to their similar appearance by light microscopy, GISTs were previously thought to be smooth muscle neoplasms and most were classified as leiomyomas, leiomyoblastomas, leiomyosarcomas or schwannomas<sup>[3]</sup>. It was in 1998, after the discovery of gain-of-function mutations in the c-KIT proto-oncogene that these tumors were reliably distinguished from other histopathological subtypes of mesenchymal tumors<sup>[1,4]</sup>. This review attempts to provide an overview of the histogenesis, molecular pathogenesis, clinical picture, investigations, surgical and non surgical management of GIST specific to the GIT.

## EPIDEMIOLOGY

GISTs represent the most common mesenchymal neoplasms of the GIT. With an annual incidence of 11-14 per 10<sup>6</sup>, they form 0.1%-3.0% of gastrointestinal malignant tumors<sup>[5,6]</sup>. The median age at diagnosis is 60 years. There is usually no predilection for either gender but some series suggest a slight male predominance. GIST occurring in the familial form is autosomal dominant<sup>[5-7]</sup>. 5% of GISTs occur in patients with neurofibromatosis type 1 syndrome, occurring mostly in the small intestine and without KIT mutations. GIST also occurs as a part of Carney triad along with paraganglioma and pulmonary chordoma in young females<sup>[6-9]</sup>.

## HISTORY

Stromal tumors were referred to as smooth muscle neoplasms of GIT but immunohistochemistry (IHC) demonstrated that these tumors lacked features of smooth muscle differentiation and, while some had markers of neuronal differentiation, some had neither<sup>[1-3,7,8]</sup>. Mazur *et al.*<sup>[3]</sup> coined the term "gastrointestinal stromal tumors" to collectively refer to a group of mesenchymal tumors of neurogenic or myogenic differentiation which lacked the immunohistochemical features of Schwann cells and did not have the ultrastructural characteristics of smooth muscle cells.

## DISCOVERY OF KIT

In 1986, a new acute transforming feline retrovirus, the Hardy-Zuckerman 4 feline sarcoma virus (HZ4-FeSV), was isolated from feline fibrosarcoma. The viral genome of HZ4-FeSV contained a new oncogene that was designated v-KIT, which encoded a transmembrane tyrosine kinase receptor called KIT. c-KIT is the cellular homologue of the oncogene v-KIT<sup>[10]</sup>. Huizinga *et al.*<sup>[11]</sup> showed that mice with mutations in the *KIT* gene lacked the network of interstitial cells of Cajal associated with Auerbach's nerve plexus and intestinal pacemaker

activity and hence it was shown that the interstitial cells of Cajal express the KIT receptor. Hirota *et al.*<sup>[4]</sup> were investigating the mutational status of c-KIT in mesenchymal tumors of the GIT and reported that GISTs contained activated c-KIT mutations, which play a central role in its pathogenesis, and that mutations of c-KIT resulted in gain of function of the enzymatic activity of the KIT tyrosine kinase.

## MOLECULAR PATHOGENESIS

### What is KIT?

KIT is a 145-kDa glycoprotein. The KIT receptor can be detected by immunohistochemical staining for CD117, which is the epitope on the extra-cellular domain of the KIT receptor. Steel factor (SLF) AKA stem-cell factor is a ligand for KIT. On binding of SLF to KIT, KIT undergoes receptor homo-dimerization, which leads to activation of KIT tyrosine kinase activity, effecting intracellular signal transduction<sup>[4,7,8]</sup>. Membrane receptor tyrosine kinase cellular signaling pathways regulate key cell functions, including proliferation, differentiation and anti-apoptotic signaling. Auto-phosphorylation of c-KIT causes ligand-independent tyrosine kinase activity, leading to an uncontrolled cell proliferation due stimulation of downstream signaling pathways. An unregulated activation can lead to various forms of cancer/benign proliferative conditions. SLF-KIT interaction is essential for development of melanocytes, erythrocytes, germ cells, mast cells and ICCs. Hence, mutations involving c-KIT produce cellular defects in hematopoiesis, melanogenesis, gametogenesis and in the interstitial cells of Cajal. Mutations of different exons of the *c-KIT* gene (exon 11, exons 9 and exon 13) cause constitutive activation of the tyrosine kinase function of c-KIT<sup>[4-9,12]</sup>.

GISTs can develop anywhere along the GI tract from the esophagus to the rectum; however, stomach (60%) and small intestine (30%) are the most common locations for GIST. Only 10% of GISTs are found in the esophagus, mesentery, omentum, colon or rectum. Up to 30% of GISTs exhibit high-risk (malignant) behavior such as metastasis and infiltration<sup>[8,9,13,14]</sup>. The metastatic pattern is predominantly intra-abdominal, with spread throughout the peritoneal cavity and to the liver. Lymph nodal invasion is uncommon. GISTs with indolent (low-risk) behavior are typically found as small submucosal lesions. True smooth muscle tumors/leiomyomas also occur throughout the GI tract but are now thought to be rare in comparison to GISTs, except in the esophagus where they are more common<sup>[6,7,9,13-15]</sup>.

## CLINICAL PRESENTATION

Only 70% of the patients with GIST are symptomatic. While 20% are asymptomatic and the tumors are detected incidentally, 10% of the lesions are detected only at autopsy. Symptoms and signs are not disease specific, they are related more to the site of the tumor<sup>[6,7,16]</sup>. Bleeding (30%-40%) comprises the most common symptom after

vague abdominal discomfort (60%-70%). Bleeding is attributed to the erosion into the GIT lumen. Bleeding occurring into the peritoneal cavity due to a ruptured GIST can lead to acute abdominal pain presenting as a surgical emergency. Bleeding into the GI tract lumen, causing hematemesis, melena or anemia, is usually more chronic on presentation. Most of the patients present with vague symptoms, such as nausea, vomiting, abdominal discomfort, weight loss or early satiety. Symptoms are usually site specific. These include dysphagia in the esophagus, biliary obstruction around the ampulla of Vater or even intussusception of the small bowel<sup>[6,7]</sup>. Lymph node metastases are uncommon in GIST. Distant metastases most commonly occur in GISTs of the peritoneum, omentum, mesentery and the liver. GISTs have a high tendency to seed and hence intraperitoneal or even scar metastases are known to occur<sup>[6,7,16]</sup>.

## **PATHOLOGY**

GIST vary greatly in size from a few millimeters to more than 30 cm, the median size being between 5 and 8 cm. Macroscopically, GIST usually has an exophytic growth and the common intra-operative appearance is that of a mass attached to the stomach, projecting into the abdominal cavity and displacing other organs<sup>[5,7,9,17]</sup>. Mucosal ulceration may be present at the summit of the lesion in 50% of cases. On gross appearance they are smooth gray and white tumors which are well circumscribed, usually with a pseudocapsule. A small area of hemorrhage or cystic degeneration and necrosis may be visible<sup>[7,18]</sup>. Gastric GISTs have a solid or nested form, often with a hyalinized stroma that shows myxoid change. GISTs in the small intestine are more often spindle than epithelioid and may show a paragangliomatous pattern. Another characteristic is the eosinophilic structures, composed of collagen, which are stained brightly with periodic acid-Schiff (PAS) stain<sup>[18,19]</sup>.

GISTs (> 95%) are positive for CD117. In 60%-70% of the patients, IHC for CD34 (mesenchymal/hematopoietic precursor cell marker) is also positive<sup>[7,8,13,15]</sup>. Vimentin and smooth muscle actin is positive in 15% to 60%. GISTs (10%-15%) have no detectable KIT or *PDGFR4* mutations [wild-type GIST (WT-GIST)]. Absence of mutations does not exclude the diagnosis of GIST<sup>[7,8,13,15,19]</sup>. DOG1 is a calcium dependent, receptor activated chloride channel protein expressed in GIST; this expression is independent of mutation type and can be used in the diagnosis of KIT-negative tumors<sup>[20,21]</sup>.

## **INITIAL EVALUATION AND WORKUP**

Due to the vague and protean presentation of GIST, initial diagnosis can be delayed. Imaging in the form of contrast enhanced computed tomography (CECT) is the modality of choice; it is used to characterize the lesion, evaluate its extent, and assess the presence or absence of metastasis at the initial staging workup. CECT is also used for monitoring response to therapy and performing follow-up surveillance of recurrence<sup>[15,18,20]</sup>. On CECT,

**Table 1 Response Evaluation Criteria in Solid Tumors**

Complete response	Disappearance of all target lesions
Partial response	At least a 30% decrease in the sum of the longest diameter of target lesions, taking as reference the baseline sum longest diameter
Progressive disease	At least a 20% increase in the sum of the longest diameter of target lesions, taking as reference the smallest sum longest diameter recorded since the treatment started or the appearance of one or more new lesions
Stable disease	Neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease, taking as reference the smallest sum longest diameter since the treatment started

Originated from [23], with permission.

GISTs appear as a large, well-defined soft tissue mass with heterogeneous enhancement. Tumors are usually of varying density and show patchy enhancement after intravenous contrast. Varying degrees of necrosis may frequently be demonstrated within the mass, more so in tumors responding to chemotherapy. Response to therapy is assessed using the Response Evaluation Criteria in Solid Tumors (RECIST) or the Choi criteria (Table 1). According to the Choi criteria, responsive tumors show a 10% decrease in tumor size and 15% decrease in tumor density on CECT. This criteria has been shown to be better than RECIST criteria in assessing the response of GIST to tyrosine kinase inhibitor (TKI) therapy<sup>[18,22,23]</sup>.

Endoscopic ultrasound (EUS) has been used in the diagnosis of GIST; it assesses the depth of invasion and is useful in obtaining a tissue sample. Preoperative percutaneous biopsy should not be used because of a significant risk of tumor rupture or dissemination<sup>[15,20]</sup>. Conventional endoscopic sampling techniques such as forceps biopsy are limited in their clinical utility given the difficulty of sampling lesions in a subepithelial location and the increased risk for perforation. The efficacy of EUS guided fine needle aspiration (EUS-FNA) has been pointed out in several studies and the reported accuracy is 80%-85%<sup>[18,24]</sup>. A clear role for EUS guided Trucut biopsy has yet to be defined, given the inconsistent results in providing adequate tissue yield. However, at present, EUS-FNA should be considered the procedure of choice to secure a tissue diagnosis of GIST<sup>[15,18,24]</sup>. EUS features of GIST which are predictive of an adequate tissue yield include a size of 10 cm, round/oval shape and location in a specific sonographic wall layer. EUS features of a high grade GIST include irregular extra-luminal borders, heterogeneous echo patterns, presence of cystic spaces and echogenic foci<sup>[25]</sup>.

GISTs are positron emission tomography (PET) avid tumors because the receptor tyrosine kinase increases the glucose transport protein signaling<sup>[20]</sup>. PET is useful in revealing small metastases which would otherwise not have been picked up on CECT<sup>[9]</sup>. It helps differentiate an active tumor from necrotic or inactive scar tissue. PET also differentiates malignant from benign tissue and recurrent



**Table 2 European Organization for Research and Treatment of Cancer metabolic response criteria for tumors evaluated with positron emission tomography**

Complete metabolic response	Complete resolution of [ <sup>18</sup> F]-FDG uptake within the tumor volume indistinguishable from surrounding normal tissue
Partial metabolic response	Reduction of a minimum of 15%-25% in tumor [ <sup>18</sup> F]-FDG SUV after one cycle of chemotherapy Reduction of a minimum of > 25% in tumor [ <sup>18</sup> F]-FDG SUV after more than one treatment cycle
Progressive metabolic disease	Increase in [ <sup>18</sup> F]-FDG tumor SUV > 25% within the tumor region, visible increase in the extent of [ <sup>18</sup> F]-FDG tumor uptake (> 20% in the longest dimension) Appearance of new [ <sup>18</sup> F]-FDG uptake.
Stable metabolic disease	Increase in tumor [ <sup>18</sup> F]-FDG SUV < 25%, decrease of < 15%. No visible increase in extent of [ <sup>18</sup> F]-FDG tumor uptake (< 20% in the longest dimension)

Originated from [26], with permission. PET: Positron emission tomography; [<sup>18</sup>F]-FDG: <sup>18</sup>F-fluoro-de-oxyglucose; SUV: Standardized uptake value.

tumor from nondescript benign changes. Changes in the metabolic activity of tumors precede anatomic changes on CECT; it is hence used to assess the response to TKI therapy. PET helps to clarify ambiguous findings seen on computerized tomography (CT) or magnetic resonance imaging and to assess complex metastatic disease in patients who are being considered for surgery. Routine use of PET for surveillance after resection is not yet recommended. The European Organization for Research and Treatment of Cancer metabolic response criteria is based on tumor evaluated with PET<sup>[9,20,22,26]</sup> (Table 2).

## PRINCIPLES OF BIOPSY AND PATHOLOGICAL ASSESSMENT

Routine preoperative biopsy is not mandatory but biopsy is necessary prior to the initiation of preoperative therapy with TKI. EUS-FNA biopsy of the primary site is preferred over percutaneous biopsy as it reduces the risk of tumor hemorrhage and intra-abdominal tumor dissemination<sup>[24,25,27,28]</sup>. Percutaneous image guided biopsy can be used while confirming the presence of metastatic disease. While assessing a specimen, a pathology report should include the anatomic location, size and mitotic rate measured in the most proliferative area of the tumor and reported as the number of mitoses in 50 high power fields (equivalent to 5 mm<sup>2</sup> of tissue). The specimen should be subjected to IHC for KIT and molecular genetic testing to identify mutations in the *KIT* or *PDGFR4* genes<sup>[8,20,27]</sup>.

## MANAGEMENT OF GIST

### Small GIST

Tumors which are less than 2 cm in the widest dimension

are defined as small GIST. They are usually discovered incidentally on endoscopy<sup>[29]</sup>. If these lesions are symptomatic, complete surgical resection is recommended. Small asymptomatic gastric GISTs (less than 2 cm) with no high-risk EUS features can be managed conservatively with endoscopic surveillance at 6 to 12 mo intervals<sup>[27-29]</sup>. Endoscopic resection of these small tumors would be another option. With the recent advent of endoscopic resection techniques, endoscopists can now remove mucosal or submucosal tumors by endoscopic mucosal resection (EMR). Complete resection of subepithelial tumors larger than 2 cm in size and those originating from the muscularis propria layer still remain difficult by EMR<sup>[30-32]</sup>. A study performed in elderly and high risk surgical patients showed that EUS guided band ligation of small duodenal tumors is a safe and efficient therapeutic method<sup>[33]</sup>.

## PRINCIPLES OF SURGERY

Surgery is the primary treatment of choice in localized or potentially resectable GIST. It is imperative to avoid tumor rupture. The tumors are fragile and should be handled with care, with an aim to achieve complete gross resection of the tumor with an intact pseudocapsule. Multivisceral and radical surgery should be avoided where possible. Segmental or wedge resection with an aim to obtain histologically negative margins is sufficient. Resection should be accomplished with minimal morbidity. Resection is not indicated for patients with an R1 resection. Lymphadenectomy is not required as GISTs have a low incidence of nodal metastases<sup>[15,18,29]</sup>.

## ROLE OF LAPAROSCOPY

Although prospective trials are lacking, small series and retrospective analyses have shown low recurrence rates, shorter hospital stay and low morbidity with a laparoscopic approach<sup>[9,15,18,29]</sup>. It has been recommended for selected GISTs present in favorable anatomic locations like the anterior wall of the stomach, jejunum and ileum. The same surgical principles as open surgery are applicable in laparoscopic surgery for GIST. The specimen is removed from the abdomen in a plastic bag to avoid spillage or seeding of port sites. Endoscopic resection of small GISTs is more controversial due to the risks of positive margins, tumor spillage and intact specimen retrieval<sup>[9,15,18,29]</sup>. During laparoscopic partial gastrectomy for GIST of the stomach, it is important to avoid an excessive surgical resection of the gastric wall as this can cause a deformity of the stomach<sup>[34-36]</sup>. Laparoscopic and endoscopic cooperative surgery (LECS) is a procedure which enables tumor resection with minimal surgical margin<sup>[35-38]</sup>. The LECS procedure involves seromuscular resection by laparoscopy with endoscopic dissection for the mucosal to submucosal layers, making it possible to standardize gastric submucosal tumor resection independent of tumor location, such as in the vicinity of the esophagogastric junction or pyloric ring<sup>[34-38]</sup>.

## IMATINIB MESYLATE

Imatinib mesylate is a tyrosine kinase inhibitor with activity against ABL, BCR-ABL, KIT, PDGFRA, PDGFRB and CSF1R. Its structure mimics adenosine triphosphate (ATP) and it binds competitively to the ATP binding site of the target kinases. This prevents substrate phosphorylation and signaling, thereby inhibiting proliferation and survival<sup>[9,15,18,27]</sup>. Patients with advanced GIST started on imatinib have shown a 35%-49% 9 year survival. The presence and the type of *KIT* or *PDGFRA* mutation status are predictive of response to imatinib. Exon 11 mutations occur in the *KIT* juxtamembrane domain and are the most common mutations in GISTs. Tumors with exon 11 mutations have better response rates to imatinib, with a longer progression free survival (PFS) and overall survival (OS). Exon 9 mutations occur in the *KIT* extracellular domain; these mutations are specific for intestinal GIST. Exon 9 mutations are associated with a decreased response to imatinib and a poorer PFS. *PDGFRA* mutations are common in gastric GIST. Mutations in *PDGFRA* affect exon 18 in the tyrosine kinase domain<sup>[9,15,18,27,39-44]</sup>. There have been multiple trials testing the most appropriate dosing of imatinib. 400 mg/d has been found to have equivalent response rates and OS compared to higher doses, which are associated with more side effects. Indications for a higher dosing (800 mg/d) include patients with an exon 9 *KIT* mutation or those with tumors which continue to progress on the standard 400 mg/d dosage<sup>[41-45]</sup>.

## NEOADJUVANT IMATINIB - RESECTABLE DISEASE

Surgery is the primary treatment for all tumors which can be resected without significant morbidity. If this is not the case, then preoperative imatinib should be considered. Imatinib is effective in reducing the size of the tumor prior to resection, increasing the likelihood of negative margins without significant morbidity<sup>[27,29,46]</sup>. Before starting a patient on neoadjuvant imatinib, a baseline CECT is recommended. The optimal duration of preoperative therapy is yet unknown. In patients responding to therapy, imatinib is continued until maximal response (defined as no further improvement between 2 successive CT scans). This can be as long as 6-12 mo but it is not always necessary to wait for a maximal response prior to surgery. Surgery is recommended when the tumor appears to have downsized to a point where complete resection can be achieved without significant morbidity<sup>[9,18,27,29,46-49]</sup>. Imatinib should be stopped just before surgery and resumed as soon as the patient is able to tolerate oral medications, regardless of the surgical margins. The recommended dose is 400 mg/d, with dose escalation to 800 mg/d advised in cases of documented mutations in *KIT* exon 9<sup>[29,44,46-50]</sup>. In cases where there is no progression, continuation of the same dose of imatinib is recommended and resection is considered. If there is tumor progression, as confirmed with CECT scan, surgery is recommended after discontinuing imatinib<sup>[29,44,46-49]</sup>.

**Table 3 Risk stratification of gastrointestinal stromal tumors**

Mitotic rate	Tumor size (cm)	Stomach	Jejunum/ Ileum	Duodenum	Rectum
≤ 5/50 HPF	≤ 2	None	None	None	None
	> 2, ≤ 5	Very low	Low	Low	Low
	> 5, ≤ 10	Low	Moderate	High	High
> 5/50 HPF	> 10	Moderate	High		
	≤ 2	None	High	NA	High
	> 2, ≤ 5	Moderate	High	High	High
	> 5, ≤ 10	High	High	High	High
	> 10	High	High		

Originated from [54], with permission. HPF: High-power fields; NA: Not available.

## ADJUVANT THERAPY

Although surgery is the therapeutic modality of choice, it does not routinely cure GIST. Complete resection is possible in approximately 85% of patients and 50% patients will develop recurrence or metastasis following complete resection<sup>[9,18,25,27,51]</sup>. The 5-year survival rate is approximately 50%, while the median time to recurrence after resection of primary high-risk GIST is 2 years. Adjuvant imatinib has been shown to improve PFS and OS in postsurgical patients. In patients who have not received preoperative imatinib and have undergone complete resection, imatinib has been found to be beneficial if continued for 36 mo, especially in patients with an intermediate or high risk of recurrence. Estimation of this risk is based on the tumor size, site, mitotic count and tumor rupture (Table 3). A survival benefit is seen in patients with a high risk of recurrence (mitotic count > 5/50 HPF, size > 5 cm, non-gastric location and tumor rupture)<sup>[27,29,35,40,51-55]</sup>. In those patients who had received preoperative imatinib and undergone a complete resection, continuation of imatinib at the same dose for 2 years following surgery is recommended. In patients with a positive resection margin, imatinib is continued/started regardless of surgical margins until disease progression is noted<sup>[27,29,50]</sup>.

## UNRESECTABLE, METASTATIC OR RECURRENT DISEASE

Imatinib has a very high likelihood of clinical benefit and a positive response in patients with documented unresectable GIST. Imatinib is indicated when primary resection would carry the risk of severe postoperative functional deficit<sup>[51]</sup>. It is also indicated in those who have a widespread metastatic disease or a recurrence after resection. There is a survival benefit of cytoreductive surgery following preoperative imatinib in patients responding to preoperative imatinib<sup>[51,55-62]</sup>. The lesion is assessed within 3 mo of initiating therapy to determine if it has become resectable. In cases where the tumor remains unresectable, imatinib is continued indefinitely until there is evi-

dence of tumor progression. Continuation of TKI therapy life-long for palliation of symptoms forms an essential component of best supportive care<sup>[9,18,27,29,51,56-62]</sup>. Options for patients with progressive disease or with widespread systemic disease and good performance status (0-2) include continuation of imatinib at the same dose, dose escalation up to 800 mg in the absence of severe adverse drug reactions or switching to sunitinib<sup>[29,44-46,51,53,55]</sup>.

## TOXICITY OF IMATINIB

The more common side effects include fluid retention, diarrhea, nausea, fatigue, muscle cramps, abdominal pain and rash. The adverse-effect profile improves with prolonged therapy. The more serious side effects include liver function abnormalities, lung toxicity, low blood counts and GI bleeding<sup>[29,44-47]</sup>. Congestive heart failure has been noted in 8.2% of patients, manageable with medical therapy. Arrhythmias and acute coronary syndromes have also been reported<sup>[63]</sup>. All the toxicities abate if imatinib is withheld. Sunitinib should be considered, after discontinuing imatinib<sup>[29,44-47]</sup>.

## RESISTANCE TO IMATINIB

Non achievement of stable disease or progression of disease within 6 mo of an initial clinical response (KIT exon 9 mutation or no detectable kinase mutation – wild-type tumors, PDGFRA exon 18) is defined as primary resistance, occurs in 10%-20% patients and relates to the mutational profile of the tumor. The majority of wild-type GISTs [pediatric GISTs (Carney Triad), NF1 GISTs, adult WT-GISTs] show primary resistance<sup>[29,52]</sup>. When there is disease progression after more than 6 mo of clinical response (new acquired kinase mutation in KIT or PDGFR that interferes with imatinib activity, secondary mutations in KIT exon 11), it is termed as secondary resistance. This has been attributed to genomic amplification and overexpression of KIT/PDGFR without new point mutations and to loss of KIT expression, accompanied by activation of an alternative tyrosine kinase or other oncogenes. Secondary resistance is also related to the acquisition of new kinase mutations<sup>[29,44,52,64,65]</sup>. Dose escalation of imatinib is the first step in overcoming drug resistance. If there is continued resistance, the use of other kinase inhibitors (sunitinib) is recommended<sup>[29,44,52,64,65]</sup>.

## SUNITINIB MALATE

Sunitinib malate is an orally administered multi-targeted receptor tyrosine kinase inhibitor which has shown significant and sustained clinical benefit in patients with imatinib-resistant or imatinib-intolerant GIST. Sunitinib has been associated with a significant improvement in median time to progression (27.3 wk *vs* 6.4 wk) and significantly greater estimated OS<sup>[66,67]</sup>. The clinical activity of sunitinib in imatinib-resistant GISTs is significantly influenced by both primary and secondary mutations in the KIT kinase domain. Sunitinib induces higher re-

sponse rates in patients with primary KIT exon 9 mutations than in those with KIT exon 11 mutations (58% *vs* 34% respectively)<sup>[27,29,66-72]</sup>. The recommended dosage of sunitinib is 50 mg orally once daily on a schedule of 4 wk on treatment followed by 2 wk off. Common adverse effects which are also dose-limiting include fatigue, nausea and vomiting. Other toxicities include hematological toxicities (anemia, neutropenia), diarrhea, abdominal pain, mucositis, anorexia and skin discoloration. Patients on sunitinib have a significant risk of developing hand-foot skin reaction, the incidence of which can be reduced by routine application of emollient lotions<sup>[27,29,68]</sup>. Hypertension is common because sunitinib targets the vascular endothelial growth factor receptor (VEGFR). Other significant toxicities involve cardiotoxicity and hypothyroidism. Close monitoring of blood pressure and left ventricular ejection fraction is essential, especially in patients with a history of heart disease or cardiac risk<sup>[72]</sup>. Routine monitoring (every 3-6 mo) of thyroid stimulating hormone levels is indicated. All of sunitinib-related toxicities can be managed with dose interruptions or reductions<sup>[68,69,72,73]</sup>.

Second-generation TKIs like sorafenib, nilotinib, dasatinib and regorafenib have shown activity in patients resistant to imatinib and sunitinib<sup>[75-88]</sup>. Results with regorafenib are most encouraging. Regorafenib is a multikinase inhibitor with activity against KIT, PDGFR and VEGFR and is well tolerated, with common adverse effects being hypertension (23%), hand-foot skin reaction (20%) and diarrhea (5%)<sup>[29,75,76]</sup>.

## PERITONEAL AND LIVER METASTASES

Patients who are medically fit with surgically accessible focally progressive disease should be considered for resection. The rationale behind this approach is the elimination of drug-resistant clones that will allow ongoing therapy with imatinib<sup>[89-94]</sup>. Debulking in the form of removal of the gross tumor followed by intraperitoneal chemotherapy with cisplatin and doxorubicin or mitoxantrone have been attempted; the median time to recurrence was increased from 8 to 21 mo with the addition of intraperitoneal chemotherapy<sup>[94-97]</sup>. Surgery in metastatic patients is a case based decision. Residual tumor resection is safe but multifocal resection is not recommended without considering the patient's performance status and personal situation<sup>[29,89-91]</sup>. When surgery may not be possible, limited evidence exists that similar benefits could be obtained with nonsurgical ablative techniques such as radiofrequency ablation or embolization<sup>[98-100]</sup>. In carefully selected patients with GIST liver metastases, radiofrequency ablation has been shown to be a safe and useful therapeutic option<sup>[100]</sup>. Liver transplantation for patients with metastatic GIST has been attempted with guarded results. Serralta *et al*<sup>[101]</sup> performed a transplant in three patients for tumors which on histopathology turned out to be GIST; all their patients had a recurrence after a median period of 3 years and survival was extended by starting them on imatinib.



## SURVEILLANCE

GISTs have unpredictable behavior and long term follow up is essential for all patients, independent of their benign or malignant characteristics. As the majority of GISTs tend to recur within the first 3-5 years, intense follow up is required during this period<sup>[18,27,29]</sup>. It is recommended both for persistent gross residual disease and for completely resected disease. Clinical examination with abdominopelvic CECT scan every 3-6 mo is the recommended surveillance protocol<sup>[18,29]</sup>.

## CONCLUSION

GISTs are the most common mesenchymal tumors of the GI system. Improved knowledge of the oncogenic drivers and resistance mechanism operant in GIST has acted as a foundation for the general understanding of the role of targeted therapies in human cancers. Surgery is the primary treatment of choice in localized or potentially resectable GIST. Surgery and imatinib form the first-line therapy and their effectiveness for the majority of patients has been revolutionary. Sunitinib is an approved second-line agent which is effective in many non-responders to imatinib therapy. Personalizing the treatment of GISTs and tailoring treatments to tumor genotype using combination therapies in order to prevent emergence of resistance is essential to optimize patient outcomes.

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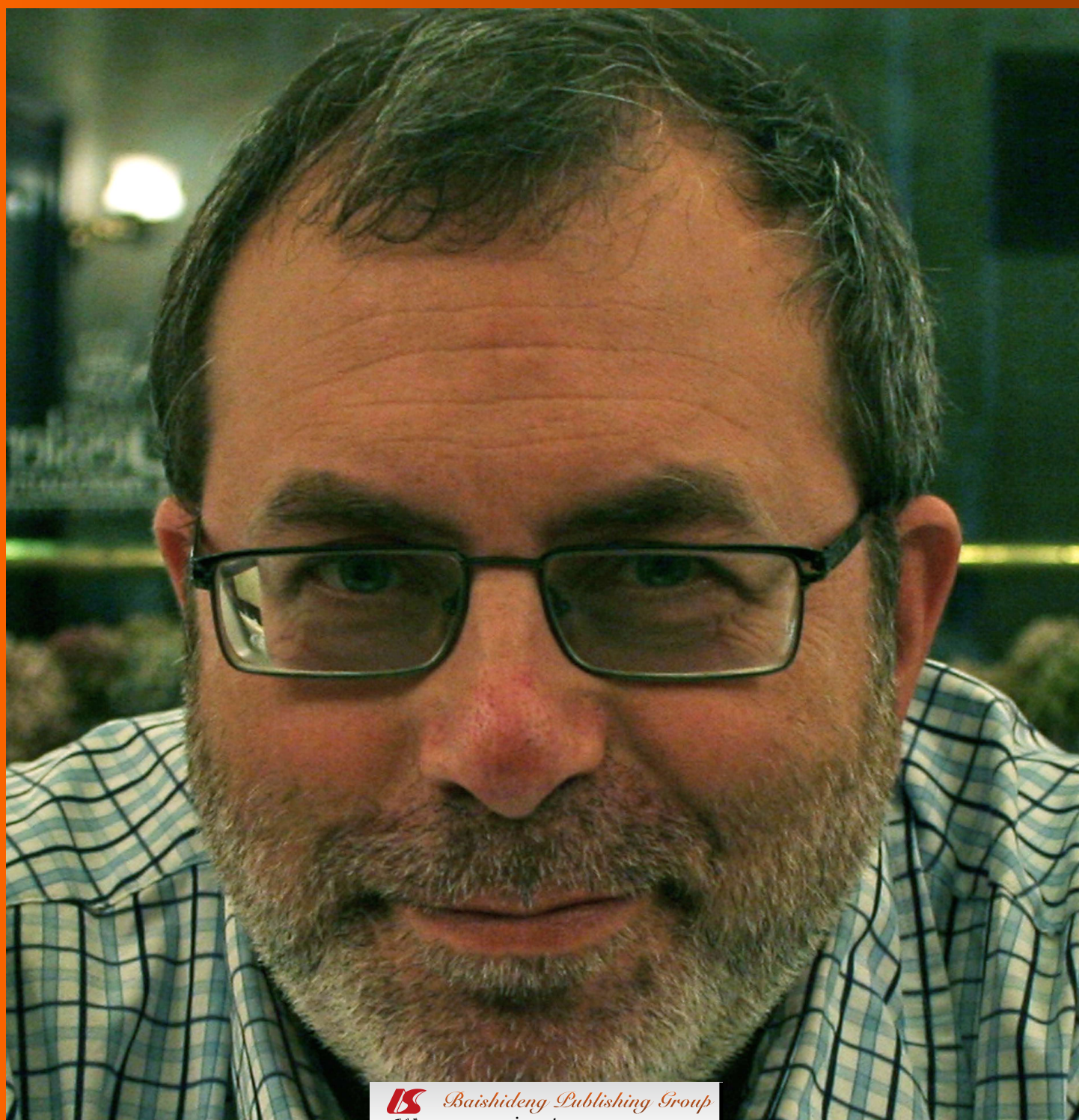
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## Hilar cholangiocarcinoma

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### Abstract

Hilar cholangiocarcinoma (HC) is a rare tumor. It accounts for 2/3 of the tumors of the biliary tract. Untreated, prognosis is very poor. Surgery is the only therapy that offers the possibility of cure but is technically very complex. With recent improvements in the therapeutic strategies applied by multidisciplinary teams, survival rates in the different series currently range from 25% to 45%. A group of experts devoted to HC (pathologists, gastroenterologists, radiologists, surgeons and oncologists) have reviewed and updated every open question in HC in a special issue.

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**Key words:** Cholangiocarcinoma; Hilar; Perihilar; Klatskin; Surgery; Cancer; Review

**Core tip:** Most remarked avances are: imaging methods have improved diagnostic sensitivity and specificity, especially for determining biliary and vascular involvement; there have been several proposals to improve the classic Bismuth-Corlette classification; pre- and post-operative care; technical aspects trying to obtain a R0 resection: widespread use of liver resection, resection of segment I and venous and arterial resection, refinement of post-operative histology and adjuvant therapies.

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### EDITORIAL

Hilar cholangiocarcinoma (HC) is a rare tumor (0.74-1.05 ×100000 inhabitants), representing 3% of gastrointestinal cancers<sup>[1,2]</sup>. It accounts for 2/3 of the tumors of the biliary tract<sup>[1]</sup>. Although HC is frequently discussed at surgical scientific meetings and arouses great interest, it has far fewer citations than other HPB cancers and several questions regarding the condition remain unanswered in the medical literature.

Untreated, the prognosis of HC is very poor. Surgery is the only therapy that offers the possibility of cure but is technically very complex<sup>[1,3]</sup>. A few years ago, the number of patients operated and resected was very low, and 5-year survival was mediocre. With recent improvements in the therapeutic strategies applied by multidisciplinary teams comprising radiologists, gastroenterologists, surgeons and oncologists, survival rates in the different series currently range from 25% to 45%<sup>[3,4]</sup>.

A group of experts devoted to HC (pathologists, gastroenterologists, radiologists, surgeons and oncologists) have reviewed and updated every open question in HC in this special issue focusing in the following areas of the management and treatment of patients with HC:

Prof. Valls *et al*<sup>[5]</sup> has updated diagnostic methods in HC: Imaging methods have improved diagnostic sensitivity and specificity, especially for determining biliary and vascular involvement. These methods include multidetector computed tomography, Cholangio-magnetic resonance, percutaneous transhepatic cholangioscopy, positron emission tomography computed tomography and staging by laparoscopy reviewed by Prof. Rotellar *et al*<sup>[6]</sup>.

Prof. Suarez-Munoz *et al*<sup>[7]</sup> have updated the classification/staging systems in HC: There have been several pro-



posals to improve the classic Bismuth-Corlette classification, among them the Memorial Sloan-Kettering Center system, the 7<sup>th</sup> edition of TNM, and the most complete system so far devised by de Oliveira *et al*<sup>[2]</sup>. Correct preoperative staging is essential to identify the patients who should be operated.

Prof. Ramos<sup>[8]</sup> has devoted his paper to perioperative care and technical aspects: Several pre-operative measures have been used to minimize risks and improve results: uni- or bi-lobar pre-operative biliary drainage, the use of probiotics, autologous blood, replacement of drained bile, portal embolization, enteral nutrition, and so on<sup>[3]</sup>. The widespread use of liver resection (above 80% in the most recent series), resection of segment I, venous resection (performed in 6%-43% of patients), arterial resection, and the non-touch technique have improved survival for many reasons, but above all because they allow an increase in R0 resections.

Prof. Serrablo *et al*<sup>[9]</sup> have resumed surgical outcomes in HC: All these technical improvements have been achieved without significantly increasing morbidity and mortality<sup>[11-3]</sup>; mortality in HC surgery currently ranges between 0% and 11.9%<sup>[3,5]</sup>.

Prof. Castellano-Megías *et al*<sup>[10]</sup> have reviewed the importance of an adequate and minucius pathologic study of surgical specimens in HC.

Dra. Ramírez-Merino *et al*<sup>[11]</sup> have updated the role of adjuvant treatment in HC: There have been advances in chemotherapy used, brachytherapy, photodynamic therapy, and so on.

All the authors hope that this special issue answered every question about HC that readers need to be answered.

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José M Ramia, MD, PhD, Series Editor

## Radiological diagnosis and staging of hilar cholangiocarcinoma

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**Author contributions:** All the authors contributed to the conception, design and interpretation of data and participated in drafting the manuscript, revised it critically and approved the final version; the detailed writing process and analysis of the data was performed by Valls C.

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**Key words:** Cholangiocarcinoma; Radiological staging; Magnetic resonance imaging; Multidetector computed tomography; Hepatic resection

**Core tip:** Hilar cholangiocarcinoma is a rare malignant tumor arising from the epithelium of the bile ducts. Surgery is still the only chance of potentially curative treatment in patients with perihilar cholangiocarcinoma. Multidetector computed tomography, magnetic resonance imaging and magnetic resonance cholangiography are useful tools, both to diagnose and stage hilar cholangiocarcinoma.

### Abstract

Hilar cholangiocarcinoma is a rare malignant tumor arising from the epithelium of the bile ducts. Surgery is still the only chance of potentially curative treatment in patients with perihilar cholangiocarcinoma. However, radical resection requires aggressive surgical strategies that should be tailored optimally according to the location, size and vascular invasion of the tumors. Accurate diagnosis and staging of these tumors is therefore critical for optimal treatment planning and for determining a prognosis. Multidetector computed tomography (MDCT), magnetic resonance imaging (MRI) and MR cholangiography are useful tools, both to diagnose and stage hilar cholangiocarcinoma. Modern imaging techniques allow accurate detection of the level of obstruction and the longitudinal and radial spread of the tumor. In addition, high-resolution MDCT and MR provide specific radiographic features to determine vascular involvement of anatomic structures, such as the hepatic artery or the portal vein, which are critical to decide the surgical strategy. Finally, radiological staging allows detection of patients with distant metastasis in the liver or peritoneum who will not benefit from a surgical approach.

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### INTRODUCTION

Carcinomas of the extrahepatic biliary tree, commonly known as hilar cholangiocarcinoma (HCCA), Klatskin tumors or perihilar cholangiocarcinomas, are rare malignancies that account for up to 3% of all gastrointestinal cancers. With an incidence rate of 0.5-2.0 cases per 100000, it is estimated that there are between 2500 and 4000 new cases per year in the United States<sup>[1]</sup>.

Although this tumor may potentially affect any location of the biliary tree, tumors involving the biliary confluence, left-hand drive (LHD) and right-hand drive (RHD) (true Klatskin tumors) are most common and account for 40%-60% of all cases. Around 2/3 of cholangiocarcinomas are hilar or extrahepatic and originate from the bile duct epithelium at the level of confluence

of the right and left ducts, whereas 30% are distal or intrapancreatic and 5%-10% are intrahepatic<sup>[2]</sup>. Due to recent improvements in radiological techniques, there is a wide range of imaging tools available for diagnosis and staging HCCA, such as multidetector computed tomography (MDCT), magnetic resonance (MR), endoscopic ultrasonography, endoscopic retrograde cholangiopancreatography (ERCP) and angiography. However, to date, there is no consensus as to the best staging algorithm. The aim of this paper is to review the results in the preoperative staging of HCCA with modern non-invasive imaging techniques, including MDCT, magnetic resonance imaging (MRI) and MR cholangiopancreatography (MRCP).

## **PATHOLOGICAL AND CLINICAL FEATURES**

Cholangiocarcinoma is a malignant tumor arising from bile duct epithelium of any portion of the biliary tree. It is the second most common primary liver cancer in the world, except for certain areas where it is more common than hepatocellular carcinoma (HCC). Traditionally, it has been classified as an intrahepatic and extrahepatic tumor according to its location with respect to the liver; this last category has been subdivided into upper, middle and distal by its location. However, recent literature classifies extrahepatic cholangiocarcinoma into perihilar and distal cancer, due to the relatively low incidence of middle bile duct cancer<sup>[2]</sup>, and the correlation of these categories with anatomic distribution and preferable treatment. Perihilar cholangiocarcinoma, also called Klatskin tumors<sup>[3]</sup>, can be defined as tumors originating on the right or left duct, near their junction or in close vicinity to the bile duct confluence, and represent two-thirds of cholangiocarcinomas<sup>[4]</sup>.

### **Pathology**

Most cholangiocarcinomas are adenocarcinomas with variable differentiation grades and fibroplasia. According to the last World Health Organization (WHO) histological classification, different types of adenocarcinoma are considered, and precursor lesions such as biliary intraepithelial neoplasia and intraductal papillary neoplasms are also included<sup>[5]</sup>. Macroscopically, carcinomas of the extrahepatic bile ducts have been divided into polypoid, nodular, scirrhous constricting and diffusely infiltrating types. These categories can provide a guide to the operative procedure, extent of resection and prognosis. However, except for the polypoid type, this division is rarely possible due to overlapping on gross features. However, the nodular and scirrhous types which tend to coexist are prone to infiltrate surrounding tissues, while the diffusely infiltrating type tends to spread linearly along the ducts.

### **Epidemiology and risk factors**

Cholangiocarcinoma occurs with a highly varying frequency in different areas of the world<sup>[6]</sup>. There are several recognized risk factors for cholangiocarcinoma,

such as primary sclerosing cholangitis (PSC), although the vast majority of cancers are seen in patients with no readily identifiable predisposing condition. PSC is an autoimmune condition that causes bile duct inflammation resulting in scarring and fibrosis. This chronic inflammation is thought to lead to dysplastic and ultimately neoplastic changes in the bile ducts. The risk of cholangiocarcinoma in patients with PSC is thought to be over a 1000-fold higher than in the general population and is thought to be 0.5%-1.0% per year or 10%-40% lifetime risk<sup>[7]</sup>. While both intrahepatic and extrahepatic cholangiocarcinoma are well-known complications of PSC in Western countries, hepatolithiasis, infections due to liver flukes and bile stasis are risk factors strongly associated with cholangiocarcinoma in Eastern Asia. However, approximately 90% of patients diagnosed with cholangiocarcinoma do not have a recognized risk factor in Western countries<sup>[8]</sup>. Other risk factors include an abnormal choledochopancreatic junction, choledocal cyst, ulcerative colitis, cirrhosis, alcoholic liver disease, type II diabetes, thyrotoxicosis, pancreatitis and possibly duodenal ulcer disease<sup>[9]</sup>.

### **Clinical features**

Obstructive jaundice is the main clinical feature and can appear relatively early, even with small neoplasms. It may progress rapidly or fluctuate. Other symptoms may include weight loss, pruritus, right-upper quadrant pain or fever and chills if cholangitis develops.

## **DIAGNOSIS OF HCCA**

### **Imaging techniques**

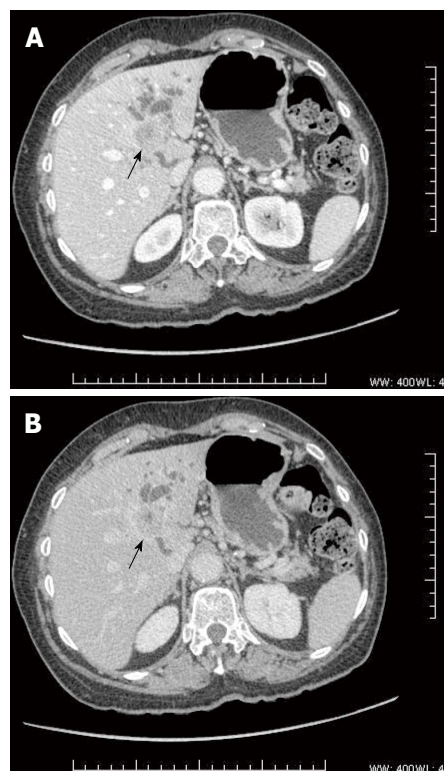
The majority of patients with HCCA present with severe painless jaundice as the initial clinical presentation. However, not every patient with jaundice and biliary obstruction at the hepatic hilus will eventually be confirmed as HCCA. In fact, almost 25% of cases turn out to have other benign conditions (such as lymphoplasmacytic cholangitis or Mirizzi syndrome) or other malignant disease (such as gallbladder cancer or nodal metastasis) that has obstructed the hepatic confluence<sup>[10]</sup>.

Initial radiological assessment is performed with sonography in most patients with perihilar biliary tract malignancies. Ultrasound rarely allows direct demonstration of perihilar biliary cancer, although indirect signs such as isolated intrahepatic dilatation can be useful in suggesting the diagnosis. However, in most cases, additional diagnostic procedures are necessary to confirm the diagnosis. The main goals of imaging in that setting will be to differentiate HCCA from other conditions leading to obstructive jaundice with intrahepatic dilatation and to perform an accurate preoperative evaluation of tumor resectability, focusing on vascular and biliary invasion as well as invasion and distant metastasis to the liver and lymph nodes. In the past, direct cholangiography combined with angiography was used to assess tumor extension. More recently, the advent of MDCT and MRCP has

dramatically changed the imaging evaluation of patients with hilar cholangiocarcinoma.

The role of cholangiography in the evaluation of hilar cholangiocarcinoma is two-fold: to assess tumoral extension to identify potentially resectable patients and to help in planning palliative biliary drainage in non-resectable patients. In this clinical setting MRCP has a number of potential advantages over ERCP and transhepatic cholangiography (THC). MRCP allows rapid non-invasive evaluation of the biliary tract without instrumentation and therefore without the risk of inducing sepsis in patients with ductal obstruction. Additionally, MRCP depicts tumoral extension along the intrahepatic branches of the biliary tree more consistently than ERCP and THC, especially in high grade stenosis<sup>[11]</sup>. In Fulcher's series<sup>[12]</sup>, MRCP allowed a more detailed visualization of the bile ducts than direct cholangiography in 3 of 4 patients who underwent both techniques. In another study, Holzknecht *et al*<sup>[13]</sup> performed a prospective comparison of MRCP and ERCP in 61 patients who underwent ERCP for a variety of clinical indications. ERCP demonstrated 36 stenosis in 33 patients (15 malignant and 21 benign). MRCP diagnosis was correct in 89% of the cases (32/36) and there were 4 false negative findings. However, MRCP was correct in all 15 patients with high-grade malignant stenosis. On a patient-by-patient basis, statistical analysis to compare the grade of stenosis indicated that differences between MRCP and ERCP were not significant. In a recent study by Yeh *et al*<sup>[14]</sup>, the efficacy of MRCP and ERCP in 40 patients with malignant perihilar obstruction, including 26 patients with Klatskin tumor, were compared. ERCP was unsuccessful in 2 patients. In this series, MRCP was superior to ERCP in delineating the anatomic extent of the tumoral lesions: 34/40 *vs* 24/38. Therefore, direct cholangiography, either endoscopic or percutaneous, has been definitively abandoned as a diagnostic tool and substituted by MRCP.

MDCT allows for faster scanning with thinner collimation and results in an improved diagnosis and staging hilar cholangiocarcinoma. CT is usually performed to assess the level of biliary obstruction (longitudinal extension) as well as involvement of liver parenchyma and vascular hilar structures (radial extension). High resolution CT allows for accurate depiction of a thickened bile duct wall and tumor spread into liver parenchyma or hilar vessels and therefore plays a key role in the diagnosis and staging of HCCA. Previous results of conventional CT in the diagnosis and staging of hilar cholangiocarcinoma have been limited. The advent of helical technology has dramatically improved the results of CT in the detection and diagnosis of Klatskin tumors, although its ability to perform an accurate staging is still limited. In the series by Tillich *et al*<sup>[15]</sup>, HCT correctly detected all hilar cholangiocarcinomas using a biphasic technique. However, its overall accuracy for predicting resectability was only 60%. In Feydy's series<sup>[16]</sup>, HCT correctly detected and localized the mass in 91% of the cases.



**Figure 1** Mass forming cholangiocarcinoma. Axial computed tomography scan in the portal phase (A) and in delayed phase (B) shows a heterogeneous hypovascular mass (arrow) with rim-like peripheral enhancement. The lesion is associated with ductal infiltration and biliary dilation.

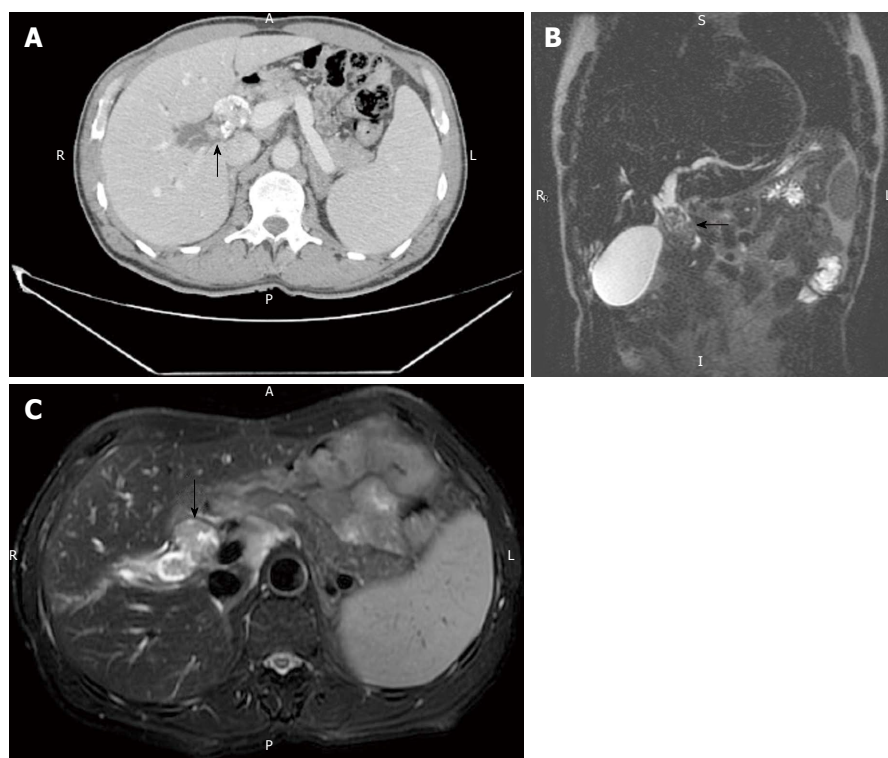
### Diagnostic features

Diagnostic features of HCCA include intrahepatic segmental biliary dilatation, periductal thickening, endoluminal lesions and direct tumor spread to the liver or adjacent vessels. Biliary dilatation is usually intrahepatic and often segmental and located proximally to an ill-defined biliary mass near the hepatic hilus. The transition between dilated and non dilated bile ducts is usually abrupt and this is a key feature for diagnosis. Therefore, these patients should not be drained before an adequate imaging study is performed.

On the basis of the Japanese Liver Cancer Group<sup>[17]</sup> classification, cholangiocarcinomas are classified into three types: mass-forming, intraductal growing and periductal infiltrating. The latter is the most prevalent in the hilar portion of the biliary tree and forms the majority of perihilar cholangiocarcinomas.

**Mass-forming cholangiocarcinoma:** In some cases, HCCA presents as a mass-forming lesion (Figure 1). This pattern of presentation includes periductal thickening and a solid tumor lesion involving the adjacent liver parenchyma<sup>[18]</sup>. Mass-forming intrahepatic cholangiocarcinoma is usually a bulky lesion with infiltrating features around the adjacent peripheral branches of the portal vein. On CT and MRI, mass-forming HCCA are usually heterogeneous hypovascular masses with rim-like peripheral enhancement in the arterial and portal phase and delayed enhancement in the equilibrium phase. These





**Figure 2** Intraductal growing cholangiocarcinoma. A: Axial computed tomography in the portal phase in a 52-year-old male shows a heterogeneously enhancing endoluminal lesion with dysmorphic calcifications (arrow). Note biliary dilatation upstream; B: Coronal thin-slab (echo spacing 4.2 ms, effective echo time 183 ms, image matrix 272 x 512, FOV 385 mm) T2-W sequence shows marked dilatation of the common hepatic duct and a hypointense endoluminal mass (arrow); C: Axial thin-slab (echo spacing 4.2 ms, effective echo time 183 ms, image matrix 272 x 512, FOV 385 mm) T2-W sequence shows a hypointense filling defect consistent with polypoid cholangiocarcinoma in the common hepatic duct (arrow).

enhancement features reflect the nature of the tumor, which is mainly desmoplastic. The bile ducts peripheral to the tumor are usually dilated because of obstruction by the tumor.

**Intraductal growing cholangiocarcinoma:** In intraductal growing cholangiocarcinoma, bile ducts can be dilated because of tumoral obstruction, increased mucin secretion or sloughed tumor debris. This pattern of cholangiocarcinoma is frequently found in papillary cholangiocarcinomas (Figure 2). On imaging, the involved bile ducts are asymmetrically dilated. Typically, an endoluminal mass is found in the segment with more intense dilatation. Intraductal cholangiocarcinoma is confined in the lumen of the dilated bile duct without direct tumoral extension to the surrounding liver parenchyma. This pattern of spread is radically different from mass-forming or periductal infiltrating cholangiocarcinoma that typically shows marked infiltration. This is related to the different histological patterns of the tumors. Intraductal growing cholangiocarcinoma appears as a nodular, well defined mass on CT or MR and typically shows intense enhancement after contrast injection. The mass is confined within the bile ducts and therefore there is preservation of the bile duct wall<sup>[18]</sup>.

**Periductal infiltrating cholangiocarcinoma:** This pattern of cholangiocarcinoma is frequently found in perihilar cholangiocarcinoma. Periductal infiltrating cholangiocarcinoma typically shows marked dilatation on imaging of the biliary tree proximal to the tumoral lesion. On CT, the involved bile ducts are diffusely narrowed or obliterated. With conventional helical or incremental CT it was extremely difficult or impossible to depict the tumor

mass on imaging studies. However, with the advent of MDCT, thin-collimation tumoral periductal involvement is almost always detectable. High-resolution CT shows tumoral involvement as an irregular periductal thickening completely obstructing or narrowing a short segment of the biliary tree around the biliary bifurcation or common hepatic duct (Figure 3).

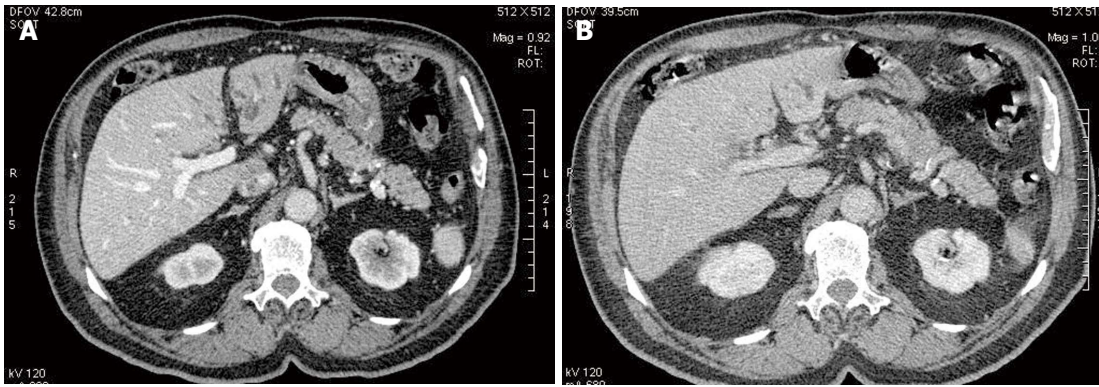
Periductal thickening is usually iso-hypo enhancing in arterial and portal phases and shows marked enhancement on delayed phase imaging (Figure 4). Occasionally periductal thickening may show brisk hyperenhancement in the arterial phase, mimicking endobiliary HCC (Figure 5). On MRCP, non union of the right and left hepatic ducts is a typical finding of infiltrating hilar cholangiocarcinoma.

### Differential diagnosis

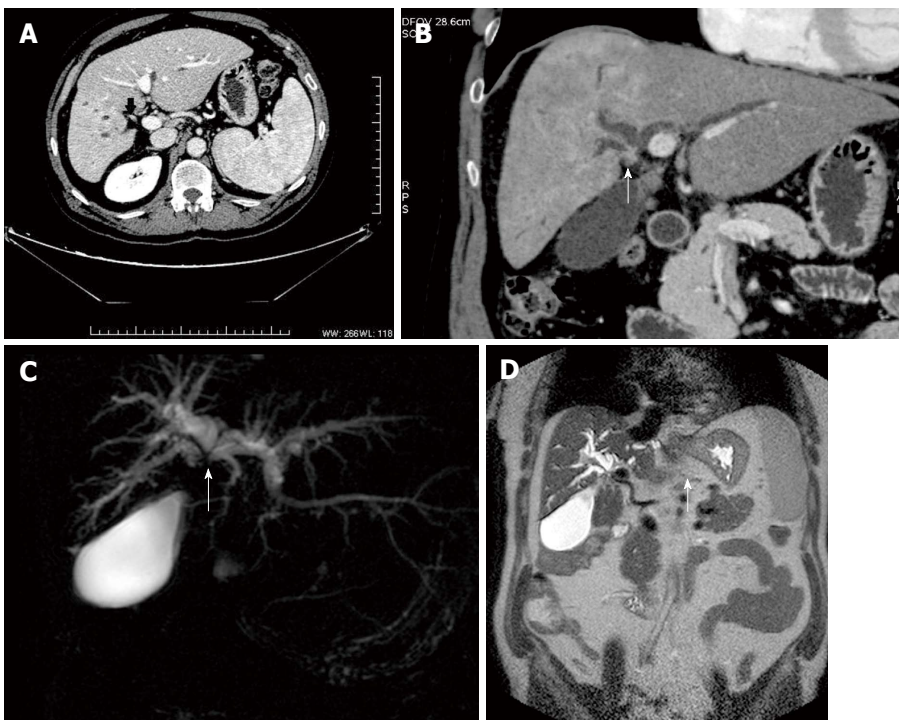
There is a wide variety of benign and neoplastic lesions at the liver hilum that may cause biliary stricture. In particular, inflammatory lesions may present with the same radiological features as those from malignant tumoral causes.

The most frequent benign lesions that may mimic cholangiocarcinoma include lymphoplasmacytic cholangiopathy (IgG4 sclerosing cholangitis), endobiliary metastases, endobiliary HCC and Mirizzi Syndrome.

Several studies have noted that approximately 14% to 25% of resected patients for cholangiocarcinoma (HCCA) prove to have a benign lesion at histopathology<sup>[19,20]</sup>. Differentiation between malignant and benign strictures in patients with suspicion of cholangiocarcinoma is often impossible before laparotomy due to overlapping of radiological and clinical features. In addition, there are no specific radiological or laboratory tests that may distin-



**Figure 3** Periductal cholangiocarcinoma multidetector computed tomography. A: Multidetector computed tomography (MDCT) in the portal phase at the level of the hepatic hilus shows an irregular periductal thickening completely obstructing the common hepatic duct consistent with periductal cholangiocarcinoma (arrow). The lesion is hypoenhancing in the portal phase; B: Delayed phase MDCT at the same level shows marked hyper-enhancement of the tumoral lesion (arrow).



**Figure 4** Periductal cholangiocarcinoma multidetector computed tomography and magnetic resonance cholangiopancreatography. A: Multidetector computed tomography in the portal phase shows periductal cholangiocarcinoma (arrow) producing complete biliary obstruction and atrophy of the right liver; B: Coronal reconstruction shows to a better advantage the scirrhous infiltrating cholangiocarcinoma slightly hyperenhancing (arrow) and the dilated bile ducts upstream; C: Coronal thick-slab (echo spacing 8.3 ms, effective echo time 1000 ms, image matrix 512 x 512, FOV 350 mm) MRCP T2-W sequence in the same patient shows marked dilatation of intrahepatic bile ducts and a signal void (arrow) at the level of the hepatic convergence and common hepatic duct consistent with malignant obstruction by cholangiocarcinoma; D: Matrix (272 x 512, FOV 385 mm) T2-W sequence shows marked dilatation of the intrahepatic ducts and a narrow stenosis at the hepatic bifurcation (arrow).

guish HCCA from benign proximal bile duct lesions.

Malignant stricture is characterized by bile-duct wall thickening greater than 1.5 mm, arterial and venous hyperenhancement of the stricture, and greater extent of proximal dilatation compared with benign lesions<sup>[21]</sup>. Different imaging studies have shown that only one feature, vascular involvement, was significantly different in benign and malignant lesions<sup>[22]</sup>.

### **Lymphoplasmacytic cholangiopathy**

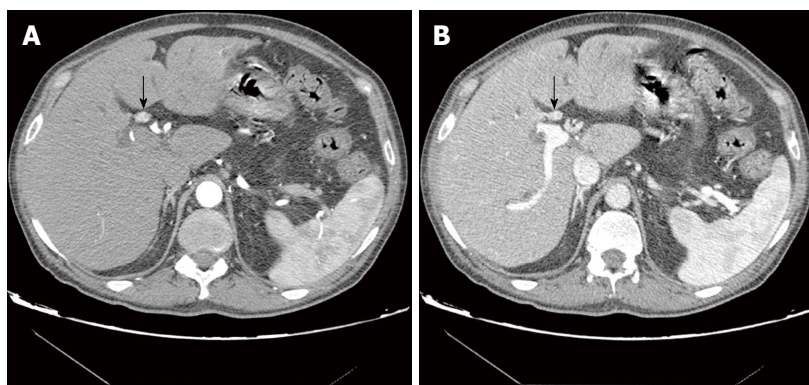
IgG4-sclerosing cholangitis is considered part of IgG4 systemic-related diseases and is commonly associated with autoimmune pancreatitis<sup>[23]</sup>. The occasional absence of pancreatic involvement in these patients has been previously reported. No strict diagnostic criteria have been described to date and diagnosis relies on a combination of clinical and histopathological findings. A typical diagnostic feature of IgG4-sclerosing cholangitis is elevation

of serum immunoglobulin G4. IgG4-sclerosing cholangitis shows clinical response to steroid therapy. Prompt initiation of steroid treatment in these patients could greatly improve outcomes, at least in part by avoiding unnecessary surgery. Both the intrahepatic and extrahepatic segments can be involved by lymphoplasmacytic infiltration characterized by transmural fibrosis which may extend to the periportal area of the liver, causing biliary stricture<sup>[24]</sup>.

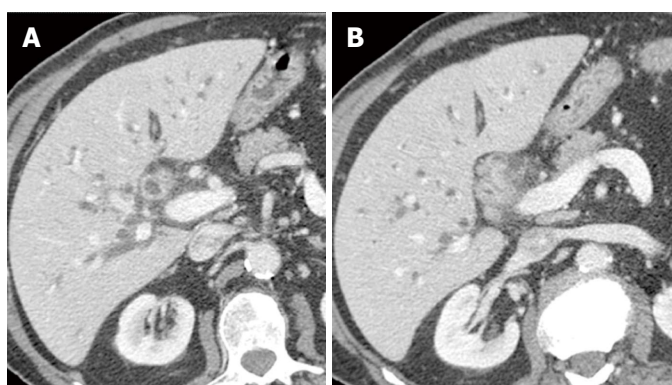
A stricture of the distal CBD is the most common abnormality of IgG4 sclerosing disease, reported in 54%-79% cases<sup>[25,26]</sup>. Associated imaging findings of autoimmune pancreatitis, such as focal or diffuse pancreatic enlargement with a peripheral ring of low attenuation and a diffusely narrowed pancreatic duct, are useful features in pointing the diagnosis of autoimmune cholangitis<sup>[26]</sup>.

However, accurate preoperative diagnosis of lymphoplasmacytic cholangitis and differentiating this condition from hilar cholangiocarcinoma is still very difficult





**Figure 5 Hypervascular periductal cholangiocarcinoma.** A: Multidetector computed tomography shows periductal cholangiocarcinoma with brisk arterial enhancement in the arterial phase (arrow); B: Portal phase multidetector computed tomography at the same level shows mild tumoral enhancement (arrow).



**Figure 6 Lymphoplasmacytic cholangiopathy multidetector computed tomography.** A: Multidetector computed tomography (MDCT) in the portal phase shows marked intrahepatic dilatation and marked irregular thickening of the common hepatic duct; B: MDCT in the same patient more caudally shows abrupt stenosis and obliteration of the bile duct lumen. Surgical exploration confirmed lymphoplasmacytic cholangiopathy.

because of the overlapping imaging features of both conditions (Figures 6 and 7). Both HCCA and LC have a similar gross macroscopic appearance related to their fibrous nature.

**Endobiliary metastases:** Neoplastic intraductal filling defects are usually considered primary intraductal biliary neoplasms until they are proved otherwise pathologically. Nevertheless, it is also well-known that extrabiliary malignant tumors, such as lung, breast, gallbladder, colon, testis, prostate, pancreas, melanoma and lymphoma, can metastasize to the bile duct and manifest as an intraductal mass too. When an intraductal lesion is found in a patient with extrabiliary malignancy, the presence of a contiguous parenchymal mass, an expansible nature of the intraductal lesion, and a history of colorectal cancer may suggest the presence of intraductal metastasis rather than double primary intraductal cholangiocarcinoma<sup>[27]</sup>.

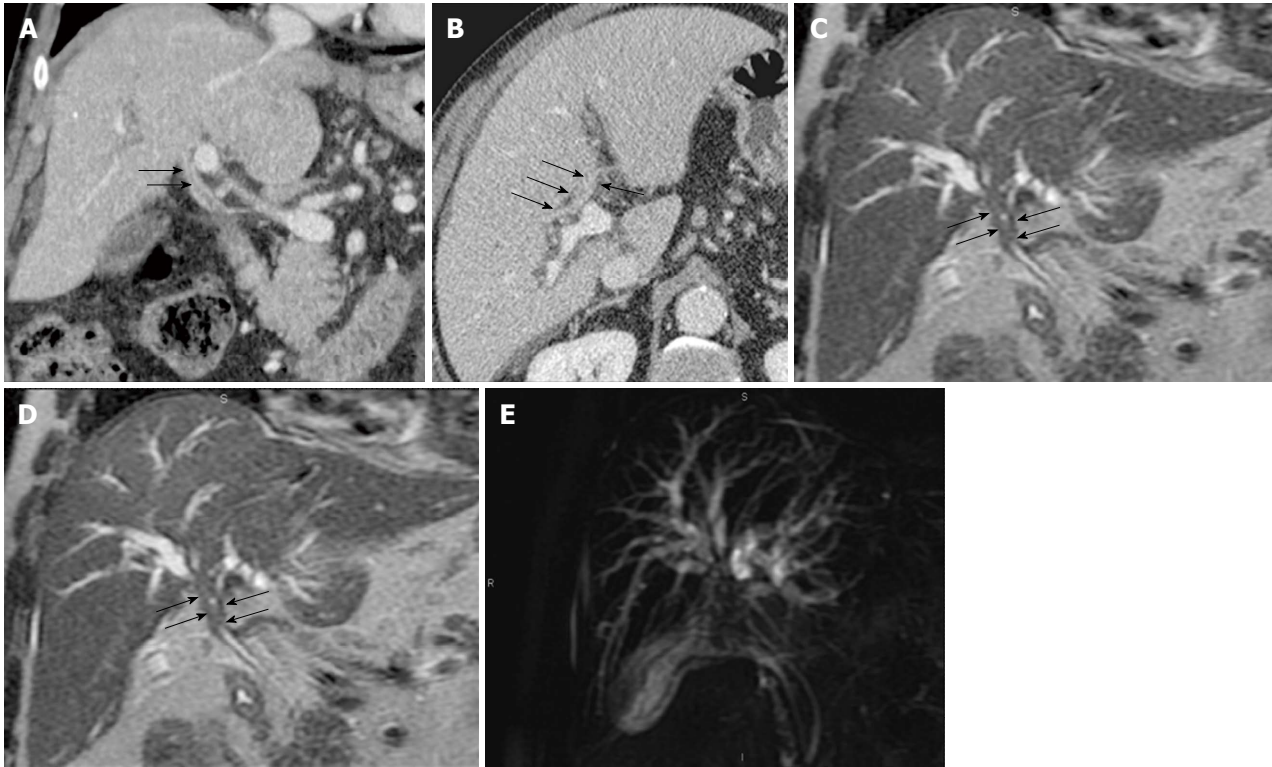
However, occasionally endobiliary metastases can be isolated without associated liver metastases and only the clinical setting may help in the differential diagnosis (Figure 8).

**HCC with endobiliary growth:** Obstructive jaundice as the main clinical feature is uncommon in patients with HCC. Only 1%-12% of HCC patients present with obstructive jaundice as the initial complaint. HCC may involve the biliary tract in several different ways: tumor thrombosis, hemobilia or endoluminal tumor extension. Jaundice in patients with HCC is usually related to diffuse tumoral intrahepatic extension with hepatic insufficiency. Filling defects in the biliary tree in a patient with

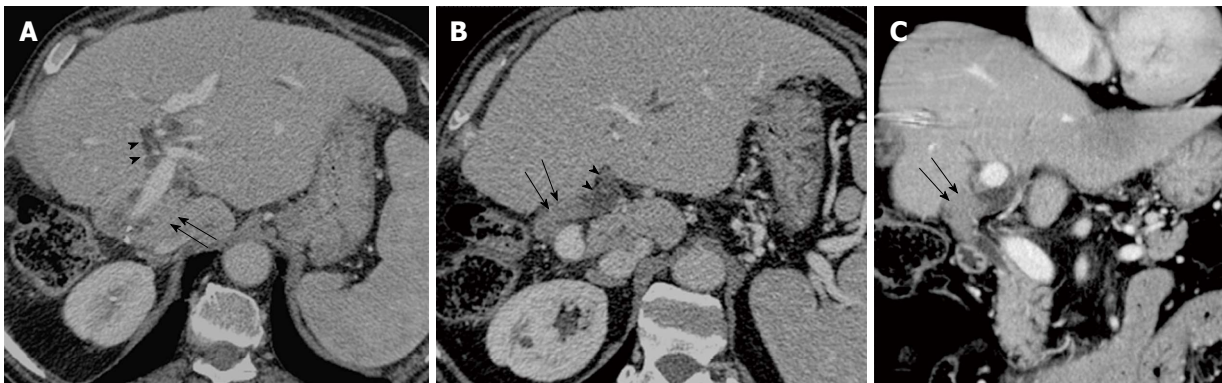
HCC may be related to blood clots, sludge or intrabiliary tumoral growth. In the absence of gallbladder stones, the most frequent cause of intrabiliary filling defects is HCC.

Intrabiliary growth of HCC shows the same enhancement pattern as the primary tumor and is hyperenhancing in the arterial phase with wash-out in portal and delayed phases. Occasionally, endobiliary growth of HCC may be found in patients without a definite hepatic mass<sup>[28]</sup>. In that setting, the differential with intraductal growing cholangiocarcinoma may be very difficult with imaging, although a clinical setting of chronic liver disease may suggest the diagnosis.

**Mirizzi syndrome:** Mirizzi syndrome is a form of obstructive jaundice caused by a bile duct stone impacted in the neck of the gallbladder or in the cystic duct. The stone and surrounding inflammation compress the common hepatic duct and results in dilation of the bile ducts upstream<sup>[29]</sup>. This is a rare complication of cholelithiasis. An accurate diagnosis is essential for proper management of a patient. From a pathophysiological point of view, Mirizzi syndrome may have two causes. In Mirizzi type I there is chronic inflammation of the gallbladder with marked contraction of the fibrous gallbladder wall that adheres to the common bile duct, resulting in fibrous stenosis of the biliary lumen. In Mirizzi type II there is a direct cholecysto-biliary fistula secondary to direct compression of large stones on the wall of the common hepatic duct. Imaging reveals dilated intrahepatic bile ducts with a normal common bile duct. The gallbladder is usually collapsed. The diagnosis



**Figure 7** Lymphoplasmacytic cholangiopathy multidetector computed tomography and magnetic resonance cholangiopancreatography. A, B: Coronal reconstructed and axial multidetector computed tomography in the portal phase show periductal thickening of the common hepatic duct and hepatic bifurcation (arrows); C: Coronal thin-slab (echo spacing 4.2 ms, effective echo time 183 ms, image matrix 272 x 512, FOV 385 mm) T2-W sequence shows marked intrahepatic biliary dilatation and an abrupt stenosis of the coronary heart disease and biliary bifurcation (arrows); D: Coronal thick-slab (echo spacing 8.3 ms, effective echo time 1000 ms, image matrix 512 x 512, FOV 350 mm) magnetic resonance cholangiopancreatography T2-W sequence in the same patient shows marked dilatation of intrahepatic bile ducts and a signal void in the hepatic bifurcation mimicking Klatskin tumor. Surgical exploration and histological study confirmed lymphoplasmacytic cholangiopathy.



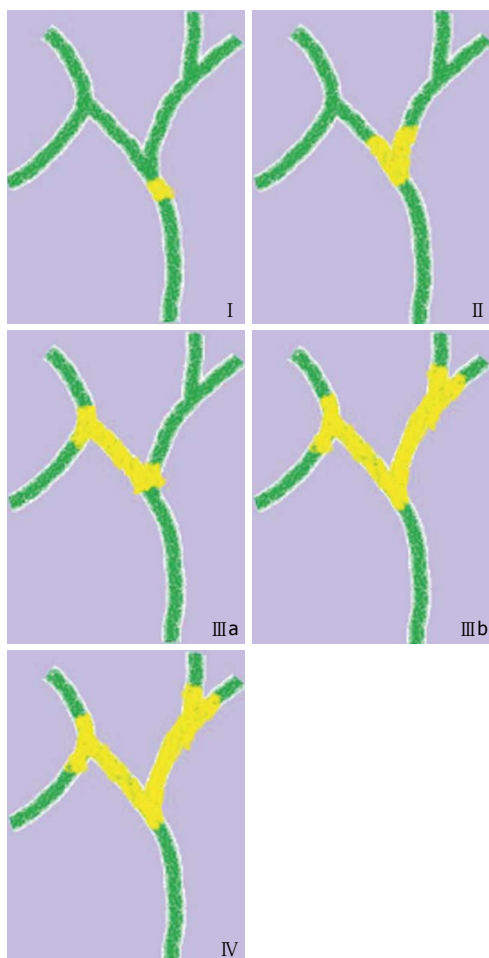
**Figure 8** Endobiliary metastases. Sixty eight-year-old patient with obstructive jaundice. The patient had been operated on for liver metastases of colorectal cancer 3 years ago (right hepatectomy). A, B: Portal phase computed tomography shows left intrahepatic biliary dilatation (arrowheads) and a solid slightly hyperenhancing endoluminal mass in the left hepatic duct; C: Coronal reconstruction shows to a better advantage the fluid density of dilated bile ducts and the solid density of the endobiliary tumor (arrows). Percutaneous fine-needle aspiration biopsy confirmed endobiliary metastasis.

may be suspected on CT when calcified bile stones are detected. However, in most cases stones are radiolucent and not detectable with CT. MRCP is the most efficient imaging technique in this setting, allowing detection of impacted stones at the gallbladder neck with compression of the hepatic duct and upstream biliary dilatation. The treatment of Mirizzi's syndrome may be a simple cholecystectomy with or without hepaticojejunostomy in case of fistula.

## PREOPERATIVE STAGING AND ASSESSEMENT OF RESECTABILITY

Cholangiocarcinoma is one of the most difficult tumors, both to stage and treat. Surgery remains the only curative treatment for HCCA. However, surgical treatment of this malignant tumor is impaired by the lack of an effective adjuvant treatment. In addition, the specific anatomic location of these tumors usually involving critical





**Figure 9** Bismuth-Corlette classification.

vascular structures makes complete resection extremely challenging. This is a critical point because long-term survival in patients with HCCA depends on complete tumor resection. Due to the infiltrative growth pattern of HCCA, thorough preoperative evaluation of the extent of tumor along the biliary tree and major vessels at the hepatic hilum is needed in order to plan adequate surgical treatment. Initial reports on surgical resection of cholangiocarcinoma involved only patients treated with biliary resection with hepaticojejunostomy with poor survival benefits<sup>[30]</sup>. Experience over recent years has shown a dramatic increase of radical surgical procedures with extended right or left hemi-hepatectomies, reporting 5-year survival rates between 25% and 40%<sup>[31]</sup>. In addition, development of sophisticated preoperative imaging techniques with multiplanar, biliary and vascular reconstructions has improved the depiction of both direct radial hepatic invasion and longitudinal intraductal extension of cholangiocarcinoma. The main problem until now has been the poor results of preoperative staging methods in order to separate potentially resectable from non-resectable patients and to avoid unnecessary surgical procedures, because benefits in terms of survival are only achieved if the tumor is completely resected.

The cornerstone of oncological resection in the liver

is to resect the whole tumor with free margins but still leave enough liver to maintain hepatic function. This is particularly difficult in HCCA due to its infiltrating nature. Preoperative staging should focus on biliary, vascular, hepatic, lymph node and extrahepatic extension.

Unresectability criteria generally include liver metastasis, distant lymph node metastasis, bilateral arterial or portal invasion, unilateral vascular invasion and contralateral lobar atrophy and distant metastases. Parenchymal invasion is not considered an unresectability criterion because a right or left hepatectomy can be performed. Bismuth stage IV is also not always considered an unresectability criterion because with an extra-Glissonian approach and hepatectomy some lesions can be safely resected.

### **Longitudinal extension of cholangiocarcinoma: Biliary staging**

Biliary extension is usually assessed with MRCP. MRCP can confidently classify cholangiocarcinoma according to the Bismuth-Corlette Classification in stages I, II, IIIa, IIIb and IV (Figure 9). Stage I includes tumor confined to the common hepatic duct. Stage II includes tumor involving the confluence of the right and left hepatic ducts without proximal biliary involvement. Stage IIIa includes tumor involving the confluence of the right and left hepatic ducts with proximal extension to the confluence of the anterior and posterior right segmental bile ducts. Stage IIIb includes tumor involving the confluence of the right and left hepatic ducts with proximal extension to the confluence of bile ducts for segments IV and II-III.

Bismuth stage IV includes tumor involving the confluence of the right and left hepatic ducts with proximal extension to both secondary confluences of the right and left hepatic ducts.

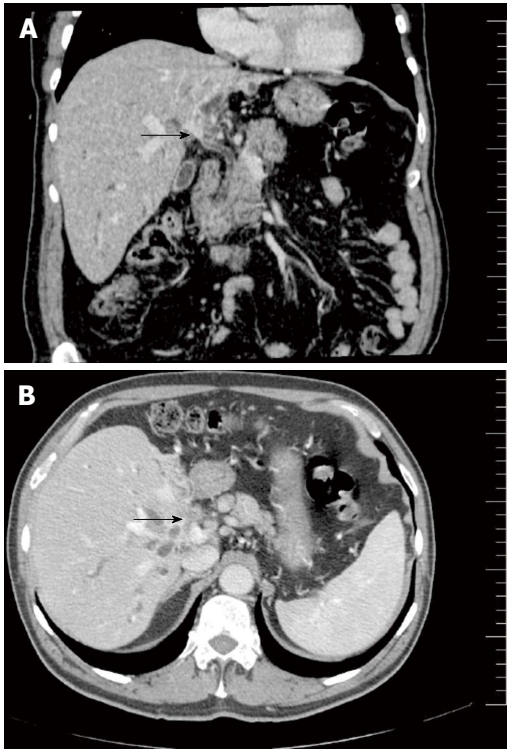
In the paper by Maselli *et al.*<sup>[32]</sup>, fifteen patients with hilar cholangiocarcinoma underwent radical surgery and preoperative MR imaging. MRCP and gadolinium-enhanced dynamic T1-W images were correlated with surgical findings in the evaluation of the extent of biliary infiltration according to the Bismuth-Corlette classification.

Radiological-pathological correlation disclosed that the assessment of the bile duct stenosis by MRCP alone was correct in 80% of the patients (12/15). MRCP underestimated tumor spread in two patients who were considered preoperatively type II and type IIIA and turned out to be type IIIA and IV respectively at surgical exploration.

MRCP overestimated the neoplastic biliary extent in another patient who was considered type IIIa preoperatively and was proven to be type II at histology. Overall accuracy for MRCP to classify tumor according to the Bismuth Classification was 80%. In the series by Lee *et al.*<sup>[33]</sup>, longitudinal biliary involvement was accurately predicted with MDCT in 84% of the patients.

### **Radial extension of cholangiocarcinoma: Vascular staging**

Vascular extension is either assessed with MDCT or gadolinium-enhanced dynamic MRI. Accurate preoperative assessment of HCCA requires thorough evaluation of

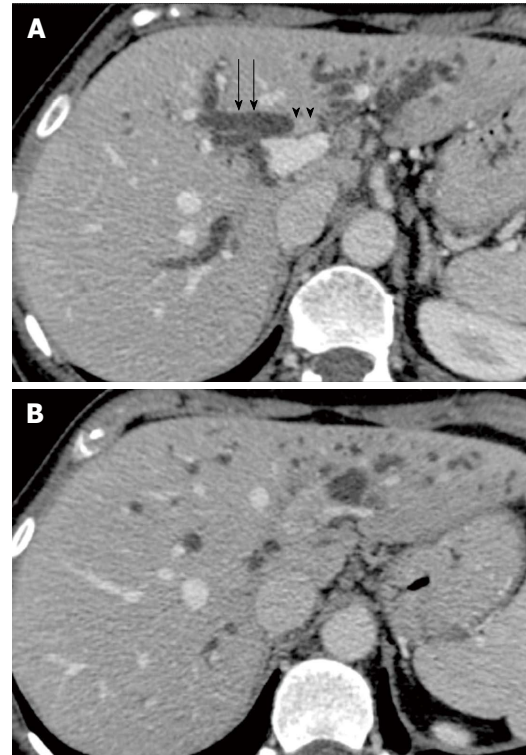


**Figure 10 Liver infiltration of cholangiocarcinoma.** A: Coronal reconstructed multidetector computed tomography shows a periductal mass at the hepatic confluence consistent with cholangiocarcinoma (arrow); B: Axial computed tomography of the same patient shows a hypoenhancing mass involving the liver parenchyma and hilar vessels (arrow) consistent with tumoral infiltration by cholangiocarcinoma.

the hepatic artery (right, left and common hepatic artery) and portal veins (right, left and main portal vein) (Figures 10 and 11).

MDCT and MRCP are useful techniques to delineate the extent of the tumor and to rule out vascular invasion and locoregional lymph node extension. Helical CT should be performed to rule out liver and lymph node metastasis as well as vascular encasement but proximal tumor extent along bile ducts is often underestimated.

On the other hand, helical CT also plays a key role in the management and staging of cholangiocarcinoma. HCT allows assessment of tumor spread to the liver or regional lymph nodes, as well as arterial or portal involvement<sup>[15]</sup>. Previous results of conventional CT in the diagnosis and staging of hilar cholangiocarcinoma have been limited. The advent of helical technology has dramatically improved the results of CT in the detection and diagnosis of Klatskin tumors, although its ability to perform an accurate staging is still limited. In the series by Tillich *et al.*<sup>[15]</sup>, HCT correctly detected all hilar cholangiocarcinomas using a biphasic technique. However, its overall accuracy for predicting resectability was only 60%. In a study by Cha *et al.*<sup>[34]</sup>, 21 patients with perihilar cholangiocarcinoma were studied with MDCT. CT correctly detected that the tumor was unresectable in 8 cases (PPV: 100%), due to vascular involvement in 8 cases and distant lymph-node metastases in 1 case. However, in 12 patients with suspected resectable disease by CT, 6 cases turned out to



**Figure 11 Portal infiltration of cholangiocarcinoma.** A: Axial computed tomography in the portal phase shows a periductal mass in the portal confluence (arrowheads) producing biliary dilatation (arrows); B: The mass extends cranially and shows encasement and infiltration of the left portal vein.

be unresectable (NPV: 50%), although none of them was related to vascular invasion (lymphadenopathy, metastasis, intraductal extension and anatomic variants).

In Maselli's series<sup>[32]</sup>, MRI correctly predicted vascular involvement in 73% of the cases. MR correctly showed arterial involvement in 88% of the cases (8/9) and had false positive findings in 12% (1/9). Portal invasion was correctly assessed in 73% of the cases (11/15) and underestimated in 20% of the cases (3/15). In 7% of the patients (1/15), portal vein invasion was overestimated and not confirmed by histology.

In the series by Lee *et al.*<sup>[33]</sup>, the accuracy of MDCT in the prediction of portal vein and hepatic artery invasion was 85.5% and 92.7% respectively. Overall accuracy of resectability was 74.5%.

In Chrysosou's series<sup>[35]</sup>, preoperative dynamic MRI with gadolinium was fully concordant with surgical data in the assessment of tumoral invasion in 77% of the veins studied (63/82) and in 58% (43/74) arteries. Concordance was better concerning the assessment of venous invasion but several cases with discordance were reported in that series. MRI showed no tumoral involvement in 5 cases but surgical exploration suggested tumoral infiltration. These veins were resected but final histological study showed no tumor infiltration.

In addition, only 60% of the veins with suspected tumor infiltration on MR and survival exploration showed definite microscopic wall invasion at histological study. However, those lesions would probably not have been

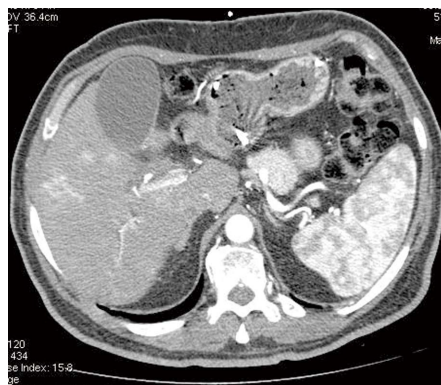
resectable without vascular resection.

Some surgical studies<sup>[36]</sup> have reported that tumoral involvement of portal veins may be overestimated by surgical exploration compared with histology because there was no adventitial tumoral invasion. These studies reflect the challenges of vascular staging of hilar cholangiocarcinoma. In most cases, there is close contact between the tumor and the venous or arterial structure and it is difficult to determine if it is invaded or not. In our experience, only complete tumor encasement (tumor contact with the vessel of more than 50%) is an adequate sign to predict tumor invasion. Despite the fact that in some cases the wall of the vein is actually not invaded microscopically by tumor, the fact is that the tumor cannot be resected without resecting the vessel. In cases of minor tumoral abutment, the patient should not be considered unresectable and surgery should be performed.

In Chrysou's series<sup>[35]</sup>, 80% of non-stented patients with tumor-to-portal vein contact of more than 90% but without stenosis on MR had invasion of the adventitia confirmed histologically. This may be a useful criterion for predicting invasion of the portal vein or its segmental branches in non-stented patients. In the same series<sup>[35]</sup>, the excellent correlation between MRI and surgery was associated with a weaker correlation between MRI and pathology. These results reflect that both MRI and surgery have limitations in differentiating true microscopic invasion from adherent perivascular fibrosis. This kind of fibrosis is especially frequent in patients with previous biliary drainage because at the hepatic hilus there is close anatomic contiguity between the vessels and the biliary tree. Therefore, in patients with suspected perihilar cholangiocarcinoma it is critical that biliary intervention should be avoided before an adequate preoperative staging is performed. Invasion of adjacent liver parenchyma is also critical in order to assess resectability of cholangiocarcinoma. In mass forming cholangiocarcinoma, parenchymal infiltration is readily seen because tumor has an eccentric location related to bile ducts. However, in periductal cholangiocarcinoma, parenchymal infiltration may be subtle and high-resolution thin collimation scanning is needed. Imaging with MDCT or MRI shows a hypovascular infiltrating tumoral mass growing beyond the duct and invading the adjacent liver parenchyma. In Masseli's series<sup>[32]</sup>, MRI accurately showed a local parenchymal invasion in 80% of the patients (Figure 12).

#### **Distant extension of cholangiocarcinoma: Distant staging**

Different imaging techniques are currently used for the preoperative staging of hilar cholangiocarcinoma. However, accurate staging remains a challenge. Distant staging is usually performed with CT or MR at the same time as doing local vascular and biliary staging, yet most evidence is available from CT. Although the quality of imaging has improved in recent years, a substantial proportion of tumors are still found to be unresectable during laparotomy despite extensive pre-operative work and only 50% of the tumors surgically explored are eventually resectable<sup>[31]</sup>.



**Figure 12 Arterial infiltration of cholangiocarcinoma.** Multidetector computed tomography in the arterial phase shows a periductal mass in the hepatic hilus completely surrounding the right hepatic artery consistent with tumor infiltration.

Recently, with the advent of functional imaging such as positron emission tomography computed tomography (PET-CT) that allows the study of the whole body, the need for additional imaging in patients with cholangiocarcinoma has arisen, in order to rule out distant metastases before radical treatment. The utility of 18F-fluorodeoxyglucose (FDG) positron emission tomography (PET scan) in the diagnosis and staging of HCCA is uncertain. Anderson *et al.*<sup>[37]</sup> showed that PET can accurately detect nodular cholangiocarcinomas as small as 1 cm with a sensitivity of 85%. However, infiltrating tumors which are more frequent are only detected in 18% of the cases. The addition of PET-CT changed the initial management in 30% of the cases by detecting unexpected distant metastasis. This study is relatively old and the results should be interpreted cautiously, especially in patients with PSC or biliary stents in place.

In a recent meta-analysis by Ruys *et al.*<sup>[38]</sup>, data of the literature concerning diagnosis and staging of hilar cholangiocarcinoma during the period 1966-2011 were reviewed. The initial search yielded 766 articles but only 16 papers, which included 448 patients, met the inclusion criteria and were actually analyzed. Most data concerned CT as the primary diagnostic tool and therefore comparison with MRI and PET-CT was not possible. Only data on CT were sufficient for pooling the findings. Pooled accuracy of CT for assessment of ductal extent of the tumor was 86%, whereas pooled sensitivity and specificity for assessment of portal vein involvement was 89% and 92% respectively. Pooled sensitivity and specificity in the assessment of hepatic artery tumoral involvement was 84% and 93% respectively. Concerning regional lymph node metastasis, pooled sensitivity and specificity were 61% and 88%. Only one study that met the inclusion criteria reported the results of PET-CT for the assessment of lymph node metastasis with a sensitivity and specificity of 42% and 80%, respectively<sup>[39]</sup>.

Concerning distant metastases, the results of the meta-analysis are somewhat limited because only one study reported on the sensitivity and specificity of incremental non helical CT on metastasis (67% and 94% respectively).



The same study reported a sensitivity and specificity of 56% and 88%, respectively for PET/CT in the detection of distant metastasis. The series by Ruys *et al.*<sup>[40]</sup> evaluated retrospectively the additional value of FDG-PET/CT in staging of hilar cholangiocarcinoma after extensive conventional preoperative work-up with CT or MR. The primary tumor was 18F-FDG-positive in 88% of patients. However, all benign lesions in the series were also FDG-positive. Sensitivity and specificity for the detection of regional lymph node metastases and distant metastases were 67% and 68%, and 33% and 96%, respectively.

To summarize, the data in the literature concerning accuracy of imaging techniques in the diagnosis and staging of hilar cholangiocarcinoma are still very limited and most studies have important methodological limitations precluding direct head-to-head comparison of different imaging modalities. The only evidence available concerns staging with CT, which has acceptable accuracy for both longitudinal and radial staging, with sensitivity and specificity ranging between 84% and 90%.

However, no solid evidence is available concerning distant staging. The sensitivity of CT regarding lymph node involvement seems to be low (61%) but this is probably not very relevant in the planning of surgical resection since lymphadenectomy is systematically performed in these patients. There are still no solid data concerning the results of the different imaging modalities in the distant staging of hilar cholangiocarcinoma.

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## Laparoscopic staging in hilar cholangiocarcinoma: Is it still justified?

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invasive procedures. Finally, SL should be performed preceding laparotomy in one session. Further studies on the benefit of SL and LUS in this subset of HCCA patients are warranted.

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**Key words:** Hilar cholangiocarcinoma; Laparoscopy; Staging laparoscopy; Laparoscopic ultrasound

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### Abstract

Radical resection remains the only potential curative therapy for hilar cholangiocarcinoma (HCCA). The aim of staging laparoscopic (SL) is to identify patients with previously undetected advanced disease who will not benefit from surgical palliation and therefore avoid unnecessary laparotomies. The accuracy of non-invasive imaging techniques has significantly improved during the last years. As a consequence, the diagnostic yield of SL of biliary tract malignancy should have decreased proportionally. At the same time, some authors have recently questioned the value of laparoscopic ultrasound (LUS) as a complement of SL. In this setting, the precise role of SL and LUS in the preoperative workup of HCCA remains unclear. As it seems undoubtedly clear that its efficacy has decreased in the last decades, there is a general consensus that the universal use of SL shouldn't be recommended anymore; SL should be performed only in selected patients with higher risk of holding unresectable disease (T2/T3 or Bismuth type 3/4 and patients with suspicion of metastases). It would also be recommended in patients with potentially resectable disease who would need preoperative

### INTRODUCTION

Radical resection, with an *en bloc* partial hepatectomy in most cases, remains the only potential curative option<sup>[1-3]</sup> for patients with hilar cholangiocarcinoma (HCCA).

Staging laparoscopy (SL) is a quick and safe minimally invasive investigation that helps to determine the presence of peritoneal disease and occult dissemination within the liver. The addition of direct contact laparoscopic ultrasound (LUS) provides the ability to assess further the local stage of the disease and to evaluate the presence of liver metastases<sup>[4]</sup>.

The accuracy of non-invasive imaging techniques is continuously improving. This improvement has resulted in highly accurate staging for many hepato-pancreato-biliary malignancies, and so, its use is nowadays recommended in selected patients. As an example, the use of SL/LUS appears to be useful as an adjunct in the preoperative staging of patients with resectable colorectal liver metastases, when peritoneal disease is suspected<sup>[5]</sup>. With regard to pancreatic cancer, the routine use of SL is not warranted any more. Instead, different studies have

determined criteria to identify the subset of patients at high risk for extended disease<sup>[6,7]</sup> in which LS still has a beneficial yield.

In spite of all the advances in preoperative imaging, evaluation of HCCA remains a challenge. Furthermore, it is not yet clearly stated whether SL should be used routinely in HCCA, or, if selectively, when.

In a recent study from the Memorial Sloan Kettering Cancer Center (MSKCC), with 380 patients, 295 were considered to have resectable disease and underwent surgery. Nevertheless, from these 295 patients that underwent exploration with curative intent, only 157 (53.2%) underwent a potentially curative resection, and ultimately only 120 (40.6%) underwent a R0 resection<sup>[8]</sup>. In this series SL was performed in patients with high risk of advanced disease, but, in spite of this approach, there was still a high rate of unnecessary laparotomies.

The purpose of this article is to review and update the available evidence to determine the precise role of SL in the diagnostic evaluation of HCCA.

## TECHNIQUE

The use of SL for optimizing resectability in HCCA has been reported from the early beginnings of laparoscopy, and naturally, the technique differs from one to another author<sup>[4,9-11]</sup>. Below is summarized a standard approach.

### Simple laparoscopic staging

SL generally begins with two trocars. A third trocar is frequently inserted to help in the inspection or biopsy procedure. The sites of the trocar insertion are usually chosen within the line of the planned incision should the disease be found resectable by laparoscopic assessment<sup>[12]</sup>.

After insertion of the first port, whether by open or closed technique, pneumoperitoneum is established, and a first general inspection performed. A second trocar is inserted, generally in the right mid-quadrant, and a careful inspection of the peritoneum is performed paying particular attention to the diaphragm, falciform ligament, liver (superior and inferior surface), porta hepatis and lesser omentum. Also pelvis is inspected, and by retracting the greater omentum, the small bowel and mesenteric root can be visualized. Any suspicious lesion is biopsied and sent for frozen section analysis<sup>[4,9]</sup>. If SL reveals previously undetected evidence of unresectability, the procedure terminates here. In any case, a sample must be taken and malignancy must be histologically confirmed.

### LUS

LUS was soon added to SL with the objective of increasing the sensitivity of the exploration<sup>[9-11]</sup>, and is nowadays routinely used in many centers.

There are several flexible-tip high-resolution types of transducers available and they usually enter through a 10 mm trocar. If needed to allow images in different planes, it can be inserted through different placed ports (and even occasionally is useful to insert an additional trocar).

A systematic scanning of the liver should be performed in order to rule out possible intrahepatic metastasis. They can appear as hyper, iso or hypoechoic lesions, and therefore, any suspicious lesion should be biopsied under ultrasound control<sup>[4]</sup>. After this, careful identification of the structures including the primary tumor in the porta hepatis is mandatory. If the quality of the study is not good enough due to inadequate probe contact, the right upper quadrant can be filled with saline solution until hepatic pedicle is covered. Another possible manoeuvre is to release the neumoperitoneum almost completely. This will help to assess the local extension of the tumor and the presence of lymph node metastases. Vascular invasion is suggested if there is loss of planes between the tumor and surrounding vessels. Metastatic lymph nodes are not well circumscribed and hypo-echoic<sup>[4]</sup>. Simply enlarged nodes may not be invaded by tumor and therefore its malignancy should be confirmed by histological study.

Patients with no evidence of disseminated disease after LS + LUS undergo laparotomy and resectability is again evaluated. At some institutions, diagnostic laparoscopy and laparotomy have to be performed in two different sessions for logistic reasons<sup>[13]</sup>.

### Findings and resectability

The concept of resectability in HCCA related to technical and oncologic variables has evolved with time and may vary from center to center<sup>[2,3,8,14]</sup>.

It is beyond the scope of this article to determine or discuss the concept of resectability for HCCA, but as a general rule it is considered according to the criteria defined by the MSKCC group<sup>[1,15]</sup>. In their studies, tumors were considered unresectable if any of the following conditions were present before surgery or at laparoscopy or laparotomy: peritoneal metastases; discontinuous intrahepatic metastases; involved lymph nodes in the periduodenal, retropancreatic, common hepatic, or celiac nodal basin; locally advanced disease secondary to main portal vein encasement or tumor extension to second-order biliary radicals bilaterally; or unilateral tumor extension to secondary biliary radicals with contralateral lobar atrophy or contralateral portal vein involvement. The presence of involved proximal porta hepatis lymph nodes is not a contraindication to resection. In addition, selected patients undergoing exploration with involvement of the portal vein generally undergo hepatectomy with resection of the portal vein.

## AVAILABLE EVIDENCE

There are not randomized trials that set the role of LS or LUS in the detection of unresectable disease in HCCA. Operable HCCA is not a common condition, and therefore the studies generally extend for long periods of time.

Table 1 summarizes the results of those studies assessing the efficacy and accuracy of SL in detecting unresectable disease in HCCA. In those series that include other kind of tumors, only the subset of patients with

**Table 1** Available studies assessing the efficacy and accuracy of staging laparoscopy in detecting unresectable disease in hilar cholangiocarcinoma

Ref.	n	LUS (yes/no)	Efficacy	Overall yield	Accuracy	Patients resected n (%)	Histology (yes/no)	Morbidity/ mortality	Period of study
Tilleman <i>et al</i> <sup>[13]</sup>	110	Yes	41.8%	Whole series: 25% -T1: 9% (2/23) -T2/T3: 36% (12/33)	72%	35 (86)	Yes	3%/0%	1993-2000
Weber <i>et al</i> <sup>[15]</sup>	56	Yes	25%		42% (14/33)	23 (41)	Yes	-	1997-2001
Goere <i>et al</i> <sup>[16]</sup>	20	No	25%		45% (5/11)	9 (45)	Yes	6%/0%	2002-2004
Connor <i>et al</i> <sup>[17]</sup>	84	Yes	24% (SL) 41.5% (+ LUS)	25% (5/20)	53%	20 (27)	Yes	-	1992-2003
Ruys <i>et al</i> <sup>[18]</sup>	175	No	14%		32%	89 (51)	Yes	-	2000-2010

LUS: Laparoscopic ultrasound; SL: Staging laparoscopic.

HCCA was selected<sup>[15,16]</sup>. Final diagnostic was confirmed by pathology after resection or by biopsy if tumoral extension was found in all the patients of all referred series<sup>[13,15-18]</sup>. In spite of all diagnostic tools used preoperatively and after undergoing SL  $\pm$  LUS, only 30%-50% of the patients were ultimately amenable to a potentially curative resection.

The efficacy and accuracy of the SL seems to have decreased throughout the years: from 41% to 72% respectively in the 2002 report by Tilleman *et al*<sup>[13]</sup> to 14% and 32% in the most recent report that was published by Ruys *et al*<sup>[18]</sup> in 2011, although in this study LUS was not performed.

With regard to the LUS, Connor *et al*<sup>[17]</sup> found in 2005 that the addition of ultrasonography increased the yield of the exploration from 24% to 41.5%. Nevertheless there is not a consensus about its use, and in some recent series LUS is not routinely used<sup>[16,18]</sup>.

The group of the MSKCC analyzed specific subgroups of patients with HCCA in an effort to identify patients with high risk of holding occult unresectable disease. In this study, accrued over a relatively short period of time (4 years) patients were classified according to a preoperative T stage system<sup>[11]</sup>. They found that the yield of SL was greater (36%) in T2-T3 tumors than in T1 tumors (9%)<sup>[15]</sup>.

Finally, there are no mortality cases reported, and morbidity-when reported-is low (3%-6%).

## PRESENT AND FUTURE OF SL IN HCCA

Preoperative evaluation of any stenosis or tumour mass at the hepatic confluence remains a significant challenge despite continuous improvements in non-invasive radiological techniques. It is sometimes difficult to differentiate between gallbladder cancer (GBC) and HCCA: in a cohort of 110 patients with supposed proximal bile duct obstruction there was doubt about the localization of the tumour in at least 20% of the cases<sup>[13]</sup>. But the difficulties lie not only in identifying the exact origin, but also the malignant or benign character of the lesion. In the referred series, resection was performed in 13 patients who turned out to have benign disease after histopathologic examination of the specimen<sup>[13]</sup>. And this is not uncommon: A previous study of 132 resections

for presumed proximal bile duct malignancy reported a 15% false positive rate<sup>[19]</sup>. Finally, most of the causes of unresectability in HCCA constitute features difficult to determine preoperatively with imaging techniques such as small peritoneal metastasis, lymph node involvement or local ingrowth. That is why HCCA assessment constitutes one of the most difficult tasks of the hepatobiliary specialized radiologists.

SL will be useful in those patients with unsuspected extended disease in whom a nonsurgical palliative procedure is of benefit, and therefore could avoid an unnecessary laparotomy. HCCA patients would therefore, at least theoretically, benefit largely from SL: there is a high rate of undetected tumoral extension and there is currently enough evidence of the benefit of the percutaneous or endoscopic placement of self expanding metal stents for palliation of unresectable cases<sup>[20]</sup>. Nevertheless, as explained below, its yield is not as high as we could expect and lower than for other hepatobiliary malignancies. But before, we will discuss about the possibility of port-site metastasis, as it was a great concern at the early beginnings of the technique. Large series of different types of oncological resection procedures have confirmed the safety of the laparoscopic approach. The rate of incisional recurrence after open surgery seems to be similar to the port-site recurrence observed. To our knowledge, there are no reported any port-site recurrence after SL in HCCA.

Regarding the evidence for the diagnostic yield of SL in HCCA, there are scarce available data, and the published series are extremely variable. Cholangiocarcinoma is a rare disease, accounting for less than 1% of all human malignancies<sup>[1]</sup>. Many clinical series, therefore, extend over a prolonged period of time, often greater than 20 years<sup>[21-23]</sup>. With regard to SL role (Table 1), one of the largest study, with 84 patients, was performed over an 11-year period<sup>[17]</sup>, during which the quality of imaging undoubtedly varied<sup>[4]</sup>. Moreover, as reported by some authors<sup>[17]</sup>, SL may have not been used universally, but with a selective approach, what makes it even more difficult to determine the real global diagnostic yield of this technique.

We find an additional difficulty when interpreting the results of the series. Most of the studies include different types of hepatobiliary<sup>[15,16]</sup> or pancreatobiliary<sup>[9,14,24,25]</sup> malignancies, and there are few studies fo-



cused exclusively in SL in HCCA<sup>[17,18]</sup>. This makes it difficult to extract the information specifically regarding HCCA. Nevertheless, we can get some interesting information from this apparent jumble of data. Although all of them are bile duct tumors (HCCA, GBC or distal cholangiocarcinomas), they have different patterns of spread that will affect their respective SL diagnostic yields. In this context, the worst rates are for HCCA<sup>[16]</sup>. In 2002 the MSKCC published an analysis of 100 patients with biliary cancers (44 GBC and 56 HCCA). The diagnostic yield in GBC was significantly superior than that obtained for HCCA (48% *vs* 25%)<sup>[15]</sup>. This is due to their particular spread tendency: GBC tends to spread with peritoneal and liver metastases, whereas the most common cause of unresectability in HCCA was vascular invasion and lymph node metastasis. SL easily identifies the first ones, but the second ones are often missed.

Due to the low overall efficacy to identify unresectable disease in HCCA (less than 30%), the MSKCC group proposed in 2001 performing SL only in those patients with higher risk of holding unresectable disease according to a preoperative staging system<sup>[1]</sup>. In their study, those patients with locally advanced but potentially resectable HCCA, the yield of laparoscopy was greater, 36% (12/33, T2/T3 tumors) *vs* 9% (2/23, T1 tumors)<sup>[15]</sup>. T2 and T3 patients would constitute therefore the HCCA patients that should undergo LS before surgical exploration.

The most recent available evidence in this subject is from 2011. Ruys *et al*<sup>[18]</sup> published a study of 175 patients with suspected HCCA who underwent SL during the past decade. As shown in Table 1, the overall yield of SL decreased from an average 25% of earlier reports to 14%. The authors consider this is likely the result of imaging techniques evolution and improvement during this period of time and therefore they conclude that the place of SL in the workup of patients with HCCA should be reconsidered. As this is the largest and more recent series published to date, it is worth taking a closer look at it. At final pathology, 12 patients showed benign disease, fact that also has an impact in the yield. Reviewing data of Ruys *et al*<sup>[18]</sup>, LUS was only performed in four (out of 175) patients. At laparotomy, they identified several unresectable cases that they declare would have been spared with a more extensive SL: twenty-one patients were identified with distant positive lymph nodes precluding a curative resection and an additional 13 patients with positive lymph nodes nearby the celiac trunk or common hepatic artery would have increased the yield of SL to 20%. It is not known if this disease could have been identified if SL was performed more thoroughly by a highly experienced surgeon or with the addition of LUS. This is a very interesting study, but the facts above discussed recommend that not definitive conclusions should be drawn from this apparent drop of yield. Nevertheless, the proportion of patients that were ultimately resected raised to 51% as opposed to 27%-45% in previous studies.

With regard to the use of LUS, it seems reasonable

that as it offers additional information, its use would therefore increase the yield of SL. In fact, several early reports soon showed this ability to increase the sensitivity of SL<sup>[9-11]</sup> in hepatobiliary and pancreatic cancers. In the study from Edinburgh<sup>[17]</sup>, LUS increased the diagnostic efficacy from 24.3% to 41.5%. As shown in Table 1, 20 patients out of 84 were identified as having unresectable disease by LS alone. Adding LUS, a further 14 patients were deemed unresectable (one with intraparenchymal metastases and 13 due to locally advanced disease). Nevertheless, this is not a consistent finding, and it remains controversial since Tillemann *et al*<sup>[13]</sup> reported a limited value of LUS in a series of 110 patients with malignant proximal bile duct obstruction (that includes an undetermined number of patients with GBC). LUS was performed in 74 patients, 12 of whom already had histologically proved metastases detected on SL alone. Among the other 62 patients, metastasis was suspected in 11 patients and locally extensive disease in eight, but histologic proof could only be obtained in one patient. The authors consider that LUS is a waste of time, as in their experience approximately half of the time was used for the LUS imaging. They explicitly recommend diagnostic laparoscopy without LUS<sup>[13]</sup>. Other authors consider the findings of LUS difficult to interpret<sup>[24]</sup>, and as previously said, some authors have abandoned its use.

A final comment about logistic aspects. There are two different subset of patients to consider. The first group of patients are those with apparent resectable disease who would need preoperative external drainage or portal vein embolization. In them, SL should be performed prior to these procedures. The early detection by SL of occult advanced disease can avoid unnecessary invasive procedures and lead to immediate institution of palliative care<sup>[16]</sup>. The second group are those patients with a potentially resectable HCCA and no need of preoperative procedures. In them, SL should be followed by laparotomy in the same session, but in some hospitals this is not always possible. As reported by Tillemann *et al*<sup>[13]</sup>, in those patients who undergo a “delayed” laparotomy, the total hospital stay was longer, as they are eventually admitted a second time. As a consequence, the potential financial benefit was not as great as would be possible.

In summary, the precise role of SL and LUS in the preoperative workup of HCCA remains unclear. What seems clear is that its yield has decreased in last years and therefore there is an agreement that its universal use shouldn't be recommended anymore. There is a general consensus to perform LS only in selected patients with higher risk of holding unresectable disease (T2/T3 or Bismuth type 3/4 and patients with suspicion of metastases). It would also be recommended in patients with potentially resectable disease who would need preoperative invasive procedures. Finally, SL should be performed preceding laparotomy in one session. Further studies on the benefit of SL and LUS in this subset of HCCA patients are warranted.

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## Risk factors and classifications of hilar cholangiocarcinoma

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Kettering cancer center proposes a staging system according to three factors related to local tumor extent: the location and extent of bile duct involvement, the presence or absence of portal venous invasion, and the presence or absence of hepatic lobar atrophy. The TNM classification, besides the usual descriptors, tumor, node and metastases, provides additional information concerning the possibility for the residual tumor (R) and the histological grade (G). Recently, in 2011, a new consensus classification for the Perihilar cholangiocarcinoma had been published. The consensus was organised by the European Hepato-Pancreato-Biliary Association which identified the need for a new staging system for this type of tumors. The classification includes information concerning biliary or vascular (portal or arterial) involvement, lymph node status or metastases, but also other essential aspects related to the surgical risk, such as remnant hepatic volume or the possibility of underlying disease.

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### Abstract

Cholangiocarcinoma is the second most common primary malignant tumor of the liver. Perihilar cholangiocarcinoma or Klatskin tumor represents more than 50% of all biliary tract cholangiocarcinomas. A wide range of risk factors have been identified among patients with Perihilar cholangiocarcinoma including advanced age, male gender, primary sclerosing cholangitis, choledochal cysts, cholelithiasis, cholecystitis, parasitic infection (*Opisthorchis viverrini* and *Clonorchis sinensis*), inflammatory bowel disease, alcoholic cirrhosis, nonalcoholic cirrhosis, chronic pancreatitis and metabolic syndrome. Various classifications have been used to describe the pathologic and radiologic appearance of cholangiocarcinoma. The three systems most commonly used to evaluate Perihilar cholangiocarcinoma are the Bismuth-Corlette (BC) system, the Memorial Sloan-Kettering Cancer Center and the TNM classification. The BC classification provides preoperative assessment of local spread. The Memorial Sloan-

**Key words:** Hilar cholangiocarcinoma; Klatskin tumor; Perihilar cholangiocarcinoma; Bile duct cancer

**Core tip:** The terminology and classification of Perihilar cholangiocarcinoma (Klatskin tumors) are sometime confusing. In the present revision, we analyze some of the risk factors identified as preneoplastic conditions, and the different systems used for staging these tumors, including the most recent consensus classification promoted by the European Hepato-Pancreato-Biliary Association.

Suarez-Munoz MA, Fernandez-Aguilar JL, Sanchez-Perez B, Perez-Daga JA, Garcia-Albiach B, Pulido-Roa Y, Marin-Camero N, Santoyo-Santoyo J. Risk factors and classifications of hilar cholangiocarcinoma. *World J Gastrointest Oncol* 2013; 5(7): 132-138 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v5/i7/132.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v5.i7.132>



## RISK FACTORS FOR HILAR CHOLANGIOCARCINOMA

Cholangiocarcinoma (CCA) is the second most common primary malignant tumor of the liver after hepatocellular carcinoma. Depending on their location is generally divided into intrahepatic or extrahepatic (distal tumours of the common bile duct and perihilar or Klatskin tumor). The classical description of this tumor corresponds to William Altemeier (1957) and Gerald Klatskin (1965) and is a subtype of CCA that stems from aberrant growth of the ductal epithelium in the extrahepatic biliary tree. Perihilar cholangiocarcinoma (PHC) or Klatskin tumor represents more than 50% of all biliary tract cholangiocarcinomas<sup>[1]</sup>.

In the United States, the incidence of this disease is rare with approximately 3000 cases diagnosed annually, and the age-adjusted incidence of extrahepatic CCA has decreased from 1.08 per 100000 to 0.82 per 100000 individuals over a 20-year period<sup>[2]</sup>. Some observed variations in the incidence of CCA and PHC may be due to a coding misclassification of these tumors in the International Classification of Diseases for Oncology (ICD-O), having been proposed a revision in order to ensure that all PHC are coded topographically to extrahepatic tumours only, rather than as currently to intra- or extrahepatic<sup>[3,4]</sup>.

A wide range of risk factors have been identified among patients with PHC including advanced age, male gender, primary sclerosing cholangitis (PSC), choledochal cysts, cholelithiasis, cholecystitis, parasitic infection, inflammatory bowel disease, alcoholic cirrhosis, nonalcoholic cirrhosis and chronic pancreatitis<sup>[5]</sup>.

One of the most influential and well-established risk factors is PSC. The prevalence of cholangiocarcinoma in patients who have PSC is 5%-15%, with an annual incidence rate of 0.6%-1.5%. In contrast to patients with ulcerative colitis, the time since diagnosis seems to have no importance. In most cases, cholangiocarcinomas are diagnosed within the first 2.5 years after the diagnosis of PSC, and prospective studies have reported that 37% of patients developing cholangiocarcinoma will do so within the first year following the diagnosis of PSC. The median age of diagnosis is in the 5<sup>th</sup> decade of life. At autopsy, CCA has been identified in as many as 40% of patients with PSC<sup>[6]</sup>.

Cholelithiasis is also a known risk factor for both intrahepatic CCA and extrahepatic CCA. In a recent large retrospective review, patients with gallstones who did not have a cholecystectomy performed had a twofold increased incidence of CCA. This increased risk subsides to the equivalent of the normal population 10 years after cholecystectomy<sup>[7]</sup>.

Bile-duct cysts are an established risk factor for CCA. Type I (solitary, extrahepatic) and IV (extrahepatic and intrahepatic) bile-duct cysts have the higher incidence. The lifetime incidence of CCA in these patients ranges from 6% to 30%. The average age at malignancy detection has been reported to be 32 years, which is younger than the age at presentation of CCA in the general popu-

lation<sup>[8]</sup>. The risk of malignancy decreases after complete choledochal cyst excision; however, these patients are still at a increased risk of developing CC compared with the general population.

The hepatobiliary flukes *Opisthorchis viverrini* and *Clonorchis sinensis* are associated with the development of CCA, particularly in Southeast Asia (fivefold increased risk of CCA and an annual incidence of 87 per 100000). They are flat worms that inhabit the bile ducts and, occasionally, the gallbladder and pancreatic duct of mammals. Both parasites increase the susceptibility of cholangiocytes to endogenous and exogenous carcinogens via chronic irritation and increased cellular turnover. Nevertheless, a recent study from Thailand, found that despite the endemicity of *Opisthorchis viverrini* (24.5% prevalence among the adult population), the lifetime risk of CCA is only 5%, which suggest other co-factor must exists, such as role of lifestyle, diet and certain polymorphisms<sup>[9]</sup>.

Another risk factor for CCA that is more common in Asian than Western countries is hepatolithiasis. It has been postulated that prolonged irritation and inflammation of the biliary epithelium by the calculi, bile stasis, and bacterial infections predispose to malignancy. CCA incidence rates of 10% in patients who have hepatolithiasis have been reported<sup>[10]</sup>.

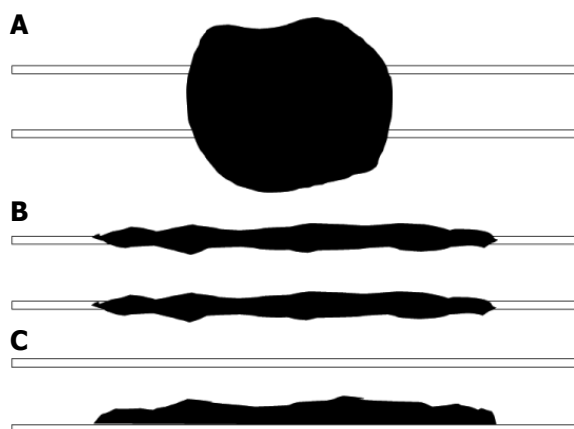
A recent study from China had stressed the importance of metabolic syndrome as potential risk factor for the development of biliary tract cancer. Cholelithiasis, triglycerides, LDL, diabetes, Apolipoprotein A and Apolipoprotein B were significantly associated with extrahepatic cholangiocarcinoma<sup>[11]</sup>.

## CLASSIFICATION OF HILAR CHOLANGIOCARCINOMA

Various terminology and classifications have been used to describe the pathologic and radiologic appearance of cholangiocarcinoma, and each describes a specific aspect of the tumor. However, some of the terminology and classifications are ambiguous and therefore confusing. In 1901, Eggel classified cholangiocarcinomas as nodular, massive and diffuse, like hepatocellular carcinoma. In 1983, Weinbren and Mutum classified cholangiocarcinoma into three types: nodular, sclerosing and papillary. Rosai, in 1996, distinguishes between polypoid and sclerosing forms. In the radiologic literature, hilar and extrahepatic cholangiocarcinomas have been classified as exophytic, infiltrating and polypoid (or papillary)<sup>[12]</sup>.

The Liver Cancer Study Group of Japan proposed in 2000 a new classification based on growth characteristics, with tumors being identified as mass-forming, periductal-infiltrating and intraductal-growing types (Figure 1). This classification describes the gross appearance, growing characteristics, and biologic behavior, and it is helpful for radiologic interpretation. According to this classification, the exophytic or nodular type matches the mass-forming type, the infiltrating or sclerosing type matches the periductal-infiltrating, and the polypoid or papillary





**Figure 1** Morphologic classification of cholangiocarcinoma. A: Mass-forming; B: Periductal-infiltrating; C: Intraductal-growing.

type matches the intraductal-growing type. The prognosis for mass-forming and periductal-infiltrating cholangiocarcinomas is generally unfavorable, whereas the prognosis for intraductal-growing types is much better after surgical resection<sup>[13]</sup>.

CCAs can be classified anatomically as intrahepatic (peripheral), perihilar (Klatskin tumor), or extrahepatic. Perihilar cholangiocarcinoma arises at the bifurcation of the hepatic ducts, whereas intrahepatic cholangiocarcinoma arises from beyond second-order bile ducts. The extrahepatic bile ducts can be further divided into proximal, middle, and distal bile ducts. The proximal extrahepatic bile duct extends from the confluence of the right and left hepatic bile ducts to the level of the cystic duct. The middle portion of the extrahepatic bile ducts extends from the cystic duct to the level of the duodenum. The distal ducts are composed of the bile duct that extends to the level of the ampulla<sup>[14]</sup>.

As in any other type of cancer, a staging system must ideally provide information about the prognosis and natural history of the disease, serve as a guide for therapy, and enable convincing comparisons of therapies among various institutions and over time. In so-called surgical diseases, a staging system is crucial for deciding between an aggressive approach (*i.e.*, chance for cure) and only palliative alternatives. Another criteria for a good staging system is its ability to identify patients for the best type of surgery (*e.g.*, local resection *vs* extensive resection or even liver transplantation).

The three systems most commonly used to evaluate PHC in most parts of the world are the Bismuth-Corlette (BC) system, the Memorial Sloan-Kettering Cancer Center (MSKCC) classification, and the American Joint Committee on Cancer (AJCC)/Union for International Cancer Control (UICC) TNM classification.

### BC classification

The BC classification<sup>[15,16]</sup> provides preoperative assessment of local spread, and classifies Klatskin tumors as Type I (proximal bile duct tumors that do not extend to the bifurcation), Type II (tumors extend to the bifurca-

tion without extension into the intrahepatic bile ducts), Types IIIa and IIIb (occluding the common hepatic duct and the right or left hepatic ducts, respectively), and Type IV (involving the confluence and both the right and left hepatic ducts) (Figure 2).

In a recent study addressed to evaluate the accuracy, sensitivity, prognostic value and impact on the management of patients with Klatskin tumors, Paul *et al*<sup>[17]</sup>, analyzing data of two centers of excellence and a meta-analysis of the literature, found that BC classification had an accuracy rate < 50%, with a low sensitivity for Type III A/III B tumors (in the 30% range), and it is not indicative of survival. Although the BC classification provides the first preoperative assessment of the possibility and extent of surgical resection, decision for laparotomy cannot be based on it, however, because does not include crucial information such as vascular encasement and distant metastases and further preoperative workup has to be made.

Another aspect to consider is that longitudinal spread pattern of a tumor can be related to gross morphology. Papillary tumors frequently present with long-range mucosal spread, while infiltrating tumors tend to show sub-epithelial extension.

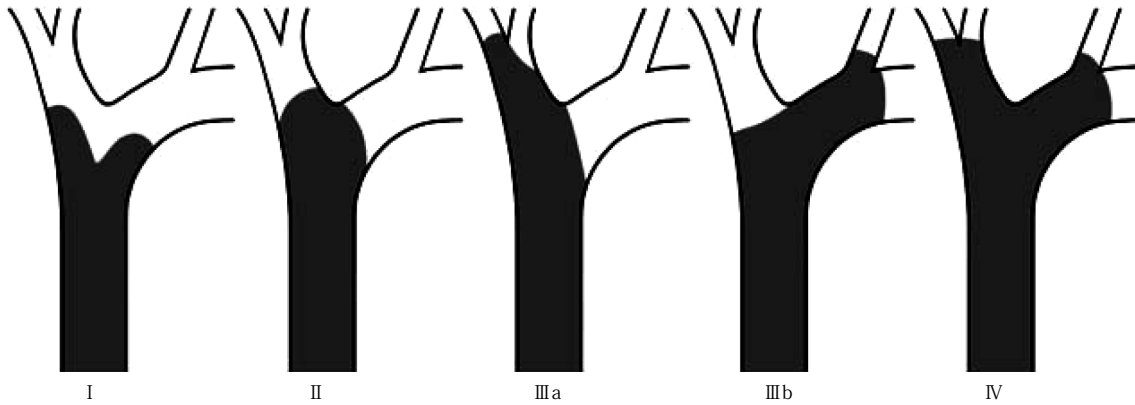
### MSKCC classification

In 1998, the MSKCC, proposed a different stage criteria for hilar cholangiocarcinoma according to three factors related to local tumor extent: the location and extent of bile duct involvement (in agreement to the BC system), the presence or absence of portal venous invasion, and the presence or absence of hepatic lobar atrophy. The initial system comprises four staging groups<sup>[18]</sup>, but was simplified three years later to the definite model which comprises three stage groupings rather than four and represents a simple combining of two stages from the earlier format (Table 1). By taking full account of local tumor extent, the proposed staging system for hilar cholangiocarcinoma accurately predicts, in author's opinions, resectability, the likelihood of metastatic disease, and survival<sup>[19]</sup>.

### TNM classification

The AJCC has recently published new staging criteria for extrahepatic bile duct tumors (Table 2)<sup>[20]</sup>. These tumors were previously grouped into proximal, middle and distal tumors but were considered as a single entity and had single TNM classification. Now, the middle group of extrahepatic bile duct tumors have been removed as the treatment of this group is similar to either proximal or distal group. Currently, extrahepatic bile duct tumors are simply classified as perihilar and distal bile duct tumors. Further, these two subgroups have different TNM staging as their pathology, treatment and prognosis is variable.

Perihilar tumors refer to those located in the extrahepatic biliary tree proximal to the origin of the cystic duct. The early stage (T1) tumor for the extrahepatic bile duct cancers is described as tumor confined to the bile duct wall. On imaging this tumor presents as wall thick-



**Figure 2 Bismuth-Corlette classification of Perihilar (Klatskin) tumors.** Type I : Proximal bile duct tumor that do not extend to the bifurcation; Type II : Tumor extend to the bifurcation without extension into the intrahepatic bile ducts; Type III a: Tumoral occlusion of the common hepatic duct and the right hepatic duct; Type III b: Tumoral occlusion of the common hepatic duct and the left hepatic duct; Type IV : Tumor involving the confluence and both the right and left hepatic ducts.

**Table 1 Memorial Sloan-Kettering Cancer Center classification**

Stage	Criteria
T1	The tumor involves the biliary confluence with unilateral involvement up to secondary biliary radicles. There is no portal vein involvement or liver atrophy
T2	The tumor involves the biliary confluence with unilateral involvement up to secondary biliary radicles. There is ipsilateral portal vein involvement or ipsilateral hepatic lobar atrophy
T3	The tumor involves the biliary confluence with bilateral involvement up to secondary biliary radicles, unilateral extension to secondary biliary radicles with contralateral portal vein involvement, unilateral involvement up to secondary biliary radicles with contralateral hepatic lobar atrophy, or main/bilateral portal vein involvement

ening of the bile duct. The low (fat) attenuation of the periductal fat is preserved. The T2 tumors are cancers that invade the periductal fat (T2a) or the liver (T2b). The proximal extrahepatic bile duct tumors may extend to the portal vein or hepatic artery.

The unilateral vascular extension is considered T3, whereas more advanced extension is considered T4. The latter (T4) includes extension into the main portal vein, common hepatic artery, contralateral vascular extension, and involvement of secondary biliary radical. Hepatic parenchymal involvement is now classified as T2 instead of T3, as patients with hepatic parenchymal involvement alone have a better prognosis compared to those with unilateral vascular involvement<sup>[21]</sup>. Distal bile duct tumors refer to those located between the junction of the cystic duct-bile duct and the ampulla of Vater. Previously these had the same AJCC classification as the proximal tumors but it has been recognized that these tumors have significant differences in the anatomy compared to the proximal lesions, which affect their resectability. Hence, these lesions have a separate TNM classification.

The nodal staging of bile ducts tumors is also different for the proximal and distal bile duct tumors. The proximal bile duct tumors have three classifications (N0, N1 and N2). N1 nodes refer to regional nodes such as

**Table 2 Perihilar bile duct tumors (American Joint Commission on Cancer Staging 7<sup>th</sup> edition)**

T1	Tumor confined to bile duct histologically
T2a	Tumor beyond the wall of bile duct into adjacent fat
T2b	Tumor beyond the wall of bile duct into liver parenchyma
T3	Tumor invades ipsilateral portal vein (R or L) or hepatic artery (R or L)
T4	Tumor invades (1) Main portal vein or its branches bilaterally (or) (2) Common hepatic artery (or) (3) The second-order biliary radicals bilaterally (4) Unilateral second-order biliary radicals with contralateral portal vein or hepatic artery involvement
Node	
Nx	Regional lymph nodes cannot be assessed.
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis (including nodes along the cystic duct, common bile duct, hepatic artery, and portal vein)
N2	Metastasis to periaortic, pericaval, superior mesenteric artery, and/or celiac artery lymph nodes
Metastasis	
M0	No distant metastasis
M1	Distant metastasis
Tumor stage AJCC staging 6 <sup>th</sup> edition	
0	Tis, N0, M0
I	T1, N0, M0
II	T2a-b, N0, M0
IIIa	T3, N0, M0
IIIb	T1 or T2 T3, N1, M0
IVa	T4, N0 N1, M0
IVb	Any T, N2, M0 Any T, any N, M1

AJCC: American Joint Committee on Cancer; R: Right; L: Left.

hilar, cystic, pericholedochal, hepatic artery, portal and posterior pancreaticoduodenal. The N2 nodes refer to distant nodes such as celiac, superior mesenteric artery, and para-aortic nodes. The presence of N2 nodes may disqualify the patient from potential curative surgery. On imaging, there are no definite criteria for the diagnosis of malignant nodes<sup>[22]</sup> and for this reason the presence of

**Table 3** Consensus classification (European Hepato-Pancreato-Biliary Association)

Label	Side location	Description
Bile duct (B)		
B1		Common bile duct
B2		Hepatic duct confluence
B3	R	Right hepatic duct
B3	L	Left hepatic duct
B4		Right and left hepatic duct
Tumor size (T)		
T1		< 1 cm
T2		1-3 cm
T3		≥ 3 cm
Tumor form (F)		
Sclerosing		Sclerosing (or periductal)
Mass		Mass-forming (or nodular)
Mixed		Sclerosing and mass-forming
Polypoid		Polypoid (or intraductal)
Involvement (> 180°) of the portal vein (PV)		
PV0		No portal involvement
PV1		Main portal vein
PV2		Portal vein bifurcation
PV3	R	Right portal vein
PV3	L	Left portal vein
PV4		Right and left portal veins
Involvement (> 180°) of the hepatic artery (HA)		
HA0		No portal involvement
HA1		Proper hepatic artery
HA2		Hepatic artery bifurcation
HA3	R	Right hepatic artery
HA3	L	Left hepatic artery
HA4		Right and left hepatic artery
Liver remnant volume (V)		
V0		No information on the volume needed (liver resection not foreseen)
V%	Indicate segments	Percentage of the total volume of a putative remnant liver after resection
Underlying liver disease (D)		Fibrosis Nonalcoholic steatohepatitis Primary sclerosing cholangitis
Lymph nodes (N)		
N0		No lymph node involvement
N1		Hilar and/or hepatic artery lymph node involvement
N2		Periaortic lymph node involvement
Metastases (M)		
M0		No distant metastases
M1		Distant metastases (including liver and peritoneal metastases)

R: Right; L: Left.

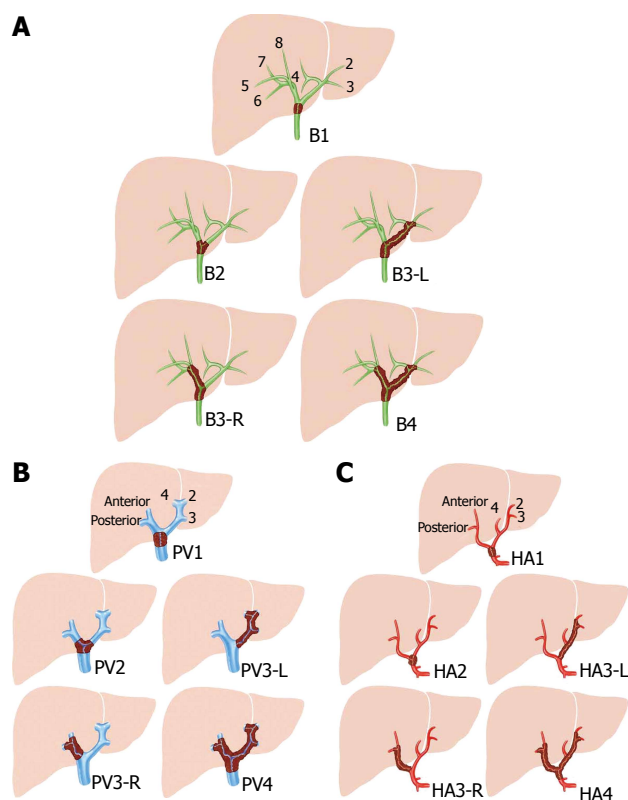
equivocal lymph nodes cannot be used as a criterion for unresectability. A node that is larger than 1 cm in minimum diameter, round in morphology and heterogeneous in attenuation or with central necrosis, is likely to be malignant. Proximity to the primary mass also increases the likelihood of malignancy. The MR diffusion weighted

images provide optimum contrast between lymph nodes and background anatomy. The M-staging for the extrahepatic biliary tumors is the similar for proximal and distal bile duct tumors. Metastases may be seen on computed tomography and magnetic resonance as soft tissue masses in the peritoneum, lungs, adrenals, liver and other sites. It is generally accepted that a fluorodeoxyglucose positron emission tomography (FDG-PET) is useful to detect distant metastases and may lead to change in management in up to 30% of patients.

Besides the stage grouping shown in Table 1, the TNM classification has additional descriptors for the residual tumor (which is labeled “R”): Rx means that the presence of the residual tumor cannot be assessed, R0 represents no residual tumor, R1 reveals a microscopic residual tumor, and R2 denotes a macroscopic residual tumor. In addition, the histological grade (“G”) is expressed as Gx (no assessment), G1 (well differentiated), G2 (moderately differentiated), G3 (poorly differentiated), or G4 (undifferentiated).

The BC classification system is possibly the system most commonly used worldwide to stage PHC, although it fails to provide other key information such as vascular encasement, lymph node involvement, distant metastases and atrophy of a part of the liver. The MSKCC system does not evaluate the presence of nodal or distant metastases or the involvement of the artery. And the TNM staging is mostly used postoperatively and therefore fails to distinguish between the various surgical options, so that its usefulness in the preoperative setting is thus limited.

A consensus conference organized by the European Hepato-Pancreato-Biliary Association in 2007, identified the need for a new staging system for perihilar cholangiocarcinoma<sup>[23]</sup>. For this reason, an international working group was constituted with the aim to design a new staging system and registry for these tumors. The results of this project were published in 2011, proposing a new classification for Klatskin tumors using some parameters from previous staging systems<sup>[24]</sup> (Table 3). The BC classification is kept for the assessment of the bile duct (which is labeled “B” for bile duct or Bismuth); the letters “a” and “b” are omitted and are replaced by “R” (for right hepatic duct) and “L” (for left hepatic duct; Figure 3A). Thus, the label indicating one of the four types (depending on the localization of the tumor) will follow “B”; for example, B2 indicates invasion of the bile duct confluence by the tumor. Additionally, the tumor size should be labeled as T1 (1 cm), T2 (1-3 cm), or T3 (3 cm). The choice of a 3-cm cutoff for T3 is based on accumulating data indicating a better prognosis for smaller tumors; this includes excellent outcomes after liver transplantation in the absence of any extrahepatic spread. The macroscopic form (which is labeled “F”) will also be recorded as the periductal or sclerosing type (sclerosing), the nodular or mass-forming type (mass), or the polypoid or intraductal type (polypoid). Often, a distinction between the sclerosing type and the mass forming type is difficult,



**Figure 3** Consensus classification from the European Hepato-Pancreato-Biliary Association. Involvement of the portal vein or hepatic artery is considered when the tumor encompasses more than 180° of the circumference. A: Biliary involvement (B), based on the Bismuth-Corlette classification; B: Portal involvement; C: Arterial involvement. Adapted from Deoliveira *et al.* [24].

and therefore, a mixed type of tumor is added (mixed).

The next factors providing information about the natural history and the choice of therapy include involvement of the vessels. In this regard, the portal vein is labeled “PV”, and the hepatic artery is labeled “HA”. The addition of “R” or “L” describes the side, right or left, with tumor involvement. It is also important to highlight when both the vein and the artery are free (HA0 and PV0, respectively).

In order to provide information related to the possibility to achieve a R0 resection in cases requiring en bloc resection of the bile duct and major hepatectomy, the staging system include a “V” (remnant hepatic volume) and a “D” (indicate the presence of an underlying disease such as fibrosis, nonalcoholic steatohepatitis, or PSC) labels, both identified as risk factors for surgery.

Lymph nodes are labeled “N”, and classified as N1 (positive periportal or hepatic artery lymph nodes) and N2 for positive para-aortic lymph nodes. Metastases, including liver and peritoneal metastases, are marked as “M”.

The staging should ideally be performed before and after surgery, and it should include all intraoperative information and results from macroscopic and microscopic examinations. In order to promote the use of this new complete, but complex, classification, an on line registry has been implemented and available at [www.cholangioca.org](http://www.cholangioca.org) (Figure 3).

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## Principles of surgical resection in hilar cholangiocarcinoma

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**Key words:** Cholangiocarcinoma; Surgery; Technique

**Core tip:** The aim of this article is to describe the surgical techniques for the treatment of hilar cholangiocarcinoma (HC). In recent years, parenchyma-preserving hepatic resections have been proposed to treat high risk surgical patients without vascular infiltration. This type of liver resection must include segments I, IVb and V. Radical surgery in patients with type I or II tumors should also include a right liver resection, except in the case of papillary HC and in high-risk surgical patients.

### Abstract

The aim of this article is to describe the surgical techniques for the treatment of hilar cholangiocarcinoma (HC). Resection with microscopically negative margin (R0) is the only way to cure patients with HC. Today, resection of the caudate lobe and part of segment IV, combined with a right or left hepatectomy, bile duct resection, lymphadenectomy of the hepatic hilum and sometimes vascular resection, is the standard surgical procedure for HC. Intraoperative frozen-section examination of proximal and distal biliary margins is necessary to confirm the suitability of resection. Although lymphadenectomy probably has little direct effect on survival, inaccurate staging information may influence post resection treatment recommendations. Aggressive venous and arterial resections should be undertaken in selected cases to achieve a R0 resection. The concept of "no-touch proposed" in 1999 by Neuhaus *et al* combine an extended right hepatectomy with systematic portal vein resection and caudate lobectomy avoiding hilar dissection and possible intraoperative microscopic dissemination of cancer cells. More recently minor liver resections have been proposed for treatment of HC. As the hilar bifurcation of the bile ducts is near to liver segments IV, V and I, adequate liver resection of these segments together with the bile ducts can result in cure.

Ramos E. Principles of surgical resection in hilar cholangiocarcinoma. *World J Gastrointest Oncol* 2013; 5(7): 139-146 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v5/i7/139.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v5.i7.139>

### INTRODUCTION

The aim of this article is to describe and discuss aspects of the surgical techniques used in the treatment of hilar cholangiocarcinoma (HC). No exhaustive survey of the results obtained by surgery will be made; this question is addressed in a separate section.

As is the case with the surgical treatment of other tumor diseases, the improvements introduced in recent decades have derived not from evidence obtained in clinical trials but from a better understanding of the pathways of tumor spread<sup>[1-11]</sup>. Furthermore, most of the publications on this subject are studies comparing the results in recent cohorts with historical data. Due to the rarity of HC, most surgical series have a long inclusion period<sup>[12,13]</sup>. As a result, changes in perioperative management techniques over the course of a study can introduce bias. All these features have complicated and delayed the introduction of new techniques and strategies in the surgical treatment of HC.

HC surgery remains one of the most technically challenging operations for hepatobiliary surgeons, due to the complex, intimate, and sometimes variable anatomical relations of the bile duct and vascular structures. Radical resection with a microscopically negative margin (R0) is the only way to cure patients with HC and is associated with marked survival advantages compared to margin-positive resections. Unfortunately, only 50%-70% of patients who undergo surgery are candidates for curative resection<sup>[14]</sup>. Over the last decades, various technical innovations have been introduced in order to increase the chances of achieving a negative resection margin, which is the only prognostic factor under the control of the surgeon. Today, resection of the caudate lobe and part of Couinaud's segment IV, combined with a right or left hepatectomy, bile duct resection, lymphadenectomy of the hepatic hilum and sometimes vascular resection, is the standard surgical procedure for HC.

In the early 1970s, Longmire<sup>[15]</sup> introduced the concept of partial hepatectomy in resection of HC. However, because of the poor postoperative outcomes, hepatic resection was not recommended during the two following decades. Starting in the 1990s, more partial liver resections were performed for HC and were routinely combined with complete excision of the caudate lobe which is now fully accepted<sup>[16-22]</sup>. Likewise, better outcomes were reported with more radical surgery. The Memorial Sloan-Kettering group found that a concomitant hepatectomy resulted in an R0 resection in 78% of the patients and it was the only independent predictor of long-term survival<sup>[14]</sup>. Today the reported resectability rate ranges between 28% and 95% and the radical resection rate varies from 14% to 95%<sup>[23-27]</sup>.

Over this period, Japanese surgeons have adopted a more aggressive approach and have achieved a higher negative margin resection rate<sup>[28]</sup>. They have also published numerous "tricks" to increase the chances of achieving radical resection.

## LAPAROSCOPIC ASSESSMENT

The goal of staging laparoscopy is to exclude peritoneal metastases and small liver metastases, for which other noninvasive tests lack accuracy. van Gulik *et al*<sup>[29]</sup> reported that laparoscopy avoided unnecessary laparotomy in 25%-40% of patients. However, the use of staging or preoperative laparoscopy for HC is not widely accepted. Regimbeau *et al*<sup>[30]</sup> observed that laparoscopic assessment was not routinely performed in France in 2008 and that the accuracy of this procedure even in selected patients appeared to be low. That series included 56 patients and only in one case was resection contraindicated due to peritoneal carcinomatosis. More recently, Ruys *et al*<sup>[31]</sup> evaluated the benefit of laparoscopic assessment in 195 patients treated from 2000 to 2010. They found that the yield and accuracy of laparoscopy were considerably lower than those reported in previous studies including one performed by their own team. Laparoscopy avoided

unnecessary laparotomy in only 14% of patients, with an accuracy of 32%.

Explanations for these changes are the impact of new imaging techniques and a better selection of patients for laparoscopy. After their review, the authors recommended that laparoscopy should be performed only in patients with Bismuth type III and IV and that it should be used preceding laparotomy in a single session.

## INTRAOPERATIVE BIOPSIES OF BILIARY MARGIN

Intraoperative frozen-section examination of proximal and distal biliary margins is necessary to confirm the suitability of resection. If invasive cancer is observed in the examination, additional resection is recommended to complete tumor removal<sup>[24,32]</sup>. However, additional resection of a positive proximal bile duct is difficult, and, usually, only a few extra millimeters can be resected. Some evidence has suggested that this additional resection of the proximal bile duct margin does not confer any survival advantage<sup>[33]</sup>. However, Ribero *et al*<sup>[34]</sup> found that median survival after additional resection of an intraoperative proximal bile duct margin was similar to that observed after primary R0 resections (30.6 *vs* 29.3). Therefore, although the available evidence is inconsistent, it seems advisable to try to complete resection whenever possible. On the other hand, this additional resection is associated with increasing incidence of postoperative biliary fistula. Finally, it should be borne in mind that intraoperative frozen section analysis of proximal bile duct margin is misleading in 9% patients.

## LYMPHADENECTOMY

Lymphadenectomy associated with resection of HC must include lymph nodes, lymphatic channels and nerves surrounding the portal vein and hepatic artery. Nodal invasion beyond the hepatoduodenal ligament, including para-aortic nodal metastases, has a dismal prognosis with a 5-year survival of 0%-12%<sup>[35,36]</sup>. Therefore, routine lymph node dissection beyond the hepatoduodenal ligament is not generally recommended<sup>[36,37]</sup>. Once HC has metastasized to lymph nodes, an extended nodal dissection can provide a more accurate staging of the disease, but cannot improve survival.

The intraoperative finding of lymph node metastases is not considered a reason for abandoning resection when lymph node metastases are confined to the hepatic pedicle or the hepatoduodenal ligament<sup>[29]</sup>. However, tumor positive lymph nodes along the common hepatic artery or celiac axis are usually considered a contraindication for resection.

Lymphadenectomy for HC is unlikely to provide any great clinical benefit. Recently, Kitagawa *et al*<sup>[35]</sup> analysed 110 patients with HC who underwent both regional and para-aortic lymphadenectomy. A median of 24 lymph

nodes were retrieved during surgery. The disease-specific survival of patients with para-aortic lymph node metastasis was similar to M1 patients, suggesting that survival is not influenced by the extent of lymphadenectomy but rather by the presence of metastatic disease. However the Nagoya group<sup>[35]</sup> reported the 5-year survival for patients with para-aortic nodal metastases to be 12.3%. The finding that long-term survival is possible in patients with para-aortic disease encouraged the authors to perform aggressive surgery with extended lymph node dissection in selected patients.

Some authors have reported changes in the extent of lymphadenectomy in successive historical periods. In the initial period<sup>[38]</sup> lymphadenectomy was regional. It was then extended to include para-aortic nodes from the level of the diaphragm to aortic bifurcation, but this was associated with high morbidity. Finally lymphadenectomy was reduced to include the para-aortic nodes from the level of the coeliac axis to the mesenteric inferior vein. However, in the elderly (> 70 years) lymphadenectomy was limited to regional nodes in order to reduce perioperative mortality.

Although lymphadenectomy probably has little direct effect on survival, inaccurate staging information may influence post resection treatment recommendations which in turn have the potential to affect outcome. The studies by the Memorial Sloan-Kettering Center<sup>[39,40]</sup> suggest that a minimum of seven lymph nodes are needed in the surgical specimen to obtain a correct staging.

## VASCULAR RESECTIONS

The role of portal vein resection (PVR) is controversial. Although portal vein bifurcation invasion used to be considered a relative contraindication for resection, more recently some surgeons have advocated a more aggressive approach<sup>[41]</sup>. Despite recent advances in diagnostic imaging techniques, portal vein invasion is still a relatively frequent intraoperative finding. Surgeons should suspect the presence of this invasion if they find severe adhesions between the tumor and the portal vein bifurcation. In this situation, combined resection and reconstruction of the portal vein is necessary to obtain a negative surgical margin.

de Jong *et al*<sup>[41]</sup> recently published the results of an international, multicenter database from seven major hepatobiliary centers. They found that 30-d postoperative mortality was higher in the cohort of patients who underwent concomitant PVR (17.6% *vs* 10.6%,  $P = 0.03$ ). However, no differences in long-term outcome were observed compared to patients who underwent hepatectomy without vein resection. The authors conclude that PVR should be undertaken when necessary to extirpate all disease.

Nagino<sup>[42]</sup> recommend PVR only when the vessel adheres to and cannot be freed from the tumor during skeletonization resection of the hepatoduodenal ligament. In contrast, Neuhaus *et al*<sup>[43]</sup> recommend routine

resection of the portal vein to achieve more radical surgery. This latter strategy will be discussed in another section. However, there are no randomized studies to support it.

Advances in surgical technique have facilitated the performance of hepatic artery resection and reconstruction during surgical treatment of HC. However, most of the studies<sup>[44-46]</sup> have shown negative results and do not recommend a combined resection of the hepatic artery for biliary cancer. However, in 2010, Igami *et al*<sup>[47]</sup> reported their experience with major hepatectomies with resection and reconstruction of the hepatic artery. In this series of 53 patients (18%) undergoing concomitant hepatic artery resection with or without PVR, only one patient died in the postoperative period and two survived more than five years after surgery.

## EXTENDED HEPATECTOMY FOR HC: NO-TOUCH CONCEPT

Extended right hemihepatectomy consists of the resection of the right liver, the inferior part of segment IV, the hilar plate, and the entire caudate lobe<sup>[48]</sup>, while extended left hemihepatectomy consists of resection of the left liver, the hilar plate of the right paramedian sector, and most of the caudate lobe. Both are coupled with complete resection of the extrahepatic bile duct and porta hepatis lymphadenectomy. The choice of side is dependent on the predominance of the tumor, but an extended right-hemihepatectomy is indicated for centrally located tumors, because of the length of each hepatic duct, the location of the hilar common bile duct in the hepatoduodenal ligament, the ease of complete caudate lobectomy and portal vein reconstruction, and the frequent involvement of the right hepatic artery<sup>[9,32]</sup>.

### “No-touch” concept

Usually, resection of the portal vein is carried out when the vein is adherent to the tumor and cannot be freed. However, even in cases where negative margins are proven histologically, local or peritoneal recurrence may occur during the follow-up period. One possible reason for recurrence is the microscopic dissemination of cancer cells during dissection of the portal vein in the hilar region, where the bile duct involved lies very close to the portal vein. In fact, the distance between the tumor and the outer layer of the adventitia of the portal vein is less than 1 mm, even in cases without portal infiltration<sup>[6]</sup>. What is more, the majority of hilar malignancies have microscopic perineural infiltration of the tumor.

Ebata *et al*<sup>[6]</sup> reported that the intraoperative macroscopic diagnosis of portal infiltration, regardless of microscopic diagnosis, was a significant prognostic factor. This result probably confirms that exposure of the tumor may occur during portal dissection, even in a case without microscopic infiltration. In the operative field microscopic invasion cannot be distinguished from adhesion and perivascular fibrosis<sup>[49]</sup>.



The concept of no-touch was proposed in 1999 by Neuhaus *et al*<sup>[50]</sup> as a result of a multivariate analysis of prognostic factors in 100 resected patients. Surgical radicality, lymphangiosis carcinomatosa, perineural sheath infiltration and histopathological grading were identified as independent prognostic variables for the entire group of patients. However, in patients with curative resection, the only independent prognostic variable was an additional resection of the portal vein bifurcation. After this, the authors decided to apply the principles of no-touch techniques to HC and combined an extended right hepatectomy with PVR and caudate lobectomy. This technique avoids the dissection of the right hepatic artery, which can easily be infiltrated by tumor, and obtains a wide tumor-free biliary margin, since the left hepatic duct measures up to 5 cm.

The goal of a no-touch technique and an *en bloc* resection can be achieved by placing vascular clamps on the left portal vein branch within the umbilical fissure as well as on the portal vein trunk, directly above the pancreatic head, and dividing the two vessels without dissecting the portal vein bifurcation. This strategy is facilitated by the anatomical characteristics of the left portal vein which runs transversely from the bifurcation to the umbilical portion. After this, an end-to-end venous anastomosis is performed. This reconstruction straightens the portal vein, avoiding the kinking frequently observed after right hepatic resections. Depending on the extent of tumor growth to the left, it may be impossible to keep the whole of segment IV. This increases the risk of postoperative liver insufficiency, but reduces the number of biliary orifices to anastomose.

In 2012, Neuhaus *et al*<sup>[43]</sup> compared the oncological results of hilar *en bloc* resection to that of major hepatectomy. The 5-year survival of patients who underwent *en bloc* resection was significantly superior (58% *vs* 29%,  $P = 0.021$ ).

## LEFT RESECTIONS

Right or extended right hepatectomy is not indicated in cases of HC extending far to the left, with atrophy of segments II and III and with vascular complications in the left hemiliver. In these situations left resections are indicated and represent about 25%-30% of all resections. Left hepatectomy is considered to be a more complicated procedure, than right hepatectomy<sup>[8,51]</sup> and requires greater skill, especially in cases involving PVR and reconstruction. Resecting the portal vein bifurcation when performing a left trisectionectomy is substantially more difficult because of the relatively short course of the right portal vein before branching. Surgical resection for Bismuth-Corlette type IIIb tumor with involvement of the portal vein bifurcation may not be feasible even in specialized centers because of the difficulty of portal vein reconstruction.

The distance from the principal biliary bifurcation to the sectional ramification in the right liver is much

shorter than in the left<sup>[52]</sup>. Furthermore, there are many anatomical variations in the right sectional bile ducts<sup>[53]</sup>. These anatomical issues may increase the difficulty of achieving tumor-free stumps for right sectional ducts during left hepatectomy compared with right hepatectomy. Furthermore, the presence of more complex biliary anastomoses increases the risk of postoperative biliary leakage.

Another oncological problem with left or left-extended hepatectomy is the need to preserve the right hepatic artery and the right portal vein, which increases the risk of tumor cell dissemination.

To confirm whether a predominantly left-sided tumor is resectable with a left trisectionectomy, the surgeon can apply a combination of manual palpation, intraoperative ultrasound and dissection along the posterior aspect of the right portal pedicle. These maneuvers could help to determine whether the tumor has extended to the posterior division of the right pedicle<sup>[54]</sup>. Lowering the hilar plate would be very useful in this exploration, because the division occurs intrahepatically; however, this dissection would be too close to the boundaries of the tumor and is not recommended.

If the right hepatic artery is infiltrated by the tumor, it must be resected *en bloc* to achieve a radial R0 resection. In this situation reconstruction of the right hepatic artery is difficult and the risk of technical failure is high. Based on the knowledge of spontaneous arterial revascularization of the liver after ligation of the proper hepatic artery, it has been proposed that pre-operative embolization of the proper hepatic artery induces development of arterial collaterals through the hepatic ligaments, providing additional arterial supply to the liver. This will facilitate the performance of a R0 resection, as the proper, left and right hepatic arteries could be totally resected without vascular anastomosis.

Yasuda *et al*<sup>[55]</sup> reported the preoperative arterial embolization of the proper hepatic artery in six patients. In all patients, arterial flow signals were detected in the liver with Doppler ultrasonography. Three weeks after embolization, surgery was performed and in all cases a R0 resection was achieved. During surgery, intraoperative Doppler ultrasonography confirmed collateral arterial blood flow in the right anterior and posterior segmental branches of the right hepatic artery. During dissection of the hepatoduodenal ligament, the bile duct can be dissected in the right liver without risk of injury to accompanying arterial branches. However, mobilization of the right liver or division of ligaments must be avoided to preserve arterial revascularization.

In the study by Shimizu *et al*<sup>[56]</sup> R0 resection was achieved in all seven patients who underwent right trisectionectomy, but in only eight of 13 patients (61.5%) undergoing left trisectionectomy. This suggests that a more extended resection from the right side, but not from the left, may provide greater potential for cure.

Some authors<sup>[57]</sup> consider that extended left hepatectomy increases the extent of resection in the periphery

of the liver, but that the oncological benefit in the perihilar region is very limited. However Nagino<sup>[42]</sup> reported that left trisectionectomy increased the number of negative proximal ductal margins compared with left hepatectomy, leading to a high proportion of R0 resections, and improving survival for patients with advanced left-sided perihilar cholangiocarcinomas<sup>[58]</sup>. Therefore, these authors recommend left trisectionectomy in such cases, even if the tumor is deemed to be resectable by left hepatectomy.

Despite the difficulties associated with left liver resections, no other treatments achieve survival rates comparable to those of surgical resection. Therefore left or extended left hepatectomy should be aggressively performed for type IIIb tumor if curative resection is possible, even in cases with portal involvement.

## STRATEGY WITH BISMUTH TYPE I AND II PATIENTS

Some authors<sup>[12,14,27]</sup> have considered that patients with Bismuth type I or II tumors can be treated with local or hilar resections including the extrahepatic suprapancreatic biliary tract. However, others<sup>[10]</sup> have recommended a left hepatectomy, because this procedure affords high resectability, is safe and provides good quality of postoperative life. Finally, others<sup>[13,32]</sup> support the indication of a right hepatectomy because the right hepatic artery passes behind the common hepatic duct and, therefore, can be infiltrated by cancer.

Bismuth type I and II HCs appear less advanced on cholangiography and are easier to resect than Bismuth type III and IV tumors. Therefore hilar resection is the procedure preferred by many surgeons. However, loco-regional recurrence may be frequent<sup>[12,15]</sup> even after R0 resections. Moreover, Seyama *et al*<sup>[26]</sup> reported better prognosis in patients with Bismuth type I and II tumors who underwent right hepatectomy with caudate lobectomy. Histologic evaluation of the right hepatic artery showed that its infiltration is infrequent, but the distance between the edge of the cancer and the arterial adventitia was very short (1 mm in many cases). Therefore without right hepatectomy the resection margin could have been positive.

In the opinion of Ikeyama *et al*<sup>[59]</sup> the surgical approach to Bismuth type I and II HCs should be based on the macroscopic tumor type seen in the preoperative study. For nodular and infiltrating HCs, right hepatectomy offers the best long-term survival, whereas for papillary tumor bile duct resection with or without limited hepatectomy is adequate unless spread of superficial cancer is discovered preoperatively.

## PARENCHYMA PRESERVING SURGERY

A reduction in morbidity and mortality after liver resection is the key strategy for improving the results of surgical treatment of HC.

Minor liver resection (three segments or fewer, according to the Couinaud nomenclature) may be one way to resolve the problem of the high mortality after major liver resections. As the hilar bifurcation of the bile ducts is near to liver segments IV, V and I, adequate liver resection of these segments together with the bile ducts can result in cure. For early tumor stage, minor resection of segments I, IVb and V has been performed to excise the tumor with adequate margins; this is termed "central liver resection" by some authors<sup>[60]</sup>.

A central hepatectomy for HC can preserve up to 35% more functional liver parenchyma than an extended hepatectomy. However, it has not been widely accepted as an alternative to extended hepatectomy because of its uncertain oncological equivalence and greater technical complexity<sup>[61,62]</sup>.

The study by Chen *et al*<sup>[63]</sup> did not find differences in cumulative survival rates between major and minor liver resection in patients with HC. Furthermore, major liver resection was associated with higher operative mortality and morbidity rates than minor resection. Chen *et al*<sup>[63]</sup> hold that major resections should be reserved for Bismuth-Corlette type III HC with vascular invasion, or type IV HC. In central liver resection for HC, many intrahepatic bile ductal openings are left behind, making the reconstruction very difficult; this is the main disadvantage of this procedure. However, using their own technique of hepatojejunal anastomosis the same authors reported a bile leak rate of only 14%<sup>[63]</sup>.

## OTHER SURGICAL "TRICKS"

### Resection of middle hepatic artery in right hepatectomy

Frequently, the middle hepatic artery (MHA) runs in close proximity to the HC. In this case, preservation of this artery in a right hepatectomy may result in a positive resection margin. On the other hand, interruption of the arterial flow could cause postoperative complications related to biliary ischemia such as the disruption of the bilioenteric anastomosis and liver abscess.

A retrospective study by Hirano *et al*<sup>[64]</sup> investigated the anatomical variations of the MHA and also assessed the safety of resection of the MHA combined with right hepatectomy, caudate lobectomy, and bile duct resection. In this study of 61 patients with hilar biliary malignancies, the perioperative outcomes in patients in whom the MHA was resected were similar to those in whom it was preserved.

Anatomic study of the microcirculation of the liver revealed that the intrahepatic bile ducts are fed by a dense surrounding vascular plexus arising from the hepatic artery<sup>[65]</sup>. After a right hepatectomy, the peribiliary plexuses of the bile ducts from the left medial and lateral sections may retain their connections through the plate system, compensating for any loss of arterial blood supply. Compensatory arterial blood supply to the area fed by the interrupted artery may also derive from intrahepatic interconnecting arterial pathways or vessels connecting the hepatic artery and the portal system.

### Portal vein arterialization

PVA has been used in patients with portal hypertension, liver transplantation and acute liver failure to solve the occlusion of the hepatic artery. In the context of HC this procedure can be used to allow radical resection in patients requiring an extended left hepatectomy who present encasement of the right hepatic artery<sup>[66]</sup>. The encasement of the hepatic artery can prevent its resection and anastomoses. The objective of the PVA is to ensure adequate oxygen delivery to hepatocytes and biliary ducts. Animal experiments have suggested that PVA can improve the microcirculation in the liver, but a sustained increase in portal pressure can promote hepatocyte apoptosis and inhibit liver regeneration<sup>[67]</sup>. Anastomoses with small arteries (< 3 mm) should reduce the risk of severe portal hypertension. PVA can be considered a salvage procedure in special situations<sup>[68]</sup>, but has some drawbacks that limit its indications.

### CONCLUSION

The goal of surgical treatment of HC should be an R0 resection. The planning of surgery should take into account the difficulties in establishing tumor boundaries intraoperatively and the close proximity to certain anatomical structures such as the caudate lobe and the right hepatic artery. Therefore, potentially radical intervention should include resection of the bile duct with lymphadenectomy of the hepatic hilum and right hepatectomy including the caudate lobe and part of segment IVb. There is evidence that systematic resection of the portal bifurcation can decrease the risk of loco-regional recurrence. However, not all authors agree on this point.

When the HC mainly involves the left liver (IIIb type) a left or left-extended hepatectomy should be performed. These interventions are technically more difficult and potentially less radical. In selected patients, resection and reconstruction of the right hepatic artery may offer the possibility of radical surgery in patients with tumors that mainly affect the left liver. Portal arterialization may be a salvage procedure in these patients when resection and reconstruction of the right hepatic artery is not possible.

In recent years, parenchyma-preserving hepatic resections have been proposed to treat high risk surgical patients without vascular infiltration. This type of liver resection must include segments I, IVb and V. Radical surgery in patients with type I or II tumors should also include a right liver resection, except in the case of papillary HC and in high-risk surgical patients.

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## Outcome of surgical resection in Klatskin tumors

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### Abstract

Cholangiocarcinomas are the second most frequent primary hepatic malignancy, and make up from 5% to 30% of malignant hepatic tumours. Hilar cholangiocarcinoma (HCC) is the most common type, and accounts for approximately 60% to 67% of all cholangiocarcinoma cases. There is not a staging system that permits us to compare all series and extract some conclusions to increase the long-survival rate in this dismal disease. Neither the extension of resection, according to the sort of HCC, is a closed topic. Some authors defend limited resection (mesohepatectomy with S1, S1 plus S4b-S5, local excision for papillary tumours, *etc.*) while others insist in the compulsoriness of an extended hepatic resection with portal vein bifurcation removed to reach cure. As there is not an ideal adjuvant therapy, R1 resection can be justified to prolong the survival rate. Morbidity and mortality rates changed along the last decade, but variability is the rule, with morbidity and mortality rates ranging from 14% to 76% and from 0% to 19%, respectively. Conclusion: Surgical resection continues to be the main treatment of HCC. Negative resection margins achieved with major hepatic resections are associated with improved outcome. Pre-resectional management with biliary drainage, portal

vein embolization and staging laparoscopy should be considered in selected patients. Additional evidence is needed to fully define the role of orthotopic liver transplant. Portal and lymph node involvement worsen the prognosis and long-term survival, and surgery is the only option that can lengthen it. Improvements in adjuvant therapy are essential for improving long-term outcome. Furthermore, the lack of effective chemotherapy drugs and radiotherapy approaches leads us to can consider R1 resection as an option, because operated patients have a longer survival rate than those who not undergo surgery.

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**Key words:** Cholangiocarcinoma; Klatskin tumor; Outcome; Pronostic factors; Survival rate

**Core tip:** Klatskin described the specific clinical characteristics in 1965, and the tumor is often referred to as Klatskin tumor. Cholangiocarcinomas (CC) are the second most frequent primary hepatic malignancy. Hilar cholangiocarcinoma (HCC) is the most common type, and accounts most of CC cases. These tumors are slowly growing, and have a tendency to local spread and infrequent distant metastases. The most common presentation is with the onset of jaundice. The majority of HCC are small infiltrating tumors. Long-term survival in patients with HCC depends critically on complete tumor resection. This work is an important update concerning outcome of surgical management in Klatskin tumors.

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### INTRODUCTION

Carcinomas arising from the confluence of the hepatic ducts were first described by Altemeier *et al*<sup>[1]</sup>. Klatskin<sup>[2]</sup>

**Table 1 Staging of perihilar cholangiocarcinoma (Green FL 2002)**

Tumor, nodes and metastases definitions
Primary tumor
Tis Carcinoma <i>in situ</i>
T1 Tumor confined to the bile duct histologically
T2 Tumor invades beyond the wall of the bile duct
T3 Tumor invades the liver, gallbladder, pancreas, and/or ipsilateral branches of the portal vein or hepatic artery
T4 Tumor invades any of the following: main portal vein or its branches bilaterally, common hepatic artery, or other adjacent structures, such as the colon, stomach, duodenum, or abdominal wall.
Regional lymph nodes
N0 No regional lymph node metastasis
N1 Regional lymph node metastases
Metastasis
M0 No distant metastasis
M1 Distant metastasis
Stage grouping
Stage 0 Tis, N0, M0
Stage IA T1, N0, M0
Stage IB T2, N0, M0
Stage IIA T3, N0, M0
Stage IIB T1, N1, M0 T2, N1, M0 T3, N1, M0
Stage III T4, any N, M0
Stage IV Any T, any N, M1

described the specific clinical characteristics in 1965, and the tumor is often referred to as Klatskin tumor. Cholangiocarcinomas (CC) are the second most frequent primary hepatic malignancy and make up from 5% to 30% of malignant hepatic tumors. Hilar cholangiocarcinoma (HCC) is the most common type, and accounts for approximately 60% to 67% of all CC cases (intrahepatic, hilar and distal)<sup>[3,4]</sup>. These tumors are slowly growing, and have a tendency to local spread and infrequent distant metastases. The most common presentation is with the onset of jaundice. The majority of HCC are small infiltrating tumors. Approximately 90% of malignant-appearing hilar strictures prove to be HCC<sup>[5]</sup>.

Adenocarcinoma is the most common histologic subtype. Three morphologic subtypes of cholangiocarcinoma have been described: sclerosing (70%), nodular (20%), and papillary (5%)<sup>[6]</sup>. Characteristics of nodular and sclerosing types may coexist.

Long-term survival in patients with HCC depends critically on complete tumor resection. In the absence of widespread disease, the likelihood of achieving a complete resection requires examination of all factors related to local tumor extent, which increasingly has become possible with non invasive imaging studies<sup>[7,8]</sup>. Tumor location and extent within the biliary tree is only one component. Additional factors that must be addressed relate to radial tumor growth and its impact on adjacent structures, specifically portal venous involvement and consequent hepatic lobar atrophy. Perihilar CC's are focused on because liver resection is required in most cases.

## STAGING AND RESECTABILITY

The TNM staging system of the American Joint Com-

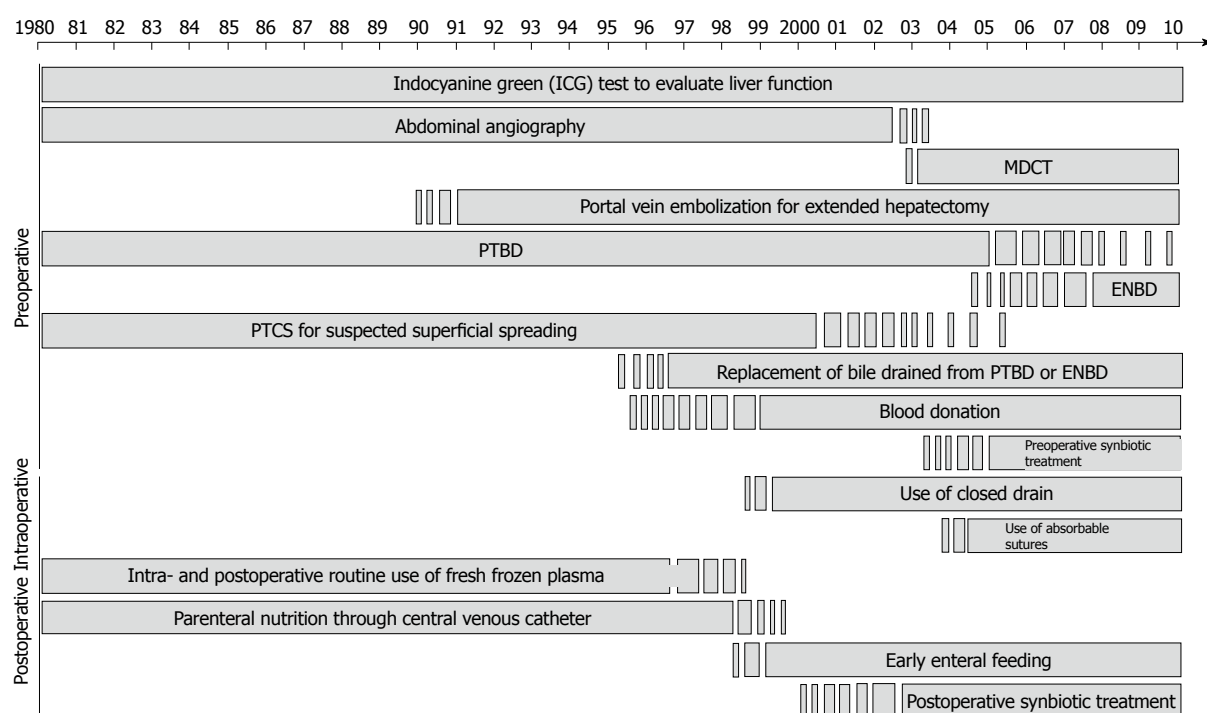
**Table 2 Memorial Sloan Kettering Cancer Centre stage**

Stage	Hilar involvement	Portal vein	Lobar atrophy
T1	Biliary confluence ± 1/2 unilateral extension to second-order biliary radicles	No	No
T2	Biliary confluence ± unilateral extension to second-order biliary radicles	+ Ipsilateral	+ Ipsilateral
	Biliary confluence + bilateral extension to second-order biliary radicles	Yes/No	Yes/No
	Biliary confluence + unilateral extension to second-order biliary radicles	+ Contralateral	Yes/No
T3	Biliary confluence + unilateral extension to second-order biliary radicles with contralateral hepatic lobar atrophy;	Yes/No	+ Contralateral
	Biliary confluence + unilateral/bilateral	Bilateral	Yes/No

mittee on Cancer (AJCC) (Table 1) is the most commonly used for staging of HCC. However, this system is based on histological criteria and does not provide information on the potential for resectability. de Jong *et al*<sup>[9]</sup> conclude that the AJCC T-classification criteria did not stratify patients with regard to prognosis and that depth of tumor invasion is a better predictor of long-term outcome. Besides that, the histologic type of tumor may also modify the staging and type of surgery required<sup>[10]</sup>.

Therefore, other staging systems have been used to predict resectability and evaluate the extent of resection. The modified Bismuth-Corlette (B-C) classification stratifies patients according to the extent of biliary involvement by tumor<sup>[11-13]</sup>. Although it does not incorporate radial tumor extension, it provides a useful preoperative terminology to describe the extension of the hepatic resection that will be necessary to encompass the longitudinal intraductal extension of HCC.

The preoperative clinical T-staging system of the Memorial Sloan Kettering Cancer Centre (MSKCC) (Table 2), as proposed by Jarnagin and Blumgart (MSKCC), defines both the longitudinal and radial extension of HCC, which are critical factors in the determination of resectability<sup>[14,15]</sup>. This staging system incorporates three factors based on preoperative imaging studies: (1) location and extent of ductal involvement; (2) presence or absence of portal vein invasion; and (3) presence or absence of hepatic lobar atrophy. Criteria for unresectable disease include: locally advanced tumor extending to secondary biliary radicles bilaterally, unilateral sectional bile ducts with contralateral portal vein branch involvement, encasement or occlusion of the main portal vein proximal to its bifurcation, and atrophy of one hepatic lobe with contralateral tumor extension to sectional bile ducts. Of note, the right bile duct is shorter and therefore more likely to be involved when the tumor appears at the confluence. Patients who have distant metastases, including metastases to lymph node groups beyond the hepatodu-



**Figure 1** Changes in pre-, intra-, and postoperative management over the course of the study period (With permission. Courtesy of Professor Nimura). ENBD: Indicates endoscopic naso-biliary drainage; MDCT: Multidetector-row computed tomography; PTBD: Percutaneous transhepatic biliary drainage; PTCS: Percutaneous transhepatic cholangioscopy.

denal ligament are also unresectable. By incorporating these criteria of resectability, the MSKCC staging system has been shown to correlate with both surgical resectability and survival, but it still is not the ideal staging<sup>[14]</sup>.

Consequently, the staging systems are not uniform and the prognostic factors that can be obtained do not allow a rigorous comparison between series. Furthermore, many series extend over a prolonged period, frequently longer than 20 years. Indeed, these reports lack a uniform approach to diagnosis, assessment of disease extent and resection, and the evaluation of the results is hence complicated. Also, most studies come from surgical departments and tend to appraise the operating findings and their results, whereas they do not contribute data of all the valued patients, which makes drawing conclusions difficult.

Characteristics of the growth pattern of HCC include: transmural invasion of bile ducts, radial extension into periductal tissue and adjacent structures, and longitudinal extension along the bile ducts in the submucosa<sup>[16]</sup>. The papillary phenotype is associated with better prognosis<sup>[17]</sup>. In contrast, longitudinal spread along the duct wall with microscopic submucosal extension is characteristic of mass-forming and periductal-infiltrating subtypes; this biologic feature often impedes obtaining histologically negative margins<sup>[18]</sup>. These tumors are often accompanied by both direct and lymphatic invasion into the periductal tissues, causing marked fibrosis and infiltration of inflammatory cells. These histologic changes give a macroscopic similarity between the tumor and peritumoral inflammatory changes that make preoperative and intraoperative biopsies diagnostically challenging. Radial extension of HCC is also common, often resulting in invasion of the portal vein, hepatic arteries and the hepatic

parenchyma adjacent to the hilar plate.

When analysing survival according to staging, Li *et al*<sup>[19]</sup> in their audit of 215 patients found that the results from univariate analyses suggest that histological grade, lymph node metastasis, vascular invasion, neuroinvasion, R1 resection and T2 or T3 stage were significant predictors for poor survival rates; by multivariate analysis, only lymph node metastasis and R1 resection were significantly associated with poor survival rates.

Series with more than 100 cases in consulted literature are scarce, and those ones that fulfil this condition cover a prolonged period of time and are retrospective. The resectability rates were highly variable, ranging between 28% and 95%, and curative resection rates ranged between 14% and 95%<sup>[4,14,15,17,20-42]</sup>. Such wide variability of resectability is probably due to heterogeneous methods of patient selection, differences in preoperative imaging techniques, and the broad range of data for inclusion in these studies. The report of DeOliveira *et al*<sup>[43]</sup> where 282 HCC patients are assessed, is one of the biggest published series of only one institution, together with that one of Nagoya group, but it covers a 31-year period and is retrospective<sup>[44]</sup>. Apart from the changes in management over the course of a long period of time, as can be seen in Figure 1, the resectability rate in that study was 62% and R0 resection was achieved only in 19% of cases<sup>[43]</sup>.

Even in high-volume centres, the resectability rate is about 30% of all patients with HCC, with the operative mortality rate ranging from 0% to 15%. After curative resection, the 1-, 3- and 5-year survival rates range from 50% to 70%, 30% to 40%, and 10% to 40%, respectively (Figure 1)<sup>[14,20,43-48]</sup>.

The major determinants of resectability include ex-



tent of vascular invasion, hepatic lobar atrophy, amount of hepatic parenchyma involved, and extent of spread within the biliary tree. Hepatic lobar atrophy with contralateral portal vein or hepatic artery encasement or contralateral tumor extension to secondary biliary radicles may preclude resection. Bilateral hepatic disease and presumed insufficient hepatic reserve preclude resection. Even with current imaging technology, accurate determination of tumor resectability pre-operatively may occur in as few as 60%-74% of patients<sup>[49,50]</sup>. Thus, a number of patients undergoing resection with curative intent will be left with a resultant R1 margin status.

## ADJUVANT TREATMENT

Adjuvant therapy for CC has not been supported by clinical evidence. Recently, gemcitabine has been shown to be active, with response rates of 8%-60% and median survival of 6-16 mo. Therefore, further studies of gemcitabine and of 5-FU plus cisplatin are warranted. For HCC, Cheng *et al.*<sup>[51]</sup> reported better survival for patients with Bismuth types III/IV tumors who received adjuvant radiotherapy after curative resection. Todoroki *et al.*<sup>[41]</sup> also showed a statistically significance of radiotherapy for R1 radical resection of stage IVa HCC. Thus, radiotherapy is potentially beneficial in patients with positive resection margins or unresectable tumors. However, Vern-Gross concluded that there is no benefit with adjuvant therapy in postoperative setting<sup>[52]</sup>.

## PREOPERATIVE BILIARY DRAINAGE

The role of preoperative biliary drainage (PBD) in jaundiced patients remains controversial<sup>[22,45,53,54]</sup>. Actually, most patients undergo biliary drainage prior to referral for resection, despite the lack of data showing a benefit. Clearly, the presence of cholangitis mandates biliary decompression, but there is no proof that routine biliary drainage in all patients facilitates resection or reduces postsurgical morbidity<sup>[55,56]</sup>. On the contrary, the available data would suggest that biliary stents are associated with greater postoperative infection complications<sup>[57,58]</sup>. Previous studies investigating this issue have been criticized for several design flaws, and whether major hepatic resection in the face of biliary obstruction is associated with a greater risk of liver failure or other complications remains an open question<sup>[59]</sup>.

Cherqui *et al.*<sup>[53]</sup> reported the results of major hepatobiliary resection without PBD in 20 patients with biliary cancer. Postoperative liver failure rate was 5%, and mortality was documented in the same patients.

PBD is associated with an increased risk of cholangitis and prolonged postoperative hospital stay, and can impede the ability to determine the extent of tumor during surgery. Cholangitis after PBD has been reported in 20%-60% of cases and may compromise subsequent surgery with patient dropout. Intraoperative bile cultures have been found to be positive in 65% of patients with

PBD, while the rate was 8% in patients without PBD. This may be associated with increased postoperative infections such as wound or intraperitoneal abscesses<sup>[60]</sup>.

However, unrelieved biliary obstruction is associated with hepatic and renal dysfunction and coagulopathy. Most patients with HCC will benefit from PBD of remnant liver to increase post-resection hypertrophy ability. Reported complications in transhepatic percutaneous catheter placement include: haemobilia, pseudoaneurysm of hepatic artery, fistula between hepatic artery and bile duct or between hepatic artery and portal vein, and catheter tract implantation metastases.

Some randomized controlled trials have revealed that biliary diversion does not improve perioperative results and increases infectious complications. But, also, none of these trials has managed to clarify the safety of major hepatic reaction for cholestatic patients with HCC<sup>[53,54]</sup>. The report of Laurent *et al.*<sup>[58]</sup> states some conditions to avoid PBD: onset of jaundice < 2-3 wk, total bilirubin < 200  $\mu\text{mol/L}$ , functional remnant liver (FRL) > 40%, neither endoscopic retrograde cholangiopancreatography nor percutaneous transhepatic cholangiography, and no sepsis. Although the results are not modified for not to drain, in agreement with other authors, undrained patients have a higher postoperative morbidity rate and transfusion requirements, and both facts are important factors of tumor recurrence. Thus, it may depend on each group's experience to determine whether to use PBD or not. It will be taken into consideration that, if the conditions described by Laurent *et al.*<sup>[58]</sup> are not fulfilled, there will be more perioperative transfusion and morbidity if the patient is not drained, which could affect the overall survival and disease-free survival rate.

## PORTAL VEIN EMBOLIZATION

Resection greater than 80% of total liver volume is associated with major complications and prolonged hospital stay in patients with normal liver function, and resection greater than 60% is associated with an increase of major complications, postoperative hepatic insufficiency and mortality in patients with impaired liver function due to chronic liver disease, chronic biliary obstruction or high-dose chemotherapy<sup>[61-64]</sup>. Preoperative portal vein embolization (PVE) was first described in 1986 and is currently used to increase FRL volume and function<sup>[65]</sup>.

Randomized controlled trials and individual institutional series support the safety and efficiency of preoperative PVE<sup>[20,61,66-69]</sup>. A potential disadvantage of PVE is that it may be difficult to determine preoperatively whether a right or left hepatectomy will be required if the tumor is located centrally in the hilum. At present, there is no evidence to support the routine use of PVE for HCC, but PVE should be considered for potentially resectable patients with normal liver function when anticipated FRL is less than 20% of the total liver volume, or for patients with compromised liver function when

anticipated FRL is less than 40% of the total liver volume. Most patients with HCC present with jaundice and are considered to have cholestasis-induced compromised liver function. There are not many data on the impact and real volume of PVE on FRL liver function increase, associated or not to biliary drainage. Only in the second period in which their series is divided do Cannon *et al*<sup>[70]</sup> use PVE in 9.1% of cases, which means 4.5% out of a total of 110 patients, and despite that use they achieve only 62% of R0 resections.

As a consequence, PVE must be assessed and chosen with precaution to avoid the frightening postoperative hepatic insufficiency, one of the main causes of mortality in these patients. Also, its application must be evaluated in accordance with a previous surgical plan, which, if uncertain, could lead us to use another type of tactic, such as associating liver partition and portal vein ligation for staged hepatectomy (ALPPS)<sup>[71]</sup>.

## LIVER RESECTION: HOW MUCH IS ENOUGH?

In the last 20 years the use of hepatic resection in patients with HCC has risen. The objective of all the techniques and of the tendency to major resection with or without resection of vessels is to obtain free resection margins. The 5-year survival rate in patients undergoing non-curative resection for HCC is below 10%<sup>[4]</sup>. The 5-year survival rate for operated patients is with curative intention 11%-41% (Figure 1). All scientific community agrees that surgical resection is the only potentially curative treatment for CC, but the disease is usually advanced at the time of diagnosis and mostly treated by chemoradiotherapy or palliative therapy, including biliary drainage or stenting. Resectability rates are low because of early infiltration of the tumor into adjacent structures such as hepatic artery, portal vein and caudate lobe. In patients treated with curative intent, an extended hemihepatectomy is often needed to achieve negative margins. Preoperative jaundice and extended procedures are important risk factors for postoperative complications<sup>[57]</sup>.

The aims of surgery in HCC are: (1) to achieve macroscopic removal of the tumor; (2) to restore satisfactorily the bile flow to the gut; and (3) to minimize postoperative liver failure or death. At the beginning of last decade, resection was possible only in 20% of cases, and the operative mortality was 10%. The median survival was only 20 mo, but resected patients enjoyed a good quality of life<sup>[3,4]</sup>. Last decade saw an aggressive approach to HCC with an increasing use of major hepatic resections<sup>[5,14,20,27,43-46,66]</sup>. The resectability rate increased to 80% with the addition of hepatic resection to bile duct resection without increasing the postoperative death rate. Bismuth *et al*<sup>[13]</sup> and Pichlmayr *et al*<sup>[72]</sup> suggested a stagewise management strategy with the prime objective of achieving complete surgical resection of the tumor without leaving behind macroscopic residual disease. Patients with Bismuth types I and II were treated by bile

duct resection. For Bismuth stage IIIa/IIIb lesions, resection of the corresponding hemiliver was recommended. However, major hepatic resection is a formidable operation in patients with a cholestatic liver and carries a high complication rate, with a morbidity of up to 81% and mortality rates of between 6% and 10% in the most advanced centres.

Vascular encasement with or without biliary obstruction may result in segment or lobar atrophy. Long-standing biliary obstruction can cause moderate atrophy, whereas concomitant portal venous compromise usually produces rapid and severe atrophy of the involved segments<sup>[3]</sup>. Approximately 30% of patients subjected to surgical exploration show evidence of lobar atrophy<sup>[15]</sup>. It is one of the problems of the PVE, together with vascular involvement not detected before embolization.

The caudate lobe is frequently involved by either direct invasion or ductal extension. Caudate bile ducts can drain to both the right and left hepatic ducts; in fact, some series have identified microscopic tumor infiltration into the caudate lobe in nearly all patients with HCC<sup>[21]</sup>. In general, the primary drainage of the caudate lobe is into the left hepatic duct<sup>[73]</sup>. For this reason, it has been alleged that the necessity to resect the caudate lobe in Bismuth type II from now on.

Ikeyama *et al*<sup>[10]</sup>, in their audit of 54 consecutive type I and II HCC resected patients, concluded that for nodular and infiltrating tumors right hepatectomy is essential; for papillary tumors, bile duct resection with or without limited hepatectomy is adequate. But the problem is that it is very difficult to know these issues preoperatively and intraoperatively. Nuzzo *et al*<sup>[74]</sup> reached the same conclusion in their audit of 440 patients, showing that pathologic factors independently predicted overall and disease-free survival at multivariate analysis.

Major hepatic resections have increased the proportion of R0 resections<sup>[4,14,29,37]</sup>, improved the outcome of disease-free survival, and decreased the prevalence of hepatic recurrence<sup>[75]</sup>. Surgical results improved in the 1990s thanks to a better ability to perform R0 resections, which is likely due to increasing use of major hepatic resection and portal resections, as well as the improvement of preoperative management concerning both prognosis and FRL preparation and care<sup>[44,75]</sup>. Recent studies have also reported an improvement in morbidity and mortality in comparison with previous decades, which probably responds to advances in overall perioperative care. Also, the improvement of preoperative management has had a consequence, as can be seen in the report of Nagino *et al*<sup>[44]</sup>.

Nonetheless, it is uncertain whether the major hepatic resection may improve the survival of patients with B-C types I or II HCC. Ikeyama *et al*<sup>[10]</sup> and Jang *et al*<sup>[76]</sup> showed survival benefit in right hepatectomy with caudate lobectomy for nodular and sclerosing tumors, but not for papillary ones. However, others have reported a non-significant difference between hepatectomy and isolated bile duct resection in B-C types I and II tumors<sup>[77]</sup>.

Regarding proximal margin, it can be stated nowadays that survival outcomes improve when bile duct resection is associated with hepatectomy, even in patients with B-C types I and II tumors<sup>[14,26]</sup>. In the series published by Jarnagin *et al.*<sup>[14]</sup> in 2001, the 5-year survival was 37% when a hepatic resection was performed (84% of R0 resections) and 0% when only a bile duct excision was performed (56% of R0 resections). The best results are obtained with a right hepatectomy, probably because this surgical technique facilitates en-bloc resection of the tumor and surrounding tissues and thereby increases radicality<sup>[26]</sup>. In the series of Neuhaus *et al.*<sup>[24,77]</sup>, the worst outcomes after hepatectomy with curative intent were obtained in patients undergoing left hepatectomy. Although Nimura defended the radical surgery of left-sided Klatskin tumors by performing a left trisectionectomy, this is characterized by high morbidity rates and by mortality rates superior to 10%<sup>[78,79]</sup>. The analysis of recurrence after R0 resection with hepatectomy shows a low frequency of local recurrence, but a high frequency of peritoneal seeding recurrence<sup>[26]</sup>. Then, manipulation of the tumor as well as biopsies may favour local recurrence, and this is the reason why some authors advise en-bloc resection including surrounding vessels, a “non-touch technique”, in order to avoid this cause of recurrence.

The hepatectomy must include the caudate lobe, since this is a frequent site of tumor recurrence when it is not included in the resection piece. However, as it happens with other “evidences” related to Klatskin tumor treatment, there are no controlled studies that support this recommendation<sup>[32,80]</sup>. Performing a perioperative biopsy of the biliary resection margin in the liver remnant is common practice for most surgeons.

In a recent report of Ribero *et al.*<sup>[81]</sup>, in the analysis of 82 cases, the group of patients who had primary R0 was compared with those patients who achieved a secondary R0 after an intraoperative additional resection, and also with the patients who were R1. The 1-, 3- and 5-year survival rates were similar in the groups with primary R0 and secondary R0, but different in R1 patients (5-year survival rate: 50%, 30.8% and 0% respectively). The authors concluded that an additional resection of a positive proximal bile duct margin, albeit associated with an increased risk of biliary fistula, offers a significant survival benefit and should be attempted whenever possible. But this Italian group does not re-operate on those patients who the pathologist changes to R1 resection in the postoperative study, and thus, although they only have 13 cases that underwent re-resection, they do not defend re-operations on patients when this occurs. However, it is necessary to take into consideration that frozen biopsy is often not concluding and that resection extension, when the biopsy is positive, is frequently impracticable<sup>[26]</sup>. This explains why perioperative biopsies in this location have low profitability. Furthermore, such resection of margin-positive proximal duct does improve survival even when a negative margin can be achieved with additional resection<sup>[82]</sup>.

## LYMPHATIC SPREAD

In addition to extension along the bile ducts, HCC often metastasizes *via* the lymphatics. Lymphatic metastases are found in 30% to 50% of patients undergoing resection<sup>[14,83,84]</sup>. Hilar and pericholedochal lymph nodes (LN) are the most commonly involved, followed by periportal, common hepatic, posterior pancreaticoduodenal, celiac and preaortic ones<sup>[85]</sup>. Metastasis in regional LN is an important prognostic factor that affects survival after the resection of an HCC<sup>[36]</sup>. Kitagawa *et al.*<sup>[73]</sup> evaluated 110 patients that underwent resection for HCC with LN dissection, including both the regional and para-aortic ones, and found that 47% of patients had no involved LN, 35% had metastases in regional LN and 17% had metastases in regional and para-aortic LN. The 5-year survival was 30% for patients with negative LN, 15% for patients with metastases in regional LN and 12% for patients with metastases in regional and para-aortic LN. Other studies have reported a worse survival in patients with LN involvement beyond the hepatoduodenal ligament, with a 5-year survival rate ranging from 0% to 6%<sup>[42]</sup>. Consequently, routine LN dissection beyond hepatoduodenal ligament is not recommended. Patients with macroscopically involved LN beyond hepatoduodenal ligament are considered to have unresectable disease, even though some surgeons resect them if they find them intraoperatively.

Only one study has presented the number of affected LN as a variable than worsens survival<sup>[86]</sup>.

## VASCULAR RESECTION

Radial growth of the tumor may infiltrate the surrounding vessels. Right hepatic artery involvement is more frequent due to its proximity to the biliary bifurcation. Contralateral artery infiltration to the hepatic resection that is to be performed is a reason for contraindication of surgical treatment. Portal involvement is present in 20%-30% of R0 resections and its preoperative identification is achieved with a precision of 85%. In the experience of Nagoya University, in approximately one third of the patients whose portal vein is resected because of apparent infiltration, this is not histologically confirmed<sup>[66]</sup>. However, most of these patients had a tumor infiltration adjacent to the vein, and the margin would have been positive without vein resection. On the other hand, vascular resection was not associated with a significant increase of morbimortality. Anyhow, resection can improve survival in some patients when R0 resection is achieved.

Encasement or occlusion of the main portal vein or vessels supplying the hepatic remnant is considered a contraindication to surgery<sup>[14]</sup>. Recent reports have shown that en-bloc resection with vascular reconstruction can achieve negative margins with a 10% perioperative mortality in selected patients.

Portal vein resection and reconstruction has been carried out in HCC with conflictive results<sup>[24,87]</sup>. Although several retrospective series have not shown difference in

**Table 3** Prognostic factors and 5-year survival rate

Hilar cholangiocarcinoma	No. of patients	Prognostic factors	Operative mortality(%)	5-yr SV (%)
Jarnagin <i>et al</i> <sup>[14]</sup>	80	Margin, hepatectomy, differentiation	10	27
Seyama <i>et al</i> <sup>[20]</sup>	58	Lymph nodes	0	40
Dinant <i>et al</i> <sup>[27]</sup>	99	Margin, resection period, lymph nodes	15	27
DeOliveira <i>et al</i> <sup>[43]</sup>	281	Margin, lymph nodes	5	10
Rea <i>et al</i> <sup>[45]</sup>	46	Lymph nodes, tumor grade, bilirubin	9	26
Silva <i>et al</i> <sup>[46]</sup>	45	Tumor stage, margin	9	11
Witzigmann <i>et al</i> <sup>[47]</sup>	60	Residual tumor status, grading	8	22
Baton <i>et al</i> <sup>[48]</sup>	59	Chemotherapy, margin, lymph nodes	5	20
Wahab <i>et al</i> <sup>[55]</sup>	243	Margin, S1 resection, lymph nodes, grading	7	16
de Jong <i>et al</i> <sup>[90]</sup>	305	Lymph nodes, margin	5	20

SV: Survival rate.

operative mortality between the patients that underwent portal vein resection and those ones that did not<sup>[24]</sup>, the impact of portal vein resection on long-term survival is less clear. Neuhaus *et al*<sup>[24]</sup> proposed portal vein resection as part of a “non-touch” resection of the tumor and surrounding tissue. Portal vein resection was identified as a positive independent prognostic factor in their multivariate analysis of patients undergoing R0 resections, when mortality within the first 60 d was excluded. Nevertheless, overall mortality within 60 d after portal vein resection was 17%, in comparison with 5% in patients without portal resection, and all the deaths occurred after non-curative resections. Other studies have reported similar or worse survival in patients undergoing portal vein en-bloc resection<sup>[22,75,88]</sup>. The role of routine portal vein resection (as stated by Neuhaus) is not likely to be clearly designed unless a randomized clinical trial is completed. However, Hemmings rejects the routine performance of this procedure and in 2012 the Nagoya group reported a 5-year survival rate of 40% in the last period of portal resection, but a morbidity of 57.3%<sup>[42-44]</sup>.

Portal resection must be recommended whenever the tumor cannot be freed from it, since the microscopic invasion of the portal vein does not seem to influence on survival when a vascular resection is carried out, whereas the macroscopic invasion does have negative results on survival.

Nishio *et al*<sup>[89]</sup> concluded that although lymph node metastasis and macroscopic portal vein involvement were independent negative prognostic factors, the 5-year survival rate obtained in patients with portal vein resection or lymph node metastasis still was about 10% (Table 3). Even in patients with both cancer invasion of the portal vein and regional lymph node metastasis, or with para-aortic lymph node metastasis, curative resection resulted in significantly longer survival than the one found in un-resected patients.

Some groups had 100% morbidity and mortality in arterial resections, although in arterial and portal combined resections mortality was 43%, and the overall percentage of positive margins was 32%<sup>[29]</sup>. de Jong *et al*<sup>[90]</sup> reported in a recent paper that combined hepatectomy, extrahepatic biliary duct resection and portal vein resection can offer

long-term survival in some patients with advanced HCC, with 17.6% mortality rate and 28% 5-year survival rate.

Some authors recommend hepatectomy with simultaneous arterial and portal vein resection. They reach 66% of R0 resection with 2% mortality rate, 54% morbidity rate and 1-, 3-, and 5-year survival rates of 78.9%, 36.3%, and 30.3%, respectively, but these data are not reproducible<sup>[86]</sup>.

Su *et al*<sup>[39]</sup>, Miyazaki *et al*<sup>[87]</sup> and Muñoz *et al*<sup>[91]</sup> reported as a conclusion that, although both portal vein and hepatic artery resection are independent poor prognostic factors after curative operative resection for locally advanced HCC, portal vein resection is acceptable from an operative risk perspective and might improve the prognosis in the selected patients, but combined hepatic artery resection cannot be justified because the 3-year survival rate is 0%.

## LIVER TRANSPLANT

Orthotopic liver transplant (OLT) is contraindicated in HCC because of disappointing long-term outcomes. However, a recent multi-institutional study in the United States, including 280 patients with earlier-stage tumors who received aggressive neoadjuvant chemoradiation, has reported that transplantation remarkably improves survival: the 1- and 5-year survival rates were 74% and 38%, respectively<sup>[92]</sup>. The Mayo Clinic protocol sets a strict selection of the patients candidates to liver transplant. Although the selection is highly rigorous and biased for patients with biologically favourable disease, the early results published by the Mayo group showed an 82% 5-year survival rate<sup>[93]</sup>. The histological analysis of resected pieces confirmed N0 and R0 state in all the patients. However, only 58% of the patients had histologically confirmed cancer.

Liver transplantation is currently done only in the setting of clinical trials. It offers the advantage of resection of all structures that may be involved by the tumor, including portal vein, bilateral hepatic ducts and atrophic hepatic lobes. Thus, total hepatectomy may permit R0 resection even in locally advanced tumors, which are beyond resection criteria. Efficacy of neoadjuvant therapy and transplantation is demonstrated by comparing results with the natural history of the disease. Untreated HCC



**Table 4** Morbidity and mortality rate and R0 resections

Ref.	Resections	R0 (%)	Morbidity	Mortality	5-yr Survival rate
Burke <i>et al</i> <sup>[3]</sup>	30	83	NA	6	45
Nakeeb <i>et al</i> <sup>[4]</sup>	109	26	47	4	11
Jarnagin <i>et al</i> <sup>[14]</sup>	80	78	64	10	26
Nimura <i>et al</i> <sup>[15]</sup>	55	84	41	6	41
Jarnagin <i>et al</i> <sup>[17]</sup>	106	77	62	8	NA
Seyama <i>et al</i> <sup>[20]</sup>	87	64	43	0	40
Kosuge <i>et al</i> <sup>[23]</sup>	65	52	37	9	33
Neuhaus <i>et al</i> <sup>[24]</sup>	80	61	55	8	22
Launois <i>et al</i> <sup>[25]</sup>	131	NA	NA	19	NA
Kondo <i>et al</i> <sup>[26]</sup>	40	95	48	0	NA
Dinant <i>et al</i> <sup>[27]</sup>	99	31	66	15	27
Gerhards <i>et al</i> <sup>[29]</sup>	112	14	65	18	NA
Hemming <i>et al</i> <sup>[32]</sup>	53	80	40	9	35
Ijitsma <i>et al</i> <sup>[33]</sup>	42	65	76	12	19
Kawarada <i>et al</i> <sup>[34]</sup>	65	64	28	2.3	26
Klempnauer <i>et al</i> <sup>[35]</sup>	151	77	NA	10	28
Miyazaki <i>et al</i> <sup>[37]</sup>	76	71	33	13	26
Nimura <i>et al</i> <sup>[38]</sup>	142	61	49	9	26
Su <i>et al</i> <sup>[39]</sup>	49	49	47	10	15
Todoroki <i>et al</i> <sup>[41]</sup>	101	14	14	4	28
DeOliveira <i>et al</i> <sup>[43]</sup>	281	62	60	5	30
Nagino <i>et al</i> <sup>[44]</sup>	574	76.5	43.1	4.7	32.5
Rea <i>et al</i> <sup>[45]</sup>	46	80	52	9	26
Nuzzo <i>et al</i> <sup>[74]</sup>	440	77.3	47.5	8.6	25.5
Ito <i>et al</i> <sup>[85]</sup>	38	63	32	0	33
Kawasaki <i>et al</i>	79	68	14	1.3	22

NA: Not available.

has a 50%-70% mortality rate within 12 mo, which is much worse than 55% 5-year survival for patients who entered the Mayo Clinic protocol and 71% 5-year survival after transplantation<sup>[94,95]</sup>.

The Cincinnati Transplant Tumor Registry reported 28% 5-year survival, with a tumor recurrence rate of 51%<sup>[88]</sup>. The Spanish liver transplant centres provided similar results, with 30% 5-year survival rate and 53% tumor recurrence rate, in 36 patients with unresectable, non-disseminated HCC<sup>[96]</sup>. As a consequence of such initial results and the limited availability of organs, HCC was perceived as a relative contraindication to OLT. Also, it is a well-known fact that 55% of HCC even in T2 stages have affected LN, which is one of the contraindications to transplant<sup>[97]</sup>.

A further complication to transplants in HCC is that, as response to postoperative radiotherapy and chemotherapy is low both in R1 and recurrence, tumors must have a more favourable biological behaviour, and if sizes bigger than 2 cm are rejected for rescue with liver transplant, then very few patients can be candidates to be transplanted<sup>[52]</sup>. It is important to remember that, out of the 281 cases analysed by DeOliveira, 58% were > 2 cm and hilar involvement occurred in 28%<sup>[43]</sup>.

Schüle *et al*<sup>[98]</sup> concluded that an acceptable survival rate could be achieved by transplantation for HCC with LN metastases as the only exclusion criterion, even if they use living donors. In this article, the authors got a 5-year survival rate of 50% in those patients with negative LN.

Nowadays, OLT cannot be considered as a standard

therapy for HCC in patients with resectable disease, but it offers a potential option to patients with underlying primary sclerosing cholangitis. Additional studies are necessary to define the role of OLT in depth.

## MORBIDITY AND MORTALITY

Due to the complex biliary and liver resections required to obtain complete tumor removal, the risks of perioperative morbidity and mortality are significant. Morbidity and mortality rates range from 14% to 76% and from 0% to 19%, respectively. Perioperative morbidity includes haemorrhage, biliary fistula, hepatic insufficiency and infectious complications. Among them, infectious complications are particularly common and account for 50% to 80% of all complications<sup>[14,42]</sup>. The postoperative liver failure and its morbidity have been joined with the extension of hepatic resection<sup>[23]</sup>. However, recent publications suggest a decrease in morbidity and mortality with the use of preoperative PVE, even in extended hepatectomies<sup>[33,42,61,69]</sup> (Table 4).

## OUTCOME OF RESECTION

Published 5-year survival rates range from 25% to 40% in recent series, and, even, it has been reported that many clinical and histological factors have a positive impact on long-term outcome, including negative histologic margin status<sup>[99,100]</sup>, concomitant hepatic resection<sup>[30]</sup>, absence of nodal involvement<sup>[14, 23,48,101]</sup>, low TNM status<sup>[56]</sup>, well-differentiated tumor grade<sup>[68]</sup>, papillary tumor morphology<sup>[36,44,80]</sup>, and lack of perineural invasion<sup>[23]</sup>. Complete resection with negative histologic margins is the only modifiable factor and, for that reason, the primary aim of surgical therapy. There is a close association between hepatic resection and negative margins<sup>[24,37,99]</sup>. The effect of R1 resection *vs* no resection on outcome has been object of discussion and analysis in surgical literature, with some recent studies that report improvement in survival after R1 resection in comparison with patients with unresectable disease<sup>[42]</sup>.

Recurrence after resection occurs quite frequently, in up to 50%-75% of cases<sup>[10,22,76]</sup>. The median recurrence time ranges from 12 to 43 mo<sup>[10,22,42,76]</sup>. Prognostic factors for recurrence-free survival include histologic grade, T and N stages, and margin status<sup>[10,22,76,102]</sup>. Since patients with recurrent disease are not candidates for curative therapy, advances in adjuvant therapy are essential to improve long-term outcome. However, the effectiveness of radiotherapy and chemotherapy is still very limited. In the report of Cherqui *et al*<sup>[53]</sup>, the authors concluded that adjuvant radiotherapy was not associated with an improvement in long-term overall survival in patients with resected HCC.

## CONCLUSION

Surgical resection continues to be the main treatment of HCC. Negative resection margins enhanced by major

hepatic resections are associated with improved outcome. Pre-resectional management with biliary drainage, PVE and staging laparoscopy should be considered in selected patients. Additional evidence is needed to fully define the role of OLT. Improvements in adjuvant therapy are essential for improving long-term outcome. Portal and node involvement worsens the prognosis and long-term survival, and surgery is the only option that can lengthen it. Furthermore, the lack of effective chemotherapy and radiotherapy treatments, at this moment, leads us to consider R1 resection as an option because these patients have a longer survival rate than patients who do not undergo resection.

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## Pathological aspects of so called "hilar cholangiocarcinoma"

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### Abstract

Cholangiocarcinoma (CC) arising from the large intrahepatic bile ducts and extrahepatic hilar bile ducts share clinicopathological features and have been called hilar and perihilar CC as a group. However, "hilar and perihilar CC" are also used to refer exclusively to the intrahepatic hilar type CC or, more commonly, the extrahepatic hilar CC. Grossly, a major distinction can be made between papillary and non-papillary tumors. Histologically, most hilar CCs are well to moderately differentiated conventional type (biliary) carcinomas. Immunohistochemically, CK7, CK20, CEA and MUC1 are normally expressed, being MUC2 positive in less than 50% of cases. Two main premalignant lesions are known: biliary intraepithelial neoplasia (BilIN) and intraductal papillary neoplasm of the biliary tract (IPNB). IPNB includes the lesions previously named biliary papillomatosis and papillary carcinoma. A series of 29 resected hilar CC from our archives is reviewed. Most (82.8%) were conventional type adenocarcinomas, mostly well to moderately differentiated, although with a broad morphological spectrum; three cases exhibited a poorly differentiated cell component resembling sig-

net ring cells. IPNB was observed in 5 (17.2%), four of them with an associated invasive carcinoma. A clear cell type carcinoma, an adenosquamous carcinoma and two gastric foveolar type carcinomas were observed.

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**Key words:** Cholangiocarcinoma; Bile duct carcinoma; Hilar cholangiocarcinoma; Perihilar cholangiocarcinoma; Klatskin tumor; Extrahepatic bile duct carcinoma; Hepatic hilum

**Core tip:** The controversy regarding the definition of hilar and perihilar cholangiocarcinoma (CC) is addressed. The authors review the main pathological features (gross and microscopic findings, immunophenotype) of hilar CC, including rare histological variants as well as precursor lesions (biliary intraepithelial neoplasia and intraductal papillary neoplasm of the biliary tract). Considerations regarding staging and other histological prognostic factors are also included. The authors also provide a series of 29 cases of resected hilar CC.

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### NOMENCLATURE AND TOPOGRAPHY

#### Considerations on the concept and classification of hilar cholangiocarcinoma

As stated in the World Health Organization (WHO) classification of tumors of the bile duct, the use and meaning of the terms hilar (and perihilar) may differ among pathologists, surgeons and radiologists<sup>[1]</sup>. The right and left hepatic ducts, their confluence and their first to third

branches are collectively called hilar and perihilar bile ducts and they are intra and extrahepatically located. The boundary between the intra and extrahepatic biliary tree has been somewhat confused in the literature. Hilar bile ducts proximal to the junction of the second-order bile ducts are intrahepatic because the peritoneum is attached there and they are called large intrahepatic bile ducts, whereas the main hilar bifurcation (*i.e.*, the right and left hepatic ducts) can be considered extrahepatic<sup>[2,5]</sup>.

Depending on its anatomical location, cholangiocarcinoma (CC) has normally been classified in intrahepatic or extrahepatic types, with extrahepatic CC further categorized in proximal (upper third), middle (middle third) and distal (lower third) subtypes<sup>[6]</sup>. Intrahepatic cholangiocarcinomas (ICC) have classically been separated into two groups: CC arising in small intrahepatic bile ducts (peripheral type CC) and CC originating from the major intrahepatic bile ducts, including the hilum (hilar type CC). CC originating from the large intrahepatic bile ducts (hilar type CC) exhibit significant clinicopathological differences from tumors of small bile duct origin (peripheral type CC)<sup>[7]</sup> and several decades ago it was suggested that it was more practical to treat such hilar carcinomas together with extrahepatic bile duct carcinomas (EBDCs) because of the similarity in symptomatology<sup>[8]</sup>. In addition, bile duct carcinoma arising near or at the confluence of the right and left hepatic ducts has also been classically known as hilar CC or Klatskin tumor<sup>[9,10]</sup>. The term hilar CC is very commonly used in a narrow sense to refer to this extrahepatic hilar bile duct carcinoma (*i.e.*, Klatskin tumor or proximal type extrahepatic CC), but hilar CC has also been used in a broad sense to refer to CCs involving the hepatic hilum regardless of their intra or extrahepatic location (*i.e.*, including intra and extrahepatic hilar CC)<sup>[11,12]</sup>. Because hilar CC is the commonest CC, the incidence of intra and extrahepatic CC in the literature has largely depended on how the hilar CC has been considered<sup>[13]</sup>.

More recently, with respect to not only anatomical distribution but also preferred surgical treatment, CC has been classified as intrahepatic, perihilar and distal types. In this classification, perihilar CC has been defined as that tumor involving or requiring resection of the hepatic duct bifurcation even if it has a significant intrahepatic component<sup>[14,15]</sup>. Some authors consider that perihilar CC is a single entity that includes all the tumors involving the hepatic hilum irrespective of whether they are extrahepatic (*i.e.*, extrahepatic hilar CC) or intrahepatic (*i.e.*, intrahepatic hilar CC) as these tumors have comparable biological behavior with similar clinical management. These authors have suggested that perihilar tumors should preferably be staged by a staging system specific for extrahepatic bile duct cancer<sup>[11]</sup>. However, other authors argue that perihilar CCs are potentially divisible in ICC involving the hepatic hilum and extrahepatic hilar bile duct carcinomas because they have found different prognoses after hepatobiliary resection<sup>[16]</sup>. Perihilar CC is considered by the American Joint Committee Cancer/Union for International Cancer Control (AJCC/UICC)

TNM system as an EBDC but the term perihilar CC is also sometimes used to refer to intrahepatic large bile duct carcinomas<sup>[1,17]</sup>. Therefore, as with hilar CC, the term perihilar CC has been used in both a broad and narrow sense.

Another pertinent issue refers to the most appropriate use of the term CC. One option is to use CC for any bile duct carcinoma originating from the small intrahepatic bile ducts until the end of the common bile duct into the ampulla of Vater. In this option, the term CC is preceded by the anatomical location, such as intrahepatic, hilar, perihilar or extrahepatic CC. The other option, assimilated by the WHO system, is to restrict the term CC for carcinomas arising in the intrahepatic bile ducts (ICC) and use the term bile duct carcinoma for the extrahepatically derived CC (*i.e.*, EBDC)<sup>[4]</sup>. According to the WHO classification, hilar CCs (*i.e.*, Klatskin tumors) are EBDC. However, the WHO system argues that, especially in locally advanced cases, their distinction from ICC of the major intrahepatic bile ducts is usually controversial and such cases could be included in the definition of perihilar ICC<sup>[1,17]</sup>. Ultimately, it is up to the pathologist to establish the topographical origin of these hilar tumors in the surgical specimen, often with the essential collaboration of the surgeon. Since the histological classification of the ICC and EBDC is somewhat different, the location of the tumor also involves applying either of these classifications. In the case of EBDC, they are histologically subclassified the same as the carcinomas of the gallbladder by the WHO system<sup>[1]</sup>.

In conclusion, several factors contribute to the designation of these tumors: topography (intrahepatic, extrahepatic, peripheral, hilar, perihilar, proximal, distal), histogenesis (small or large duct), preoperative staging by radiology (of interest for surgical approach-resectable, unresectable), definitive staging (of prognostic interest and for additional oncological treatment approach) with pathological staging of the resected tumor in the surgical specimen (intraepithelial, intraductal, invasive) and clinical aspects (obstructive or not). Ideally, all these parameters should be incorporated into the final diagnosis. With respect to the histological designation, we recommend naming this tumor biliary duct carcinoma (CC), specifying the exact location (for example: bile duct adenocarcinoma-CC-of the common hepatic duct and its confluence).

The different use of the term hilar CC complicates the review of its pathological features in the literature. In addition, many reports are confounded by the inclusion of patients with tumors arising from other locations of the biliary tract (for example, including mid and distal bile duct tumors).

## MACROSCOPIC PATHOLOGY

Hilar CC has been grossly classified in polypoid or papillary, nodular, scirrhous constricting or nodular-infiltrating, and diffusely infiltrating types, each type showing different resectability and prognosis<sup>[18,19]</sup>. The polypoid or papillary type protrudes to the lumen in a bland and

friable cauliflower-like fashion. The nodular type is characterized by a firm, gray-white tumor bulging from the mucosa, with the border of the tumor to the adjacent tissue fairly well defined. Both papillary and nodular types have evident tumorous lesions. On the contrary, scirrhous constricting or nodular-infiltrating type exhibits only a slight protuberance of the mucosa and involves the thickness of the wall with distortion or annular fibrous constriction. In the diffusely infiltrating type, the tumor appears as an ill-defined stricture of the duct due to a hard fibrous thickening of the duct wall, with more linear extension than the scirrhous constricting type<sup>[19]</sup>. Nodular, and especially nodular-infiltrating and diffusely infiltrating types, usually infiltrate more intensively than polypoid (papillary) type and more frequently exhibit submucosal extension at the proximal border that may make their resection difficult. On the contrary, mucosal extension at the proximal border is observed more often in nodular and even more in papillary tumors. These macroscopic classifications present difficulties in practice because many tumors may have overlapping features. The papillary type has the best prognosis, is more often resectable and less invasive, and usually corresponds to well differentiated papillary tumors. Therefore, hilar CC can be classified macroscopically, making a major distinction between papillary and non-papillary (*i.e.*, nodular-sclerosing) types<sup>[9]</sup>.

## MICROSCOPIC PATHOLOGY

### **Conventional histology and immunohistochemistry**

Most of hilar CC or Klatskin tumors histologically correspond to well to moderately differentiated biliary type adenocarcinomas<sup>[1,20]</sup>. These biliary type EBDCs are histologically very similar to those arising in intrahepatic large bile ducts (perihilar ICC)<sup>[1,3]</sup>. They are characterized by tubules or glands in a typical desmoplastic stroma with variable inflammatory response. There may also be solid nests and cords in less differentiated cases and same papillary groups are often seen on the surface. Tumor cells are columnar to cuboidal with moderate amount of clear to eosinophilic cytoplasm. Nuclei are generally small, although a major grade of nuclear atypia is also possible. They typically produce numerous peri and intraneural invasions. They also tend to produce lymphatic invasion, with venous invasion less frequent<sup>[11,21,22]</sup>. Perineural invasion is a specific route of invasion in bile duct carcinomas and it is an important prognostic factor<sup>[23]</sup>. Spread by direct invasion to periductal hilar tissues, portal vein branches, hepatic arterial branches and adjacent liver tissue are common findings in hilar CC<sup>[5,24]</sup>. Although hilar CC has traditionally been considered a slow-growing locally invasive tumor, different reports have found lymph node involvement in 30%-50% of patients on exploration and 20% had involvement of distant sites<sup>[11,22,25,26]</sup>.

Biliary duct adenocarcinomas can be histologically graduated based on their degree of glandular or tubular differentiation. The previous edition of WHO classification established a quantitative grading system in

which well, moderate and poorly differentiated biliary duct adenocarcinomas were composed of, respectively, 95%, 40%-94% and 5%-39% of glands<sup>[21,27]</sup>. However, the current WHO classification does not mention any quantitative criteria<sup>[1]</sup>. The College of American Pathologists (CAP) has proposed a quantitative grading system in which greater than 95%, 50%-95% and less than 50% of tumor composed of glands corresponds to, respectively, well, moderate and poorly differentiated bile duct adenocarcinomas<sup>[28]</sup>. We believe it is advisable to use a degree of differentiation based on cytological atypia in addition to architectural pattern (tubular, papillary, solid, single cells).

Immunohistochemically, cytokeratin (CK) 7 is nearly always positive in hilar CC, like the rest of the CC. The majority of perihilar ICC and EBDC also express CK20 (80% in case of hilar tumors), most commonly with a low or moderate labeling index. On the contrary, peripheral ICC is CK20 negative in just over half of the cases<sup>[29]</sup>.

Several mucin-related glycoproteins and oncoproteins, such as carcinoembryonic antigen (CEA), human mucin (MUC) type 1 (MUC1) and 5AC (MUC5AC), B72.3 and CA 19-9 are normally expressed, although they may be focal<sup>[30,31]</sup>. Surface proteins such as CEA or MUC1 may be related to anti-adhesion molecular functions that promote the release of cells from the tumor and facilitate tissue invasion. CEA is generally limited to the apical membrane in biliary duct benign cells but it is commonly detected in the cytoplasm of biliary duct adenocarcinoma cells, more intensely in advanced tumors or poorly differentiated adenocarcinomas<sup>[32]</sup>. MUC1 is expressed in the majority of EBDC, with cytoplasmic expression more frequently observed in invasive lesions. MUC1 expression is related to poor differentiation, locoregional tumor progression, metastases to the liver and poor outcome<sup>[33,34]</sup>. In contrast, MUC2 (an intestinal-type secretory mucin) is expressed in less than half of the cases (usually with low or moderate labeling index), is highly expressed in well-differentiated adenocarcinomas, and is inversely related to tumor progression and poor outcome<sup>[34]</sup>.

Variably dispersed endocrine cells, immunoreactive for neuroendocrine markers such as synaptophysin and chromogranin, may be observed in just under a third of the EBDC, especially in well to moderate differentiated tumors<sup>[21,35]</sup>.

*p53* is a tumor-suppressor gene very commonly mutated in different human tumors. DPC4 is another tumor-suppressor gene, known to be inactivated in around 55% of pancreatic adenocarcinomas and less often in other tumors. Immunohistochemically, *p53* overexpression and loss of DPC4 expression have been observed in, respectively, 25% and 15% of hilar CC. On the contrary, carcinoma of the distal bile duct exhibited a higher frequency of *p53* overexpression and DPC4 inactivation, in proportions more similar to those observed in pancreatic adenocarcinoma<sup>[36,37]</sup>. The reported rates of K-ras mutations in EBDCs has also shown lower frequencies in proximal compared to distal EBDC<sup>[38-40]</sup>. These findings suggest that the molecular mechanisms in the tumorigenesis along the biliary tract might be different, reflecting



different etiologies, whereas distal bile duct and pancreas would share similar molecular alterations. However, the reported rates of p53 and K-ras alterations have differed widely in different studies<sup>[41]</sup>. The immunohistochemical reported results of HER-2/neu (c-erbB-2) overexpression in EBDC has also varied considerably. More recent studies have observed c-erbB-2 overexpression in 4% to up to one third of EBDC<sup>[42-45]</sup>.

### Non conventional histology: histological variants

Hilar CC rarely corresponds to other histological variants. Adenosquamous carcinoma exhibits variable amount of malignant squamous cells with keratin pearls and/or intercellular bridges and a glandular component identical to conventional adenocarcinoma. Either of the two components can be predominant. The squamous areas are positive for high molecular weight cytokeratins, CK5/6, p63 and S100A2, these markers being negative in the adenocarcinoma component<sup>[31,46]</sup>. The survival time for patients with adenosquamous carcinoma seems to be significantly worse when compared with adenocarcinoma of the bile ducts. An inverse relationship between the proportion of the squamous component and patient survival has been observed<sup>[46,47]</sup>. Currently, the WHO classification makes no comment on the need for a minimum amount of squamous cell component required for the diagnosis of adenosquamous carcinoma of the extrahepatic bile ducts<sup>[1]</sup>. Squamous cell carcinoma of the hilar bile duct is very rare. The presence of any amount of glandular component excludes this diagnosis. The majority of reported cases were already advanced at the time of diagnosis so it has been assumed that its prognosis is poor<sup>[48,49]</sup>. Malignant transformation of squamous metaplasia in biliary epithelium has been suggested for the origin of squamous cell carcinoma and adenosquamous carcinoma, whereas histopathological alteration from adenocarcinoma to squamous cell carcinoma has been suggested as an alternative etiology for adenosquamous carcinomas<sup>[48]</sup>.

Mucinous (colloid) adenocarcinoma is characterized by prominent extracellular or stromal mucin deposition. In the literature, mucinous (colloid) adenocarcinoma of the extrahepatic bile ducts is normally described as the invasive component related to some papillary non-invasive neoplasms<sup>[50,51]</sup>. Intestinal type adenocarcinoma is composed of tubular glands closely resembling those of colonic adenocarcinomas. It has been pointed out that some conventional bile duct carcinomas (*i.e.*, pancreatobiliary type) can exhibit a somewhat pseudostratified appearance with tall columnar cells or some expression of intestinal makers, such as MUC2 and CDX2, which may have led to classifying these cases as intestinal type adenocarcinoma<sup>[30]</sup>. In any case, pure mucinous adenocarcinoma and intestinal adenocarcinoma are very rare in the bile ducts<sup>[20,30]</sup>.

Clear cell carcinoma of the extrahepatic bile ducts is composed of tumor cells with clear cytoplasm containing PAS-positive diastase-labile cytoplasmic granules. It can exhibit trabecular, nesting, glandular and sheet pattern in variable proportions and must be differentiated from

metastatic renal cell carcinoma. The presence of areas of conventional biliary adenocarcinoma and the immunohistochemical study permit the correct diagnosis. For instance, primary clear cell carcinoma of the hilar ducts express CK7, whereas this is not the case for renal clear cell carcinoma<sup>[52,53]</sup>.

Gastric foveolar type hilar CCs are well-differentiated tumors with tubular glands lined by slender tall columnar cells with mucin-containing cytoplasm and basal nuclei with small nucleoli, resembling gastric foveolar cells. Long and irregular tubular glands, nuclear pseudostratification and minor foci of less differentiated tumor cells can also be observed<sup>[1,54]</sup>. Very recently, three hilar CCs with pyloric gland phenotype have been described as a new morphological variant of EBDC. Although they were extremely differentiated, their infiltrative pattern and perineural invasion facilitated the diagnosis and their clinical behavior was similar to that of conventional biliary adenocarcinoma. They exhibit complex glands with a stellar pattern that seems to be a unique feature of this distinctive variant. Immunohistochemically, this variant coexpress MUC5AC (gastric foveolar mucin) and MUC6 and is negative for MUC2 and CDX2. This immunophenotype is similar to that observed in foveolar adenocarcinomas<sup>[20]</sup>.

Other carcinomas that have very rarely been described in the extrahepatic bile ducts are signet ring cell carcinoma, carcinosarcoma and undifferentiated carcinoma<sup>[1,20,55]</sup>.

### Premalignant lesions

Currently, two premalignant lesions related to the development of CC are known. They are referred to in the WHO classification of biliary tumors with the names of biliary intraepithelial neoplasia (BilIN) and intraductal papillary neoplasm of the biliary tract (IPNB)<sup>[1]</sup>. They are usually found in the intrahepatic large bile ducts and extrahepatic ducts (including the hilar bile ducts) and are precursors of some ICC and EBDC. BilIN e IPNB are postulated to be in many respects the counterpart of respectively, pancreatic intraepithelial neoplasm (PanIN) and intraductal papillary mucinous neoplasm (IPMN) of the pancreas (IPMN-P) because they share common morphological features and biological behaviors<sup>[51]</sup>.

Previously known as atypical biliary epithelium, biliary dysplasia or carcinoma *in situ*, BilIN are flat or low-papillary lesions, therefore only recognizable microscopically. Currently, BilIN are classified into three histological grades based on the degree of atypia, suggesting a spectrum of lesions with increasing neoplastic potential. Diagnostic criteria of consensus, for which a moderate interobserver agreement among experienced pathologists has been observed, are available<sup>[56,57]</sup>. BilIN-1 lesions are most commonly flat, with cellularity only slightly increased and round or oval nuclei only slightly enlarged. BilIN-2 are flat, pseudo or micropapillary lesions with loss of cellular polarity, nuclear pseudostratification and enlargement of the nuclei with hyperchromasia and irregular nuclear membrane. BilIN-3 cytologically resemble carcinoma (includes the lesion previously called

carcinoma *in situ*), mostly being pseudo or micropapillary, sometimes with budding of small cluster of cells into the lumen, with large hyperchromatic nuclei with severe membrane irregularities<sup>[57]</sup>. When accompanied by invasive lesions, BilIN is known to progress to conventional CC (*i.e.*, tubular adenocarcinoma) with biliary type phenotype. This pathway is characterized by MUC2-/CK7+/CK20-, with an increased expression of MUC1 along with the disease progression. MUC2 and CK20, reflecting the intestinal type, is rarely observed in this lineage<sup>[58]</sup>.

Intraepithelial carcinoma (BilIN-3, carcinoma *in situ*) have been described in 10% to 75% of invasive EBDC. When observed associated with invasive carcinoma, these intraepithelial malignant cells might belong to a primary carcinoma *in situ* or could be due to cancerization of the surface epithelium from the invasive carcinoma. Extensive intraepithelial spread (also called superficial spread) have been defined as the presence of intraepithelial carcinoma 20 mm or more in length from the margin of the main lesion to the proximal or distal side and has been observed in 13%-18% of EBDC<sup>[18,59]</sup>. The majority of cases with extensive intraepithelial spread are histologically well differentiated papillary tumors. In resected specimens, the presence of extensive intraepithelial spread has been observed to be associated with a better postoperative prognosis, although it might be related to late relapses of the tumor in the bile duct stump.

Biliary papilloma, biliary papillomatosis, papillary CC, CC of the intraductal growth type, mucin hypersecreting CC, mucin hypersecreting bile duct tumors, mucin ball-producing EBDC and others are different terms to designate a constellation of biliary papillary tumors that currently are considered to belong to a single tumor entity called IPNB<sup>[60-62]</sup>. The incidence of IPNB among all bile duct carcinomas range from 7% to 38%<sup>[63]</sup>. IPNB produce papillary projections macroscopically evident into the lumen of the bile ducts. Around a third of the IPNB secrete mucus grossly visible into the lumen (IPNB with excess mucin secretion or mucin secreting biliary tumor)<sup>[1,64]</sup>. Infrequently, IPNB appears as a cystic tumor (IPNB with prominent cystic changes or cystic variant of IPNB)<sup>[64,65]</sup>.

Histologically, IPNB are composed of papillary fronds with delicate fibrovascular cores, with or without gland formations that may be lined with four different cell types: pancreatobiliary, intestinal, gastric and oncocytic type. The most frequent phenotype is the pancreatobiliary, characterized by columnar cells with moderate amphophilic cytoplasm and enlarged nuclei resembling biliary epithelium (MUC1+, MUC2-, MUC5AC+ being the most common immunophenotype). Next in frequency are the intestinal and gastric types, with different outcomes in terms of its prevalence. The intestinal type show columnar cells and goblet cells resembling intestinal epithelium (MUC1-, MUC2+, MUC5AC+), whereas the gastric type is composed of columnar epithelial cells with abundant cytoplasmic mucin that resemble the gastric foveolar epithelium (MUC1-, MUC2-, MUC5AC+). The

oncocytic type (MUC-/+, MUC2+, MUC5AC+) is considered a variant of the pancreatobiliary type and is the rarest form. In some cases it may be difficult to identify the type only by its microscopic appearance as it is necessary to carry out its classification on the basis of immunohistochemistry<sup>[60,62,63,66]</sup>. Although pancreatobiliary type is the most common phenotype, the majority of IPNB with macroscopically visible mucin secretion (mucin producing biliary tumors) are intestinal type (also referred as columnar type). These mucin secreting papillary tumor, also known as biliary tract IPMN, are the IPNB who most resemble IPMN of the pancreas pathologically, especially the main pancreatic duct type<sup>[61,67,68]</sup>. Recently, a rare case of pseudomyxoma peritonei preceded by IPNB has been described<sup>[69]</sup>.

IPNB exhibit a spectrum of architectural complexity and cytological atypia but, unlike BilIN, there are no well-defined criteria for their grading. Instead, consensus criteria for IPMNP have been applied to grade IPNB. Accordingly, the current WHO system classifies IPNB into low, intermediate and high grade<sup>[1,58]</sup>.

Most IPNB (70%-80% of cases) are associated with a component of invasive adenocarcinoma<sup>[63]</sup>. Invasive papillary carcinoma has been the classical name for this invasive adenocarcinoma. However, currently this tumor is better named "intraductal papillary neoplasm with associated invasive adenocarcinoma". In fact, invasive papillary structures are very rarely observed, the invasive component associated with IPNB being a conventional type (tubular or pancreatobiliary type) adenocarcinoma in the majority of cases, although can also frequently be a colloid carcinoma and other invasive type components may occasionally be seen<sup>[58,67]</sup>. During carcinogenesis from IPNB to invasive carcinoma, most of IPNB are characterized by an intestinal immunophenotype (MUC1-/MUC2+) which is commonly conserved in associated colloid carcinomas, whereas the majority of tubular adenocarcinoma related to IPNB acquires MUC1 expression (MUC1+, MUC2+). MUC1 is also expressed in the majority of biliary duct adenocarcinomas not related to IPNB. The CK7+/CK20+ pattern is the commonest both in tubular and colloid carcinomas related to IPNB. CK20 is most frequently expressed in invasive adenocarcinoma associated with IPNB compared to CC not related to IPNB<sup>[58,62]</sup>. It has been suggested that invasive tumors arising from IPNB have a better prognosis than invasive carcinomas not related to IPNB. However, it is now believed that evolution depends largely on the stage of the invasion and histological type, the prognosis being better in colloid carcinomas. Minimally invasive papillary carcinomas (*i.e.*, with only superficial stromal infiltration) of the extrahepatic bile duct have been found to have a good prognosis, similar to noninvasive papillary carcinomas<sup>[50,70]</sup>.

Recently, two cases have been described of so called biliary intraductal tubulopapillary neoplasm (ITPN) in the hilar bile ducts. It has been proposed that biliary ITPN is a distinct tumor entity with a suggested origin from pre-existing peribiliary cysts<sup>[71]</sup>.

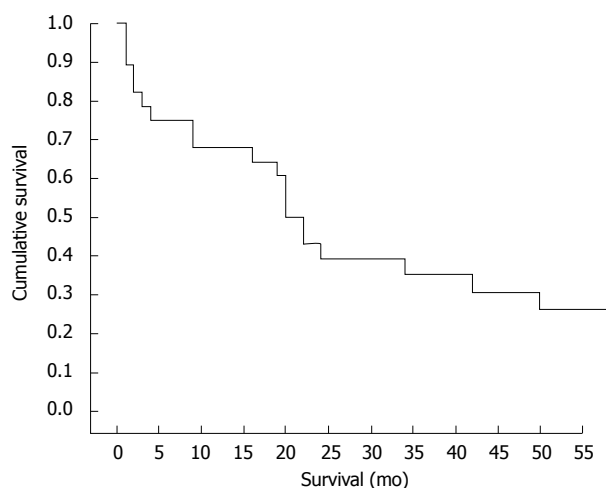
**Table 1** Observed survival of a series of 29 patients with resected hilar cholangiocarcinoma

Period (yr)	Observed survival (95%CI)
1	68.4 (51.3-85.5)
2	39.6 (21.5-57.7)
3	35.6 (17.7-53.5)
4	31.2 (13.5-48.9)
5	22.3 (6.2-38.4)

**Table 2** Histological types of 29 cases of perihilar bile duct carcinomas *n* (%)

Histology	
Adenocarcinoma, biliary type	24 (82.8)
Without IPNB	20 (69)
Well or moderately differentiated	18
Poorly differentiated with signet ring cells	2
With IPNB	4
Well or moderately differentiated	3
Poorly differentiated with signet ring cells	1
IPNB high grade (papillary carcinoma)	5 (17.2)
Without invasive carcinoma	1 (3.4)
With invasive carcinoma, biliary type	4 (13.8)
Well-moderately differentiated	3
Poorly differentiated with signet ring cells	1
Adenocarcinoma, gastric foveolar type	2 (13.8)
Adenocarcinoma, clear cell type	1 (3.4)
Adenosquamous carcinoma	1 (3.4)
Total number of cases	29

IPNB: Intraductal papillary neoplasm of the biliary tract.

**Figure 1** Survival curve of a series of 29 patients with resected hilar cholangiocarcinoma.

### Staging and other histological prognostic factors

There are different staging schemes to evaluate hilar CC: the modified Bismutt-Corlette system<sup>[72]</sup>, the Memorial Sloan-Kettering Cancer Center classification<sup>[73]</sup> and the more recently described new proposal by the International CC Group for the Staging of Perihilar CC<sup>[74]</sup>. Currently, the system most widely used by pathologists to stage these tumors after surgical resection is the pathological

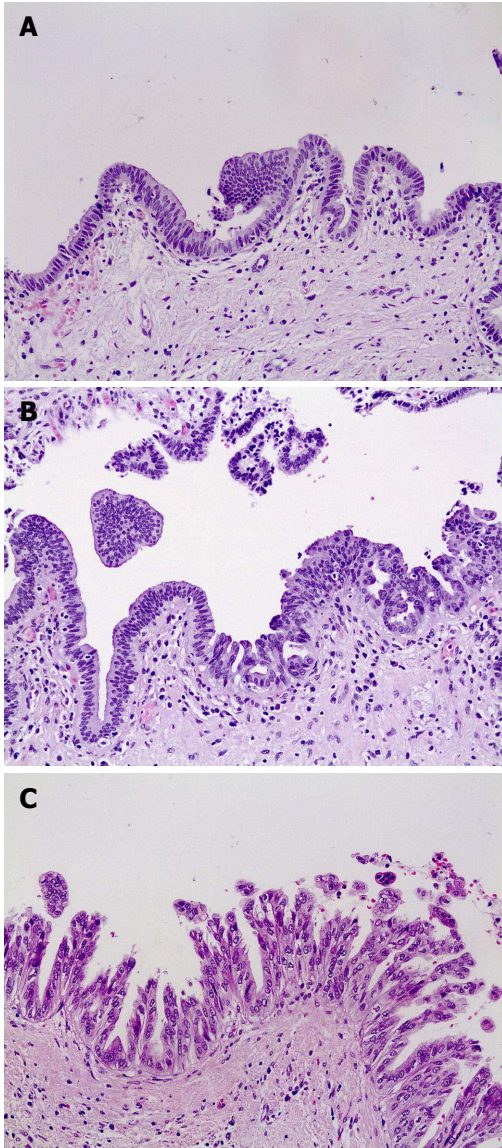
**Table 3** Other histological features in 29 cases of perihilar bile duct carcinomas *n* (%)

Gross type	
Sclerosing	10 (34.5)
Nodular	8 (27.6)
Nodular-sclerosing	6 (20.7)
Papillary	2 (6.9)
Papillary-nodular	2 (6.9)
Papillary-sclerosing	1 (3.4)
BillIN	
BillIN-1 and/or 2	10 (34.5)
BillIN-1	9 (31)
BillIN-2	5 (17.2)
BillIN-3 ( <i>in situ</i> carcinoma)	6 (20.7)
Lymphatic invasion (L1)	9 (31)
Venous invasion (V1)	11 (37.9)
Perineural invasion	23 (79.3)
T-staging (according to AJCC/UICC (7th ed)	
pTis	1 (3.4)
pT1	1 (3.4)
pT2a	10 (34.5)
pT2b	12 (41.4)
pT3	5 (17.2)
Lymph node status:	
Positive	7 (24.1)
Negative	16 (55.2)
No lymph nodes histologically studied	6 (20.7)
Margin status	
Negative (R0)	12 (41.4)
Positive (R1) (invasive carcinoma)	17 (58.6)
Bile duct margin	10 (34.5)
Radial margin	11 (37.9)

BillIN: Biliary intraepithelial neoplasia; AJCC/UICC: American Joint Committee Cancer/Union for International Cancer Control.

TNM included in AJCC/UICC TNM classification<sup>[5]</sup>. The AJCC/UICC establishes three different staging systems for intrahepatic, perihilar and distal bile duct carcinomas. Proximal or perihilar CC (Klatskin tumors) are defined anatomically by the AJCC/UICC as tumors located in the extrahepatic biliary tree proximal to the origin of the cystic duct, which may extend proximally into either the right or left hepatic ducts, or both. Recently, some problematic aspects that may arise when applying the pathological TNM for EBDC have been revised, looking for opportunities for improvement<sup>[75]</sup>. For instance, perihilar CC is considered pT1 if it is confined to the bile duct, with extension up to the muscle layer or fibrous tissue, and pT2a when it invades beyond the wall of the bile duct to the surrounding adipose tissue. However, the muscle layer is only well-defined in the very distal common bile duct and in many bile ducts there are no hallmarks to determine where the ducts end, especially in the setting of fibrosis which often accompanies these tumors. As an alternative, some authors have proposed the use of the depth of invasion as part of the T-staging of EBDC. They have found that the cutoff points of 5 and 12 mm separate patients with EBDC into three groups with different lengths of survival. For hilar CC, tumor depth  $\geq 5$  mm was predictive of poor survival in one study<sup>[76,77]</sup>. pT3 and pT4 definitions involve determining whether the portal vein and hepatic artery or their branches are affected, as

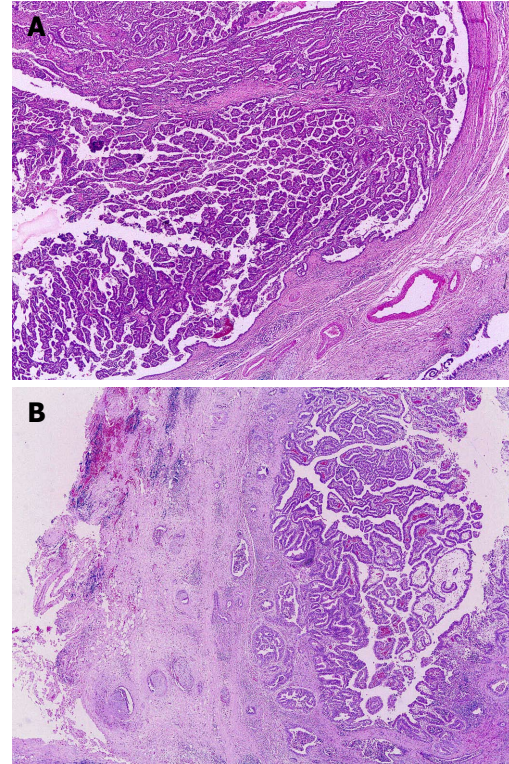




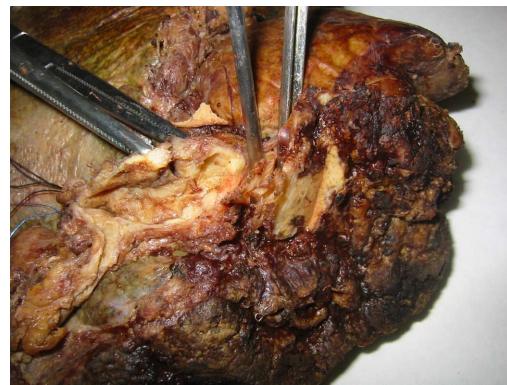
**Figure 2 Biliary intraepithelial neoplasia.** A: Biliary intraepithelial neoplasia (BillIN) 1 (HE stain,  $\times 200$ ); B: BillIN 2 (HE stain,  $\times 100$ ); C: BillIN 3 (HE stain,  $\times 100$ ).

well as the secondary biliary radicals. However, this assessment may be inaccessible to the pathologist unless some of these structures are specifically marked by the surgeon<sup>[75]</sup>. Positive lymph nodes represent one of the most relevant prognostic factors<sup>[11,14]</sup>. Regional lymph node metastases (nodes along the cystic duct, common bile duct, hepatic artery and portal vein) are considered pN1, whereas periaortal, pericaval, superior mesenteric artery and celiac artery lymph nodes are assigned pN2<sup>[5]</sup>. To avoid an incorrect staging, lymph nodes should be referred properly identified with regards to their origing to the pathologist.

The incidence of positive surgical resection margins in patients treated surgically with curative intent is very variable (9%-74%). The affected ductal resection margins by invasive carcinoma has a strong adverse effect on patient survival, whereas being affected by severe dysplasia or carcinoma *in situ* does not seem to have such a pernicious effect, although it could be responsible for some



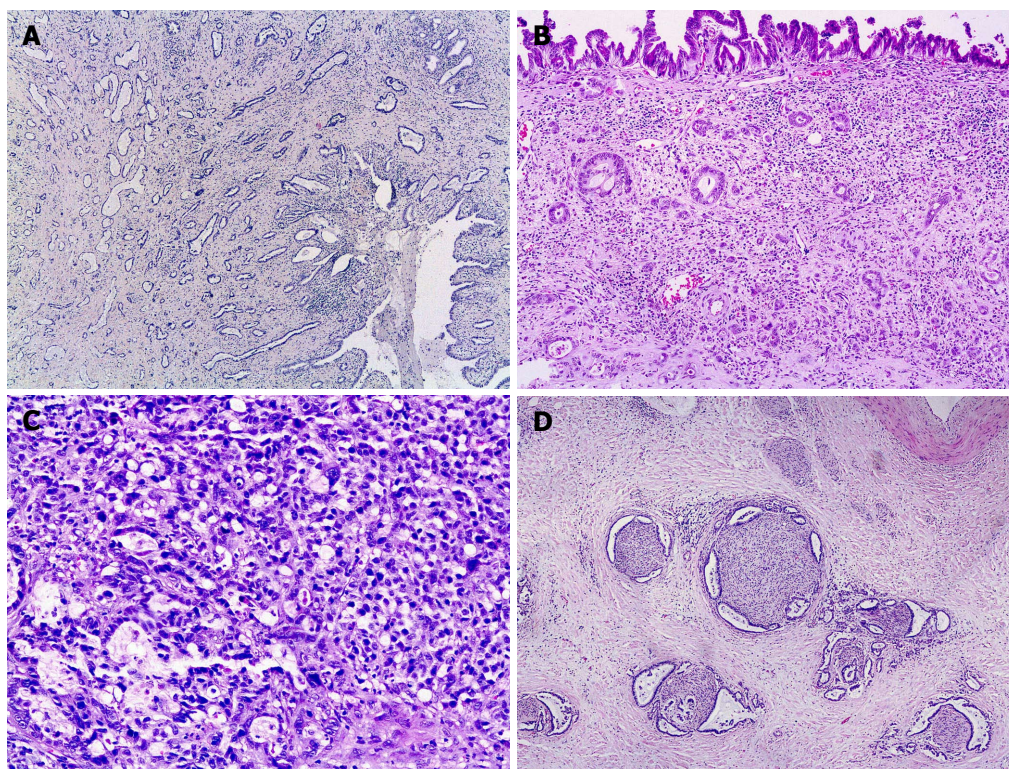
**Figure 3 Intraductal papillary neoplasm of the biliary tract.** A: Intraductal papillary neoplasm of the biliary tract (IPNB) without invasion, classically named biliary papillomatosis (HE stain,  $\times 20$ ); B: IPNB with associated invasive carcinoma, previously named invasive papillary carcinoma (HE stain,  $\times 20$ ).



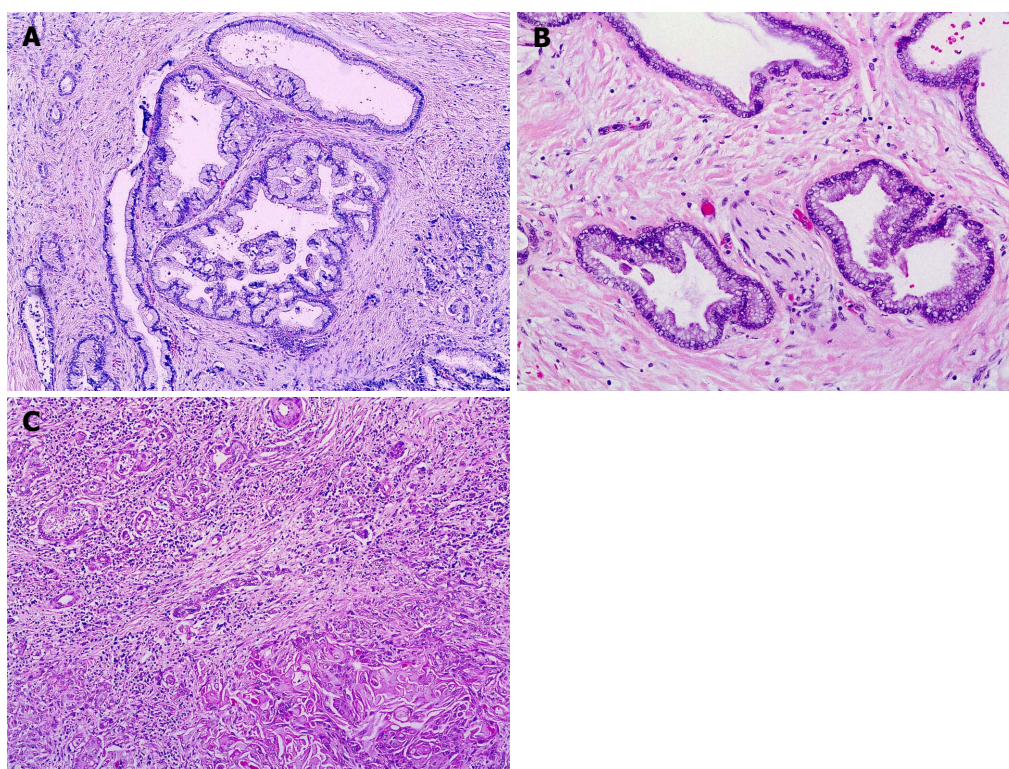
**Figure 4 An example of a nodular-sclerosing cholangiocarcinoma in the common, right and left hepatic ducts.**

late recurrences in the stump. This suggests that carcinoma *in situ* could take several years to become invasive in the stump<sup>[14,15,25,78,79]</sup>. The dissection margin has been defined as the remaining surgical cleavage plane with the adjacent hilar structures. Although it seems that less emphasis has been placed on dissection margin, this radial margin should be taken into consideration. Positivity of the dissection margin in hilar CC has been observed to be considerably higher compared to the ratio of positivity of the ductal margins<sup>[22]</sup>. In addition, intraoperative examination by frozen sections is very useful for obtaining a ductal resection margin free of invasive carcinoma (the distinction between dysplasia, carcinoma *in situ* and





**Figure 5** Examples of the broad morphological spectrum of hilar bile duct carcinoma. A: Well differentiated biliary type adenocarcinoma (HE stain,  $\times 40$ ); B: A case with well defined tumor glands interspersed with poorly differentiated small tumor groups and single tumor cells (besides, biliary intraepithelial neoplasia 3 can be observed on the duct surface) (HE stain,  $\times 100$ ); C: A case with poorly differentiated cell component with resembling signet ring cells (HE stain,  $\times 200$ ); D: Perineural invasion by well differentiated glands (HE stain,  $\times 40$ ).



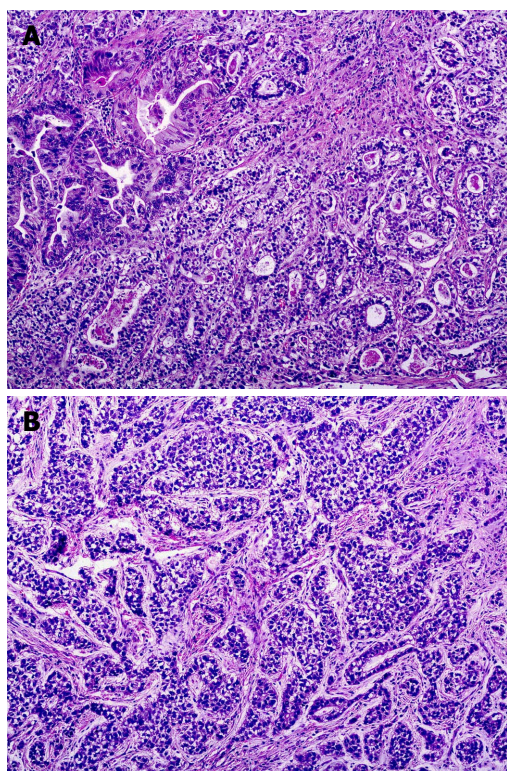
**Figure 6** Histological variants. A: Gastric foveolar type carcinoma (HE stain,  $\times 100$ ); B: Very well differentiated glands of a gastric foveolar type carcinoma near a nervous fascicle (HE stain,  $\times 200$ ); C: Adeno-squamous carcinoma showing the glandular and squamous component (HE stain,  $\times 100$ ).

reactive changes in frozen sections is less reliable) but is not really feasible for the dissection margin in most cases. Another issue concerns the minimal distance required between tumor and resection margin. Some authors have observed that a distance of tumor less than 5 mm from the transverse surgical margin is associated with the worst prognosis. In Japan, a distance of 5 mm has been proposed to define R0 resections, although the TNM

AJCC/UICC system does not make any consideration in this regard<sup>[26,80,81]</sup>. We prefer to follow the notion of R0 according to its definition by the AJCC/UICC literally, so we only consider R0 if the tumor is not at the margin. In addition, we add the distance between the tumor and the margin in the report.

Other histological factors that have been adversely associated with prognosis in univariable and, in some cases,





**Figure 7** Histological variants: clear cell carcinoma (HE stain,  $\times 100$ ). A: An area with glandular pattern; B: An area with trabecular pattern.

multivariable analysis, are the high tumor grade, the presence of vascular, lymphatic or perineural invasion<sup>[11,23,28]</sup> and nodular or sclerosing gross features (*vs* papillary tumors)<sup>[9]</sup>. Some histological subtypes like adenosquamous and squamous carcinoma could have greater malignant potential than conventional biliary type adenocarcinoma<sup>[47,49]</sup>. According to the CAP protocol for perihilar CC, high-grade tumors, such as signet-ring cell carcinomas, small cell carcinomas and undifferentiated carcinomas, are associated with a poorer prognosis compared with conventional adenocarcinoma<sup>[28]</sup>.

#### Contribution of a series of hilar CC from our institution

A total of 71 patients with extrahepatic non ampullary biliary duct carcinoma were obtained from the files of the Department of Pathology, Hospital “12 de Octubre” in Spain between January 1999 to December 2011. After excluding distal bile duct tumors and cases with only incisional biopsies or cytology samples, we reviewed the surgical specimens of 29 patients, 17 males and 12 females, aged 47 to 82 years (median age, 68 years; mean age, 67.5 years) with hilar CC. The observed survival was 39.6% and 22.3% at one and three years. The survival curve is shown in Table 1 and Figure 1. A summary of the histological features is given in Tables 2 and 3.

With respect to T-staging, in three cases the tumor extended to the cystic duct and/or gallbladder, although the main tumor mass was located in the hilar bile ducts. This situation is not covered by the current TNM system for perihilar CC.

The majority of the tumors (82.8%) were conventional type adenocarcinomas (biliary or pancreatobiliary), most of them well to moderately differentiated (21 cases, 72.4%). A component of *in situ* carcinoma BiIN-3 was present in 6 of the cases (20.7%) (Figure 2). Five cases exhibited IPNB (17.2%), four of them with an associated invasive carcinoma (Figure 3). We consider it more appropriate to call these latter cases adenocarcinoma with associated IPNB rather than invasive papillary carcinoma given that they did not infiltrate with a papillary pattern but with a conventional appearance. Four and one IPNB, respectively, showed a biliary and an intestinal differentiation. All the IPNB, including the one without an invasive component, exhibited a high cytological grade of dysplasia. With respect to their macroscopic appearance, two grossly papillary cases correspond to the IPNB without associated invasive carcinoma and one case of IPNB with associated superficial invasive carcinoma (*i.e.*, micro-invasive carcinoma o pT1). Cases with papillary-nodular pattern (2) and papillary-sclerosing pattern (1) pertain to the remaining observed cases of invasive carcinoma associated to IPNB. Among non-papillary tumors, the separation between nodular and sclerosing types was a somewhat subjective in practice (Figure 4).

Histologically, conventional adenocarcinomas exhibited a broad morphological spectrum from one case to another and even within the same tumor, with cuboidal or tall cells, different sized glands, tubules, cords and single cells. Desmoplastic stroma was constantly present, at least in some tumor areas. Some cases were extremely well differentiated. In these cases, the diagnosis was aided by their infiltrative appearance in the surgical specimen; however, the diagnosis of these cases in small biopsies would be extremely difficult. We observed three cases with a poorly differentiated cell component resembling signet ring cells, one of them somewhat histiocytoid. In addition, we observed a biliary type adenocarcinoma with taller cells with greater nuclear stratification resembling intestinal adenocarcinoma, although with other glands showing biliary features clearly (Figure 5).

With respect to non-conventional histology, two well differentiated tumors belonged to the gastric foveolar type (although this variant, as well as the more recently described pyloric type would need studies of larger series for better characterization). In addition, a clear cell type carcinoma and one case of adenosquamous carcinoma was observed (Figures 6 and 7).

Most reviewed cases (23 cases, 79.3%) showed perineural invasion, which in most cases was extensive (Figure 5). Lymphatic and venous vessel invasion was observed in 9 (31.0%) and 11 (37.9%) cases, respectively. Lymphatic invasion especially showed a significant subjectivity in its assessment, in part due to tissue shrinkage around many neoplastic groups.

Seventeen specimens (58.6%) exhibited involvement of the surgical margins, with the bile duct margin positive in 10 (34.5%) and radial margin positive in 11 (37.9%) cases. Radial margin involvement frequently occurred in the periductal soft tissue and in some cases at the liver parenchyma.

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## Chemotherapy for cholangiocarcinoma: An update

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### Abstract

Cholangiocarcinomas (bile duct cancers) are a heterogeneous group of malignancies arising from the epithelial cells of the intrahepatic, perihilar and extrahepatic bile ducts. Patients diagnosed with cholangiocarcinoma must be evaluated by a multidisciplinary team and be treated with individualized management. First of all, it is very important to define the potential resectability of the tumor because surgery is the main therapeutic option for these patients. Overall, cholangiocarcinomas have a very poor prognosis. The 5-year survival rate is 5%-10%. In cases with a potentially curative surgery, 5-year survival rates of 25%-30% are reported. Therefore, it is necessary to increase the cure rate from surgery, exploring the survival benefit of any adjuvant strategy. It is difficult to clarify the role of adjuvant treatment in localized and locally advanced cholangiocarcinomas. There are limited data and the role of adjuvant chemotherapy/chemoradiation in patients with resected biliary tract cancer is poorly defined. The most relevant studies in the adjuvant setting are one from Japan, the well known ESPAC-3 and BILCAP from the United Kingdom and a meta-analysis. We show the results of these trials. According to medical oncology guidelines, postoperative adjuvant therapy is widely recommended for all patients with intrahepatic or extrahepatic cholangiocarcinoma who have microscopically positive resection margins, as well as for those with a

complete resection but node-positive disease. Clinical trials are ongoing. The locally advanced cholangiocarcinoma setting includes a heterogeneous mix of patients: (1) patients who have had surgery but with macroscopic residual disease; (2) patients with locally recurrent disease after potentially curative treatment; and (3) patients with locally unresectable disease at presentation. In these patients, surgery is not an option and chemoradiation therapy can prolong overall survival and provide control of symptoms due to local tumor effects. Nowadays, no neoadjuvant therapy can be considered a standard approach for the treatment of patients with cholangiocarcinoma. There are promising results and randomized trials are needed in patients with a metastatic cholangiocarcinoma. In systemic therapy, no single drug or combination has consistently increased median survival beyond the expected 8-12 mo. It is always recommended that patients enrol in clinical trials. Clinical trials have shown that the more standard chemotherapy for a first line regimen of gemcitabine plus cisplatin (or oxaliplatin as a potentially better tolerated agent) is superior to gemcitabine alone. Leucovorin-modulated 5-fluorouracil, capecitabine monotherapy or single agent gemcitabine are reasonable options for patients with a borderline performance status. After progression in patients with an adequate performance status, active regimens that could be considered include gemcitabine plus capecitabine, or erlotinib plus bevacizumab, for second line treatment.

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**Key words:** Chemotherapy; Cholangiocarcinoma; Review; Oncology; Gemcitabine

**Core tip:** Cholangiocarcinomas (bile duct cancers) are an heterogeneous group of malignancies arising from the epithelial cells of the intrahepatic, perihilar and extrahepatic bile ducts. Leucovorin-modulated 5-fluorouracil, capecitabine monotherapy or single agent gemcitabine are reasonable options for patients with a borderline performance status. After progression in patients with an adequate performance status, ac-

tive regimens that could be considered for second line treatment include gemcitabine plus capecitabine or erlotinib plus bevacizumab.

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## INTRODUCTION

Cholangiocarcinomas (bile duct cancers) are malignancies arising from the epithelial cells of the intrahepatic, perihilar and extrahepatic bile ducts.

Bile duct cancers are a heterogeneous group. Overall, cholangiocarcinomas have a very poor prognosis<sup>[1]</sup>. The 5-year survival rate is 5%-10%. In cases which undergo a potentially curative surgery, the reported 5-year survival rates are 25%-30%<sup>[2,3]</sup>. In metastatic disease, no single drug or combination has consistently increased median survival beyond the expected 8-12 mo. Different outcomes present depending on the specific location. Other prognosis factors to consider are: perihilar had particularly poor prognosis; margin status; vascular invasion; lymph node metastases; transmural extension to the gallbladder; hepatic venous invasion; histology (papillary ones have a better prognosis); gender (female is better); albumin level ( $< 3$  g/dL is adverse); bilirubin level ( $> 10$  g/dL is adverse)<sup>[4,5]</sup>.

## TREATMENT OF LOCALIZED CHOLANGIOCARCINOMAS: ROLE OF ADJUVANT TREATMENT

Patients diagnosed with cholangiocarcinoma must be evaluated by a multidisciplinary team and be treated with individualized management.

First of all, it is absolutely vital to assess the potential resectability of the tumor since surgery represents the main therapeutic option for these patients. Even in patients who undergo complete surgical resection, prognosis is poor and relapses are frequent. The most common relapse pattern is local; distant metastases are less common but not rare, typically a hepatic or peritoneal recurrence<sup>[6]</sup>.

So, it is necessary to increase the cure rate from surgery, exploring the survival benefit of any adjuvant strategy. Postoperative adjuvant therapy is widely recommended for all patients with intrahepatic or extrahepatic cholangiocarcinoma who have microscopically positive resection margins, as well as for those with a complete resection but node-positive disease.

The role of adjuvant chemotherapy/chemoradiation in patients with resected biliary tract cancer is poorly defined. Although it is widely used and recommended

in guidelines from medical oncologist expert groups, the survival benefit of any adjuvant strategy has never been proven in specifically designed randomized clinical trials. Most of the recommendations are primarily based on practice patterns at some institutions and on retrospective studies from single center experiences<sup>[7,8]</sup>. There are limited data from multiple retrospective series and small phase II trials from single institutions, but most of these studies are not randomized and with heterogeneous patient populations often combine gallbladder cancers with pancreatic cancers with intrahepatic and extrahepatic cholangiocarcinomas.

The optimal treatment strategy in the adjuvant setting has not been determined. There are no randomized phase III clinical trial data to support a standard adjuvant regimen. There are phase II trials that support the following regimens: gemcitabine/cisplatin; gemcitabine/oxaliplatin; gemcitabine/capecitabine; capecitabine/cisplatin; capecitabine/oxaliplatin; 5-fluorouracil (5-FU)/oxaliplatin; 5-FU/cisplatin; and for monotherapy with the single agents gemcitabine, capecitabine and 5-FU<sup>[9,10]</sup>.

Following complete surgical resection of intrahepatic or extrahepatic cholangiocarcinoma, postoperative adjuvant therapy is widely recommended for all patients who have microscopically positive resection margins and for all those who have node-positive disease. This is the recommended management suggested in guidelines from expert groups but the actual survival benefit of any adjuvant strategy for cholangiocarcinomas has not been proven in well-designed randomized trials. There are limited data from multiple retrospective series that suggest superior outcomes for patients with postoperative chemoradiotherapy compared to historical series of patients who did not undergo the treatment<sup>[11,12]</sup>.

In a recent retrospective review of the period of 1995-2005 at a single institution, of the patients treated for biliary tract cancer, only 6.5% received adjuvant chemotherapy alone and another 6.5% received chemoradiation<sup>[13]</sup>. In another retrospective analysis which used the Surveillance Epidemiology and End Results (SEER) database to investigate patients with gallbladder cancer from 1992-2002, only 17% of the 2325 patients in the surgical cohort received adjuvant chemoradiation<sup>[14]</sup>.

There are few studies that evaluate the use of adjuvant chemotherapy alone in patients with biliary tract cancer. Some groups<sup>[15]</sup> have reviewed the available data on both chemotherapy and targeted therapies for biliary carcinoma and, with conventional chemotherapy, a response rate ranging from 10% to 40% has been reported.

There are other studies: one from Japan, the well-known European Study Group for Pancreatic Cancer-3 and BILCAP from the United Kingdom and a meta-analysis. The first is a single multi-institutional randomized trial from Japan which compared postoperative chemotherapy [two courses of mitomycin C plus infusional 5-FU, followed by prolonged oral administration of 5-FU until tumor progression] vs surgery alone in 508 patients with resected pancreatobiliary malignancies, 139



were cholangiocarcinomas. Lymph node metastases were present in 84% and 88% of the patients randomly assigned to chemotherapy and surgery alone, respectively. In the subset of patients with bile duct cancer, 5-year overall survival (5yOS) was not significantly better with chemotherapy (27% *vs* 24%). When the results were stratified according to surgical margins, chemotherapy did not significantly improve outcome in patients undergoing non curative resection (5yOS: 8% *vs* 16%), while there was a statistically non significant trend towards better 5yOS among patients with a potentially curative resection (41% *vs* 28%)<sup>[14]</sup>.

Secondly, the European Study Group for Pancreatic Cancer-3 trial, the largest randomized trial was conducted in patients with resected periampullary adenocarcinomas. Four hundred and twenty-eight patients with periampullary malignancies (96 bile ducts) were randomly assigned to one of three arms: observation, 6 mo of leucovorin-modulated FU or 6 mo of single agent gemcitabine. The use of adjuvant treatment was associated with a potential advantage but was not statistically significant (median 43 mo *vs* 35 mo, HR 0.86, 95%CI: 0.66-1.11), but multivariate analysis, correcting for prognosis factors, found a statistically significant survival benefit for chemotherapy, specifically for gemcitabine, and with a better safety profile. These results must be considered hypothesis generating for further studies<sup>[17]</sup>.

The other important study from the United Kingdom is the BILCAP study<sup>[18]</sup>. BILCAP is a multi-center prospective, randomised phase III trial which is trying to examine the role of adjuvant chemotherapy with oral fluoropyrimidine (capecitabine) in patients following potentially curative surgical resection of a biliary tract cancer. Since 2006, patients in England and Wales with a macroscopically complete surgical resection are randomised to receive either adjuvant chemotherapy with capecitabine or observation. BILCAP is the most successful adjuvant study in biliary tract cancer and is on target to complete accrual early in 2013.

The meta-analysis includes the Japanese trial, two SEER registry analyses and 17 retrospective series, which includes 6712 patients, of whom 1797 received some form of adjuvant therapy. There were 8 studies of radiotherapy (RT) plus chemotherapy, 3 of chemotherapy alone, and 9 of RT alone. Only one study included intrahepatic cholangiocarcinoma. In this meta-analysis, the improvement in five year survival with any adjuvant therapy was not statistically significant [pooled odds ratio (OR) = 0.74, 95%CI: 0.55-1.01] compared with surgery alone. The results were similar when gallbladder and bile duct cancers were analyzed independently. However, the survival benefit from adjuvant therapy was statistically significant when the data from the two large registry series (*n* = 1233 patients) were excluded (OR = 0.53, 95%CI: 0.39-0.72). The benefits of adjuvant therapy were modality-dependent. In a combined analysis of gallbladder and bile duct cancers, there was a significant survival benefit for chemotherapy (OR = 0.39, 95%CI: 0.23-0.66)

and chemoradiotherapy (OR = 0.61, 95%CI: 0.38-0.99) but not RT alone (OR = 0.98, 95%CI: 0.67-1.43). Pooled data from nine studies, in which at least 50% of the patients had nodal or margin positivity, confirmed a statistically significant overall survival advantage for any adjuvant therapy in node-positive disease (OR = 0.49, 95%CI: 0.30-0.80). The majority of these patients (77%) had received chemotherapy alone, while the remainder underwent chemoradiotherapy. Similarly, a significant benefit for any adjuvant therapy was shown for patients with margin-positive disease (OR = 0.36, 95%CI: 0.19-0.68)<sup>[19]</sup>.

While this analysis supports current practice (*i.e.*, adjuvant therapy for high risk subgroups with bile duct cancer), it does not resolve the question of the best treatment strategy for high risk patients or adequately address the benefit of adjuvant therapy for patients with low risk (*i.e.*, node negative) disease.

## GUIDELINE RECOMMENDATIONS

### *The National Comprehensive Cancer Network*

**For extrahepatic cholangiocarcinoma:** For patients with resected, margin-negative extrahepatic cholangiocarcinoma with negative regional nodes, observation, fluoropyrimidine or gemcitabine-based chemotherapy or fluoropyrimidine-based chemoradiotherapy are acceptable options; for patients with carcinoma *in situ* at the margins or positive margins with invasive disease, fluoropyrimidine-based chemoradiotherapy followed by additional fluoropyrimidine or gemcitabine chemotherapy; for positive regional lymph nodes, fluoropyrimidine or gemcitabine-based chemotherapy<sup>[20]</sup>.

**For intrahepatic cholangiocarcinoma:** For no residual local disease, no adjuvant therapy recommendations are made. For patients with positive margins, options include re-resection, ablation, fluoropyrimidine or gemcitabine-based chemoradiotherapy, or fluoropyrimidine or gemcitabine-based chemotherapy.

### *Guidelines from the European Society of Medical Oncology*

For treatment of either intrahepatic or extrahepatic cholangiocarcinoma, the following are suggested: supportive care or palliative chemotherapy and/or radiotherapy after a non curative resection and consideration of postoperative chemoradiotherapy as an option after complete surgical resection<sup>[21]</sup>.

Efforts should also be made to conduct randomized clinical trials in which the individual disease entities are evaluated separately. They are desperately needed in this area and several are now ongoing. Clinical trial participation is especially encouraged.

## NEOADJUVANT THERAPY

Nowadays, no neoadjuvant therapy can be considered a standard approach for the treatment of patients with



cholangiocarcinoma; usually, they are patients with jaundice and a poor functional status at presentation and where a neoadjuvant strategy of preoperative chemoradiotherapy to convert to a potentially resectable disease is difficult. However, there are promising results that suggest the potential benefit of this approach for selected patients in the following small reports.

In an early series of nine (out of a total of 91) patients with extrahepatic cholangiocarcinoma who underwent preoperative chemoradiotherapy prior to exploration, three had a pathological complete response while the remainder showed different degrees of histological response to treatment. Margin negative resections were possible in all nine patients compared to only half of those who did not receive neoadjuvant therapy<sup>[22]</sup>. The benefit of neoadjuvant chemoradiotherapy was also suggested in a report of 45 patients undergoing concurrent chemoradiotherapy in resected extrahepatic cholangiocarcinoma, of whom 12 were treated neoadjuvantly. Three had a complete pathological response and 11 were able to undergo a complete (R0) resection. Despite having more advanced disease at presentation, patients who received neoadjuvant chemoradiotherapy had longer five year survival (53% *vs* 23%) and rates of grade 2 to 3 surgical morbidity were no higher (16% *vs* 33%) compared with those treated in the postoperative setting<sup>[23]</sup>. These are promising results that support the need for randomized trials to test this strategy of preoperative chemoradiotherapy.

## TREATMENT OF LOCALLY ADVANCED CHOLANGIOCARCINOMA

The term “locally advanced cholangiocarcinoma” includes a heterogeneous mix of patients: patients who have had surgery but with macroscopic residual disease; patients with locally recurrent disease after potentially curative treatment; patients with locally unresectable disease at presentation.

Between 50% and 90% of patients with cholangiocarcinoma present with locally unresectable disease. The prognosis for patients with locally unresectable or recurrent disease is very poor, typically measured in months. The goals of palliative therapy are relief of symptoms (pain, pruritus, jaundice) and improvement in quality of life. There is no role for tumor debulking in these cases. In this setting, chemoradiation therapy can prolong overall survival and provide control of symptoms due to local tumor effects.

The optimal regimen remains uncertain but expert groups suggest fluoropyrimidine or gemcitabine based chemotherapy, like the regimens for advanced disease. In some centers, with its convenience, capecitabine 825 mg/m<sup>2</sup> twice daily during the 5 wk of RT, or even weekly infusional 5-FU (225 mg/m<sup>2</sup> daily for 5 wk of RT) is used, following an additional 4 mo of chemotherapy alone with capecitabine alone 1000 mg/m<sup>2</sup> twice daily for 14 d of every 21 d. In cases of very aggressive tumors

or with multiple positive nodes, some prefer the use of gemcitabine plus oxaliplatin concurrent with radiotherapy.

There are three different modalities included in radiotherapy: (1) external beam irradiation (EBRT) delivered either by conventional approaches or with conformal treatment planning techniques; (2) brachytherapy with iridium-192; and (3) stereotactic radiotherapy.

Conventional dose EBRT (with or without systemic chemotherapy) may relieve pain and contribute to biliary decompression. At one year, 60%-75% of patients are free of locoregional disease progression and median survival approximates 7 to 12 mo<sup>[24]</sup>. However, local failure remains the first site of disease progression in 50%-75% of cases. Higher dose RT approaches that use either a combination of transcatheter brachytherapy plus EBRT, three-dimensional conformal radiation therapy or intensity modulated radiation therapy with or without chemotherapy may be associated with better local control and possibly prolonged survival<sup>[25]</sup>. Technical advances over the past few years have created the ability to deliver more precise, highly conformal radiation treatment to the tumor, maximally sparing adjacent normal tissues. The enhanced capability to spare such normal tissues now permits the safe delivery of a single or limited number of high dose radiation fractions to a target, whereas in the past, small fractions of daily radiation were typically used to spare normal tissues. Approaches such as these are referred to as stereotactic body radiotherapy or stereotactic body radiosurgery, although many current approaches no longer utilize an external stereotactic localization method. Experience is limited. One report included 27 patients with unresectable cholangiocarcinoma who underwent stereotactic body radiotherapy (45 Gy in three fractions) as the sole form of therapy<sup>[26]</sup>. At a median follow-up of 5.4 years, only two were still alive and the median progression-free and overall survival was 6.7 and 10.6 mo, respectively. While local control was maintained in 84% of patients at one year, six had severe duodenal/pyloric ulceration and three developed duodenal stenosis.

No randomized trial has compared any of these newer radiotherapy techniques to conventional EBRT alone or fluoropyrimidine-based chemoradiotherapy using conventional fractionation. Furthermore, the possibility of higher rates of long-term toxicity<sup>[27]</sup> has tempered enthusiasm for these approaches.

## SYSTEMIC THERAPY FOR ADVANCED CHOLANGIOCARCINOMA

Several regimens of chemotherapy are active for the treatment of advanced cholangiocarcinoma. Evidence is inconsistent because the literature regarding treatment results with specific regimens is limited because most series are small and reports consist of a mix of bile duct cancers, gallbladder cancer, ampullary cancer and either pancreatic or hepatocellular cancers. The most active agents are 5-FU, gemcitabine, cisplatin and oxaliplatin.

In patients with advanced biliary tract cancer, the

survival benefit for chemotherapy over best supportive care alone was suggested in a trial that randomly assigned 90 patients with advanced pancreatic or biliary cancer (37 with bile duct cancer) to 5-FU-based systemic chemotherapy with leucovorin and etoposide *vs* best supportive care alone (median survival 6 *vs* 2.5 mo, respectively)<sup>[28]</sup>.

Chemotherapy combinations and single agents have been evaluated in clinical studies in the metastatic setting, as reviewed by Hezel *et al*<sup>[10]</sup>.

Chemotherapy combinations with activity demonstrated in phase II clinical trials include: gemcitabine plus cisplatin; gemcitabine plus capecitabine; gemcitabine plus oxaliplatin; capecitabine plus oxaliplatin; capecitabine plus cisplatin; and 5-FU plus cisplatin.

It is known that gemcitabine is the main cytostatic for bilio-pancreatic cancer. Additional support for gemcitabine as an anchor drug for the treatment of advanced biliary cancer comes from these results: Firstly, a recent pooled analysis of 104 trials of patients with advanced biliary tract cancers that showed that the subgroup receiving a combination of gemcitabine and platinum-based agents had the greatest benefit<sup>[29]</sup>. Then, a retrospective review of 304 patients with advanced cholangiocarcinoma who received gemcitabine, a cisplatin-based regimen or a fluoropyrimidine-based regimen, showing that patients receiving a gemcitabine-based regimen had a lower risk of death<sup>[30]</sup>.

Most importantly, the superiority of the combination gemcitabine-cisplatin regimen is shown in the recently published ABC Trial. A multicenter, randomized controlled phase III study, which enrolled 410 patients with locally advanced or metastatic cholangiocarcinoma (*n* = 242), gallbladder (*n* = 148) and ampullary (*n* = 20) cancers, demonstrated that the combination of gemcitabine and cisplatin improved overall survival and progression free survival by 30% more than gemcitabine alone. Median overall survival was 11.7 *vs* 8.1 mo (HR 0.64, 95%CI: 0.52-0.80) and median progression free survival was 8.0 mo *vs* 5.0 mo (HR 0.63, 95%CI: 0.51-0.77), both in favor of the combination arm. Based on these results, the combination gemcitabine plus cisplatin is considered as the standard of care as first-line chemotherapy for biliary tract cancer treatment<sup>[31]</sup>.

## NEW AGENTS COMBINATION

There are currently several trials investigating the role of molecularly targeted therapies in biliary tract cancers. Combinations with TKI antiEGFR such as erlotinib, other antiEGFR such as cetuximab, antiangiogenics such as bevacizumab *etc.* are being examined. Although encouraging data are emerging with the use of targeted therapies, further efforts and additional data with randomized trials are needed to improve treatment options for patients.

## CONCLUSION

In conclusion, patients with localized and locally advanced cholangiocarcinomas must be treated in a multi-

disciplinary team. Surgery is the main therapeutic option for these patients but it is necessary to improve results and survival benefit. It is difficult to clarify the role of adjuvant treatment. In medical oncology guidelines, postoperative adjuvant therapy is widely recommended for all patients with intrahepatic or extrahepatic cholangiocarcinoma who have microscopically positive resection margins, as well as for those with a complete resection but node-positive disease. There are clinical trials ongoing. In systemic therapy, gemcitabine plus cisplatin has been shown to be superior to gemcitabine alone, but this regimen has not been compared head to head with other gemcitabine-based combinations. It is always recommended that patients enrol in clinical trials. Nevertheless, if a patient is not a candidate for a clinical trial or if one is not available, we suggest gemcitabine plus cisplatin (or oxaliplatin as a potentially better-tolerated agent) for a first line regimen for patients with a good performance status. Leucovorin-modulated 5-FU, capecitabine monotherapy or single agent gemcitabine are reasonable options for patients with a borderline performance status. After progression in patients with an adequate performance status, active regimens that could be considered include gemcitabine plus capecitabine or erlotinib plus bevacizumab for second line treatment.

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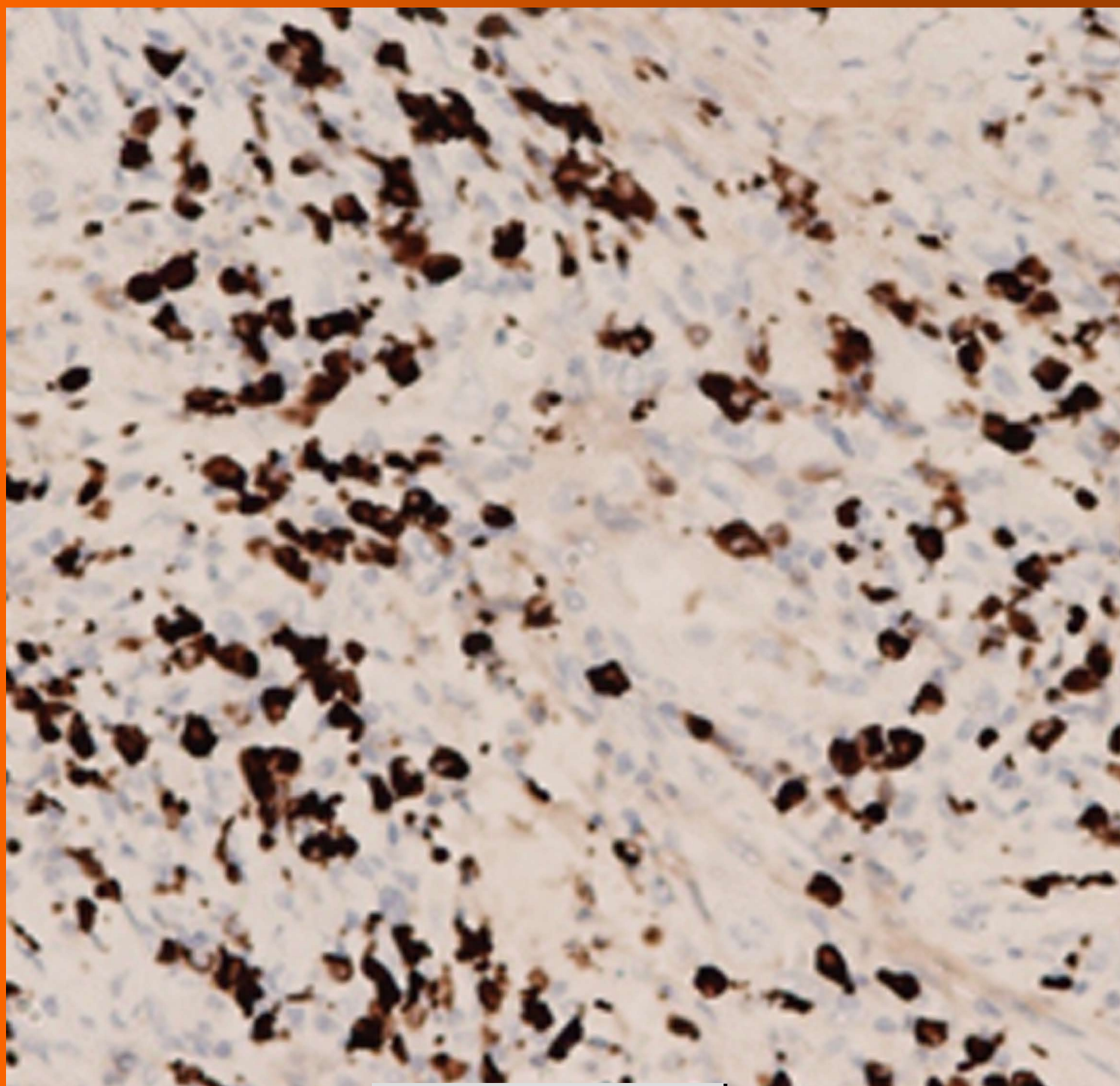
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## AIM AND SCOPE

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## Neuroendocrine tumor metastatic to the orbit treated with radiotherapy

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**Key words:** Neuroendocrine tumor; Carcinoid tumor; Orbital metastases; Diplopia; Radiotherapy

**Core tip:** Neuroendocrine tumors are rare neoplasms that only uncommonly metastasize to the orbit. Given these tumors are associated with prolonged survival despite dissemination, maintaining quality of life by providing early diagnosis and effective treatment to preserve vision and comfort is a fundamental issue. We report the case of a patient who 2 years after the diagnosis of metastatic neuroendocrine tumor to the liver presented with right-sided diplopia. She was found to have metastatic neuroendocrine tumor to the right orbit and was successfully treated with radiotherapy. Eighteen months later, she remains well and with no visual loss.

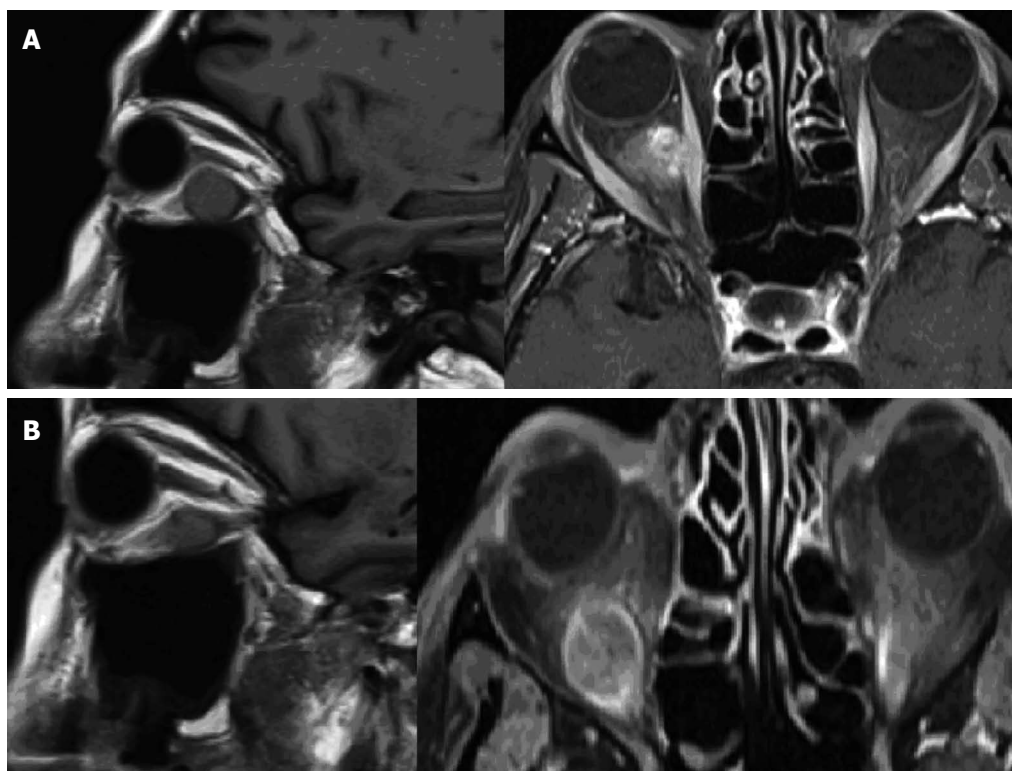
### Abstract

Neuroendocrine tumors are rare neoplasms that infrequently metastasize to the orbit. Given that patients with these tumors may have prolonged survival despite dissemination, maintaining quality of life by providing early diagnosis and effective treatment to preserve vision and comfort is a fundamental issue. We report the case of a 79-year old woman who presented with well-differentiated metastatic neuroendocrine tumor to the liver with no carcinoid syndrome and was started on intramuscular long-acting octreotide with disease stabilization. Two years later she developed right-sided diplopia associated with mild eye discomfort, proptosis and reddening. An magnetic resonance imaging showed a 2.1 cm mass in the right orbit and further biopsy confirmed a neuroendocrine tumor metastasis. The patient was treated with a four-week course of stereotactic radiotherapy to the right orbital metastasis (4000 cGy in 20 fractions) with minor conjunctivitis as the only side effect. Eighteen months later, she remains well with no visual loss.

Peixoto RD, Lim HJ, Cheung WY. Neuroendocrine tumor metastatic to the orbit treated with radiotherapy. *World J Gastrointest Oncol* 2013; 5(8): 177-180 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v5/i8/177.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v5.i8.177>

### INTRODUCTION

Carcinoid tumors are low grade neoplasms usually arising from neuroendocrine cells of the bronchial and gastrointestinal tracts<sup>[1,2]</sup>. Although carcinoid tumors metastasize in 50%-75% of patients with the most common sites being lymph nodes, liver, and bones<sup>[1-3]</sup>, metastases to the eyes and orbits have only rarely been reported<sup>[3-6]</sup> and are believed to occur through hematogenous spread *via* carotid and ophthalmic artery. Improved patient survival, vigilant surveillance and advances in diagnostic tests have led to increased detection of orbital metastases. We re-



**Figure 1** Magnetic resonance imaging of the head. A: Prior to radiation therapy showing a 2.1 cm × 1.4 cm × 1.9 cm mass in the inferomedial right orbit, located between the orbital floor and inferior rectus muscle; B: After radiation therapy showing reduction in the size of the orbital metastases (1.5 cm × 1.5 cm × 1.2 cm).

port the case of a patient who 2 years after the diagnosis of metastatic neuroendocrine tumor to the liver presented with right-sided diplopia. She was found to have metastatic neuroendocrine tumor to the right orbit and was successfully treated with radiotherapy.

## CASE REPORT

JVD is a 79-year-old Black woman who presented with small bowel obstruction. A computerized tomography (CT) scan showed a mesenteric mass with possible central necrosis as well as multiple low attenuating hepatic lesions. This prompted an ultrasound-guided fine needle aspiration of the liver nodule which confirmed a well-differentiated neuroendocrine tumor (< 2 mitoses per 10 hpf, Ki-67 index 8%, and strongly positive for synaptophysin and chromogranin). Additional investigations demonstrated an elevated 24-h urine 5-Hydroxyindoleacetic acid (133 μmol/d) but normal chromogranin A (27 U/L). An octreotide scan revealed increased uptake within the mesentery as well as the liver. The patient was placed on intramuscular long-acting octreotide 20 mg every 4 wk. A subsequent CT scan showed mild interval progression, prompting an increase in dose of octreotide to 40 mg every 4 wk. Follow-up CT scans showed stable disease. The patient did not report any carcinoid symptoms.

Approximately 2 years after her initial diagnosis, the patient developed right-sided diplopia associated with

mild discomfort. On examination, right-sided proptosis and reddening were noted. She underwent a magnetic resonance imaging (MRI) of the head, which showed a homogeneously enhancing well-circumscribed 2.1 cm × 1.4 cm × 1.9 cm mass in the inferomedial right orbit, located between the orbital floor and inferior rectus muscle (Figure 1A). The radiographic findings were highly supportive for metastatic orbital spread from her original carcinoid tumor and a further orbital aspiration biopsy confirmed the diagnosis of low-grade neuroendocrine tumor. She underwent a four-week course of stereotactic radiotherapy to the right orbital metastasis (4000 cGy in 20 fractions) with minor conjunctivitis as the only complication.

At that time she was found to have progression of the disease in the liver as well as a new focus of metastases in the left ventricular myocardial wall. Soon after the completion of palliative radiation therapy to the right orbital metastasis, she was started on palliative Capecitabine and Temozolamide. Three months later a new MRI showed reduction in the size of the orbital lesion to 1.5 cm × 1.5 cm × 1.2 cm and there was improvement of the diplopia (Figure 1B). Eighteen months after receiving radiation therapy the patient remains stable with only mild diplopia and without any vision deterioration.

## DISCUSSION

Carcinoid tumors are mostly low grade neoplasms arising



from neuroendocrine cells of the bronchial and gastrointestinal tracts and rarely from other tissues<sup>[1,2]</sup>. Although originally thought to be of low malignant potential, clinically evident carcinoid tumors metastasize in 50%-75% of patients with the most common sites being surrounding lymph nodes, the liver, and bone<sup>[1-3]</sup>. Carcinoid tumor metastases to the eye and orbit have been reported only rarely<sup>[3-6]</sup> and typically occur through hematogeneous spread *via* carotid and ophthalmic artery. However, improved patient survival, vigilant surveillance and advances in diagnostic tests have led to increased detection of orbital metastases.

Classically, the diagnosis of ocular metastases is mainly suspected on the basis of a prior history of neuroendocrine tumor, evidence of other systemic metastases, and/or clinical symptoms of carcinoid syndrome<sup>[3,4]</sup>. Diplopia (48%), pain (42%), and visual loss (30%) are the commonest symptoms at presentation, whereas proptosis (63%), strabismus (62%), and visual loss (41%) are the most frequent signs<sup>[7]</sup>. MRI is considered the diagnostic image of choice in evaluating suspected orbital metastases.

Data regarding survival after the diagnosis of ocular metastatic carcinoid tumor are scarce. In a series of 13 cases, the overall survival was 72% at 5 years and 38% at 10 years<sup>[8]</sup>. Given that patients with carcinoid tumors may have prolonged survival despite dissemination, maintaining quality of life by providing early diagnosis and effective treatment to preserve vision and comfort is a fundamental issue<sup>[9]</sup>. Although primary orbital tumors require total surgical excision, metastases to the uvea and the orbit are frequently associated with widespread disease, in which case other therapeutic modalities appear to be more appropriate<sup>[2]</sup>. In the largest series consisting of 13 patients with carcinoid orbital metastases, 4 underwent exenteration, 5 had radiotherapy after tumor debulking, 2 had radiotherapy alone, and 2 had local radiotherapy with receptor-targeted chemotherapy<sup>[8]</sup>.

The generally accepted treatment of orbital metastatic disease is with irradiation and/or chemotherapy<sup>[10]</sup>, although serial observation may be appropriate in asymptomatic slow-growing tumors without vision deterioration. External beam irradiation may be an effective and noninvasive tool in selected cases<sup>[11,12]</sup>, especially for single and symptomatic lesions. Side effects may include skin erythema, conjunctivitis, corneal ulceration, cataract formation, retinopathy and neuropathy<sup>[13,14]</sup>. Data regarding tumor response and symptom relief of ocular metastatic carcinoid tumor after palliative radiotherapy is non-existent.

Another potential treatment option for metastatic carcinoid tumors to the orbit is radiopharmaceuticals. Its main advantage is the treatment of disseminated lesions in a variety of sites, and the dose may be repeated as required. Regression of choroidal metastases after chemotherapy has also been described<sup>[15]</sup>. One case of successfully treated choroidal metastases from a bronchial carcinoid with xenon arc photocoagulation and proton beam irradiation has also been reported<sup>[16]</sup>. In addition,

the combination of chemotherapy with external beam radiotherapy may be associated with symptomatic improvement and stabilization of the lesions with minimal adverse effects<sup>[5]</sup>.

In conclusion, metastatic neuroendocrine tumors to the orbit are rare, but usually associated with important symptoms that compromise patients' well-being. Because neuroendocrine tumors tend to have an indolent course with prolonged survival, early treatment of orbit metastases may help maintain quality of life.

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## Cholangiocarcinoma developed in a patient with IgG4-related disease

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**Author contributions:** Douhara A and Mitoro A designed the report; Mitoro A was the attending doctor for the patient; Otani E, Furukawa M, Kaji K, Uejima M and Yoshiji H discussed the pathogenesis; Douhara A, Mitoro A, Sawai M, Yoshida M and Yamao J performed endoscopic examinations; Fukui H organized the report; and Douhara A wrote the paper.

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### Abstract

A 77-year-old man with jaundice and a pancreatic head tumor was referred to our hospital in August 2006. The initial laboratory tests, computed tomography (CT) scan, magnetic resonance imaging (MRI), and endoscopic retrograde cholangiopancreatography suggested IgG4-related cholangitis and autoimmune pancreatitis. Oral prednisolone (PSL) was then administered. This treatment reduced the size of the pancreatic parenchyma, and the lower common bile duct (CBD) returned to its normal size. Thus, the oral PSL was gradually tapered to a maintenance dose. In February 2010, a CT scan and MRI showed segmental wall thickening and stenosis of the middle CBD, the progression of which led to extrahepatic obstructive jaundice. We suspected the emergence of a cholangiocarcinoma rather than the exacerbation of the IgG4-related sclerosing cholangitis because the stricture of the CBD

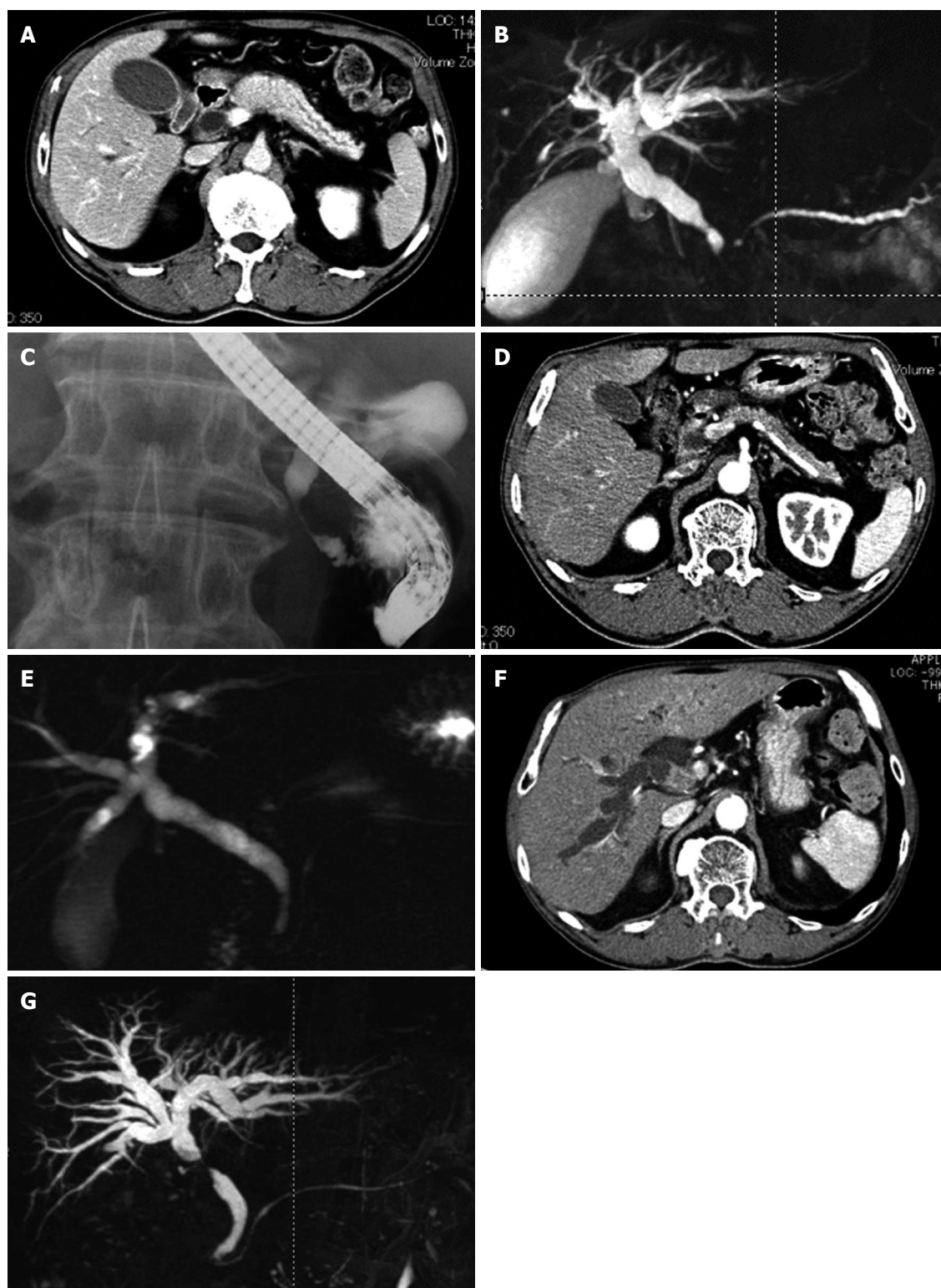
was short and localized. Then, a percutaneous transhepatic biliary drainage was performed. The biopsy specimens obtained *via* the percutaneous transhepatic tract indicated an abnormal glandular formation, suggesting the presence of a moderate, well-differentiated adenocarcinoma. The gross examination, microscopic examination and immunohistochemical analysis of the pancreaticoduodenectomy specimen suggested that a cholangiocarcinoma developed from the IgG4-related sclerosing cholangitis.

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**Key words:** IgG4; Sclerosing cholangitis; Autoimmune pancreatitis; Cholangiocarcinoma

**Core tip:** Chronic biliary inflammation and cholestasis are risk factors for cholangiocarcinoma. However, an association of IgG4-related sclerosing cholangitis with cholangiocarcinoma has not been previously demonstrated. To date, only two report described have neoplasia in the bile duct in patients with IgG4-related sclerosing cholangitis. Risk factors for cholangiocarcinoma include chronic biliary inflammation and cholestasis, both of which can contribute to the malignant transformation of cholangiocytes in patients with sclerosing cholangitis. Because of a long duration of overt IgG4-related sclerosing cholangitis and the affected location of the patient's cholangiocarcinoma, we presumed a cause-and-effect relationship between the sclerosing cholangitis and bile duct cancer.

Douhara A, Mitoro A, Otani E, Furukawa M, Kaji K, Uejima M, Sawai M, Yoshida M, Yoshiji H, Yamao J, Fukui H. Cholangiocarcinoma developed in a patient with IgG4-related disease. *World J Gastrointest Oncol* 2013; 5(8): 181-185 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v5/i8/181.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v5.i8.181>



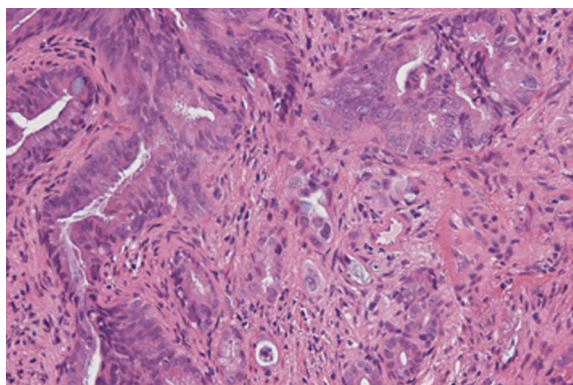
**Figure 1 Radiologic findings.** A: Enhanced computed tomography showing diffuse enlargement of the pancreas with capsule-like rim and segmental wall thickening and enlargement of the lower common bile duct (CBD); B: Magnetic resonance imaging revealing narrowed main pancreatic duct and narrowed lower CBD; C: Endoscopic retrograde cholangiopancreatography showing narrowed lower CBD; D: Enhanced computed tomography (CT) showing that swelling and capsule-like rim of pancreas disappeared after corticosteroid treatment; E: Magnetic resonance imaging showing that stenosis of bile duct disappeared after corticosteroid treatment; F: CT scan showing segmental wall thickening with enhancement of the middle CBD; G: MRI revealing stenosis of the middle CBD.

## INTRODUCTION

Autoimmune pancreatitis (AIP) is a type of chronic pancreatitis characterized by an inflammatory process that

presents with unique clinicopathologic features, including enlargement of the pancreas and pancreatic ductal narrowing, elevated levels of IgG or IgG4, dense lymphoplasmacytic infiltration, storiform fibrosis, obliterative





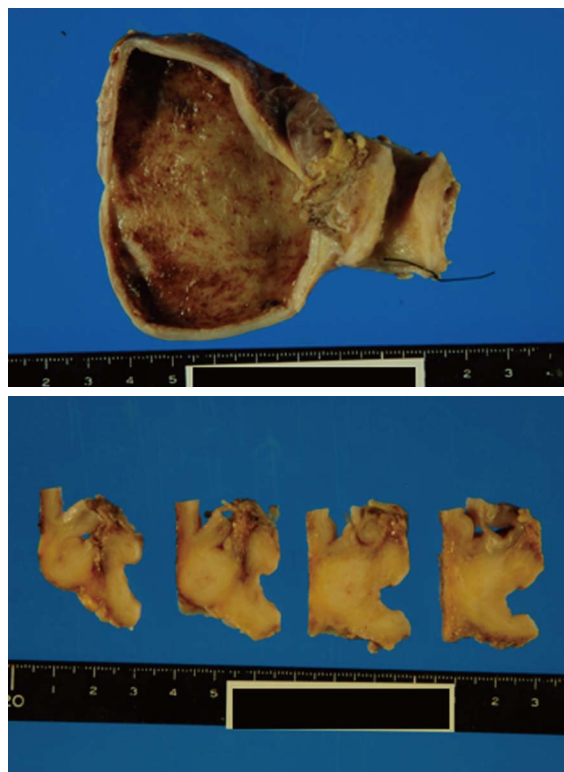
**Figure 2 Histologic findings of the biopsy specimens.** The biopsy specimens obtained via the percutaneous transhepatic tract showing abnormal glandular formation, suggesting moderate, well-differentiated adenocarcinoma.

venulitis, and a favorable response to steroid therapy<sup>[1,2]</sup>. Most patients (65%-75%) develop painless jaundice<sup>[3,4]</sup>, resulting from stenosis in the intrapancreatic portion of the common bile duct (CBD) induced by both extrinsic compression of the inflamed pancreatic head and inflammatory changes of the CBD itself<sup>[4,5]</sup>. Distal CBD strictures and irregular narrowing of the main pancreatic duct usually improve after steroid therapy<sup>[3]</sup>.

An association of primary sclerosing cholangitis (PSC) with malignancy, *i.e.*, cholangiocarcinoma, is well-established with an incidence rate of 1.5% per year<sup>[6]</sup>. However, an association of IgG4-related sclerosing cholangitis with invasive carcinoma of the biliary duct has not been demonstrated. Recently, a case of biliary intraepithelial neoplasia in a background of sclerosing cholangitis and autoimmune pancreatitis<sup>[7]</sup> and a case of intrahepatic cholangiocarcinoma in a background of sclerosing cholangitis<sup>[8]</sup> have been described. Chronic biliary inflammation and cholestasis are risk factors for cholangiocarcinoma; however, cholangiocarcinoma in a background of IgG4-related sclerosing cholangitis has rarely been reported. The department of radiology at our university reported this case in terms of the interesting computed tomography (CT) scan and magnetic resonance imaging (MRI), findings in a study by Rinsho-hoshasen<sup>[9]</sup>. Here, we present a case of cholangiocarcinoma that developed 4 years after the diagnosis of IgG4-related sclerosing cholangitis in regards to its intriguing clinical course from the viewpoint of the attending physician.

## CASE REPORT

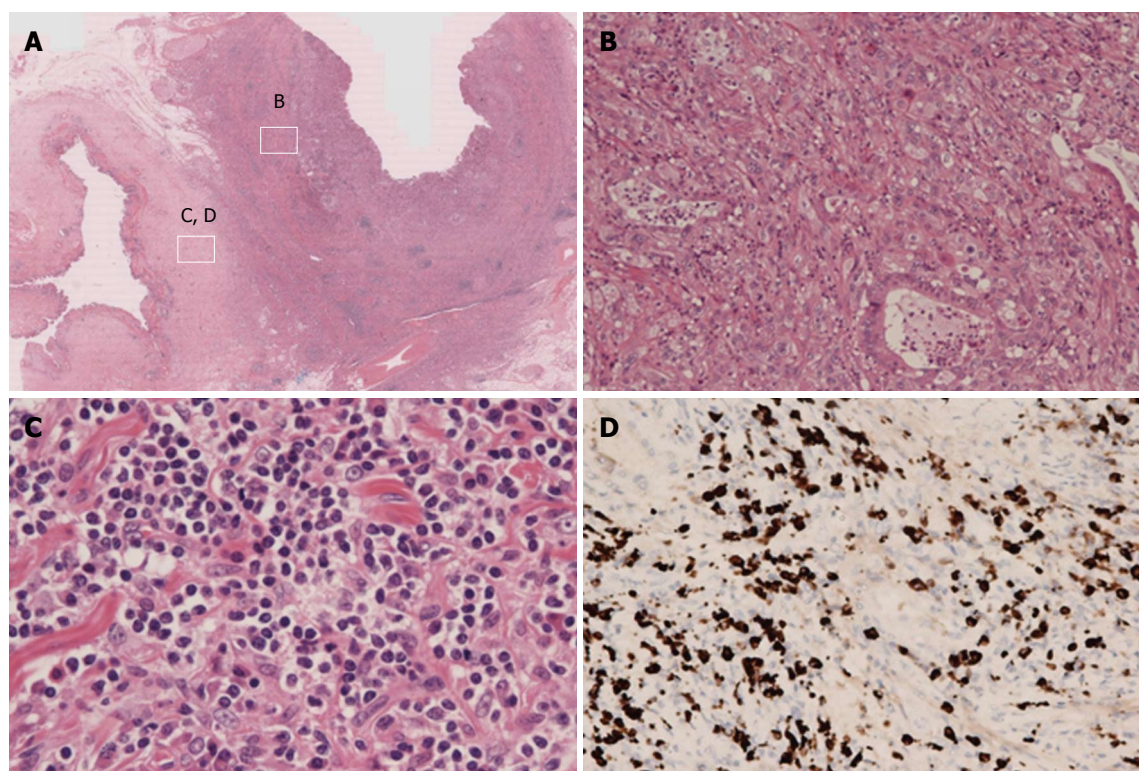
The patient was a 77-year-old man with jaundice and a pancreatic head tumor. He was referred to our hospital in August 2006. The physical examination revealed icteric sclerae without abdominal pain. The initial laboratory tests showed 2.3 mg/dL total bilirubin (normal level: 0.2-1.5 mg/dL), 742 IU/L aspartate aminotransferase (normal level: 12-32 IU/L), 545 IU/L alanine aminotransferase (normal level: 5-36 IU/L), 1104 IU/L gamma-glutamyltransferase (normal level: 11-69 IU/L), 1350 IU/L alkaline phosphatase



**Figure 3 Gross findings of the pancreaticoduodenectomy specimen.** The wall of the middle common bile duct was markedly thickened without cancer invasion to serosa.

(normal level: 80-260 IU/L), 1663.4 mg/dL IgG (normal level: 860-1800 mg/dL), 299 mg/dL IgG4 (normal level: 4.8-105 mg/dL), and an ANA of 160 (normal level: < 40). The CT scan showed a diffuse enlargement of the pancreas with a capsule-like rim and segmental wall thickening and an enlargement of the lower CBD (Figure 1A). The MRI revealed a narrowed main pancreatic duct and narrowed lower CBD (Figure 1B). The endoscopic retrograde cholangiopancreatography (ERCP) also confirmed the narrowed lower CBD (Figure 1C). The laboratory and radiologic findings suggested IgG4-related cholangitis and autoimmune pancreatitis. Oral PSL (30 mg, 0.6 mg/kg per day) was then administered for 2 wk. The treatment reduced the size of the pancreatic parenchyma, and the lower CBD returned to its normal size. The normal enhancement pattern in the pancreatic parenchyma was recovered (Figure 1D, E), and oral PSL was gradually tapered to a maintenance dose of 2.5 mg/d over a period of 2 mo.

In February 2010, a CT scan showed segmental wall thickening with enhancement of the middle CBD (Figure 1F). An MRI revealed stenosis of the middle CBD (Figure 1G), and its progression led to extrahepatic obstructive jaundice. We suspected the emergence of a cholangiocarcinoma rather than the exacerbation of the IgG4-related sclerosing cholangitis because the stricture of the CBD was short and localized. We failed in our attempt at ERCP-guided tissue diagnosis of the biliary stricture. The biopsy guided by percutaneous transhepatic cholangiography was difficult because the intrahepatic bile duct was



**Figure 4** Histologic findings of the pancreaticoduodenectomy specimen. A: Microscopic examination of the specimen; B: There was abnormal glandular formation, suggesting moderate, well-differentiated adenocarcinoma from mucosa to subserosa (HE  $\times 40$ ); C: Lymphoplasmacellular infiltrate with lymph follicles and obliterative phlebitis were present around the common bile duct (CBD) cancer (HE  $\times 400$ ); D: Immunohistochemistry was performed to identify IgG4-positive plasma cells. More than 10 IgG4-positive plasma cells/high power field were detected in the CBD (IgG4  $\times 400$ ).

not sufficiently dilated. Because informed consent was obtained, we increased the PSL dose from 2.5 to 15 mg/d for a differential diagnosis. A month later, the CBD stricture persisted and led to the dilation of the intrahepatic bile duct. Finally, a percutaneous transhepatic biliary drainage was performed. The biopsy specimens obtained *via* the percutaneous transhepatic tract showed an abnormal glandular formation, suggesting a well-moderate differentiated adenocarcinoma (Figure 2).

A pancreaticoduodenectomy was performed. At gross examination of the pancreaticoduodenectomy specimen, the wall of the middle CBD was markedly thickened without cancer invasion of the serosa (Figure 3). At microscopic examination, there was abnormal glandular formation, suggesting a moderate, well-differentiated adenocarcinoma from the mucosa to the subserosa in the middle CBD (Figure 4B). Lymphoplasmacellular infiltrate with lymph follicles and obliterative phlebitis were present around the CBD cancer (Figure 4C). Immunohistochemistry was performed to identify IgG4-positive plasma cells. More than 10 IgG4-positive plasma cells/high power fields were detected in the CBD (Figure 4D). Thus, we arrived at the diagnosis of cholangiocarcinoma developed from IgG4-related sclerosing cholangitis.

## DISCUSSION

IgG4-related sclerosing disease is a systemic disease char-

acterized by a dense lymphoplasmacytic infiltrate rich in IgG4-positive plasma cells and the storiform fibrosis of various organs. Major clinical manifestations of this disease are apparent in the pancreas, bile duct, gall bladder, salivary glands, retroperitoneum, periorbital tissues, kidneys, lungs, lymph nodes, meninges, aorta, breasts, prostate, thyroid, pericardium, and skin. While IgG4-related sclerosing cholangitis and AIP can both appear as a tumor mass mimicking cholangiocarcinoma or pancreatic cancer, these diseases have not been demonstrated to be associated with an increased risk of developing cancers of the bile duct or pancreas.

To date, only a few cases of synchronous autoimmune pancreatitis and pancreatic ductal adenocarcinoma have been reported<sup>[10,11]</sup>. One report described an epithelial atypia in the common bile duct (suggestive of biliary intraepithelial neoplasia) concurrent with a bile duct affection caused by autoimmune pancreatocholangitis<sup>[8]</sup>. One report described an adenocarcinoma in the intrahepatic bile duct in a patient with IgG4-related cholangitis<sup>[7]</sup>.

Risk factors for bile duct cancer include chronic biliary inflammation and cholestasis<sup>[12]</sup>, both of which can contribute to the malignant transformation of cholangiocytes in patients with sclerosing cholangitis. In patients with PSC, the lymphocytic infiltration is dominant near the lumen of the extrahepatic bile duct and is followed by erosion and damage of the surface epithelium. Furthermore, a multistep carcinogenesis from biliary intraep-



ithelial neoplasia (BilIN: equivalent to biliary dysplasia) has been suggested<sup>[13]</sup>. However, for patients with IgG4-related sclerosing cholangitis, the inflammation and fibrosis are observed in the whole layer of the extrahepatic bile duct and the surface epithelium is less damaged compared to patients with PSC. A recent study showed that a significant K-ras mutation occurs most frequently in the pancreatobiliary regions of patients with AIP<sup>[14]</sup>, although no relationship of this mutation with carcinogenesis is known. The mechanism of carcinogenesis from IgG4-related sclerosing cholangitis still needs to be investigated. Because our patient had a long duration of overt IgG4-related sclerosing cholangitis and the location of the patient's cholangiocarcinoma was in the affected bile duct, we presumed a cause-and-effect relationship between the IgG4-related sclerosing cholangitis and the bile duct cancer in this case. Until now, reports of cholangiocarcinoma with a histological background of IgG4-related sclerosing cholangitis have been rare, but it appears that these reports will increase as the concept of IgG4-related sclerosing cholangitis becomes well known.

In conclusion, if unspecific morphological changes in the bile duct of a patient appear during the course of IgG4-related sclerosing cholangitis, proactive pathological examination is needed to detect potential cholangiocarcinomas at an early stage.

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# World Journal of *Gastrointestinal Oncology*

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**REVIEW**

- 186 Primary hepatocellular carcinoma and metabolic syndrome: An update

*Rahman R, Hammoud GM, Almashhrawi AA, Ahmed KT, Ibdah JA*

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## Primary hepatocellular carcinoma and metabolic syndrome: An update

Rubayat Rahman, Ghassan M Hammoud, Ashraf A Almashhrawi, Khulood T Ahmed, Jamal A Ibdah

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ciation with obesity, diabetes mellitus, hyperlipidemia, and hypertension. Current understanding of pathophysiology, clinical features, treatments, outcomes, and surveillance of hepatocellular carcinoma in the background of metabolic syndrome and non-alcoholic fatty liver disease is reviewed. With the current epidemic of metabolic syndrome, the number of patients with non-alcoholic fatty liver disease is increasing. Subsequently, it is expected that the incidence and prevalence of HCC will also increase. It is very important for the scientific community to shed more light on the pathogenesis of HCC with metabolic syndrome, both with and without cirrhosis. At the same time it is also important to quantify the risk of hepatocellular carcinoma associated with the metabolic syndrome in a prospective setting and develop surveillance recommendations for detection of hepatocellular carcinoma in patients with metabolic syndrome.

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**Key words:** Liver; Hepatocellular carcinoma; Metabolic syndrome; Non-alcoholic fatty liver disease; Obesity

### Abstract

Hepatocellular carcinoma (HCC) is the most common primary liver malignancy. The incidence of hepatocellular carcinoma has increased dramatically by 80% over the past two decades in the United States. Numerous basic science and clinical studies have documented a strong association between hepatocellular carcinoma and the metabolic syndrome. These studies have documented that, in most patients, non-alcoholic fatty liver disease is the hepatic manifestation of the metabolic syndrome, which may progress to hepatocellular carcinoma through the cirrhotic process. However, minority of patients with non-alcoholic fatty liver disease may progress to hepatocellular carcinoma without cirrhosis. This review summarizes the current literature of the link between hepatocellular carcinoma and metabolic syndrome with special emphasis on various components of the metabolic syndrome including risk of asso-

**Core tip:** Hepatocellular carcinoma is a common malignancy with dismal outcome. The metabolic syndrome has been implicated for the recent increase in hepatocellular carcinoma. Numerous studies have shown a strong association between hepatocellular carcinoma and the metabolic syndrome. This review summarizes the current literature linking hepatocellular carcinoma and the metabolic syndrome.

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## INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary liver malignancy with increasing incidence and prevalence both nationally and internationally that was evident more than a decade ago<sup>[1]</sup>. According to International Agency for Research on Cancer (IARC), HCC has high fatality worldwide with overall ratio of mortality to incidence of 0.93<sup>[2]</sup>. The incidence of HCC has increased dramatically by 80% in the last two decades in the United States<sup>[3]</sup>. This phenomenon was also observed in many of the developed countries of the world<sup>[4]</sup>. The trend of increased incidence of HCC from hepatitis C virus is predicted to plateau by 2020 with no significant changes of other known causes<sup>[5]</sup>. Most of the high risk entities of HCC including hepatitis B virus, hepatitis C virus, and alcohol are well defined. However, 5%-30% of the HCC cases do not have any identifiable risk factor<sup>[6]</sup>. Moreover, some studies have indicated that even up to 50% cases of HCC may not have any readily identifiable risk factor<sup>[7,8]</sup>. The majority of these “cryptogenic” HCC in the United States and many other developed countries are now widely attributed to the metabolic syndrome, specially its hepatic manifestation non-alcoholic fatty liver disease (NAFLD)<sup>[9]</sup>.

Metabolic syndrome (MetS), a cluster of metabolic abnormalities is now considered a major public health issue worldwide. It is particularly important in the developed countries because of the alarming obesity epidemic. MetS is also considered to be the central association of the current epidemic of diabetes and cardiovascular diseases<sup>[10]</sup>. It is estimated that 25% of the United States population meet the diagnostic criteria of MetS<sup>[11]</sup>. According to the Third National Health and Nutrition Examination Survey (NHANES III) criteria, about 47 million people have metabolic syndrome in the United States and the number is increasing at an alarming rate<sup>[12]</sup>. In the background of increasing “cryptogenic” HCC, MetS and NAFLD, it is important to review the relationship between HCC and the MetS.

## EPIDEMIOLOGY OF HCC

Worldwide, HCC is the fifth most common cancer in men and the seventh most common cancer in women according to the IARC. Most of the disease burden of HCC resides in developing countries such as East Asia, South East Asia, and sub-Saharan Africa, where almost 85% of the cases occur. The overall gender ratio of male: female is 2.4. Low incidence rates are estimated in developed countries, with the exception of Southern Europe where the incidence in men is significantly higher than in other developed regions. Worldwide, there was an estimated 694000 deaths from HCC in 2008 (477000 in men, 217000 in women), making it the third most common cause of death from cancer. The geographical distribution of HCC mortality rates is similar to that observed for the incidence rates indicating more or less

similar outcomes across the world<sup>[13]</sup>.

In the United States, the average age of diagnosis for HCC is 63 years (62 years for males, and 69 years for females). The age-adjusted incidence rate is 7.7/100000 per year. The age-adjusted death rate from HCC is 5.5/100000 per year. It is estimated that the median age at death for HCC is 68 years of age. However, gender, race and ethnic disparities exist in the incidence and mortality rates of HCC in the United States. Table 1 summarizes the incidence and mortality rates of HCC according to race, ethnicity and gender based on cases diagnosed in 2006-2010 from 18 Surveillance Epidemiology and End Results (SEER) geographic areas<sup>[14,15]</sup>.

The Centers for Disease Control and Prevention (CDC) examined all HCC cases (48596) diagnosed during 2001-2006 that were reported to the National Program of Cancer Registries (NPCR) or SEER from 45 cancer registries (covering 90.4% of the United States population). As shown in Table 2<sup>[16]</sup>, the data document that the incidence rate of HCC is on the rise in both genders. During this period, the annual percentage change (APC) for males (3.6%) was significantly higher than the APC for females (2.3%). The largest significant increase in HCC incidence rates were among Non-Hispanic Whites (APC = 3.8%), African American (APC = 4.8%), and persons aged 50-59 years (APC = 9.1%)<sup>[16]</sup>.

## METABOLIC SYNDROME

The World Health Organization (WHO) was the first to identify MetS as a global problem and took initiative to propose a definition and diagnostic criteria. The WHO used insulin resistance (IR) as the major criteria for defining the MetS. However, in clinical practice it was difficult to quantify or qualify insulin resistance across the world<sup>[17]</sup>. Subsequently in 2001, the National Cholesterol Education Program expert panel on the detection, evaluation and treatment of high blood cholesterol in adults (Adult Treatment Panel III or ATP III) defined the metabolic syndrome by the presence of three parameters of the following criteria: hyperglycemia, hypertriglyceridemia, low HDL, abdominal obesity and hypertension<sup>[18]</sup>. Recently, the International Diabetes Federation (IDF) adopted a definition with emphasis on central obesity in MetS such that central obesity plus two additional factors are required in order to diagnose the MetS<sup>[19]</sup>. Table 3 summarizes the criteria used to define the metabolic syndrome over time.

The prevalence of MetS varies worldwide depending on the geographic location, socioeconomic background, culture and ethnicity. It is estimated that the prevalence of MetS is about 14% in China, 26% in South Asia, 19% in Australia, 9% in France and 18% in Italy. Although prevalence of obesity as defined by the WHO is relatively low in Asia compared to western countries, metabolic syndrome is growing into a significant public health problem. Comparative studies indicate that metabolic



**Table 1** Incidence and mortality rates of hepatocellular carcinoma according to race/ethnicity and gender, reported in Surveillance Epidemiology and End Results database 2006-2010

Race/Ethnicity	Incidence rate per 100000		Mortality rate per 100000	
	Male	Female	Male	Female
All races	11.9	4.0	8.3	3.4
Non-Hispanic White	10.4	3.5	7.6	3.2
African American	15.1	4.5	11.8	4.1
Hispanics	18.3	6.9	12.3	5.4
Asian/Pacific Islander	21.4	8.2	14.4	6.0
American Indian/Alaska Native	20.6	7.7	13.2	6.1

responses to obesity may be greater in South and East Asians than their western counterparts at given body mass indexes (BMI)<sup>[10,20]</sup>.

The prevalence of MetS was evaluated in adults in the United States participating in the third National Health and Nutrition Examination Survey (NHANES III, 1988 to 1994). The overall prevalence was 22%, with an age-dependent increase (6.7, 43.5, and 42.0% for ages 20 to 29, 60 to 69, and > 70 years, respectively)<sup>[12]</sup>. Data from NHANES 1999 to 2000 demonstrate that the prevalence has continued to increase, particularly in women. The unrelenting increase in the prevalence of obesity in the United States suggests that the current prevalence of the metabolic syndrome is now very likely higher than that estimated from 1988-1994 NHANES III data<sup>[11]</sup>. According to CDC in 2008, the obesity rate among adult Americans was estimated at 32.2% for men and 35.5% for women; these rates were roughly confirmed again for 2009-2010. Recent data indicate that 34% of the adult in the United States met the criteria for MetS and the rate of increment was equal in both sexes<sup>[21]</sup>. This rapidly increasing prevalence of obesity among adults in the United States will lead to even higher rates of the MetS now and in the near future<sup>[22]</sup>. Study has shown that susceptibility to obesity cannot simply be attributed to the combination of genetic and environmental factors, but can also be triggered by influences on a baby's development during intrauterine period, including mother's dietary habit. A mother's nutrition while pregnant can cause important epigenetic changes that contribute to her offspring's risk of obesity during childhood<sup>[23]</sup>.

## HEPATOCELLULAR CARCINOMA AND THE METABOLIC SYNDROME

HCC is one of the most common malignancies in the world, with more than 500000 new cases per year<sup>[24]</sup>. Data from SEER which covers 28% of the United States population, reported a total of 4032 cases of HCC in 2001. This number increased by 27% to 5122 in 2005 and by 60% to 6464 in 2009<sup>[14]</sup>. It is predicted that the total number of HCC will continue to increase in the future<sup>[25]</sup>. Since the association is not readily identifiable in a significant percentage of HCC cases, it was postulated,

**Table 2** Changes in incidence rate of hepatocellular carcinoma from 2001 to 2006

Incidence rate/100000	2001	2006
Overall	2.7	3.2
Male	4.5	5.4
Female	1.2	1.4

and now well established, that the MetS is contributing to the development of HCC. With the current rising epidemic of obesity and MetS in the general population, it is established that MetS is responsible for HCC cases with unaccounted association<sup>[26]</sup>. The prevalence of MetS is paralleling the epidemic of obesity in the United States. Obesity and MetS are well documented risks factors associated with NAFLD, a metabolic hepatic disorder that can progress to nonalcoholic steatohepatitis (NASH) and fibrosis. A subset of aggressive NAFLD can lead to cirrhosis and HCC. Worldwide, it is estimated that there are 400 million obese individuals, among whom 75% have NAFLD. Up to 20% have NASH and over 5-10 years, 33% of whom will develop cirrhosis<sup>[27,28]</sup>. In another study, among patients with NAFLD followed for a mean of 8 years, the occurrence of cirrhosis was 20% and the incidence of HCC was 1%<sup>[29]</sup>. It is estimated that the prevalence of NAFLD is 3-10 times higher than the prevalence of hepatitis C virus (HCV) in the United States ranging from 5.5% to 31%<sup>[30,31]</sup>. Besides being the most rapidly increasing cause of cancer death in the United States, the economic burden of HCC is also enormous with an estimated cost of more than 437 million dollars per year<sup>[32]</sup>. It is very imperative for the medical care providers to appreciate the association of MetS and HCC with appropriate surveillance for the high risk population groups.

## HEPATOCELLULAR CARCINOMA RISK FACTORS ASSOCIATED WITH THE METABOLIC SYNDROME

### Obesity

Many malignancies have been directly or indirectly associated with obesity and HCC is among these established malignancies<sup>[33,34]</sup>. Meta-analysis of 11 studies conducted in United States, Europe and Asia demonstrated that both overweight (RR = 1.07, 95%CI: 1.01-1.15) and obesity (RR = 1.85, 95%CI: 1.44-2.37) were associated with development of HCC<sup>[35]</sup>. Even in patients with chronic HBV and HCV, coexisting obesity has been associated with increased risk for HCC by more than 100-fold<sup>[36]</sup>. A Large prospective trial showed that obesity has influenced disease progression, and increased weight is associated with overall cancer mortality. In a prospective study of United States adults, BMI > 35 kg/m<sup>2</sup> negatively impacted overall mortality from HCC with a relative risk (RR) of 1.68 times in women and 4.52 times in men. This was the highest for any malignancy analyzed

**Table 3** Criteria used to define the metabolic syndrome

Diagnostic criterion	WHO (1999)	ATP (2005)	IDF (2006)
Abdominal obesity	BMI - Waist/hip ratio > 0.9 (men) or > 0.85 (women) or BMI $\geq$ 30 kg/m <sup>2</sup>	Central - Waist $\geq$ 102 cm (men) or $\geq$ 88 cm (women)	Central - Waist $\geq$ 102 cm (men) or $\geq$ 88 cm (women)
Hypertension	$\geq$ 140/90 mmHg	$\geq$ 130/85 mmHg or drug treatment for hypertension	$\geq$ 130/85 mmHg or drug treatment for hypertension
Fasting glucose	IPG/HOMA	$\geq$ 5.6 mol/L	$\geq$ 6.1 mol/L
Hypertriglyceridemia	$\geq$ 1.7 mmol/L (150 mg/dL) or drug treatment for elevated triglycerides	$\geq$ 1.7 mmol/L (150 mg/dL) or drug treatment for elevated triglycerides	$\geq$ 1.7 mmol/L (150 mg/dL) or drug treatment for elevated triglycerides
Low HDL cholesterol	Not used	< 1.0 mmol/L (40 mg/dL) (men); < 1.3 mmol/L (50 mg/dL) (women) or drug treatment for low HDL	< 1.0 mmol/L (40 mg/dL) (men); < 1.3 mmol/L (50 mg/dL) (women) or drug treatment for low HDL
Micro albuminuria	Used	Not used	Not used

ATP: Adult Treatment Panel; BMI: Body mass index; HDL: High-density lipoprotein; IDF: International Diabetes Federation; IPG/HOMA: Impaired plasma glucose/homeostatic model assessment; WHO: World Health Organization.

in the study<sup>[37]</sup>. Comparable conclusions were drawn in both Danish and Korean studies analyzing large cohorts of obese patients<sup>[38,39]</sup>. SEER-Medicare data analysis from 1993 to 2005 showed that adjusted odd ratio (OR) of obesity for HCC was 1.93 (95%CI: 1.71-2.18,  $P < 0.0001$ )<sup>[40]</sup>. The Metabolic Syndrome and Cancer Project (Me-Can) from Norway, Sweden and Austria examining 578700 subjects showed RR of 1.39 (95%CI: 1.24-1.58) for obesity in the development of HCC<sup>[25]</sup>. Obesity is also an independent predictor of HCC in obese transplanted patients (4% *vs* 3.4%)<sup>[34]</sup>.

Moreover, in a large retrospective cohort of 342 consecutive patients who underwent liver transplantation for hepatocellular carcinoma, BMI was found to be an independent predictor of micro vascular invasion<sup>[41]</sup>. Nonetheless, review of the United Network of Organ Sharing database on all liver transplantations performed in the United States, showed that obesity was an independent predictor of HCC in patients with alcoholic cirrhosis and cryptogenic cirrhosis, but not for those with cirrhosis of other associations<sup>[34]</sup>.

### Diabetes mellitus

Diabetes mellitus (DM) is an independent risk factor for the development of HCC. Analysis of 2061 patients with HCC showed a significant increase in the development of HCC (OR 2.87, 95%CI: 2.49-3.3) in the background of DM regardless of the presence of other risk factors. There was a significant positive interaction between obesity and HCV ( $P < 0.0001$ ) for HCC<sup>[42]</sup>. Multiple European studies showed RR of 4.5 of HCC in male patients, with a lower, but still significant RR of 1.86 in female patients with DM<sup>[43-45]</sup>. Moreover, a large longitudinal study analyzing 173643 DM and 650620 non-DM controls over a period of 10- to 15-year revealed a RR of 2. This risk estimation even persisted after exclusion of the patients with viral hepatitis, alcohol use, or fatty liver disease<sup>[46]</sup>. DM was established as an independent risk factor for the development of HCC in 12 cohort studies after adjustment for infectious and alcoholic associations<sup>[47]</sup>. In a large population based study

with 615532 DM patients and 614871 controls, the overall hazard rate for the development of HCC in males and females was 32.76 and 17.41 per 10000 patients-years, respectively. Furthermore, in a recent Italian case-control study including 185 HCC cases and 404 controls, diabetes and obesity were positively associated with HCC risk, with ORs of 4.33 (95%CI: 1.89-9.86) and 1.97 (95%CI: 1.03-3.79), respectively<sup>[48]</sup>. DM with cirrhosis demonstrated the highest risk of HCC development (RR = 82.25, 95%CI: 76.84-94.58)<sup>[49]</sup>. SEER-Medicare data analysis from 1993 to 2005 showed that adjusted OR of DM for HCC was 2.9 (95%CI: 2.71-3.1,  $P < 0.0001$ )<sup>[40]</sup>. The Me-Can from Norway, Sweden and Austria examining 578700 subjects showed RR of 2.13 (95%CI: 1.55-2.94) for DM in the development of HCC<sup>[25]</sup>.

### Hyperlipidemia

Hyperlipidemia is an integral part of the MetS. Although there have been several studies examining the association of HCC with MetS, only few studies reported the association of HCC with hyperlipidemia individually. Hyperlipidemia, in many instances is closely related with the central mechanism of insulin resistance in MetS. SEER-Medicare data analysis from 1993 to 2005 showed that adjusted OR of dyslipoproteinemia for HCC was 1.35 (95%CI: 1.26-2.45,  $P < 0.0001$ )<sup>[40]</sup>. The Me-Can from Norway, Sweden and Austria examining 578700 subjects showed RR of 0.85 (95%CI: 0.65-1.10) in the development of HCC<sup>[25]</sup>. Although not well understood, this discrepancy between Me-Can study<sup>[25]</sup> and SEER-Medicare data analysis<sup>[40]</sup> could be secondary to the short follow up period in the Me-Can study.

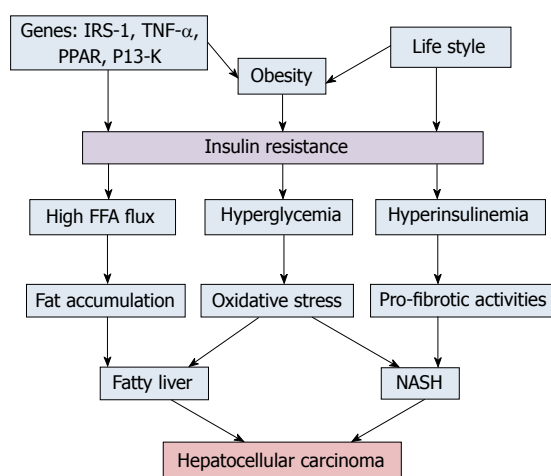
### Hypertension

Similar to hyperlipidemia, hypertension is one of the parameters of the metabolic syndrome. Very few studies examined the individual association of HCC with hypertension. SEER-Medicare data analysis from 1993 to 2005 showed that adjusted OR of hypertension for HCC was 2.22 (95%CI: 2.04-2.42,  $P < 0.0001$ )<sup>[40]</sup>. In a study from

**Table 4 Association of different components of metabolic syndrome and the development of hepatocellular carcinoma**

Author	Type of Study	Risk Parameter	Obesity	DM	Hyperlipidemia	HTN
Larsson <i>et al</i> <sup>[35]</sup>	Meta analysis	RR	1.85			
Calle <i>et al</i> <sup>[37]</sup>	Prospective	RR	4.52 (Male) 1.68 (Female)			
Welzel <i>et al</i> <sup>[40]</sup>	Retrospective	OR	1.93	2.9	1.35	2.2
Borena <i>et al</i> <sup>[25]</sup>	Prospective	RR	1.39	2.13	0.85	2.08
Turati <i>et al</i> <sup>[48]</sup>	Retrospective	OR	1.97	4.33		
Davila <i>et al</i> <sup>[42]</sup>	Retrospective	OR		2.87		
Lagiou <i>et al</i> <sup>[43]</sup>	Prospective	RR		4.5 (Male) 1.86 (Female)		
El-Serag <i>et al</i> <sup>[46]</sup>	Prospective	RR		2		
Tomimaru <i>et al</i> <sup>[55]</sup>	Prospective	RR		82.2 (with cirrhosis)		

RR: Relative risk; OR: Odd ratio; DM: Diabetes mellitus; HTN: Hypertension.



**Figure 1 Pathogenesis of hepatocellular carcinoma in the background of metabolic syndrome.** PPAR: Peroxisome proliferator-activated receptors; NASH: Nonalcoholic steatohepatitis; FFA: Free fatty acid; IRS-1: Insulin receptor substrate 1; TNF- $\alpha$ : Tumor necrosis factor  $\alpha$ .

a single center, among 209 NBNC-HCC patients, 38% had hypertension, and 11% had hyperlipidemia<sup>[50]</sup>. The Me-Can from Norway, Sweden and Austria examining 578700 subjects showed RR of 2.08 (95%CI: 0.95-4.73) for hypertension in the development of HCC<sup>[25]</sup>.

## PATHOPHYSIOLOGY OF HEPATOCELLULAR CARCINOMA ASSOCIATED WITH THE METABOLIC SYNDROME

Hepatocellular carcinoma generally arises in the background of cirrhosis (Figure 1). Factors that are associated with development of the metabolic syndrome and HCC maybe inherently linked (Table 4). It is well postulated and established that insulin resistance is the principal dominator that links all the components of MetS. Insulin resistance exerts a major role in the development of NAFLD even in lean subjects with appropriate glycemic control<sup>[51]</sup>. Insulin resistance leads to fat accumulation in the hepatocytes by lipolysis and hyperinsulinemia. Aberrant adipose tissue accumulation, release of pro-inflammatory

cytokines, inhibition of anti-inflammatory cytokines and lipotoxicity collectively promote and propagate both systemic and hepatic insulin resistance, leading to hyperinsulinemia<sup>[52]</sup>. Multiple mechanisms have been proposed that may work simultaneously and complementary to each other to provide a tumor promoting environment in MetS. This may distinguish the pathogenesis of HCC related to NAFLD from that of infectious and alcoholic associations<sup>[53,54]</sup>. Hyperinsulinemia results in increased insulin growth factor-1 (IGF-1) which has important proliferative and antiapoptotic effects. IGF-1 promotes angiogenesis through increased vascular endothelial growth factor production, which in turn leads to cancer cell proliferation. Upregulation in IGF-1/IRS1 pathway has been shown to contribute to the pathogenesis of HCC<sup>[55]</sup>. Likewise, peroxisome proliferator-activated receptors (PPARs) regulate a network of genes encoding protein involved in fatty acids uptake, enzymes required for the  $\beta$ -oxidation of fatty acids, and enzymes required for ketogenesis. PPARs play an important role in fatty liver, and its involvement in carcinogenesis has been clarified. Abnormal stimulation of PPAR- $\alpha$  has been shown to induce HCC in animal models<sup>[56]</sup>.

Expansion of adipose tissue in obesity may lead to release of pro-inflammatory cytokines. Visceral fat accumulation has been shown to be an independent risk factor for HCC recurrence after curative treatment<sup>[57]</sup>. Further, Interleukin-6 (IL-6) has been linked to obesity-associated inflammatory response such that it activates STAT3 potentiating cell proliferation and anti-apoptotic mechanisms. Tumor necrosis factor (TNF) activates pro-oncogenic pathways including JNK, NF- $\kappa$ B, mTOR, and the extracellular signal-regulated kinases<sup>[58,59]</sup>. In experimental models, both TNF and IL-6 strongly promote HCC growth induced by diethyl nitrosamine in mice. Both dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression<sup>[60]</sup>.

Adiponectin (an anti-inflammatory cytokine) is expressed at reduced levels in MetS and NAFLD, which may not sufficient to suppress endotoxin-mediated inflammatory signaling. On the other hand, high circulating levels of leptin in NAFLD exert pro-inflammatory

and pro-fibrogenic effects in NAFLD<sup>[61-63]</sup>. In addition, there is evidence that lipid peroxides and free radicals are elevated in MetS, which may cause oxidative injury, endoplasmic reticulum stress, mitochondrial dysfunction, and apoptosis<sup>[64]</sup>.

## CLINICAL FEATURES OF HCC IN THE BACKGROUND OF METABOLIC SYNDROME

NAFLD leading to cirrhosis can predispose to HCC. There are several reports that patients may even develop HCC from steatosis without cirrhosis. However, the degree of developing HCC from NAFLD is less in comparison to infectious and alcoholic associations<sup>[65]</sup>. Patients who develop HCC in the background of MetS are predominantly males. The average age of diagnosis is older than HCC secondary to other causes; and HCC secondary to NAFLD is generally well differentiated with early stages at diagnosis<sup>[53]</sup>. An analysis of 87 Japanese patients with HCC in the background of MetS showed a median age of 72 years, and the male patients appear to develop HCC at a less-advanced stage of liver fibrosis<sup>[66]</sup>. Similar results were also confirmed by another Japanese study<sup>[67]</sup>. Male predominance, older age at diagnosis, and early stages were also found in a prospective study comparing 34 NASH cases with HCC with 348 NASH patients without HCC<sup>[68]</sup>. A recent study from China examined 169 patients with NAFLD associated HCC. The result showed 73% male predominance with average age of diagnosis of 67 years, 99% had at least one component of MetS, 76% with solitary nodule (mean 3.4 cm) and most of the patients were well or moderately differentiated. In more than 40% patients, HCC developed in the absence of cirrhosis<sup>[69]</sup>. Comparable results were reported by different studies with different population groups<sup>[70-72]</sup>.

## TREATMENT AND OUTCOMES OF HCC IN THE BACKGROUND OF METABOLIC SYNDROME

Unfortunately there is no specific recommendation for treatment of HCC developing in the background of MetS. It is thought that HCC secondary to MetS may have better prognosis than its other counterparts partly because of early diagnosis with favorable prognostic markers. Because of lack of specific guidelines at present, HCC secondary to MetS are treated like HCC from other major associations. Insulin-sensitizing therapy may also improve the outcome of HCC. Metformin therapy is associated with lower mortality in diabetic patients with early stage HCC after radiofrequency ablation<sup>[73]</sup>. In another study, 100 diabetic patients with hepatitis C virus and cirrhosis were prospectively followed for 2.3-8.3 years and evaluated for the development of HCC, liver-related

death, or liver transplantation. The 5-year HCC development was lower in the group receiving metformin than in the group without metformin (9.5% *vs* 32.1%;  $P = 0.001$ ). Multivariate analysis showed that metformin treatment was independently associated with decreased HCC development (HR = 0.19;  $P = 0.023$ ) and liver-related death or transplantation in those patients (HR = 0.22;  $P = 0.049$ )<sup>[74]</sup>. Several other studies also demonstrated that the use of insulin-sensitizing agents in diabetes may reduce the risk of HCC development<sup>[75-77]</sup>. The role of rosuvastatin and ursodeoxycholic acid in the treatment of NASH or NAFLD and in prevention of NASH or NAFLD associated HCC is well studied<sup>[78,79]</sup>. A recent systematic review and meta-analysis showed a 50% reduction in HCC incidence with metformin use (OR = 0.50, 95%CI: 0.34-0.73). However, thiazolidinediones did not modify the risk of HCC (OR = 0.54, 95%CI: 0.28-1.02)<sup>[80]</sup>. Moreover, post-hoc analysis of randomized controlled trials did not reveal any significant association between antidiabetic medication use and risk of HCC although there was considerable heterogeneity across studies<sup>[80]</sup>.

## SURVEILLANCE FOR HCC IN METABOLIC SYNDROME

HCC still remains one of the malignancies with higher mortality ratio<sup>[2]</sup>. With better imaging studies, improvement of surgical techniques, and targeted therapy, the prognosis for early stage HCC is improving. But for advanced HCC the prognosis still remains poor. There are well established recommendations for surveillance of the patients with infectious risk factors and cirrhosis for HCC<sup>[81]</sup>. Evidence on metabolic syndrome as a risk factor for development of HCC, especially in the background of DM and obesity, is growing rapidly. With the ongoing epidemic of obesity, increased number of patients with DM, and the overall increase in the incidence and prevalence of MetS, it is imperative to identify the high risk groups for the development of HCC with MetS and provide appropriate surveillance strategies. The patients, who develop cirrhosis in the background of NAFLD, may be under surveillance as per current recommendations. But the question remains whether surveillance in patients who have NAFLD, without evidence of cirrhosis, is appropriate.

Most of the time, NAFLD will progress to cirrhosis before the development of HCC. But in a very small number of cases, it may progress to HCC without cirrhosis in the background of MetS. With about one third (34%) of the United States adult population meeting the criteria of MetS, surveillance for HCC may cause a significant health related economic issue in this regard<sup>[22]</sup>. But nevertheless, there should be an urgent and collaborative process to resolve this issue in the near future.

## SUMMARY

HCC is a common malignancy with dismal outcome.



The associations of HCC have been identified in great details. With the recent increase in the incidence of HCC without any indefinable causes, major efforts have been undertaken to identify more causative factors. The metabolic syndrome, especially with obesity and DM, has been implicated for the recent increase in HCC. Numerous basic science and clinical studies have shown a strong association between HCC and the metabolic syndrome. These studies have documented that NAFLD, to a large extent, is the hepatic manifestation of MetS and may progress to HCC through the cirrhotic process. It has also been shown that in a very small fraction of patients with NAFLD, HCC may develop without evidence of cirrhosis.

With the current epidemic of MetS, the number of patients with obesity and DM is increasing. Subsequently, it is expected that the incidence and prevalence of HCC will also increase. It is very important for the scientific community to shed more light on the pathogenesis of HCC with MetS, both with and without cirrhosis. At the same time it is also important to quantify the risk of HCC associated with the MetS in prospective setting and develop surveillance recommendations to detect HCC for patients with MetS.

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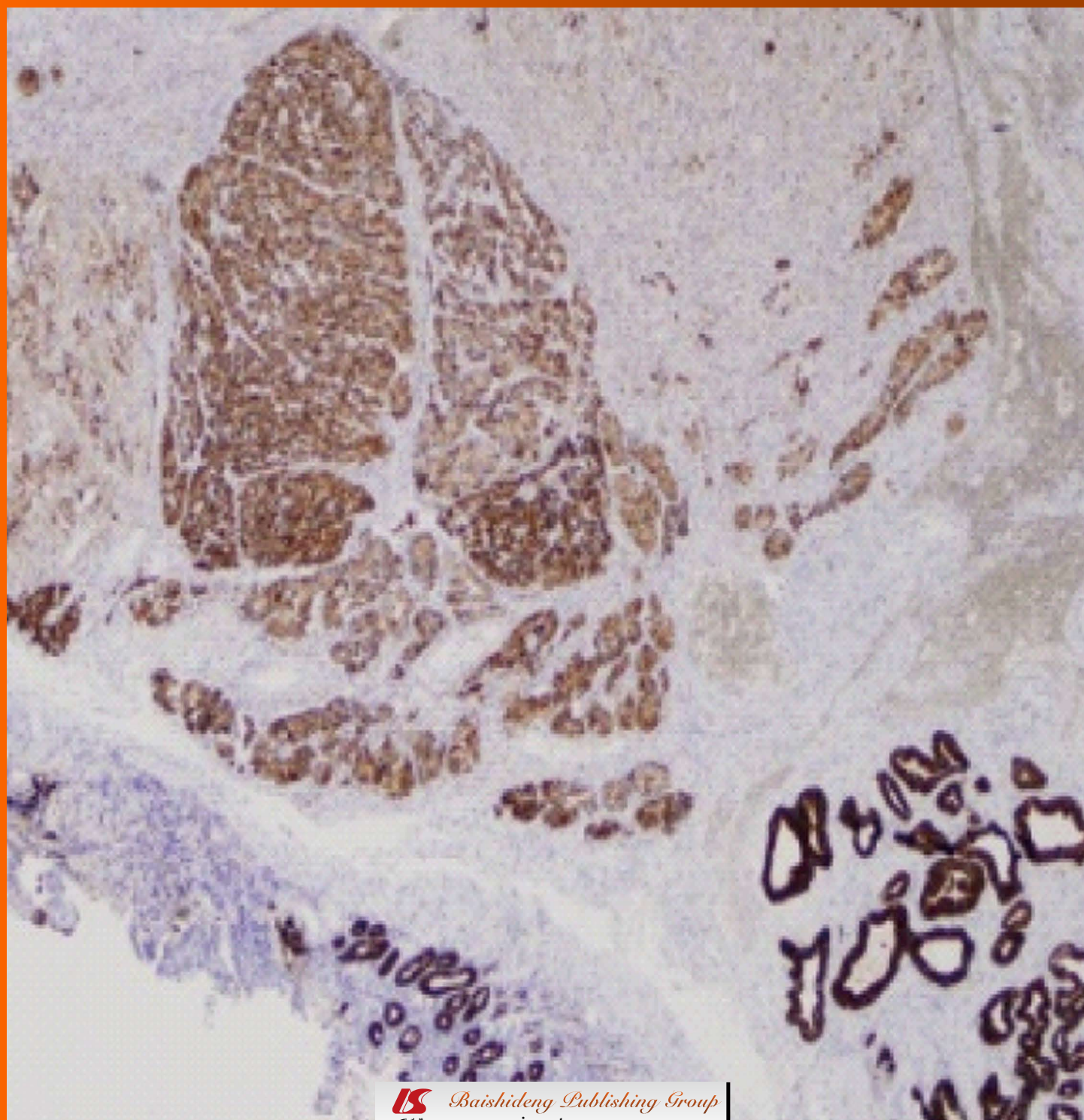
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# World Journal of *Gastrointestinal Oncology*

*World J Gastrointest Oncol* 2013 October 15; 5(10): 195-197





**CASE REPORT**

- 195** Obstructive jaundice due to a rare periampullary tumor  
*Sathyamurthy A, Choudhary A, Ng D, Okponobi S, Diaz-Arias A, Grewal A, Hammoud GM*

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## Obstructive jaundice due to a rare periampullary tumor

Anjana Sathyamurthy, Abhishek Choudhary, Dennis Ng, Shuaib Okponobi, Alberto Diaz-Arias, Ajitinder Grewal, Ghassan M Hammoud

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### Abstract

Gangliocytic paraganglioma is a rare neuroendocrine tumor predominantly arising in the second part of the duodenum with rare local recurrence or metastasis to regional lymph nodes. A 92-year-old female presented with obstructive jaundice. On exam she had pale conjunctiva and icteric sclera. Abdominal examination revealed tenderness in the upper abdomen. Laboratory data was consistent with obstructive jaundice. Computed tomography of the abdomen revealed a dilated gall bladder and a common bile duct (CBD) with no evidence of liver lesions or pancreatic head mass. Endoscopic ultrasonography revealed a 1 cm isoechoic submucosal nodule at the periampullary area, dilated CBD (9 mm), a prominent pancreatic duct (4.1 mm) and a hydropic gall bladder with no stones. Endoscopic retrograde cholangiopancreatography was performed to relieve obstruction and showed a 1 cm periampullary mass which underwent an en-bloc snare resection. Histopathology

analyses with immunohistochemical stains were positive for cytokeratin, synaptophysin, S-100 protein, neuron specific enolase and negative for actin and desmin consistent with periampullary gangliocytic paraganglioma. Periampullary gangliocytic paraganglioma is a rare benign tumor of the small bowel. Common presentation includes abdominal pain and obstructive jaundice which should be included in differential diagnosis of obstructive jaundice. Endoscopic resection is a curative therapy in the absence of local invasion or distant metastasis.

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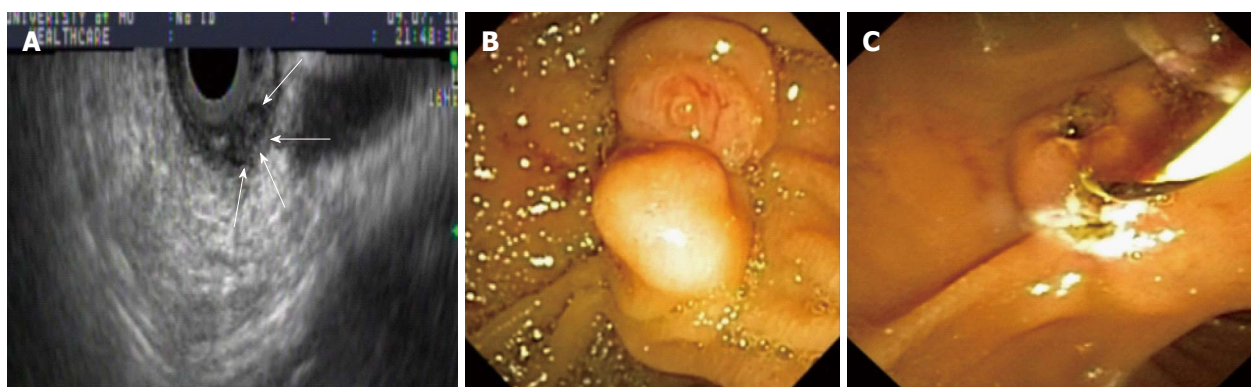
**Key words:** Gangliocytic paraganglioma; Periampullary tumor; Spindle-shaped; Epithelioid; Ganglion cells; Jaundice; Duodenum; Endoscopic mucosal resection

**Core tip:** This case report shed some light on a rare cause of obstructive jaundice in elderly patients. The disease is rare but should be considered in the differential diagnosis of biliary obstruction. The literature provided summarizes several outcomes of case presentation with this disorder and provide input on some of the aggressive feature of this disorder.

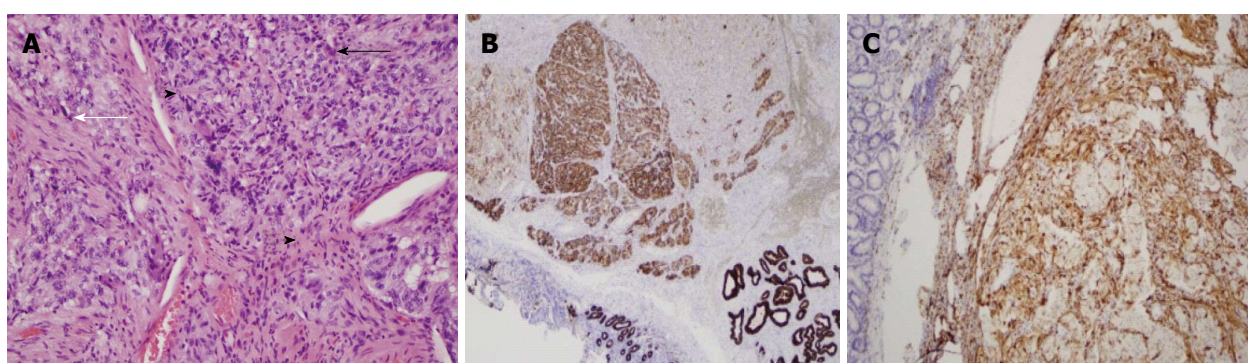
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### INTRODUCTION

Gangliocytic paraganglioma is a rare neuroendocrine tumor predominantly arising in the second part of the duodenum with rare local recurrence or metastasis to regional lymph nodes. We present a case of a 92-year-old female with abdominal pain, obstructive jaundice and a mass in the second portion of the duodenum, near the papillary region.



**Figure 1** Endoscopic images of periampullary tumor prior and after endoscopic mucosal resection. A: A 1 cm × 1.5 cm isoechoic, submucosal nodule near the major ampulla (arrows); B: Periampullary submucosal nodule with a normal overlying mucosa; C: Lesion post endoscopic mucosal resection.



**Figure 2** Histologic characteristics of the gangliocytic paragangliomas. A: Submucosal location of the peri-ampullary tumor (H and E, original magnification × 2); Epithelioid cells (black arrow) with surrounding spindle cells (white arrow). Ganglion-like cells present (arrow heads) (H and E, original magnification × 20); B: Immunohistochemistry of tumor showing positivity for cytokeratin in the epithelioid cells (original magnification × 4); C: Immunohistochemistry of tumor showing S-100 positivity of the spindle cell component (original magnification × 10).

## CASE REPORT

A 92-year-old female presented with upper abdominal pain associated with nausea, vomiting and jaundice for 4 d. She had no history of fever, chills, melena, hematemesis or weight loss. Her past medical history was significant for diabetes, hypertension, gastroesophageal reflux, chronic renal insufficiency and hypothyroidism.

Physical examination revealed pale conjunctiva and icteric sclera with no cervical lymphadenopathy. Abdominal examination revealed tenderness in the upper abdomen with no rebound tenderness. She had no hepatosplenomegaly or palpable masses. Bowel sounds were present. Laboratory data were significant for hemoglobin 9.7 g/dL, total bilirubin 2.4 mg/dL, aspartate aminotransferase 166 U/L, alanine aminotransferase 465 U/L, and alkaline phosphatase 515 U/L. Her white cell count, serum amylase and lipase were normal. Computed tomography of abdomen revealed a dilated gallbladder and common bile duct (CBD = 9 mm) with no evidence of liver lesions or pancreatic head mass. Endoscopic ultrasound (EUS) revealed a well-defined, 1 cm × 1.5 cm heterogeneous, isoechoic, periampullary submucosal nodule (Figure 1A). The nodule appears to cause an extrinsic compression of the CBD at the ampullary orifice. The CBD was dilated at 10 mm in

diameter and the pancreatic duct (PD) appeared mildly prominent and measured 4.1 mm in diameter. The gallbladder appeared hydropic with no stones. The lesion did not appear to invade the CBD, PD or muscularis propria layer of the duodenal wall (Figure 1A). Endoscopic retrograde cholangiogram with biliary sphincterotomy was performed to relieve jaundice and showed a 1.5 cm × 2.0 cm periampullary nodule that partially obstruct the orifice of the major papilla (Figure 1B) which underwent en-bloc endoscopic mucosal resection with electrocautery snare (Figure 1C). Upon follow up, jaundice resolved once resection of the lesion was performed. There were no lymph nodes seen on EUS examination. Histopathology analyses with immunohistochemical stains were positive for cytokeratin (Figure 2B), synaptophysin, S-100 protein (Figure 2C), neuron specific enolase and negative for actin and desmin confirming the diagnosis of periampullary gangliocytic paraganglioma (Figure 2A). The margins were free of tumor and there were no histologic findings of aggressive behavior such as mitosis and/or pleomorphism.

## DISCUSSION

Gangliocytic paragangliomas are exceedingly rare tumors that arise in close proximity to the papilla of Vater and



**Table 1** Summary of case reports, findings and outcome from selected publications

Author	Presentation	Endoscopic findings	Outcome	Conclusion
Kwon <i>et al</i> <sup>[5]</sup>	56 yr old male with melena	EGD-tumor of ampulla of Vater with bleeding on surface	Pancreaticoduodenectomy	If followed up after a diagnosis, local excision can be curative, avoiding surgery or lymph node dissection
Okubo <i>et al</i> <sup>[6]</sup>	61 yr old male with epigastric pain and melena	EGD, ERCP, EUS-tumor of papilla of Vater	Pylorus-preserving Pancreaticoduodenectomy and lymph node dissection; Lymph nodes positive	Do not limit to local resection, as disease recurrence, lymph node involvement or distant metastases may occur
Witkiewicz <i>et al</i> <sup>[7]</sup>	38 yr old female with right upper quadrant abdominal pain	EGD-mass in duodenum near ampulla of Vater	Endoscopic excision of mass followed by pylorus-preserving pancreaticoduodenectomy as margin was positive	It may recur or metastasize; hence pancreaticoduodenectomy with lymph node dissection might be indicated for large lesions with infiltrative margin or lesions with pleomorphism and mitoses
Morita <i>et al</i> <sup>[8]</sup>	53 yr old male with incidental finding on EGD	EUS-submucosal tumor in the 3 <sup>rd</sup> -4 <sup>th</sup> layer	Endoscopic mucosal resection	Endoscopic removal is an alternative to surgical resection if no local or distant invasion
Sakhuja <i>et al</i> <sup>[9]</sup>	33 yr old male with obstructive jaundice	ERCP-periampullary growth	Pancreaticoduodenectomy	Recognize and diagnose this rare benign entity (with 3 components on H and E sections)
Evans <i>et al</i> <sup>[10]</sup>	56 yr old male with epigastric pain, vomiting and obstructive jaundice	EGD-pedunculated ampullary tumor	Pylorus-preserving total pancreatectomy	Benign entity-2 yr post procedure no recurrence of tumor

EGD: Esophagogastroduodenoscopy; ERCP: Endoscopic retrograde cholangiopancreatography; EUS: Endoscopic ultrasound.

90% are found in the second part of the duodenum<sup>[1]</sup>. The disease is common in the 5<sup>th</sup> decade and the incidence is slightly higher in males with M:F ratio 1.8:1. Gangliocytic paragangliomas are epithelial (submucosal) tumors with three histological cell types namely epithelioid, ganglion and spindle cells<sup>[2]</sup>.

Typical presentation is abdominal pain, gastrointestinal outlet obstruction and bleeding. However, obstructive jaundice is not common. In our case, jaundice was presumed secondary to mechanical obstruction of the ampullary orifice by the tumor. Immunohistochemistry is positive for cytokeratin, synaptophysin, neuron specific antigen and S-100 protein<sup>[2]</sup>. Endoscopic ultrasonography is useful for preoperative differential diagnosis such as gastrointestinal stromal tumors, carcinoids and periampullary adenoma. The disease generally follows a benign course with rare invasive growth patterns and lymph node metastasis<sup>[3,4]</sup>.

Histologic findings such as increase mitosis, pleomorphism, infiltrative margin and lymph node metastasis are suggestive of potential malignant features<sup>[5,6]</sup>. If feasible, endoscopic resection is a curative therapy in the absence of local invasion or distant metastasis. Table 1 shows summary of case reports, findings and outcome from selected publications<sup>[5-10]</sup>.

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**CASE REPORT**

- 198** Striking similarities in genetic aberrations between a rectal tumor and its lung recurrence

*Rahma OE, Burotto M, Do Canto LM, Germanos AA, Haddad BR, Marshall JL*

- 204** Rare complication of percutaneous endoscopic gastrostomy: Ostomy metastasis of esophageal carcinoma

*Sousa AL, Sousa D, Velasco F, Açucena F, Lopes A, Guerreiro H*

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**APPENDIX** I-V Instructions to authors

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## Striking similarities in genetic aberrations between a rectal tumor and its lung recurrence

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### Abstract

We are reporting on a colorectal cancer patient with the longest disease-free interval ever published, where chromosomal microarray analysis was used to confirm the link between the primary and metastatic lesions. This rare case reports on a patient with late recurrence of colorectal cancer in the lung 19 years after its initial diagnosis. We used high-resolution array CGH (aCGH) to analyze the genetic aberrations of both the primary rectal and the recurrent metastatic lung lesions. Interestingly, we found striking similarities between the two

lesions, despite the 19 years disease-free interval. In addition, most of the genes that were previously reported to be associated with a high recurrence score showed copy number gains by aCGH in one or both lesions. Our findings suggest that aCGH may be a helpful tool in analyzing the origin of metastases and underline the need for a better understanding of the characteristics of rectal tumors that have a late recurrence potential.

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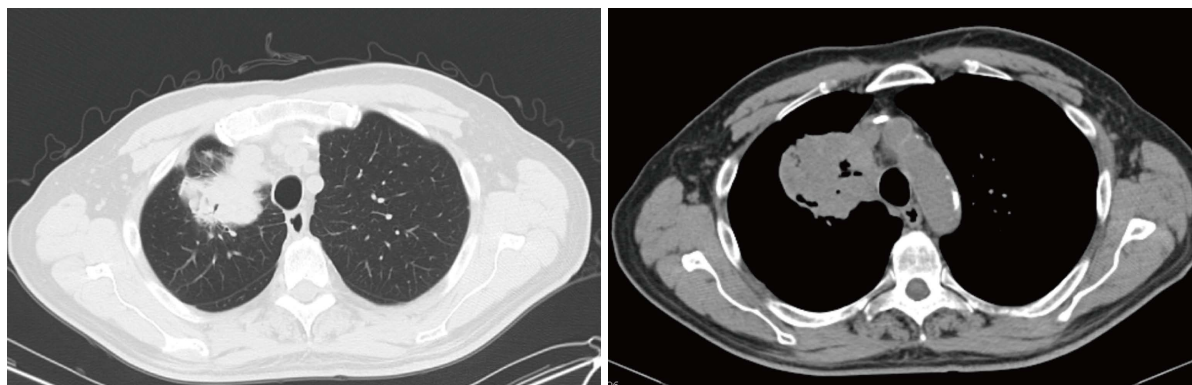
**Key words:** Colorectal cancer; Genetic aberrations; Delayed; Recurrence; High-resolution array CGH.

**Core tip:** The role of genetic profiling in determining the risk of recurrence in colorectal cancer has been under serious investigation. This case report not only represents the longest rectal cancer disease-free interval in the literature, but also applies genetic analysis as a tool to confirm the similarity of the original and metastatic tumor and to predict the risk of recurrence.

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### INTRODUCTION

Colorectal cancer (CRC) has a high incidence worldwide with more than 1.2 million new cases diagnosed in 2008<sup>[1]</sup>. The 5-year overall survival in the United States for all stages is 61%<sup>[2]</sup>. Rectal cancer accounts for approximately 30% of CRC cases<sup>[3]</sup>. The treatment of



**Figure 1** Chest computed tomography-scan demonstrating a 7.9 cm × 7.8 cm mass in the right upper lobe and right sided mediastinal and hilar lymphadenopathy.

localized rectal tumors differs from colon tumors in that it involves a multidisciplinary approach that includes surgery, radiation and chemotherapy<sup>[4]</sup>. The goal of neoadjuvant or adjuvant treatment is to decrease local and distant recurrence of the disease<sup>[5]</sup>. As of today, we do not have predictive biomarkers that indicate when a particular patient will benefit from systemic chemotherapy or, more importantly, in which cases the tumor will recur. Preliminary molecular tools have been developed to help predict which patient is more likely to experience disease recurrence and eventually die from the disease<sup>[6,7]</sup>. Despite these efforts we only partially understand the complexity of rectal cancer, clonal evolution and dormancy of micro-metastatic disease<sup>[8,9]</sup>. In this report we present the case of a long term survivor of CRC with a delayed recurrence almost two decades later.

## CASE REPORT

An 81-years-old Caucasian male initially presented with rectal bleeding in 1991. He underwent a colonoscopy with biopsy that was later tested and revealed a wild type K-ras moderately differentiated adenocarcinoma. The patient was diagnosed with stage IIIB (T3N1M0) rectal cancer and treated with surgical resection and colostomy followed by chemoradiation with fluorouracil (5-FU) and leucovorin. He subsequently underwent a colostomy reversal and remained in remission with no evidence of disease until 2011 when he developed a cough and was found to have a lung mass in the right upper lobe (RUL) and right sided mediastinal and hilar lymphadenopathy (Figure 1). A PET scan showed the RUL mass to be 8.2 cm × 7.3 cm with SUV of 14.5, and confirmed the right-sided mediastinal and hilar lymphadenopathy, in addition to a 1.2 cm nodule in the left costophrenic angle with no FDG activity. He underwent bronchoscopy and biopsy that revealed a wild type K-ras adenocarcinoma of colonic primary, CDX2/CK20 positive and TTF1/CK7/CD58 negative. The tumor characteristics were consistent with the primary tumor. The patient had a colonoscopy that only showed friable rectal mucosa with no evidence of malignancy. Accordingly, the pa-

tient was treated with FOLFOX (5-FU, oxaliplatin and leucovorin) in combination with bevacizumab for his recurrent metastatic rectal cancer. He received 10 cycles of FOLFOX/bevacizumab. The oxaliplatin was stopped due to cumulative neuropathy and he was switched to capecitabine and bevacizumab. The patient had a good response to chemotherapy by PET scan that showed a decrease in the RUL mass size (from 8.2 to 6.6 cm) and SUV (from 14.5 to 4.6), in addition to a decrease in the bilateral hilar and subcarinal lymph nodes' uptake. Given the patient good response to chemotherapy he subsequently underwent right upper and middle lobectomies in July 2012. His pathology showed metastatic adenocarcinoma with extensive necrosis consistent with his known primary colorectal carcinoma. Given the dormancy of his disease for so many years, the options were presented to the patient including watchful waiting versus maintenance chemotherapy with capecitabine for 1-2 years. The patient opted not to proceed with more treatment and to be monitored with regular CT-scans.

## Genetic aberrations in the metastatic lung lesion compared to the primary rectal carcinoma

In order to compare the DNA copy number changes in the metastatic lung lesion to the changes in the primary tumor, we evaluated both lesions by high-resolution aCGH analysis using an Agilent® platform (SurePrint G3 Human CGH Microarray Kit 8x60K, Agilent, Santa Clara, CA). Genomic DNA was isolated from formalin-fixed paraffin embedded tumor tissues using a standard phenol-chloroform laboratory protocol and cleaned with a MinElute® Reaction Cleanup Kit (Qiagen, Valencia, CA). Commercially available, pooled, normal control DNA (Promega, Madison, WI) was used as a reference DNA. aCGH experiments were performed according to the manufacturer's protocol with minor modifications. In brief, tumor and reference DNA were labeled using enzymatic labeling (Agilent, Santa Clara, CA), hybridized for 40 h at 65 °C, washed, and immediately scanned using Agilent Scanner (G2505C). Data were extracted using Agilent Feature Extraction 10.7.3.1 software, and analyzed with Agilent Workbench 7.0 software. High-

**Table 1** Genetic aberrations in the primary rectal tumor, the metastatic lung lesion, and both lesions by array comparative genomic hybridization analysis

	Chr	Cytoband	Base pair		Aber
			Start	Stop	
Recurrent metastatic lung lesion	chr1	p34.2-p34.1	40022181	45250726	G
	chr1	q21.1- q44	143700072	245804497	G
	chr2	p25.3-p11.2	32444	89387655	G
	chr2	q11.2-q37.3	96143358	241478888	G
	chr4	q32.1- q35.1	156452014	186681608	L
	chr6	p25.3-p11.1	200350	58722020	G
	chr7	p12.3-p11.1	49282714	57498383	G
	chr7	q11.21-q22.1	62291739	99905860	G
	chr7	q36.1-q36.3	150049339	158781397	G
	chr8	p23.1- p12	12627630	32499834	L
	chr8	p12-p11.21	32882718	42971936	G
	chr8	q11.21-q24.3	48549253	146250824	G
	chr9	q33.2-q34.3	124984647	139633014	G
	chr10	q22.3-q24.2	80370579	101360302	L
	chr10	q26.3	131868597	134682710	L
	chr11	p15.5-p11.12	974637	48986659	G
	chr13	q12.11-q34	18556982	113766081	G
	chr15	q25.3-q26.3	83411251	96875147	G
	chr19	p13.3-p13.11	318892	19154766	G
	chr20	q11.21-q13.33	29436537	62320720	G
	chrX	p11.23-p11.1	48639378	57116899	G
	chrX	q11.1-q28	61980262	154886101	G
	chrY	p11.31-p11.2	2716461	8521949	L
	chrY	q11.21-q11.221	13208776	17558012	L
Primary rectal lesion	chr1	q21.1-q44	143700072	243198779	G
	chr2	p25.3-p11.2	698239	89387655	G
	chr2	q11.1-q37.3	95562654	241301905	G
	chr3	p26.3-p11.1	134711	90336752	G
	chr3	q11.2-q29	95063426	197289184	G
	chr6	q11.1-q27	63002508	170700061	G
	chr7	p22.3-p11.2	524935	55936992	G
	chr7	q11.21-q36.3	62291739	158602499	G
	chr8	p23.3-p12	369418	32621998	L
	chr8	p12-p11.21	32705506	42971936	G
	chr8	q11.1-q24.3	47800500	146024209	G
	chr9	p24.3-p13.2	319684	37451026	G
	chr11	p15.5-p11.2	2121540	46490960	G
	chr13	q11-q34	18361637	113964366	G
	chr20	q11.21-q13.33	29352138	62343283	G
	chrX	p22.33-p11.1	2719027	58068490	G
	chrX	q11.1-q28	61848414	154561665	G
	chrY	p11.31-p11.2	2716461	10511314	L
	chrY	q11.21-q11.23	12593244	27176992	L
Common aberrations between the two lesions	chr1	q21.1-q44	143700072	243198779	G
	chr2	p25.3-p11.2	698239	89387655	G
	chr2	q11.2-q37.3	96143358	241301905	G
	chr7	p12.3-p11.2	49282714	55936992	G
	chr7	q11.21-q22.1	62291739	99905860	G
	chr7	q36.1- q36.3	150049339	158602499	G
	chr8	p23.1- p12	12627630	32499834	L
	chr8	p12-p11.21	32882718	42971936	G
	chr8	q11.21-q24.3	48549253	146024209	G
	chr11	p15.5-p11.2	2121540	46490960	G
	chr13	q12.11-q34	18556982	113766081	G
	chr20	q11.21-q13.33	29436537	62320720	G
	chrX	p11.23-p11.1	48639378	57116899	G
	chrX	q11.1-q28	61980262	154561665	G
	chrY	p11.31-p11.2	2716461	8521949	L
	chrY	q11.21-q11.221	13208776	17558012	L

Chr: Chromosomes; Aber: Aberrations; G: Gain; L: Loss.

resolution aCGH analysis showed that both lesions share a large number of similar aberrations (Table 1 and Figure 2). Review of these aberrations revealed that many of them have been reported to be very common in colorectal cancers (*e.g.*, segments with copy number increase on chromosomes 13, 7, 8q, and 20q)<sup>[9,10]</sup>, thus supporting the conclusion that the lung lesion is a recurrent metastasis from the primary rectal lesion.

## DISCUSSION

This case represents an atypical course for rectal cancer, with prolonged disease-free survival of about 19 years prior to the manifestation of disease recurrence in the form of metastatic disease to the lung.

Our aCGH results are consistent with other studies showing similar patterns of chromosomal imbalances in primary colorectal tumors and their corresponding pulmonary metastasis<sup>[11,12]</sup>. While we realize that aCGH analysis reveals the DNA copy number changes in tumor cells and not the exact origin of these cells, specific trends and patterns of genetic aberrations have been reported to be associated with specific tumor sites and types<sup>[10,13]</sup>.

O'Connell *et al*<sup>[14]</sup> identified a recurrence risk score based on the expression of 12 genes (seven cancer-related genes and five reference genes). Six of the seven cancer-related genes were grouped into two biological pathways: cell cycle control (KI-67, C-MYC, MYBL2) and stromal response (BGN, FAP, INHBA), and the seventh gene (GADD45B) may regulate the activity of the stromal response genes. Interestingly, while we have not evaluated the expression of these genes in the primary rectal tumor or the recurrent lung metastatic lesion, we have noticed that most of those genes show copy number gains by aCGH in one or both lesions. Specifically, BGN (Xq28), FAP (2q24.2), C-MYC (8q24.21), and MYBL2 (20q13.12) have copy number gain in both the rectal and lung lesions; INHBA (7p14.1) has copy number gain in the rectal lesion; GADD45B (19p13.3) has copy number gain in the lung lesion; and KI-67 (10q26.2) has no changes in the copy number in either lesions.

Approximately 30% of patients with colorectal carcinoma who undergo primary curative surgical resection experience recurrent disease<sup>[15,16]</sup>. Several predictive factors for recurrence have been reported including: primary site (rectum *vs* colon), advanced stage, invasion of contiguous organs, and presence of perforation<sup>[16]</sup>. The most frequent sites of recurrence are liver and lungs (33% and 22%, respectively), with the majority of these recurrences occurring in the first two years after surgery<sup>[17]</sup>. In a retrospective study by Galandiuk *et al*<sup>[18]</sup>, the median time to recurrence for patients who had undergone curative resection for stage III colorectal cancer was 16.7 mo. Likewise, another retrospective study by Obrand *et al*<sup>[17]</sup> reported an average time for distant recurrence of 22.9 mo.

It was established in the early 90's that adjuvant



**Figure 2 Common aberrations between the rectal tumor and the lung metastasis.** The abnormalities are summarized by the colored bars (blue for the colon tumor and orange for the lung metastasis). The bar is to the right of the tracing when there is DNA gain and to the left of the tracing when there is DNA loss. The length of the bar delineates the area of the chromosome involved.

therapy with fluorouracil and radiation in rectal cancer patients with locally invasive or regional nodal involvement reduces the risk of cancer recurrence and improves the overall survival<sup>[18]</sup>. More recently the German Rectal Cancer Trial established preoperative chemoradiotherapy as the standard of care in locally advanced rectal cancer showing a lower pelvic relapse rate (6% *vs* 13%) with no change in 10-years disease-free survival (68%) or overall survival (60%) compared to postoperative chemoradiation treatment<sup>[19]</sup>. Our patient was treated prior to the era of preoperative chemoradiation therapy and therefore received postoperative chemoradiation therapy. It would be difficult to determine whether the prolonged remission time in this case is due to the administration of adjuvant chemoradiation therapy or simply due to this patient's unique tumor biology.

Late recurrence of colorectal cancer has been reported in small series. Recently, Ishii *et al*<sup>[21]</sup> reviewed 16 cases of colorectal cancer recurrence after a disease-free interval of 5 years or more. The median disease-free interval was 10 years with a range of 5-16 years. Shimoda *et al*<sup>[22]</sup> reported the longest recurrence interval in the literature of 16 years in a rectal cancer patient who had recurrent solitary metastatic ileal cancer. To our knowledge, the

case we are reporting here represents the longest disease-free survival of 19 years in recurrent colorectal cancer after surgical resection.

The 5-years survival of patients with untreated metastatic disease is less than 5%<sup>[22]</sup>. Pulmonary metastasectomy in a select group of patients has a positive effect on survival (5-year survival rate of up to 50%)<sup>[23]</sup>. Accordingly, recurrent disease in this case was treated with preoperative chemotherapy followed by surgical resection. Whether patients with metastasectomies should receive perioperative chemotherapy remains controversial<sup>[24]</sup>.

This case identified striking similarities in genetic aberrations between a primary rectal tumor and its lung recurrence after long disease-free survival. Indeed, it reflects a lack of our full understanding of the tumor microenvironment. The mechanism responsible for recurrence following years of “dormancy” of the cancer cells deserves further investigation, in order to identify a subgroup of colorectal cancer patients that should be treated differently and, perhaps, should have prolonged surveillance. Focusing research efforts on outliers such as this case may help identify fundamental biological patterns that would also help in the treatment of more traditional patients.



## COMMENTS

### Case characteristics

An 81-years-old male with a history of resected rectal cancer presented with cough.

### Clinical diagnosis

Dullness to percussion and decrease breath sounds over the upper lobe of the right lung.

### Differential diagnosis

Lung mass, lung abscess, pneumonia.

### Laboratory diagnosis

WBC 8.20 k/uL; HGB 12.10 gm/dL; CEA 1.20 ng/mL; metabolic panel and liver function test were within normal limits.

### Imaging diagnosis

CT/PET scan showed right upper lobe mass (8.2 cm × 7.3 cm) with SUV of 14.5, and right-sided mediastinal and hilar lymphadenopathy, in addition to a 1.2 cm nodule in the left costophrenic angle with no FDG activity.

### Pathological diagnosis

Bronchoscopy and biopsy revealed a wild type K-ras adenocarcinoma of colonic primary, CDX2/CK20 positive and TTF1/CK7/CD58 negative.

### Treatment

The patient was treated with FOLFOX (5-FU, oxaliplatin and leucovorin) in combination with bevacizumab.

### Related reports

The tumor biology of colorectal cancer of is not very well understood and we do not have predictive biomarkers that indicate when a particular patient tumor will recur.

### Term explanation

High-resolution array CGH is a molecular cytogenetic method that is used for analyzing DNA copy number aberrations which is applied to detect genomic abnormalities in cancer.

### Experiences and lessons

This case report not only represents the longest rectal cancer disease-free interval in the literature, but also applies genetic analysis as a tool to confirm the similarity of the original and metastatic tumor and to predict the risk of recurrence.

### Peer review

This article applies genetic analysis to confirm the origin of a recurrent rectal tumor and to predict the risk of recurrence.

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## Rare complication of percutaneous endoscopic gastrostomy: Ostomy metastasis of esophageal carcinoma

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**Key words:** Enteral nutrition; Gastrostomy; Esophageal neoplasms; Neoplasm metastasis; Ostomy

**Core tip:** We present this case to alert endoscopists to the possibility of metastatic cells in the ostomy when the percutaneous endoscopic gastrostomy is the chosen method for the nutrition support of the patient with head and neck cancer. This is a very rare but a possible complication so, in these situations, probably we must think about other possibilities of tube placement namely using an overtube, the introducer method or performing a surgical gastrostomy.

Sousa AL, Sousa D, Velasco F, Açucena F, Lopes A, Guerreiro H. Rare complication of percutaneous endoscopic gastrostomy: Ostomy metastasis of esophageal carcinoma. *World J Gastrointest Oncol* 2013; 5(11): 204-206 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v5/i11/204.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v5.i11.204>

### Abstract

The authors present the case of a 55-year-old male with a stage III (T4N1M0) squamous-cell esophageal carcinoma, who underwent percutaneous endoscopic gastrostomy (PEG). The pull method of tube placement was used. Five months after the procedure, the patient was referred to the hospital with a hard palpable tumour at the ostomy site. The histologic exam revealed an abdominal wall metastasis of the esophageal cancer. The authors present this case because of the rarity of metastasis in ostomy after placement of PEG in patients with tumours located in the head and neck. In this particular context and judging by the rarity of situation, the clinical impact of this phenomenon is limited. Nevertheless, metastasis in ostomy site could be prevented by the push method, laparoscopy or laparotomy.

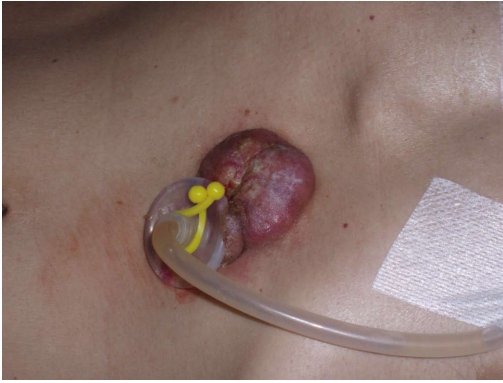
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### INTRODUCTION

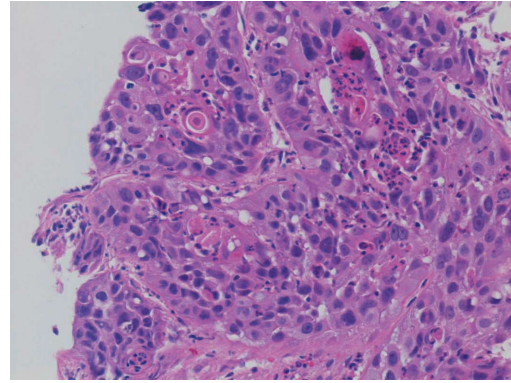
A percutaneous endoscopic gastrostomy (PEG) is a way of long-term enteral nutrition, simple to perform, safe and highly accepted in patients with head and neck tumours<sup>[1,2]</sup>. The pull method of the tube placement, described by Gauderer *et al*<sup>[1]</sup> in 1980, is the most commonly used in clinical practice. A potential, but very rare complication is the metastasis of malignant cells from the primary tumour to the ostomy<sup>[3,4]</sup>. It is believed that direct implantation of the tumour by instrumentation is the explanation for this phenomenon; however, hematogenous metastasis is also a possible mechanism<sup>[5]</sup>.

### CASE REPORT

The authors describe the case of a 55-year-old man



**Figure 1** Tumor at the site of ostomy.



**Figure 2** Biopsy of the tumor at the site of ostomy: squamous cell carcinoma-hematoxylin and eosin stain (x 200).

that presented to the emergency room with dysphagia to solids, with progressive worsening in the last week to semi-liquids, late regurgitation of undigested food, non-selective anorexia and weight loss. Relevant social history included heavy smoking and chronic alcoholism.

He underwent upper endoscopy which revealed an infiltrating neoplasm with almost complete stenosis of the upper third of the esophagus (21-26 cm), with poorly differentiated squamous cell carcinoma histology.

The computerized tomography showed neoplastic involvement of a higher level, occupying the left vallecule and the left piriform sinus with extrinsic anteroposterior compression of the trachea and presence of ipsilateral internal jugular lymphadenopathies.

The bronchoscopy showed extrinsic compression of the upper third posterior wall of the trachea with intact mucosa. The throat examination revealed neoformation at the left piriform sinus with decreased hemilarynx motility, without affecting the airway.

It is, therefore, a case of a man with a stage III (T4N1M0) squamous cell carcinoma of the esophagus with indication for chemotherapy (cisplatin + 5 - fluorouracil) and palliative radiotherapy.

As the patient presented with significant symptoms, several dilations with through-the-scope balloons and Savary dilators were attempted and the proximal area of the tumour was destroyed with argon-plasma coagulation. The PEG insertion was selected as a long-term pathway for nutritional support. The pull method is the most frequently used for PEG insertion both generally as in our institution, thus being the selected method.

Five months after the placement of PEG, the patient was referred to the Gastroenterology Department after presenting with a hard palpable tumor at the ostomy site, with approximately 10 cm in diameter (Figure 1). Biopsy of the mass confirmed identical histology to the primary carcinoma (Figure 2). The patient died approximately 1 mo after this finding.

## DISCUSSION

The use of PEG as a means of nutritional support for patients with head and neck tumors is one of the clearest

indications for this procedure. This is a safe technique however, complications may occur, ranging between 6% and 30% in different series, being skin infections the most common<sup>[2,6,7]</sup>.

We report a very rare but potential complication: metastasis of malignant cells from the primary tumour site to the ostomy. In the literature review about 50 cases of metastasis in ostomy are described, after placement of PEG in patients with head and neck tumours<sup>[8]</sup>. The pull method was used in our patient, as in most other cases reported in literature<sup>[5,8,9]</sup>. The time from tube placement to ostomy metastasis range between 3 and 18 mo<sup>[10]</sup>. However, when head and neck tumours metastasize to the site of ostomy, even with extensive tumor resection, patient survival is low<sup>[11]</sup>. Since the implantation of the tumor by direct instrumentation is the more likely explanation of metastasis, this complication might possibly be prevented by alternative PEG methods, such as introducer method, laparoscopy or laparotomy, or by using an overtube<sup>[4,5,10]</sup>. However, due to the rarity and clinical impact of this situation we consider that the practical relevance of the using of these methods will be low.

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## Surgery for colorectal liver metastases: The evolution of determining prognosis

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### Abstract

Despite improvements in the multi-modality treatment of colorectal liver metastasis (CRLM), survival after resection remains varied. Determining prognosis after surgical resection has historically been predicated on preoperative clinicopathological factors such as primary tumor stage, carcinoembryonic antigen levels, number of liver metastases, presence of extrahepatic disease, as well as other factors. While scoring systems have been developed by combining certain preoperative factors, these have been inconsistent in accurately determining prognosis. There has been increasing interest in the use of biologic and molecular markers to predict prognosis following CRLM. The role of markers such as KRAS, BRAF, p53, human telomerase reverse transcriptase, thymidylate synthase, Ki-67, and hypoxia inducible factor-1 $\alpha$  and their correlation with accurately predicting survival after surgical resection have been supported by several studies. Furthermore, other elements such as pathological response to chemotherapy and the presence of circulating tumor cells have shown promise in accurately determining prognosis after resection for colorectal liver metastasis. We herein review past, present,

and possible future markers of prognosis among colorectal cancer patients with liver metastasis undergoing resection with curative intent.

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**Key words:** Colorectal; Metastasis; Prognosis; Risk score; Molecular markers; Outcomes

**Core tip:** Historically, prognosis after resection has been largely assessed based on preoperative clinicopathologic features. Data validating the prognostic value of patient and tumor specific factors have been mixed, with many recent studies showing these scoring systems to correlate poorly with survival. Rather, there has been an emerging interest in biological or molecular markers of prognosis to more effectively assess patient prognosis after resection of colorectal liver metastasis. In this review, we discuss past, present, and possible future markers of prognosis among colorectal cancer patients with liver metastasis undergoing resection with curative intent.

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### INTRODUCTION

Colon cancer is the 3<sup>rd</sup> most common cancer worldwide. It has an estimated incidence of 42.5 per 100000 with over 140000 estimated new cases expected in the United States in 2013 as reported by the Center for Disease Control<sup>[1,2]</sup>. About 14%-25% of patients with colorectal cancer will have liver metastasis at presentation and up to

**Table 1** Studies of prognostic clinicopathological factors

Study	Primary stage	CEA level	Size of major metastasis	Number liver metastases	Disease free interval	Extrahepatic disease	Surgical margins
Scheele <i>et al</i> <sup>[11]</sup>	P	NA	P	NP	P	P	P
Nordlinger <i>et al</i> <sup>[10]</sup>	P	P	P	P	P	NA	P
Fong <i>et al</i> <sup>[9]</sup>	P	P	P	P	P	P	P
Mann <i>et al</i> <sup>[12]</sup>	P	P	P	NP	NP	NA	NA
Rees <i>et al</i> <sup>[15]</sup>	P	P	P	P	NA	P	P
John <i>et al</i> <sup>[21]</sup>	NP	P	NP	NP	NA	NP	P
Doci <i>et al</i> <sup>[27]</sup>	P	NP	NP	NP	NP	NA	NA
Hughes <i>et al</i> <sup>[34]</sup>	P	P	P	P	P	NA	NA
Gayowski <i>et al</i> <sup>[37]</sup>	P	NA	NP	P	NP	P	P

CEA: Carcinoembryonic antigen; P: Prognostic; NP: Non prognostic; NA: Non available.

60% of patients will develop metastasis at some point after diagnosis<sup>[3-6]</sup>. Surgical resection remains the only hope for cure. Contemporary series have demonstrated that surgical therapy for colorectal liver metastasis (CRLM) is associated with a low operative mortality of 1% to 2%<sup>[7,8]</sup>. The reported 5- and 10-year survival does vary, however, ranging from 25% to 74% (median 38%) and 9% to 50% (median 26%), respectively, depending on the era from which the data were reported and the underlying patient population. Historically, prognosis after resection has been largely assessed based on preoperative clinicopathologic features. Data validating the prognostic value of patient and tumor specific factors have been mixed, with many recent studies showing these scoring systems to correlate poorly with survival. Rather, there has been an emerging interest in biological or molecular markers of prognosis to more effectively assess patient prognosis after resection of CRLM. In this review, we discuss past, present, and possible future markers of prognosis among colorectal cancer patients with liver metastasis undergoing resection with curative intent.

## CLINICAL MARKERS

Numerous clinical prognostic factors have been identified in an attempt to estimate overall prognosis among patients with CRLM. The most relevant factors have been included in clinicopathological scoring systems, proposed in the late 90s and now widely used by many clinicians<sup>[9,10]</sup>. The role of each of these factors in determining the prognosis of patients with CRLM is still, however, a matter of some debate. Furthermore, there remains no consensus regarding which of these clinicopathological factors has the “best” prognostic value (Table 1).

### Primary tumor stage

Advanced primary tumor stage has been considered a negative prognostic factor by multiple investigators. Scheele *et al*<sup>[11]</sup> initially proposed a correlation between the primary tumor grade and overall survival (OS) as well as disease free survival (DFS). Primary tumor stage was later incorporated into clinical prognostic scoring systems<sup>[9,10]</sup>. Specifically, Fong *et al*<sup>[9]</sup> proposed the stage of the primary tumor as an adverse prognostic factor, concluding that

the nodal status of the primary cancer was highly predictive of outcome<sup>[9,12-15]</sup>. A subsequent meta-analysis reported an association between primary tumor stage, nodal metastasis, and worse outcomes following resection of CRLM<sup>[13-15]</sup>. Tranchart *et al*<sup>[16]</sup> similarly noted that primary tumor lymph node metastasis was an independent predictor of adverse OS and DFS. Previously Bennett *et al*<sup>[17]</sup> analyzed the prognostic value of perihepatic lymph node micrometastases in patients with CRLM. Patients with at least one perihepatic lymph node with metastases had a shorter recurrence free survival.

### Preoperative carcinoembryonic antigen level

The role of carcinoembryonic antigen (CEA) as a robust predictor of long-term survival following resection of CRLM remains poorly defined<sup>[9,10,12,18-22]</sup>. Among many patients, CEA can be an effective marker to monitor for recurrence, as well as to assess response to systemic therapy<sup>[18,21]</sup>. CEA levels can correlate with the radiological response to preoperative chemotherapy; however, other data have suggested that the absolute change in CEA level with chemotherapy may not correlate with long-term outcome<sup>[23]</sup>. As a pre-operative prognostic factor, Mann *et al*<sup>[12]</sup> reported that CEA levels did correlate with 5-year survival (CEA levels < 200 ng/mL: 48.9% *vs* > 200 ng/mL: 0.0%). Other studies have similarly noted that preoperative CEA > 200 ng/mL was an independent factor of poor OS and disease specific survival (DSS), respectively<sup>[18,21]</sup>. In a one study, Park *et al*<sup>[19]</sup> looked at both tissue CEA and serum CEA concentration after resection for CRLM and noted that CEA expression was an independent prognostic factor for OS and DFS. Of note, patients with elevations in both tissue CEA expression and serum CEA had a worse OS and DFS compared with patients who had only one CEA category elevated<sup>[19]</sup>. Despite these data, other studies have noted that CEA level was not a significant predictor of survival or recurrence after hepatic resection for metastatic colorectal cancer<sup>[24-27]</sup>. The reason for the disparate finding from various studies may be due to the different cut-off values used for CEA, as well as differences in how the statistical models were constructed (*e.g.*, which other competing risk factors were put into the model, how many patients in any given study had a particular factor, *etc.*).

### Number of liver metastases

Several studies have reported that a higher number of CRLM lesions is a poor prognostic factor<sup>[28-32]</sup>. A recent large meta-analysis examining nearly 10000 patients reported a 5-year survival of only 17.1% for patients with four or more CLMs<sup>[28]</sup>. Other studies have found no difference in survival based on the number of tumors with 5-year survival ranging from 40%-50% regardless of tumor burden<sup>[9,27,29-32]</sup>. The reason for these differences may be related to patient selection, differences in surgical approach (resection only, resection plus ablation, *etc.*), as well as differences in the use of neoadjuvant chemotherapy. For example, in a study by Pawlik *et al.*<sup>[33]</sup> the 5-year survival among patients with 4 or more CRLM was 50.9%, however many of the patients had been pretreated with neoadjuvant chemotherapy and response to neoadjuvant therapy was strongly associated with survival. As such, the impact of tumor number on prognosis needs to be considered in light of other important clinical and therapeutic information. While the limit of hepatic involvement that precludes a patient from being “operable” is still a matter of debate, the general consensus is that tumor number should not be used as an absolute contraindication to surgery. When all the lesions can be resected with a microscopically negative margin (R0) in the setting of an adequate future liver remnant (FLR), surgery should at least be contemplated. Considering that as the number of tumor metastases increases, a curative resection becomes more technically challenging, the number of liver tumors may impact survival when all tumors are not able to be completely removed.

### Size of liver metastases

The size of the largest metastasis is another clinical factor that has long been considered a prognostic factor. Mann *et al.*<sup>[12]</sup> reported that 5-year survival was 51.6% among patients undergoing surgery for CRLM  $\leq 5$  cm compared with 27% for those patients with a tumor  $> 5$  cm. In other studies, patients with CRLM measuring  $> 5$  cm were similarly noted to have a worse survival<sup>[15]</sup>. Specifically, Aldrighetti *et al.*<sup>[22]</sup> reported that patients with a CRLM lesion measuring  $> 5$  cm had a survival of only 18.8% *vs* 30% for patients with smaller tumors. In a separate study, Rees *et al.*<sup>[15]</sup> similarly reported that CRLM diameter  $> 5$  cm was an independent predictor of survival. As such, tumor size  $> 5$  cm has been adopted by several investigators as a predictor of adverse long-term outcome, evidenced by the inclusion of tumor size in multiple clinical scoring systems<sup>[9,11,14,34-36]</sup>. However, several other studies have been unable to find any differences in recurrence and survival with relation to tumor size<sup>[20,24,27,37]</sup>. Modern era chemotherapeutic agents are now frequently able to cytorreduces or downsize metastasis. In this context, it is not clear if tumor size continues to hold important prognostic information. Response to chemotherapy - as evidenced by change in tumor size - may be a more important and relevant prognostic marker than initial CRLM tumor size<sup>[38,39]</sup>.

### Synchronous metastases and disease free interval

Approximately 25% of patients have a synchronous presentation of their primary tumor and CRLM at the time of diagnosis<sup>[1]</sup>. Some authors have found an association between the presence of synchronous metastasis and a worse prognosis<sup>[9-11,34,40]</sup>, while others have not noted that synchronous presentation has an effect on survival<sup>[12,20,27]</sup>. Similarly, there is no consensus regarding the impact of disease-free interval on outcomes. Some authors have reported that a short disease-free interval did not impact disease-free or OS<sup>[12]</sup>, however other investigators consider disease-free interval a reliable prognostic factor<sup>[9,22]</sup>. Fong *et al.*<sup>[9]</sup> concluded that disease-free interval of  $< 12$  mo after resection of the colorectal primary was predictive of adverse outcomes, and included this factor in the clinical risk score. Tan *et al.*<sup>[18]</sup> similarly noted that a disease-free interval  $< 12$  mo was an independent predictor of disease-specific survival (DSS) at 3 years. The prognostic role of disease-free interval is still controversial. One reason why the impact of disease-free interval may have changed over time is that there is more effective adjuvant treatment for patients with advanced colorectal cancer. More effective chemotherapy may prolong the disease-free interval among these patients and may contribute to why studies conducted in the past might not be comparable to the ones conducted in the era of modern chemotherapy.

### Extrahepatic disease

Traditionally extrahepatic disease (EHD) has been considered a contraindication to hepatectomy for CRLM due to the unfavorable prognosis previously noted in multiple studies<sup>[10,34,41-43]</sup>. While the presence of EHD has clear prognostic implications, the impact of the extent and location of the EHD and its effect on prognosis has been debated. In a study by Elias *et al.*<sup>[44]</sup>, the investigators argued that the total number of metastases was more prognostically important than the site of EHD. While other groups have shown that multiple EHD sites is clearly associated with a worse survival<sup>[45,46]</sup>, the site of EHD also has prognostic importance. Specifically, Pulitanò *et al.*<sup>[46]</sup> noted that the location of EHD was associated with prognosis, as patients having pulmonary metastasis had the best prognosis and patients with retroperitoneal/aortocaval lymph node metastasis had the worse prognosis. Pulmonary metastasectomy has been demonstrated to prolong survival in selected patients and has a clear benefit in patients with solitary or oligometastatic disease<sup>[47-49]</sup>. Specifically, 5-year survival after pulmonary resection of colorectal metastasis has been reported to be as high as 48.0%<sup>[50]</sup>. In contrast, regional lymph node involvement has been correlated with a worse survival, with observed 5-year OS of 25% for pedicular, 0% for celiac, and 0% for para-aortic lymph node involvement<sup>[51]</sup>.

### Surgical margin status

Microscopically negative surgical margins (R0) have traditionally been considered an important prognostic factor

**Table 2** Survival based on the clinical risk cumulative score (adapted from Fong *et al*<sup>[9]</sup>)

Cumulative score	1 yr	2 yr	3 yr	4 yr	5 yr	Median, mo
0	93	79	72	60	60	74
1	91	76	66	54	44	51
2	89	73	60	51	40	47
3	86	67	42	25	20	33
4	70	45	38	29	25	20
5	71	45	27	14	14	22
Prognostic factor	Score 0	Score 0	Score 0	Score 1	Score 1	Score 1
Node-positive primary	negative	negative	negative	positive	positive	positive
Disease-free interval	≥ 12 mo	≥ 12 mo	≥ 12 mo	< 12 mo	< 12 mo	< 12 mo
Number of liver metastases	1	1	1	> 1	> 1	> 1
Size of major liver metastases	≤ 5 cm	≤ 5 cm	≤ 5 cm	> 5 cm	> 5 cm	> 5 cm
CEA (ng/mL)	< 200 ng/mL	< 200 ng/mL	< 200 ng/mL	> 200 ng/mL	> 200 ng/mL	> 200 ng/mL

CEA: Carcinoembryonic antigen.

following resection of CRLM. Most authors have indeed reported that an R1 (microscopically positive) and R2 (macroscopically positive) margin are associated with worse long-term OS<sup>[9,15,20,21,52-55]</sup>. While there has been some lack of consensus as to what constitutes a “truly” microscopically negative margin<sup>[56-59]</sup>, Pawlik *et al*<sup>[60]</sup> demonstrated in a large cohort of patients that margin width > 1 mm was not associated with overall risk or pattern of recurrence. Kokudo *et al*<sup>[30]</sup>, using a sensitive genetic analysis detecting *KRAS* and *p53* mutations, found micrometastases in the liver parenchyma surrounding CRLM in only 2% of patients, all within 4 mm of the tumor border. Andreou *et al*<sup>[61]</sup> did report that it was important to achieve an R0 margin as patients who had an R1 resection were noted to have a worse outcome. Some investigators have argued, however, that it is biology, not millimeters that dictate prognosis following resection<sup>[62]</sup>. Specifically, these investigators note that margin status is often confounded by the extent of intrahepatic disease. Patients with a larger intrahepatic tumor burden are most at risk for an R1 margin; it is these patients who also have worse overall tumor biology and overall recurrence. To this point, de Haas *et al*<sup>[23]</sup> did not find a difference in OS among patients undergoing an R0 *vs* R1 resection. These data may suggest that, in an era of more effective chemotherapy options, leaving microscopic disease behind may result in increased local failure but not necessarily a worse OS. The impact of margin status on outcomes may therefore be influenced by patient and tumor factors, as well as the utilization of chemotherapy<sup>[61]</sup>. Regardless of the impact of margin status on prognosis, complete macroscopic and microscopic removal of all lesions with negative resection margins should remain the gold standard in the surgical treatment of CRLM<sup>[23]</sup>.

### Operative and post-operative factors

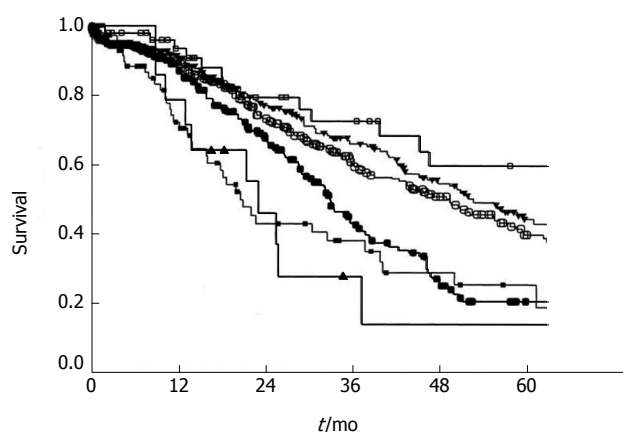
There is no consensus regarding the impact of blood loss, transfusion, or postoperative complications on survival following resection of CRLM<sup>[63-66]</sup>. The most convincing prognostic factor seems to be the effect of infections and other postoperative complications<sup>[40,67]</sup>. Specifically, Mavros *et al*<sup>[68]</sup> reported that postoperative complications

were independently associated with decreased long-term survival after surgery for CRLM with curative intent. The effect of complications on long-term survival may be due to the immune modulating effects of sepsis, impaired immune system and consequent metastatic spread. Moreover, a high rate of complications, longer hospital stays and the delayed wound healing may cause a postponement or avoidance of necessary adjuvant treatments, which in turn may have implications for long-term survival.

### Clinical scoring system

One of the first preoperative prognostic scoring systems was described by Nordlinger *et al*<sup>[10]</sup> in 1996. In this scoring system, one point was given to each of the following factors: age, size of largest metastasis, CEA level, stage of the primary tumor, disease-free interval, number of liver nodules, and resection margin<sup>[10]</sup>. Subsequently, Fong *et al*<sup>[9]</sup> proposed a “clinical risk score” to predict long-term outcome and recurrence. In a cohort of 1001 patients treated with resection of CRLM, the authors identified 5 criteria as significantly impacting prognosis: nodal status of the primary tumor, disease-free interval, number of hepatic metastases > 1, preoperative CEA level > 200 ng/mL, and size of the largest metastasis > 5 cm<sup>[9]</sup>. One point was assigned to each factor (Table 2) and the total score was reported to be highly predictive of long-term outcome (Figure 1). This score has been widely utilized; while some groups have validated the scoring system, other investigators have questioned its prognostic accuracy<sup>[12,25,26,69-72]</sup>. In a separate study, Iwatsuki *et al*<sup>[73]</sup> proposed a different prognostic score that included tumor number ≥ 3, tumor size > 8 cm, time to hepatic recurrence ≤ 30 mo as well as the presence of bilobar tumors. The prognostic score, calculated by summing these prognostic factors, was suggested to predict 5-year survival. When comparing the Fong score<sup>[9]</sup> with other described clinical scoring systems, including the Nordlinger score<sup>[10]</sup>, Iwatsuki score<sup>[73]</sup>, Mayo Clinic scoring system and Basingstoke index, several authors have found that only the Fong and the Iwatsuki scores provide a statistically significant stratification of disease specific survival<sup>[9,10,15,70,71,73,74]</sup>. In 2008, the Memorial Sloan Kettering Cancer Center (MSKCC) proposed





**Figure 1** Survival after hepatic resection stratified by the clinical risk score. Open box: score 0 ( $n = 52$ ); filled triangle: score 1 ( $n = 262$ ); open circle: score 2 ( $n = 350$ ); filled circle: score 3 ( $n = 243$ ); filled box: score 4 ( $n = 80$ ); open triangle: score 5 ( $n = 14$ ).  $P < 0.0001$  (from Fong *et al.*<sup>[9]</sup>). Used with permission.

the first nomogram for predicting disease-specific survival for the individual patient<sup>[75]</sup>. The nomogram appears to better represent characteristics of individual patients, for instance incorporating the true preoperative CEA value rather than applying an arbitrary cutoff value<sup>[76]</sup>.

The ultimate clinical value of these prognostic scoring systems remains debatable. In a study by Nathan *et al.*<sup>[77]</sup>, the authors reported a c-statistic of only 0.5 to 0.6 for many of the scoring systems. The authors postulated that the moderate-to-poor accuracy of the staging systems was related to the inability to account for neoadjuvant treatments, varying R0 resection rates, as well as differences in establishing categorical cutoff values for continuous data fields (*e.g.*, CEA level  $> 200$  ng/mL, and size of the largest metastasis  $> 5$  cm, *etc.*). Moreover, despite some external validation, these scores are based on single-institution cohorts and have not been modified based on newer developments in treatments. Lastly, the variations observed in the OS of patients with similar prognostic scores suggest that other factors may play a role in determining survival after resection of CRLM, most intriguingly patient-specific biological and molecular factors<sup>[78]</sup>.

## BIOLOGICAL, PATHOLOGICAL, AND MOLECULAR MARKERS

Recently, attention has turned to the use of biological and molecular markers as a more accurate means to predict long-term outcomes. Patient and tumor specific markers may provide more accurate predictions of survival after hepatic resection for colorectal metastasis (Table 3).

### Tumor response to preoperative chemotherapy on imaging

Preoperative chemotherapy is increasingly being used, especially among patients with advanced CRLM. Preoperative “conversion” chemotherapy has allowed many previously unresectable patients to be treated and converted/down-

sized so that surgery becomes possible<sup>[79,80]</sup>. In some centers, neoadjuvant therapy for patients with resectable disease is also frequently being used<sup>[32,79]</sup>. The use of preoperative systemic chemotherapy provides the opportunity to assess response. Response to chemotherapy has been shown to improve 5-year survival from 35% to 85% in one study when compared with patients who did not receive chemotherapy<sup>[38]</sup>. Adam *et al.*<sup>[32]</sup> reported a 30% increase in 5-year survival among patients who underwent hepatectomy after an objective tumor response *vs* patients who had tumor progression while receiving neoadjuvant chemotherapy. Similarly, recurrence-free survival (RFS) has been shown to be influenced by tumor response. In a study by Gruenberger *et al.*<sup>[39]</sup>, patients who had a response to chemotherapy had a RFS of 24.7 mo *vs* only 3 mo for patients with progressive disease.

Most commonly, response to chemotherapy can be assessed by standard cross-sectional imaging using the Response Evaluation Criteria in Solid Tumors (RECIST) criteria. RECIST allows for the assessment of changes in the standard cross-sectional diameter of lesions. Oxaliplatin- and irinotecan-based cytotoxic chemotherapeutic regimens may result in radiographic “shrinkage” of tumors. In contrast, the use of biologic or targeted agents, such as bevacizumab, can sometimes be difficult to assess using RECIST criteria on cross-sectional imaging. For example, in a phase III study examining the addition of bevacizumab to oxaliplatin-based chemotherapy for metastatic colorectal cancer, the investigators noted an improved progression-free survival without affecting RECIST-defined response rates<sup>[81]</sup>. In a separate study, Chun *et al.*<sup>[82]</sup> reported that morphological changes in CRLM lesions - rather than RECIST changes - were prognostic with regard to long-term outcomes.

### Tumor response to preoperative chemotherapy on pathology

In addition to assessment on preoperative imaging, tumor response can be assessed on pathological examination after extirpation of the tumor. Andreou *et al.*<sup>[61]</sup> reported on the effect of pathological response to neoadjuvant chemotherapy in achieving negative margins. Patients with a minor pathologic response to preoperative chemotherapy ( $\geq 50\%$  residual viable tumor cells) had significantly worse OS (5-year OS rate 46% after R0 resection *vs* 0% after R1 resection). In a study by Adam *et al.*<sup>[83]</sup>, complete pathological response (CPR) was similarly correlated with an increase in overall 5-year survival from 45% to 76%. This finding was subsequently confirmed by correlating pathologic response, considered as mean of the percentage of cancer cells remaining within each tumor, with 5-year overall survival. In a separate study, Blazer *et al.*<sup>[84]</sup> reported on 305 patients who underwent preoperative irinotecan- or oxaliplatin-based chemotherapy, followed by resection of CRLM. In this group of patients, 9% had a complete response (no residual cancer cells), 36% a major response (1% to 49% residual cancer cells), and 55% a minor response ( $\geq 50\%$  residual cancer cells). The residual tumor was assessed semiquantitatively, estimating the proportion of residual

**Table 3** Studies of prognostic biomarkers

Study	No. of patients	Positive case (%)	Biomarker	Correlation with survival
Nash <i>et al</i> <sup>[110]</sup>	188	27	KRAS	Independent predictor of poor survival (HR = 1.9)
Nash <i>et al</i> <sup>[110]</sup>	188	62	Ki-67	Independent predictors of poor survival (HR = 2.6)
Teng <i>et al</i> <sup>[113]</sup>	292	2.1	BRAF	Independent prognostic biomarker after metastasectomy (HR = 6.245, <i>P</i> < 0.003)
Smith <i>et al</i> <sup>[26]</sup>	66	36	Ki-67	Ki-67 correlate with survival ( <i>P</i> = 0.04)
Smith <i>et al</i> <sup>[26]</sup>	66	35	hTERT	Htert correlate with survival ( <i>P</i> = 0.0001)
Dòmont <i>et al</i> <sup>[25]</sup>	201	43	hTERT	Independent predictor of poor survival (RR = 2.03, <i>P</i> < 0.0001)
Gonen <i>et al</i> <sup>[123]</sup>	156	Not reported	TS	Independent predictor of poor survival (RR = 4.22, <i>P</i> < 0.01)
Costa <i>et al</i> <sup>[137]</sup>	104	Not reported	TLI	High TLI independently Predicted decreased DFS ( <i>P</i> = 0.035)
Nitti <i>et al</i> <sup>[128]</sup>	69	64	p53	Independent predictor of poor survival (RR = 2.53, <i>P</i> = 0.008)
Mehta <i>et al</i> <sup>[144]</sup>	50	30	FGA	A high FGA is an independent predictor of survival ( <i>P</i> = 0.01)
Shimomura <i>et al</i> <sup>[136]</sup>	64	31	HIF-1 $\alpha$	High HIF-1 $\alpha$ is an independent risk factor for recurrence

hTERT: Human telomerase reverse transcriptase; TS: Thymidylate synthase; TLI: Thymidylate labeling index; DFS: Disease free survival; FGA: Fraction of genome altered; HIF-1 $\alpha$ : Hypoxia inducible factor-1 $\alpha$ .

cancer cells in relation to the tumor area, comprehensive of areas of chemotherapy-related tissue injury, tumor necrosis, fibro-collagenous proliferation, and other reparative changes. Survival was strongly correlated with pathologic response: 5-years survival was 75%, 56%, and 33% for patients with a complete response, major response, or minor response respectively<sup>[84]</sup>.

A semi-quantitative analysis of the proportion of viable cancer cells, however, is limited due to the difficulty in determining the baseline percentage of tumor cell before preoperative chemotherapy. Therefore, it could be that this type of pathological response would have a better prognostic role than a predictive one based on response to chemotherapy<sup>[82,84]</sup>. Interestingly Tanaka *et al*<sup>[85]</sup> found that a complete pathological response in all the metastases is not necessary to obtain a correlation with OS. The authors showed that patients with multiple metastases and complete response in some of those tumors still experienced a higher OS and DFS compared with pathologic non-responders. The “best” OS was, however, noted among those patients in whom all CRLM lesions showed a complete response<sup>[85]</sup>.

Tumor regression grading, as well as tumor thickness at the tumor-normal interface, have been proposed as prognostic histopathological factors<sup>[83-90]</sup>. Based on the tumor regression scheme proposed for esophageal carcinoma, Rubbia-Brandt *et al*<sup>[90]</sup> described a pathological grading system for CRLM<sup>[90]</sup>. In this schema, tumor regression was characterized by fibrosis overgrowing on tumor cells, decreased necrosis, and the presence or absence of tumor glands at the periphery of liver metastases. Based on this, a tumor regression grade (TRG) score, ranging from 1 to 5, was proposed and subsequently shown to correlate with DFS<sup>[91]</sup>. Maru *et al*<sup>[89]</sup> recently introduced the idea of using tumor thickness measured at the tumor-normal interface as a new prognostic factor for therapy response and survival. Greater tumor thickness predicted shorter recurrence-free survival: 70% for patients with a tumor

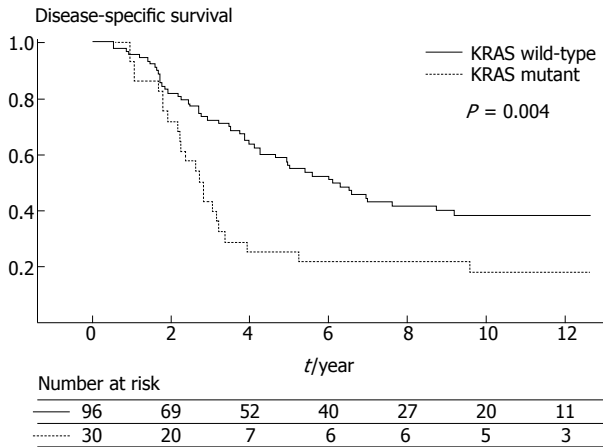
thickness of < 0.5 mm, 51% for patients with a tumor thickness between 0.5 mm and 5 mm, and 35% for patients with a tumor thickness of  $\geq$  5 mm<sup>[89]</sup>.

Other factors noted on pathology beyond response to preoperative therapy may impact prognosis. Rudolf Virchow hypothesized in 1863 that the origin of cancer was at sites of chronic inflammation. Today, the causal relationship between inflammation, innate immunity and cancer is widely acknowledged. Nonetheless, many of the molecular and cellular mechanisms mediating this relationship still remain unresolved<sup>[92,93]</sup>. Okano *et al*<sup>[94]</sup> reported that patients with dense tumor infiltrating lymphocytes (TIL) surrounding metastatic liver survived longer than patients with weak TILs after hepatic resection. Canna *et al*<sup>[95]</sup> recently examined the relationship between local and systemic inflammatory responses and outcomes in patients undergoing resection of colorectal cancer. A low tumor CD4+ T-lymphocyte infiltrate was associated with an elevated circulating C-reactive protein (CRP) and both were associated with a poor outcome. Furthermore CRP was superior to tumor T-lymphocytic infiltration in predicting cancer specific survival<sup>[95]</sup>. There is increasing evidence that a host's inflammatory response to tumor (IRT) is associated with recurrence and lower survival in patients undergoing potentially curative resection for colorectal cancer<sup>[96,97]</sup>. Similar studies have shown worse OS and DFS in patients with an elevated preoperative CRP > 10 mg/L and neutrophil to lymphocyte ratio (NLR) > 5:1<sup>[93,95-100]</sup>. NLR > 5:1 has been shown to be an independent predictor of recurrence and worse survival in patients undergoing resection for CRLM<sup>[101]</sup>.

## MOLECULAR MARKERS OF PROGNOSIS

### KRAS, BRAF

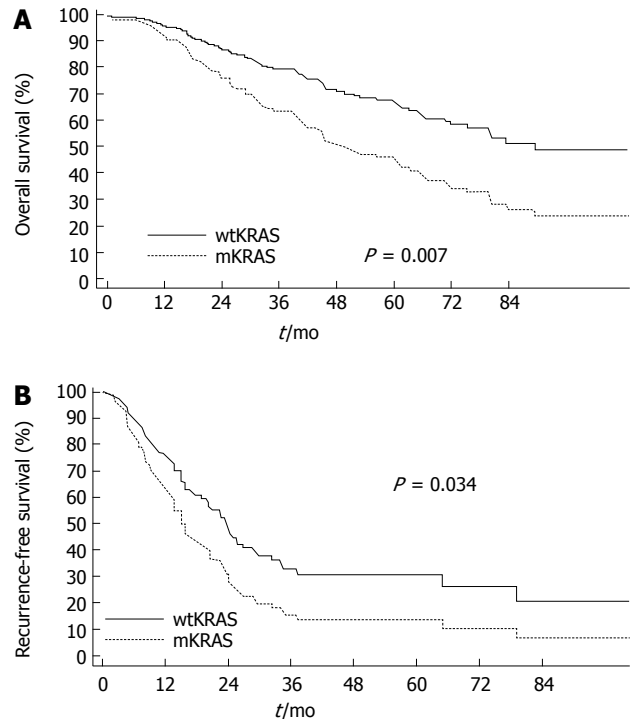
KRAS, along with HRAS and NRAS, belongs to a family of GTPases. When activated, KRAS can induce a cascade of mitogen-activated protein kinases (MAPKs) that



**Figure 2** Disease specific survival after liver resection stratified by KRAS mutation (from Nash *et al*<sup>[110]</sup>). Used with permission.

transfers signals from the cell membrane *via* the cytoplasm into the nucleus. The ras gene products activate proteins in the Raf family, which consists of the ARAF, BRAF and RAF-1 members<sup>[102]</sup>. Mutations of the *KRAS* gene predicts resistance to epidermal growth factor receptor (EGFR)-targeted monoclonal antibodies, and acquired resistance to anti-EGFR therapies may be due to the late switch in *KRAS* mutational status<sup>[103,104]</sup>. The reported prevalence of *KRAS* mutations in liver metastases varies from 15% to 44%. While several studies have reported no statistically significant association between *KRAS* mutation and metastatic progression, proliferative index, or survival has been reported<sup>[105-109]</sup>, Nash *et al*<sup>[110]</sup> did report a prevalence of 27% *KRAS* mutation in liver metastasis and noted an independent association between *KRAS* mutation and worse survival after liver resection (Figure 2). In a separate study, Karagkounis *et al*<sup>[111]</sup> reported *KRAS* and BRAF analysis performed on 202 patients undergoing surgery for CRLM at the Johns Hopkins Hospital. In this study, the authors noted that *KRAS* mutations were found in approximately one third of patients, while BRAF mutations were found in only 2% of patients undergoing surgery for CRLM. *KRAS* status was an independent predictor of overall and recurrence-free survival (Figure 3); the low incidence of BRAF mutation limited assessment of its prognostic impact<sup>[111]</sup>. Other studies, however, have noted BRAF mutation to be an independent prognostic factor of worse survival following resection of CRLM, as well as a poor prognostic factor for colon cancer patients of various stages<sup>[102,112-114]</sup>. *KRAS* status may not only predict overall recurrence, but perhaps also the pattern of recurrence. Vauthey *et al*<sup>[115]</sup> recently reported that RAS mutation was predictive of early lung recurrence after curative resection of CRLM.

As the MAP kinase signaling pathway is involved in the inflammatory cascade, Huang *et al*<sup>[112]</sup> described the role of the activated MAP kinase pathway and CRP in liver metastases. This study demonstrated the significance of both specific C reactive protein (CRP) single nucleotide polymorphisms (SNP) and mutations in *KRAS*/BRAF



**Figure 3** Overall (A) and disease-free (B) survival after hepatic surgery for colorectal liver metastasis depicted by *KRAS* mutation status (multivariate Cox model) (wtKRAS, wild type *KRAS*; mKRAS, mutated *KRAS*) (from Karagkounis *et al*<sup>[111]</sup>). Used with permission.

in liver metastases with respect prognosis after resection of CRLM<sup>[116]</sup>. CRP SNP rs7553007 and *KRAS* mutations were found to be independent prognostic factors for CRC patients with synchronous liver metastasis<sup>[112]</sup>.

### hTERT

Telomerase is a ribonucleoprotein enzyme responsible for the replication of telomeres, preventing cell senescence and death. Telomerase has two core functional components: the catalytic subunit of hTERT (with telomere-specific reverse transcriptase activity) and a telomerase RNA template. hTERT is the rate-limiting component of telomerase complex and its expression correlates with telomerase activity<sup>[117]</sup>. Despite the growing evidence that hTERT is predictive of response to neoadjuvant chemoradiation among patients with rectal cancer, the prognostic role of hTERT among patients with resected CRLM has not been well studied<sup>[114,115]</sup>. Fong *et al*<sup>[9]</sup> and Smith *et al*<sup>[26]</sup> did compare the prognostic value of the Fong clinical scoring system *vs* markers of cell proliferation, such as hTERT. In this study, the authors noted that hTERT correlated better with survival than predictions based on the clinical risk score<sup>[9,26]</sup>. The independent prognostic value of hTERT has subsequently been validated as predictor of worse overall survival among patients with surgically resected CRLM<sup>[25,118,119]</sup>.

### Thymidylate synthase

Thymidylate synthase (TS), the target enzyme of fluoro-

uracil (FU)-based chemotherapy, is commonly reported to correlate with response to systemic therapy and survival<sup>[120-123]</sup>. A few small studies have suggested that TS gene overexpression might be associated with poor prognosis in patients undergoing resection of CRLM<sup>[120-122]</sup>. A separate study by Gonen *et al.*<sup>[123]</sup> confirmed that TS was an independent poor prognostic factor for OS and progression-free survival in a multivariate analysis using data from a large cohort of patients with resected CRLM. Interestingly, this same group also analyzed tumor mRNA and confirmed that tumor TS expression was associated with lower RFS and disease specific survival<sup>[123]</sup>. Other authors have also noted that TS seems to correlate with the clinic risk score in patients who undergo resection for CRLM<sup>[124]</sup>.

### p53

p53 is a tumor suppressor gene with a central role in controlling the cell cycle and apoptosis through regulation of Bax activity. p53 mutation correlates with the development of CRLM, as well as increased metastatic burden<sup>[125]</sup>. While such findings have raised interest in the potential of p53 as a predictive biomarker, data on the prognostic role of p53 in patients with resected CRLM has yielded mixed and inconclusive results<sup>[126]</sup>. Bellucco *et al.*<sup>[127]</sup> showed a lower median survival among patients with p53-positive tumors with synchronous unresectable CRLM treated by hepatic artery infusional chemotherapy. These data were later confirmed in patients undergoing curative hepatic resection for CRLM, as p53 protein status was the single best predictor of survival (median survival: p53 wild type, 93 mo *vs* p53 mutated 27 mo)<sup>[128]</sup>. Similarly, 3- and 5-year survival were better among patients with p53 wild type CRLM<sup>[128]</sup>. Tanaka *et al.*<sup>[129]</sup> also showed that mutated p53 remained an independent prognostic factor for worse survival after hepatectomy based on a multivariate analysis. In contrast, Yang *et al.*<sup>[130]</sup> reported a separate study in which patients with p53 mutated CRLM actually had a better long-term survival after liver resection compared with patients who had wild type p53 tumors. Thus, the results of p53 on prognosis are conflicting and the actual role of p53 in defining long-term outcome remains to be determined.

### Ki-67

Ki-67 is a proliferation marker, present in the nucleus during cellular proliferation. Due to its correlation cellular proliferation, Ki-67 has been identified as a possible predictive factor of outcome after liver resection of CRLM. Weber *et al.*<sup>[29]</sup> conducted a large single-institutional study showing that Ki-67 labeling index was a reliable prognostic factor of survival among patients with resected CRLM. The prognostic impact of Ki-67 was subsequently confirmed on a meta-analysis that identified Ki-67 overexpression as a strong predictor of survival<sup>[131]</sup>. In a comparison between the Fong clinical scoring system<sup>[9]</sup> and the expression of Ki-67 as prognostic factors, Smith *et al.*<sup>[26]</sup> concluded that both Ki-67 correlated better with survival than the clinical score.

### Hypoxia inducible factor-1 $\alpha$

Hypoxia inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) is a transcription factor involved in crucial aspects of cancer biology, including angiogenesis, cell survival, glucose metabolism and invasion<sup>[132]</sup>. Recent studies have shown that inflammation induces HIF-1 $\alpha$  activity<sup>[132-135]</sup>. Moreover, constitutive activation of Ras-MAPK pathway and the PI3K-AKT pathway, or loss of function of tumor suppressor protein, as p53, regulate HIF-1 $\alpha$  activity. Recently Shimomura *et al.*<sup>[136]</sup> evaluated the clinical significance of HIF-1 $\alpha$  expression in CRLM. The authors concluded that overexpression of HIF-1 $\alpha$  is an independent risk factor for cancer recurrence after curative resection for CRLM<sup>[136]</sup>. With new confirmatory studies, HIF-1 $\alpha$  may prove to be an important prognostic factor for survival after CRLM resection.

### Miscellaneous Markers (p21, H-thymidine labeling index and markers of angiogenesis)

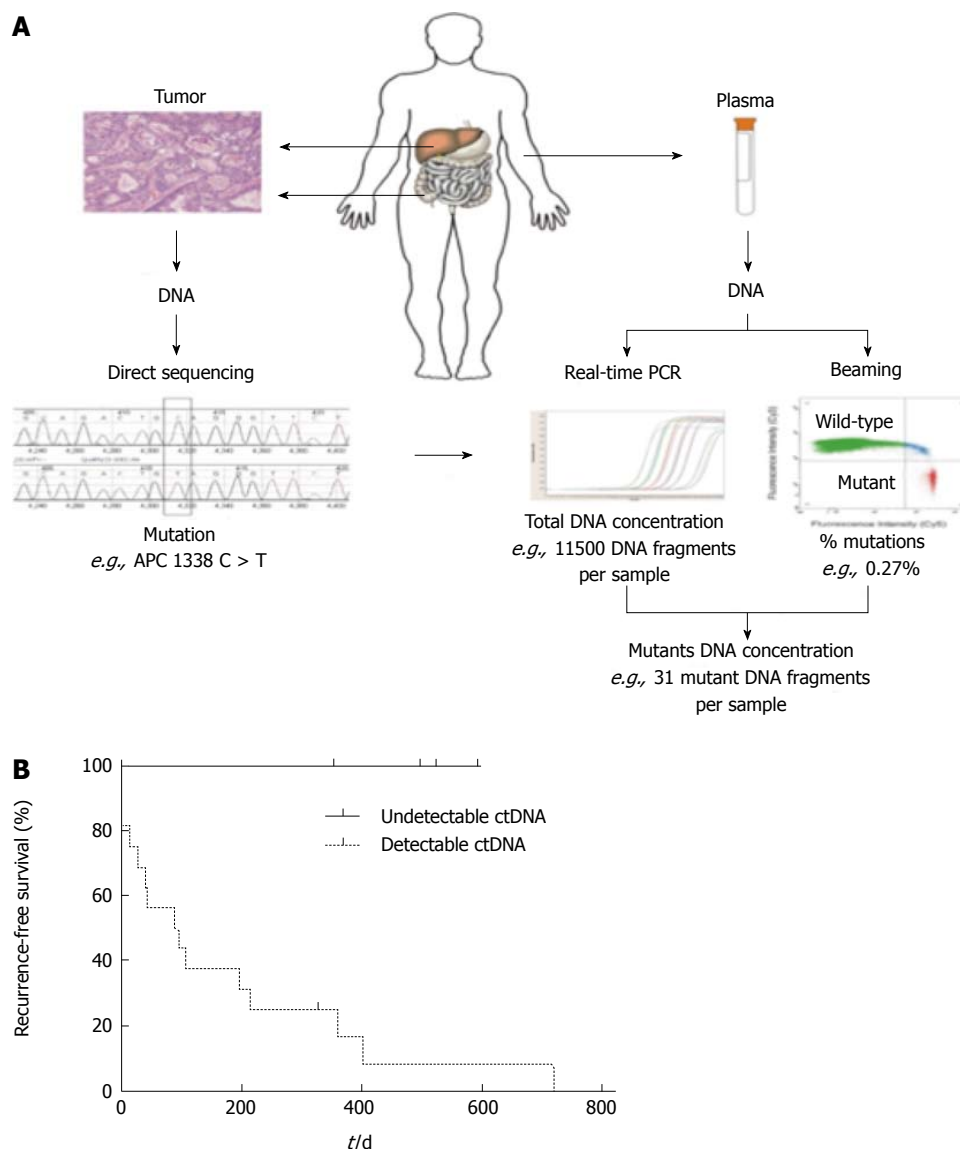
Studies have been unable to correlate prognosis between p21, a cyclin-dependent-kinase inhibitor and a key effector of p53 anti-proliferative activity, and OS in patients with CRLM<sup>[126]</sup>. Similarly, only one study has analyzed the relation between 3H-thymidine labeling index (TLI) and clinical outcome<sup>[137]</sup>. In this study, the authors did find that TLI correlated relapse at 4 years following surgery<sup>[137]</sup>. There is insufficient data to evaluate the prognostic value of markers of angiogenesis and thrombospondin-7.

### Circulating tumor cells and circulating tumor DNA

Circulating tumor cells (CTC) and disseminated tumor cells (DTC) may serve as prognostic factors for tumor relapse after potentially curative resection of CRLM. Some investigators have suggested that intraoperative manipulation of the tumor may increase CTC and DTC, spreading malignant cells and causing an increase in intrahepatic or extrahepatic tumor recurrences<sup>[138]</sup>. The data on this hypothesis are scarce and there is no consensus regarding the matter. While most studies analyzing CTC and DTC have largely focused on their prognostic value in the setting of primary colorectal cancer, a few studies have examined their role in the setting of CRLM. Vogelaar *et al.*<sup>[139]</sup> demonstrated that patients free of DTC in their bone marrow assessed by RT-PCR had a significantly better DFS and OS after resection of CRLM. A recent meta-analysis investigated the association between outcomes in patients with resected CRLM and tumor cells in the blood or bone marrow<sup>[140]</sup>. Specifically, in a cohort of 1329 patients (16 studies), the authors reported strong evidence suggesting that CTC correlated with worse OS and DFS. Of note, patients with detectable CTC had a 2 fold risk of progression or recurrence and a 2.5 fold increased risk of death compared with patients who had no CTC detected<sup>[140]</sup>.

Another developing field of cancer research is the detection of tumor-derived circulating mutant DNA. Circulating tumor DNA (ctDNA) represents a small part of the circulating DNA, making detection challenging. Recently Diehl *et al.*<sup>[141]</sup> proposed a multistep approach to quantify ctDNA in patients with metastatic colorectal cancer un-





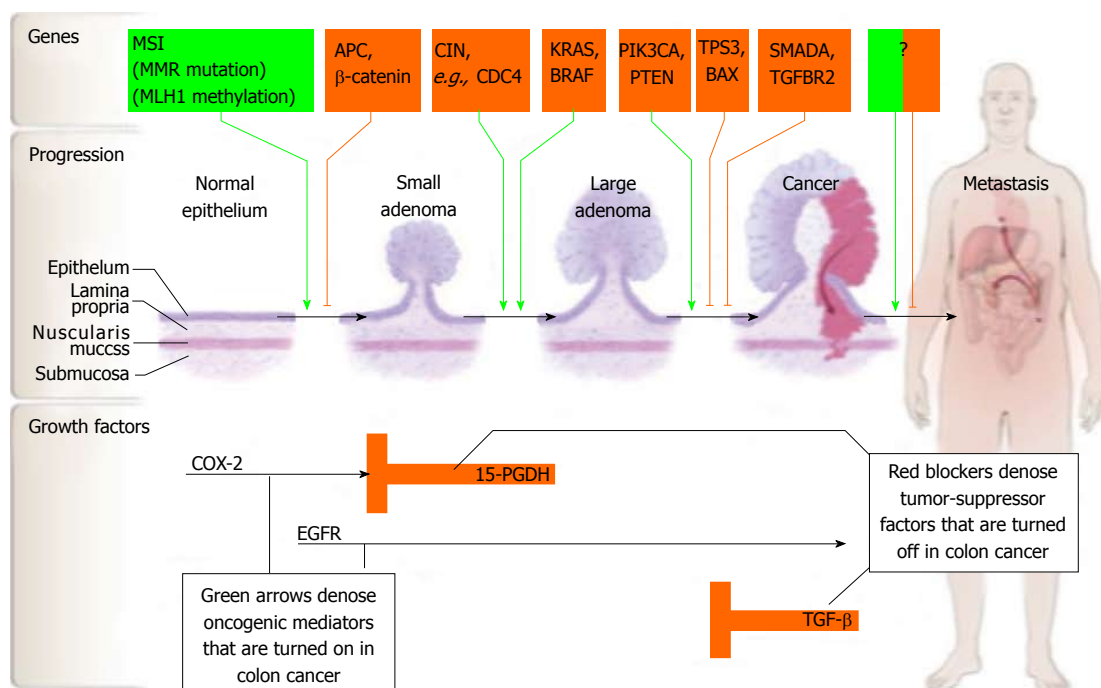
**Figure 4 Patients with detectable ctDNA following resection of colorectal liver metastasis, recurrence was universal.** A: Depiction of process by which ctDNA is detected and amplified from the specimen and plasma of patients; B: Representative flow cytometric data of ctDNA of one subject who underwent resection of colorectal liver metastasis. Note that the notable difference in recurrence-free survival in subjects with detectable vs undetectable ctDNA (from Diehl *et al*<sup>[141]</sup>). Used with permission. PCR: Polymerase chain reaction.

dergoing surgery and receiving chemotherapy. Using this beaming technique, the authors were able to detect ctDNA in a subset of patients following resection of CRLM. Among those patients with detectable ctDNA following resection of CRLM, recurrence was universal (Figure 4). In addition, the investigators noted a significant difference in DFS among patients with and without detectable ctDNA. Although preliminary in nature, these results suggest that ctDNA might be a promising prognostic factor of outcome following resection of CRLM.

### Genetic integrity

The development and progression of colorectal cancer is a multistep process leading to the accumulation of genomic alterations that occur over the lifetime of a tumor<sup>[142]</sup> (Figure 5). The loss of genomic integrity, in terms

of gross chromosomal aberrations and abnormalities of nuclear DNA content (aneuploidy), has been examined in relation to long-term outcome. In the early 1990's, Cady *et al*<sup>[143]</sup> found that aneuploidy was an independent prognostic factor, negatively impacting DFS. More recently, Metha *et al*<sup>[144]</sup> used an array-based comparative genomic hybridization to investigate the association of DNA copy number alterations with survival in patients with CRLM resected with curative intent. The total fraction of genome altered (FGA) in the metastases was noted to be an independent predictor of survival in patients with resected hepatic colorectal cancer metastases. In addition, the authors described a direct proportionality between level of FGA and probability of survival<sup>[144]</sup>. Although genetic instability seems to be correlated with tumor aggressiveness in primary colorectal cancer, it is not clear yet if it has a



**Figure 5** Carcinogenesis of colorectal cancer (from Markowitz *et al*<sup>[145]</sup>). Used with permission. EGFR: Epidermal growth factor receptor; TGF: Transforming growth factor; COX-2: Cyclooxygenase-2.

prognostic value in following resection of CRLM<sup>[126]</sup>.

## CONCLUSION

Survival following resection of CRLM varies and is dependent on clinical, tumor, and molecular factors. Accurate predictors of prognosis are important for patients, as well as providers. While some preoperative clinicopathologic factors are associated with outcome, the emergence of biologic and molecular markers may allow for a more individualized approach to prognosis. Factors such as KRAS, BRAF, TS, hTERT, Ki-67 can help predict long-term prognosis following CRLM. In addition, more recent data on CTC and ctDNA holds for a more sensitive and powerful metric of prognosis near the time of surgery for CRLM. With more accurate markers of prognosis in the future, a greater emphasis on patient-specific treatments and prognostic information will hopefully continue to emerge.

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## Pneumo-CT assessing response to neoadjuvant therapy in esophageal cancer: Imaging-pathological correlation

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### Abstract

Pneumo-computed tomography (PnCT) is a technique primarily developed and used to study stenotic lesions of the esophagus, gastroesophageal junction and stomach for pre-surgical planning. It helps to define both upper and lower borders of neoplasms located in the aforementioned areas. It achieves maximum lumen distension with CO<sub>2</sub> highlighting thickened areas of the esophageal wall, thus allowing an accurate quantification of their extents. Although there are other alternatives for distension (oral contrast agents, water and effervescent granules), they may be suboptimal. Patients with locally advanced esophageal cancer have a dismal prognosis despite surgical resection. Therefore, neoadjuvant treatment strategies using radiation therapy and chemotherapy were developed to improve survival. Neoadjuvant therapy improves esophageal tumor prognosis in a substantial proportion of patients, and the use of imaging techniques is mandatory to detect their response. PnCT combined with virtual endoscopy and multiplanar reconstruction enhances morphologic details in esophageal cancer, and thus would allow an

improved assessment of response to neoadjuvant treatment. Therefore, more information could be provided to assess the efficacy of pre-surgical treatment. We describe the potential use of PnCT to assess the response to neoadjuvant therapy in esophageal cancer with an imaging pathologic correlation.

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**Key words:** Esophagus; Cancer; 64-multidetector computed tomography; Neoadjuvant treatment; Assessment response

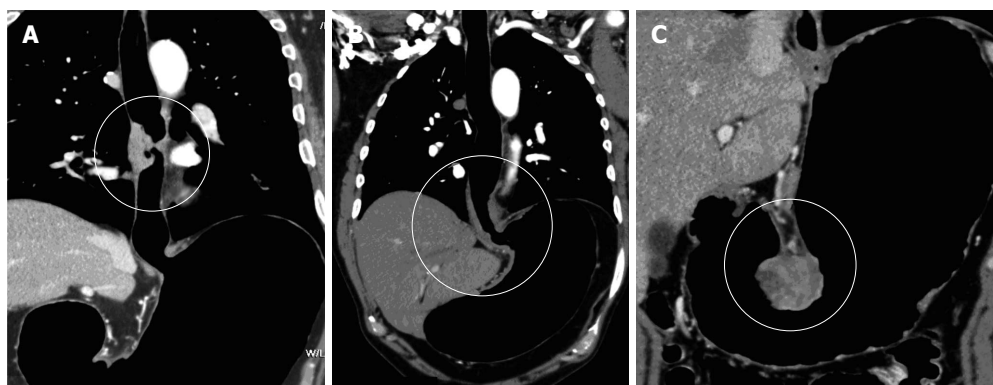
**Core tip:** Pneumo-computed tomography may be a useful technique to monitor neoadjuvant therapy response as it enhances morphologic details. Besides, it provides key information for surgical planning as it helps to define both upper and lower borders of esophageal or gastro-esophageal neoplasms in a single examination.

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### INTRODUCTION

Esophageal carcinoma is the sixth most common cause of cancer deaths worldwide<sup>[1]</sup>. There are approximately 14000 new cases of esophageal cancer per year in the United States, half of which are adenocarcinomas<sup>[2]</sup>. It is currently the most rapidly increasing cancer in the United States and Western Europe<sup>[3]</sup>. Prognosis is poor, with an overall survival of less than 10% within 5 years<sup>[4,5]</sup>. There-





**Figure 1** Pneumo-computed tomography in esophageal and gastric neoplasms. A: Pneumo-computed tomography (PnCT) multiplanar reconstructions (MPR) coronal soft tissue window reconstruction depicting involvement of mid-thoracic esophagus. Note detailed demonstration of location; B: PnCT MPR coronal soft tissue window reconstruction depicting involvement of the gastroesophageal junction; C: PnCT MPR coronal soft tissue window shows a GIST tumor arising from gastric wall.

fore, there is need to improve the survival rate for patients with this disease, by earlier diagnosis when prognosis is more favorable, and/or by improving its therapy<sup>[6]</sup>.

Neoadjuvant treatment strategies have been developed to improve survival<sup>[7]</sup>. Under current standards, chemoradiation is administered preoperatively in the majority of patients with advanced locoregional disease<sup>[8]</sup>. Thence, the ability to predict response to this combined therapy is clearly desirable.

Despite the importance of accurate pre-operative staging of esophageal neoplasm, there isn't a well accepted method to do so by imaging<sup>[9]</sup>. The spectrum of diagnostic modalities most commonly used besides computed tomography (CT), includes endoscopic ultrasound, positron emission computed tomography (PET)/CT and magnetic resonance imaging (MRI). The strength of endoscopic ultrasound is in its role in the initial staging of esophageal cancer. One weakness is its inaccuracy for staging after neoadjuvant therapy because of its inability to distinguish inflammation and fibrosis from residual cancer<sup>[10]</sup>.

Even though PET/CT is primarily indicated to look for distant metastases, it has also been used as a noninvasive test to evaluate response after neoadjuvant therapy before surgical resection<sup>[11,12]</sup>. One of its drawbacks lies in the fact that it does not provide accurate information for surgical planning because of lack of distension of the esophageal lumen.

MRI studies using routine clinical protocols have demonstrated a limited ability to evaluate esophageal anatomy<sup>[6]</sup>. Dynamic contrast-enhanced MRI is an emerging approach that needs further validation to be used in daily practice.

PneumoCT (PnCT) is a recently described technique that optimizes tumor visualization in the esophageal wall, gastroesophageal junction (GEJ) and stomach<sup>[13]</sup> (Figure 1). It achieves maximum lumen distension, highlighting thickened wall areas<sup>[13,14]</sup>.

The purpose of this review is to present a correlation between PnCT findings and post surgical pathology in order to evaluate the response of esophageal cancer to

neoadjuvant therapy.

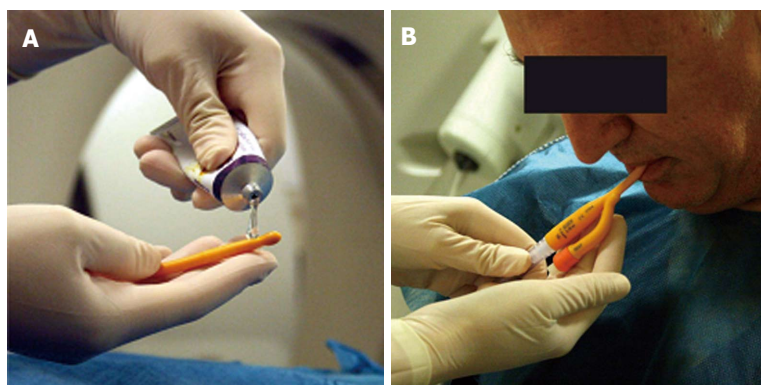
First, we discuss the basic technique of how to perform PnCT. Second, we describe the reconstruction steps followed by the classification and CT parameters used to assess response to neoadjuvant therapy. Finally, we present clinical examples of pre- and post-neoadjuvant cases with imaging-pathological correlation.

## PNCT TECHNIQUE

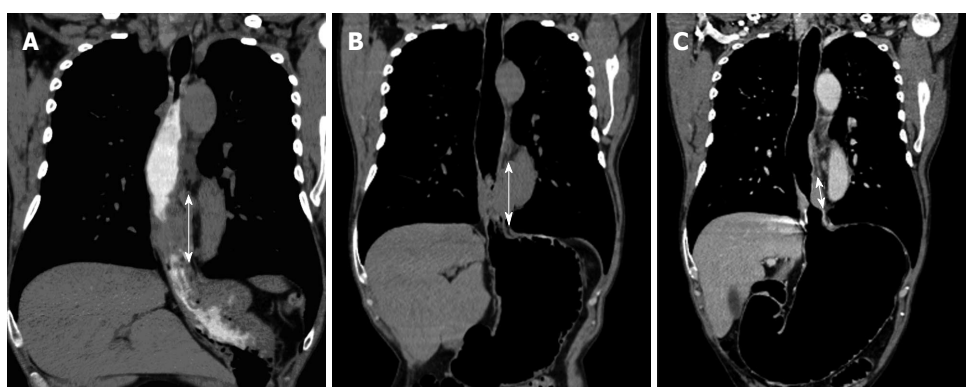
Patients are given before their imaging appointment an information sheet describing the procedure in detail including mention of slight discomfort that may be experienced by the esophageal distension. Patients with an 8-h pre-procedural fasting are received in the radiology department by a nurse, and a peripheral venous line is placed. Once in the CT suite, after administration via spray of a local anesthetic a lubricated Foley catheter is introduced transorally or transnasally, placing its distal tip below the cricopharyngeal muscles (Figure 2).

Continuous and sustained supply of CO<sub>2</sub> is maintained during the CT acquisition, with a pressure between 10 and 20 mmHg. We use the same CO<sub>2</sub> pump as in CT-Colonography (Protocol pump, PROTOCOL2L, E-Z-EM, Inc., Lake Success, NY, United States). Patients are instructed to hold the air and avoid burping during the procedure.

Pn6CTs are performed at our institution with Aquilion 64-row multidetector computed tomography (MDCT) (Toshiba Inc, Tokyo, Japan) with the following technical parameters: 0.5 mm slices, 0.25 mm table feed, 50 mAs, 120 kV, 0.75 s rotation time and 0.875 pitch. Anterior and lateral scout views are obtained to protocol the scans. We perform two cervico-thoraco-abdominal phases, the first non-enhanced and the second enhanced. The time required for each acquisition is approximately of 8 seconds. Nonionic iodinated contrast (Iobitridol, Xenetix® 350; Guerbet, France) at a dose of 1 mL/kg is infused using an automatic injection pump at a flow of 2.5 mL/s. No oral contrast is used. We haven't experienced a major adverse events specific to the CO<sub>2</sub> insufflation. The typical effective radiation dose is 18 mSv.



**Figure 2** Pneumo-computed tomography technique. A: The tip of the Foley catheter is lubricated with gel anesthesia; B: The Foley catheter is introduced transorally.



**Figure 3** Comparison of different distention options in the same patient with diagnosis of adenocarcinoma. A: Distension with positive oral contrast, the double arrow shows the extent of the lesion, which is larger than b and c, thus overestimating the neoplasm size; B: Distension with effervescent granules, the double arrow shows the tumor extent which is larger than c, still overestimating its size; C: Distension with pneumo-computed tomography technique, the double arrow shows the extent of the wall thickening which is shorter than a and b in accordance with the surgical specimen.

## DISTENSION OPTIONS

Other well-known alternatives for esophageal and gastric lumen distension (*i.e.*, oral contrast agents or effervescent granules) are used in daily practice<sup>[15]</sup>. However, distension may be suboptimal due to rapid transit of contrast and the required esophageal distension cannot always be achieved<sup>[16]</sup>. Oral contrast enhancement may generate confusing images, with the same density as the tumor<sup>[17,18]</sup>. Moreover, a suboptimal distension can cause distortion both in the quantification of the extension and in the degree of wall thickening (Figure 3).

## RECONSTRUCTION STEPS AND INTERPRETATION

Once acquired, images are sent to a Vitrea 2 working station (Vital Images, Inc, Minnesota, United States) for evaluation. Multiplanar reconstructions (MPR) and curved MPRs are performed with different window settings in order to characterize the lesion. In addition, we performed 3D reconstructions with different window settings (surface-shaded and transparent modes similar to the images obtained in single- and double-contrast barium studies). These images are easy to understand and allow visualization of the tumor. Finally, we obtain fly through views of the esophageal lumen and generate en-

doluminal views akin to esophagoscopy, to further assess lesion morphology.

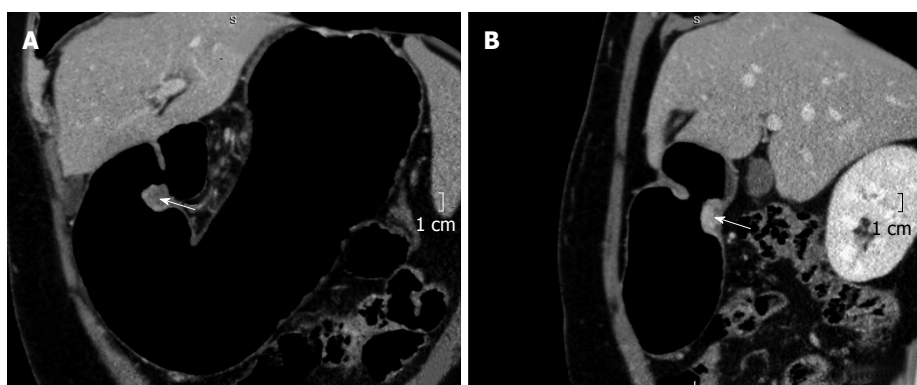
A description of shape and location of the lesion as well as measurements of size and wall thickening are performed. Also, we evaluate for presence of periesophageal fat stranding, adenopathy, and extraesophageal disease. Information regarding panoramic and longitudinal extent of the esophageal lesion is obtained.

## INDICATIONS

This technique was originally developed for GEJ stenosis, since the GEJ is a known difficult area to distend with traditional double contrast esophagograms<sup>[13,14]</sup>. The obtained gastric distension led to an adequate definition of both the upper and lower borders of GEJ lesions and in turn proved to be helpful for surgical planning<sup>[13,14]</sup>.

We then extended its use to all cases of esophageal stenosis impeding the passage of an endoscope and also to cases where endoscopy was contraindicated. The latter include cases in which a noninvasive method for endoluminal lesion characterization, stenosis grading, and beyond stricture visualization of the esophagus and/or the stomach is needed.

Palliation therapy with self-expandable stenting is the method of choice<sup>[19]</sup> both in unresectable esophageal tumors due to distant metastasis or local invasion, and



**Figure 4** Adenocarcinoma of the pylorus. A: Coronal multiplanar reconstructions (MPR) reconstruction, the arrow shows the tumor and its relationship with the pylorus; B: Sagittal MPR reconstruction, the arrow shows the tumor and its relationship with the pylorus.

**Table 1** Dworak classification for gastrointestinal tumors

Dworak classification	
Dworak grade 0	Is define as no regression
Dworak grade 1	Is define as prevalence of active cells and fibrosis/necrosis
Dworak grade 2	Is define as many of fibrosis or necrosis with active cells easier to find
Dworak grade 3	Is define as scarce neoplastic cells, hard to detect
Dworak grade 4	Is define as absence of tumor cells

in high-risk patients. In these patients, the definition of both upper and lower limits of the lesion in the longitudinal axis provided by PnCT allowed determining the stent graft length and the need for a valved stent. Thus no further barium studies are required.

Although the stomach can be well distended with other contrasts, such as water, milk, effervescent granules, the pyloric area is also a known difficult area to distend. Due to the optimal distension obtained with PnCT, we began to use this technique for distal stomach pathology with suspected pyloric involvement (Figure 4).

### TOMOGRAPHIC PARAMETERS USED TO ASSESS RESPONSE TO POST-NEOADJUVANT THERAPY

Esophageal tumors exhibit increased expression of pro-angiogenic factors such as basic fibroblast growth factor (bFGF) and vascular endothelial growth factor<sup>[20,21]</sup>. This form of malignancy is associated with greater vascularization and has a higher microvascular density compared with normal esophageal tissue and precancerous lesions<sup>[20-23]</sup>. These properties suggest a valid pathologic basis upon which quantifying the density with PnCT could not only detect esophageal tumors and but also evaluate their response to neoadjuvant therapy.

The following CT parameters are measured to evaluate (or restage) response to neoadjuvant therapy: wall thickening, density, and presence of adenopathy.

We compare the maximum diameters of pre- and

post-neoadjuvant therapy for wall thickening evaluation. To analyze its density, we make the same pre- and post-neoadjuvant therapy comparison by placing a region of interests (ROI) at the same site of greatest wall thickening, excluding areas of low density representing wall necrosis. Finally, we compare adenopathy sizes as well before and after neoadjuvant therapy.

### IMAGING-PATHOLOGIC CORRELATION

The use of neoadjuvant therapy began to be practiced for rectal cancer<sup>[24]</sup>. Its use was extended to esophageal and other gastrointestinal cancers<sup>[24]</sup>. Since neoadjuvant therapy changes the internal structure of tumor, pathologists have been faced to reconsider the staging of these neoplasms resected after receiving chemo-radiation. The appearance of fibrosis and necrosis confirms the action of therapy and constitutes the basis for several new staging classifications<sup>[24]</sup>.

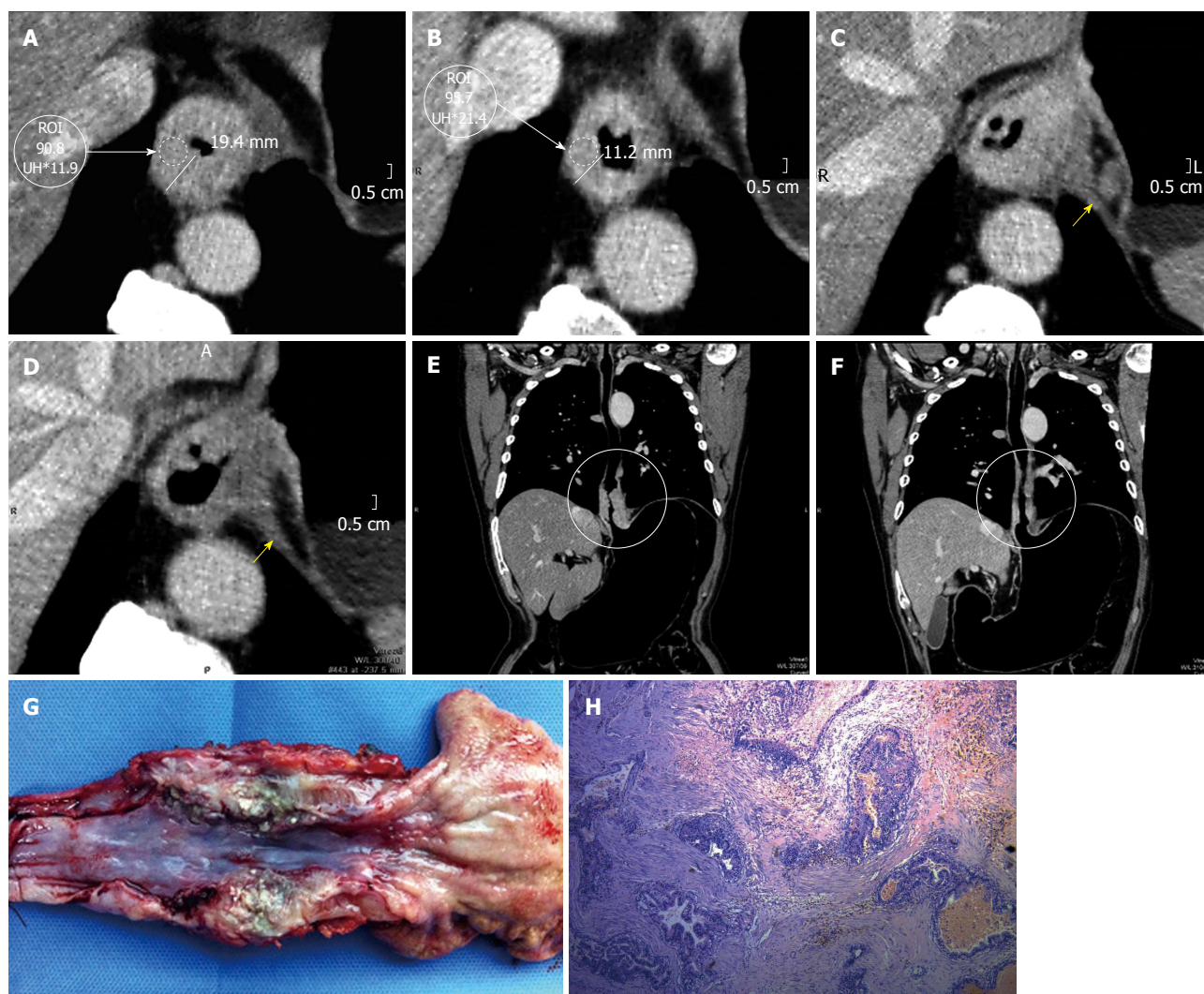
In 1997, Dworak *et al*<sup>[24]</sup> presented a pathological classification for rectal cancer based mainly on the difficulty of highlighting viable tumor cells in a fibronectrotic stroma. Thus, five categories were identified (Table 1). Due to its usefulness, this classification, has also been extrapolated to other gastrointestinal tumors<sup>[25]</sup>.

### CORRELATION BETWEEN THE PN64MDCT AND DWORAK CLASSIFICATION

We use clinical examples to show the change observed in the CT findings comparing pre- and post-therapy PnCT scans with correlation with the Dworak classification obtained after surgery.

Wall thickening persistence is evident in the pre- and post-neoadjuvant therapy measure comparison at the same level (Figure 5). Placing the ROI on the site of maximum wall thickening, the density in Hounsfield Units (HU) increases from (90.8 HU) before to 95.7 HU after therapy. The increase in the density may be given by the persistence of active cells containing proangiogenic factors (Figure 5). Additionally, an adenopathy monitor-





**Figure 5 Advanced adenocarcinoma. Dworak grade I, prevalence of active cells.** A: Axial pre neoadjuvant therapy image. Both wall thickness and density are measured; B: Axial post-neoadjuvant therapy image reveals persistence of the wall thickening and almost no variation in density; C: Axial pre-neoadjuvant therapy image, the arrow is pointing to a lymphadenopathy; D: Axial post-neoadjuvant therapy image reveals resolution of the adenopathy; E: Coronal multiplanar reconstructions (MPR) pre-neoadjuvant therapy reconstruction. The circle shows the long axis compromise of the tumor; F: Coronal MPR post-therapy reconstruction. The circle shows the long axis of the tumor and its precise location; G: Surgical specimen of total esophagectomy and upper polar gastrectomy. Open piece shows an important thickening of the lower esophagus, at squamo-columnar junction recognizes a polypoid lesion and poorly defined borders. The remaining gastric mucosa presents edematous folds; H: Section shows at gastroesophageal junction one degree injuries ranging from low-grade dysplasia to invasive adenocarcinoma. The primary lesion is below the squamocolumnar junction. Generally infiltrates shaped surface (submucosa) with occasional foci in muscle layer and extends in the form of multiple separate foci below esophageal epithelium. Proximal margin: adenocarcinoma foci observed at adventitia and submucosa layers. Tumor is seen infiltrating striated muscle tissue.

ing can be performed, determining its evolution.

On the other hand, the pre- and post- comparison of the wall thickness in Figures 6 and 7 at the same level demonstrate a decrease of over 50% in the measured density, for instance before 85.7 HU and after 36.4 HU chemotherapy and radiotherapy (Figures 6-8). This decrease in density could be explained by the presence of extensive areas lacking proangiogenic factors. The disappearance of the lymphadenopathy can also be observed.

In patients without response to neoadjuvant therapy (Dworak I), there was no change in the density of the tumor when we compared the PnCT pre- and post- therapy. This finding could be explained by the presence in the active cells of bFGF and vascular endothelial growth factor. Conversely, there is reduction in the thickness and

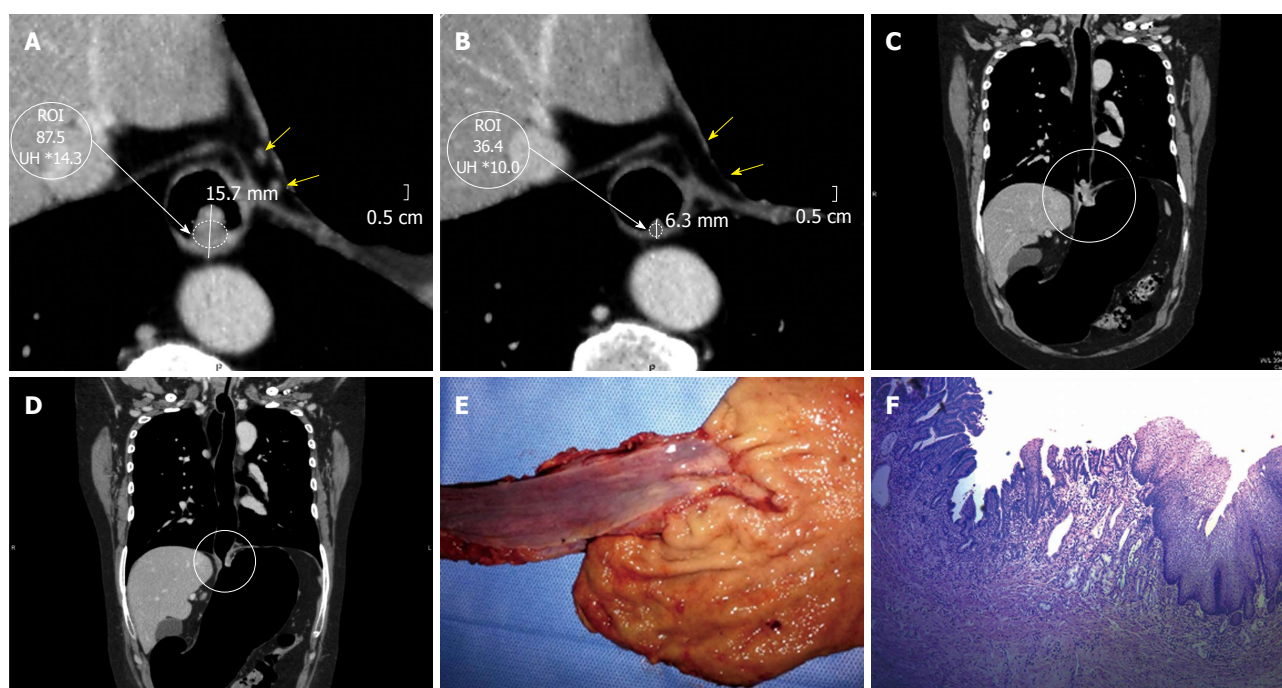
in the Hounsfield Units density (between 33% and 46% less) of the tumor in those patients with a good response to therapy, correlating well with a significant regression in the post-surgical pathological staging of Dworak III or IV neoplasms.

## CONCLUSION

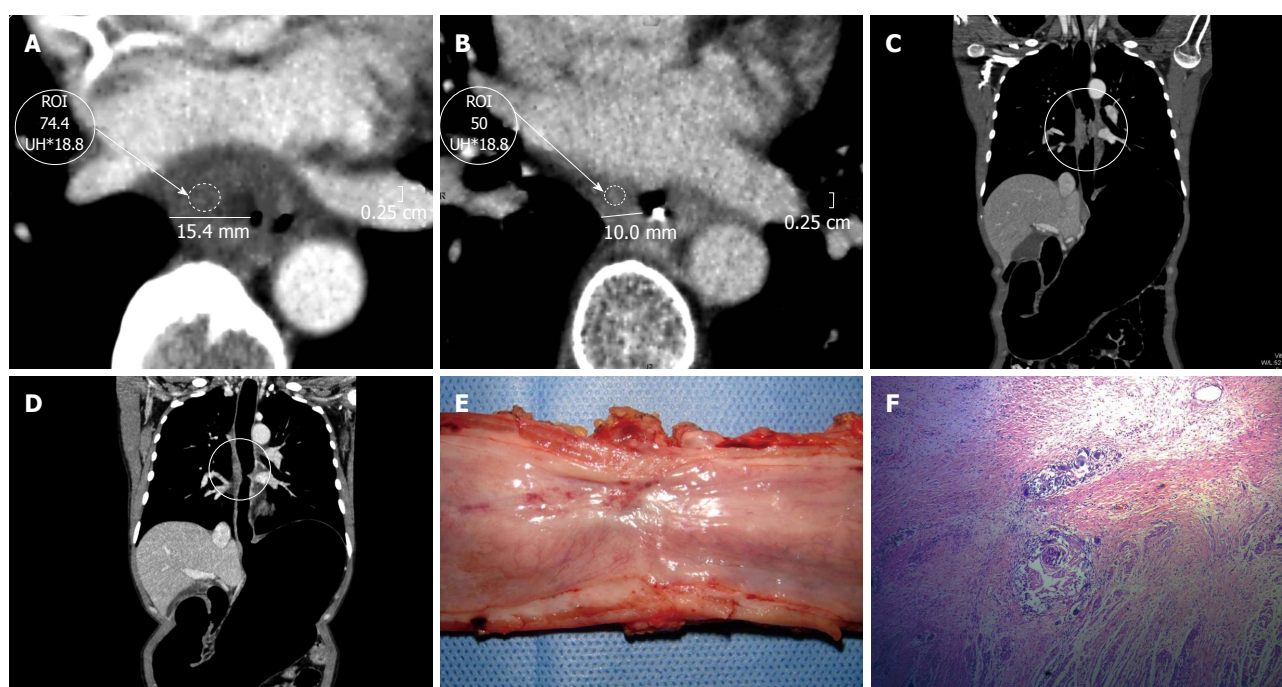
PnCT is a useful non-invasive imaging technique for evaluating esophageal and gastroesophageal tumors, allowing a precise evaluation of their size, location, local extension and regional adenopathy a single examination<sup>[13,14]</sup>.

Further, PnCT provides key pre-surgical planning information, since it defines both upper and lower borders of neoplasms located in the GE junction<sup>[13,14]</sup>.

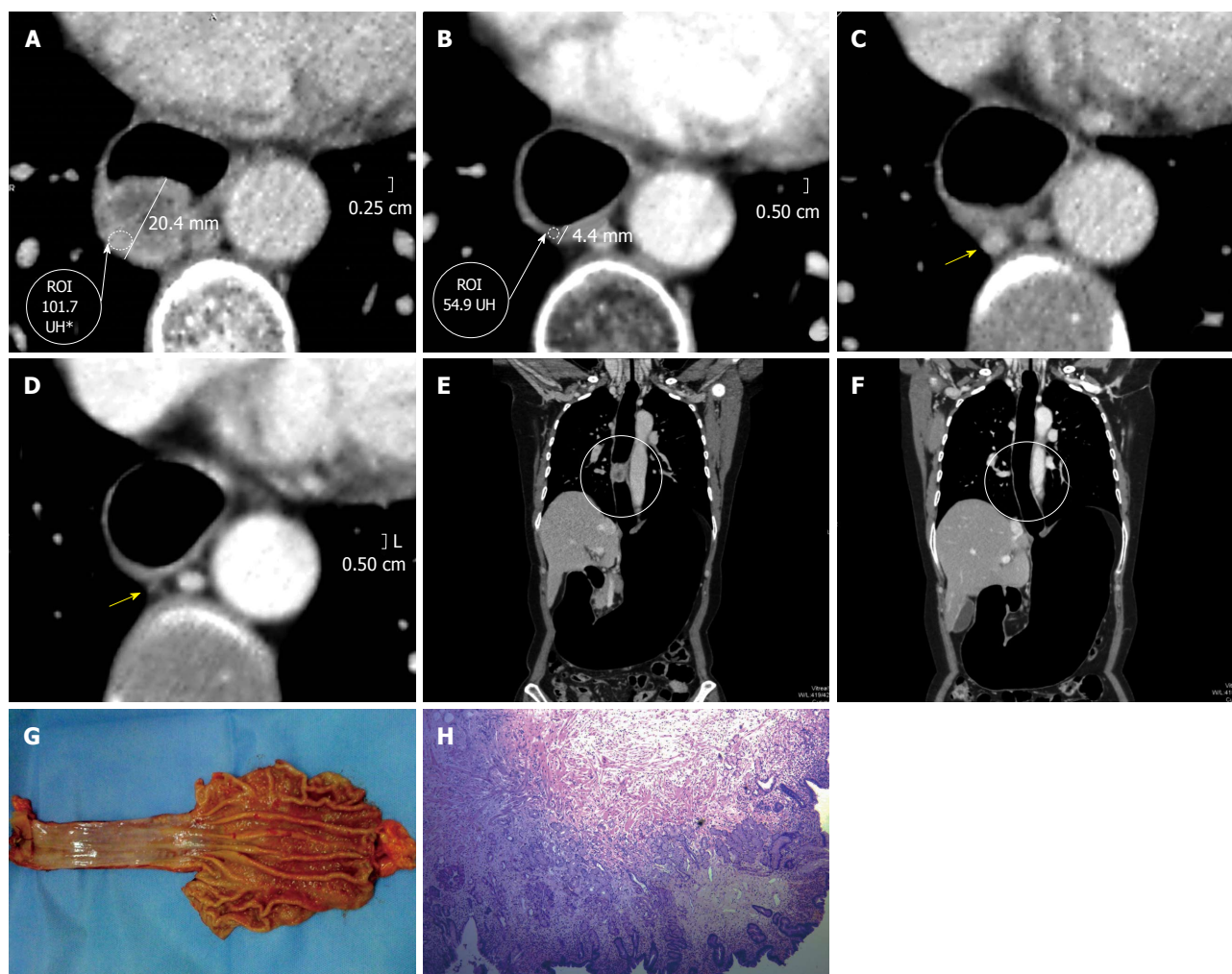




**Figure 6** Advanced adenocarcinoma of the gastroesophageal junction. Dworak grade 2, large areas of fibrosis or necrosis with active cells. A: Axial pre-therapy image. Both wall thickness and density are measured. Yellow arrows indicate two small lymph nodes; B: Axial post-therapy image reveals a clear decrease of tumor size and in density. Lymph nodes are not visible; C: Coronal multiplanar reconstructions (MPR) pre-therapy reconstruction. The circle shows the long axis of the tumor; D: Coronal MPR after therapy reconstruction. Note marked decrease in tumor size; E: Surgical specimen of total esophagectomy and upper polar gastrectomy correlating well with the lesion demonstrated with pneumo-computed tomography. Open piece, is recognized at the level of the gastro-esophageal junction an indurated area with diminished mucosal folds and elevated edges with whitish nodule and firm consistency; F: Submucosal layer with nodular accumulations of atypical epithelial cells scant cytoplasm that are arranged in small tubular structures, cords or dispersed. Nodules are found at the distal esophagus and cardia underlying mucosa. Linfovascuales tumor emboli are observed. In small area is observed submucosal fibrosis, chronic inflammation and congestion. At the level of the gastro-esophageal junction is observed extensive intestinal metaplasia to intramucosal carcinoma focus. Preserved oxyntic gastric mucosa.



**Figure 7** Epidermoid carcinoma. Dworak grade 3, scarce neoplastic cells. A: Axial pre-neoadjuvant therapy image. Both wall thickness and density are measured; B: Axial post-neoadjuvant therapy image reveals a clear decrease of the wall thickening and density; C: Coronal multiplanar reconstructions (MPR) pre-neoadjuvant therapy reconstruction. The circle shows the long axis of the tumor; D: Coronal MPR post-neoadjuvant therapy reconstruction with decrease in tumor size; E: Surgical specimen of total esophagectomy. Open piece, shows thickening at the middle third of the esophagus with an ulcerated area; F: The sections shows at submucosal layer a nodular accumulation of atypical epithelial cells with vesicular nuclei and scant cytoplasm that are arranged in small tubular structures. Also the submucosa presents fibrosis, chronic inflammation and congestion. Mucosal layer shows conserved squamous epithelium and focal fibrosis regression suggesting changes.



**Figure 8 Epidermoid carcinoma of the thoracic esophagus [CME #5].** Dworak grade 4, absence of tumor cells. A: Axial pre-neoadjuvant therapy image. Both wall thickness and density are measured; B: Axial post-neoadjuvant therapy image reveals a clear decrease of the wall thickening and density; C: Axial pre-neoadjuvant therapy image, the arrow is pointing to adenopathy; D: Axial post-neoadjuvancy image reveals disappearance of adenopathy; E: Coronal multiplanar reconstructions (MPR) pre-neoadjuvant therapy reconstruction. The circle shows the long axis of the tumor; F: Coronal MPR reconstruction post-neoadjuvant therapy. The neoplasm is no longer detected; G: Surgical specimen of total esophagectomy and upper polar gastrectomy. Open piece, is recognized at the level of the gastro-esophageal junction an area of white-depressed with elevated edges; H: Squamous epithelium with acanthosis, conserved cell polarity. Fibrohistiolysis in lamina propria and submucosa with lymphocytic infiltrate. Absence of atypical cells. At the level of the gastroesophageal junction shows a sector with intestinal metaplasia in the stomach side, negative for dysplasia.

We also demonstrate the PnCT findings correlate with the Dworak pathologic classification and could allow assessing the response of esophageal neoplasms to neoadjuvant therapy. This is crucial regarding the overall prognosis and therapeutic strategy.

Our next goal is to prove in a prospective, blinded and randomized study the accuracy of PnCT to assess response to neoadjuvant therapy in esophageal neoplasms and its correlation with post resection pathological staging.

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## Rare presentation of post-transplant lymphoproliferative disorder isolated to gastroesophageal junction

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**Author contributions:** All authors were involved in management of the patient and writing and editing of this case report; Shana'ah A provided the pathology photographs that are in this article; Oza VM, Walker J, Johnson A and Haverkos BM were involved with admitting, and managing this patient thru the hospitalization.

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### Abstract

Post transplant lymphoproliferative disorder (PTLD) represents a life threatening disorder occurring after transplantation, ranging from a polyclonal mononucleosis like illness to a monomorphic high grade neoplasm with cytologic and histopathologic evidence indicative of transformation to lymphoma. PTLD of diffuse large B-cell lymphoma (DLBCL) subtype, isolated to the esophagus is a rare diagnosis. We describe the first case of an immunocompromised adult patient diagnosed with DLBCL-PTLD limited to his esophagus without an associated mass or locoregional lymphadenopathy on imaging since the institution of the revised

Cheson criteria, which includes positron emission tomography-computed tomography as the standard staging modality. Even more unique to our case was the suggestion of underlying cytomegalovirus (CMV) gastritis leading to a hypothesis about a less well understood relationship between CMV and Epstein Barr virus (EBV). In the post transplant setting, immunocompromised state, or EBV positive state, upper gastrointestinal symptoms should prompt investigation with an upper endoscopy (EGD). Additionally, specific to our case, the fact that the patients' presentation was suspicious for CMV gastritis raises the possibility that the CMV infection predated his PTLD increasing his risk of acquiring PTLD. This reemphasizes the importance and diagnostic utility of early screening with EGD in patients after transplantation.

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**Key words:** B-cell lymphoma; Esophageal; Lymphoproliferative; Post-transplant; Epstein barr virus

**Core tip:** Post transplant lymphoproliferative disorder (PTLD) are a heterogeneous group of lymphoid proliferations associated with immunosuppression following solid organ transplantation (SOT) or allogeneic hematopoietic stem cell transplantation (HSCT). PTLD associated with B-cell esophageal lymphoma is a rare diagnosis. Our patient was identified to have isolated PTLD at the gastroesophageal junction, which has not been reported in the modern era of positron emission tomography-computer tomography. Previous reported cases describe esophageal lymphoma associated with symptoms of dysphagia, odynophagia or esophagoduodenoscopy (EGD) findings that revealed an associated mass or locoregional lymphadenopathy. However, given the variation in presentation in post-transplant and immunocompromised patients, gastrointestinal symptoms should prompt further investigation with an EGD.



Haverkos BM, Oza VM, Johnson A, Walker J, Shana'ah A. Rare presentation of post-transplant lymphoproliferative disorder isolated to gastroesophageal junction. *World J Gastrointest Oncol* 2013; 5(12): 230-234 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v5/i12/230.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v5.i12.230>

## INTRODUCTION

Post-transplant lymphoproliferative disorder (PTLD) represents a life threatening disorder occurring after transplantation, ranging from a polyclonal mononucleosis like illness to a monomorphic high grade neoplastic disease with cytologic and histopathologic evidence indicative of non-Hodgkin as well as Hodgkin's lymphoma. Monomorphic disease includes diffuse large B-cell lymphoma (DLBCL), Burkitts lymphoma, plasma cell myeloma/plasmacytoma, and rarely T-cell neoplasms<sup>[1]</sup>. The majority of PTLD following both allogeneic HSCT and SOT are of B cell origin, and are usually associated with the Epstein Barr virus (EBV). In the pediatric population, the highest incidence of lymphoma has been observed during the first year after transplantation with 47% of cases occurring within 6 mo, 62% within 1 year, and 90% within 5 years of transplant<sup>[2]</sup>. In adults slightly more than 50% of patients are diagnosed beyond 12 mo with a predilection for EBV associated tumors to occur sooner than EBV negative tumor types. In children at ten years, the relative risk of lymphoma is 11.8 fold greater higher than in persons in the non-transplant population<sup>[3]</sup>. The incidence of PTLD is generally higher among pediatric patients as compared to adults. Among renal transplant recipients, the prevalence of PTLD has been reported to be about 5%<sup>[4]</sup> and recent studies suggest that risk factors for partial or no clinical remission are multiple site disease, performance status, and non-detection of EBV in the tumor<sup>[5]</sup>.

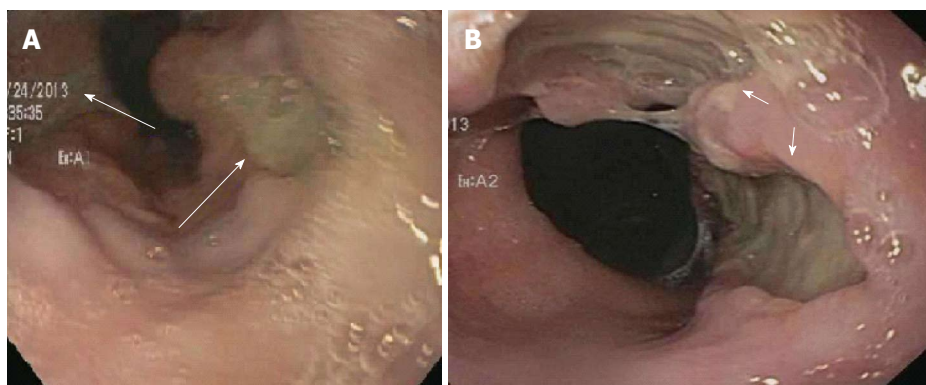
The clinical features of PTLD are multiple and varied and can range from asymptomatic EBV seroconversion or peripheral blood viremia to monoclonal B cell proliferation with nodal, extra-nodal, and disseminated disease<sup>[6]</sup>. In a retrospective review of 78 PTLD patients at the University of Michigan the majority were identified with extensive symptomatic disease: extranodal disease (79%), poor performance status (68%), elevated lactate dehydrogenase (71%), and advanced stage by Ann Arbor criteria (68%)<sup>[1]</sup>.

The esophagus is one of the more infrequent sites for gastrointestinal lymphomas, usually accounting for < 1% of cases<sup>[7]</sup>. Furthermore, fewer than 30 cases of lymphoma limited to the esophagus have been reported<sup>[8]</sup>. Approximately 2/3 of these cases are diffuse large B cell lymphomas. Our case represents the first documented case of PTLD of DLBCL type with isolated gastroesophageal involvement without local or disseminated lymphadenopathy in the modern revised Cheson criteria staging era.

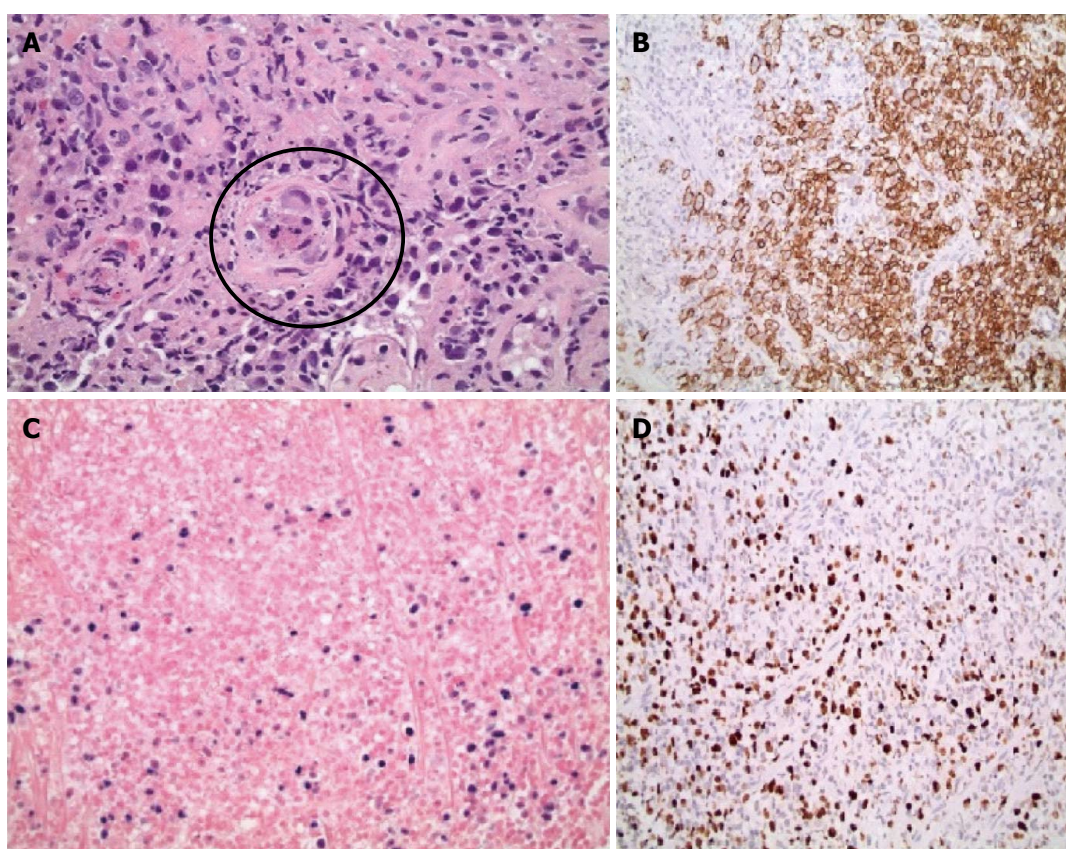
## CASE REPORT

We present the case of a 25-year-old African American male with a history of gastroesophageal reflux disease (GERD) and systemic lupus nephritis status post living related donor kidney transplantation, 3 years prior, on chronic immunosuppression presented to the emergency department for evaluation of four weeks of progressive retrosternal burning, nausea, vomiting and odynophagia. He had previously been diagnosed six years prior with GERD and was placed on daily proton pump inhibitor (PPI) therapy, but had discontinued PPI use after symptoms resolved. He denied solid and/or liquid dysphagia, but reported an approximate ten pound weight loss because of worsening symptoms. There was no history of any fevers, chills or night-sweats. At the time of admission immunosuppression for his renal transplant included Prednisone, Sirolimus and Mycophenolate mofetil. Admission labs were within normal limits with the exception of a lipase level of 130 U/L (normal range of 18-51 U/L). Gastroenterology service was then consulted for further evaluation. An upper endoscopy (EGD) was pursued to evaluate for cytomegalovirus (CMV), Herpes Simplex virus (HSV) and/or candidal esophagitis. EGD revealed Los Angeles grade A esophagitis with two small cratered esophageal non-bleeding ulcers at the gastroesophageal (GE) junction (Figure 1A). The gastric antrum was also mildly erythematous. Given the broad differential diagnosis for esophagitis including non-infectious as well as infectious etiologies biopsies were obtained from the gastroesophageal (GE) junction. Gastric biopsies were also obtained to evaluate for *Helicobacter pylori* infection.

Biopsy results of the stomach only demonstrated mild chronic gastritis with reactive epithelial changes whereas biopsies of the GE junction demonstrated ulceration, a diffuse infiltrate of large atypical cells with irregular nuclei and prominent nucleoli as well as prominent necrosis and rare CMV inclusions within an endothelial cell (Figure 2A). Based on immunohistochemical and *in-situ* hybridization stains the large lymphoma cells were strongly and uniformly positive for CD20 (Figure 2B), CD30, CD45, PAX5, BCL2, BCL6, MUM1, and *in-situ* hybridization for Epstein Barr virus encoded RNA (EBER) (Figure 2C). The Ki-67 stain was positive in 100% of large cells consistent with a high proliferation fraction (Figure 2D). The large B cells were negative for CD5, CD10, CD15, BCL1, and GCET1. The pathology was diagnostic of a post-transplant lymphoproliferative disorder, DLBCL type expressing a non-germinal cell immunophenotype. A CMV immunostain did not demonstrate positive results due to loss of the rare endothelial cell on deeper levels. The patient was subsequently started on valganciclovir for treatment of CMV esophagitis (given CMV inclusions and ulcers) and Hematology/Oncology service was consulted for further management and treatment of PTLD-DLBCL type. He subsequently underwent staging workup to evaluate this new diagnosis, which



**Figure 1** Esophageal ulcers (arrows) noted on upper endoscopy on initial presentation (A), and repeat endoscopy 10 d later shows significantly enlarged ulcers (arrows) (B).

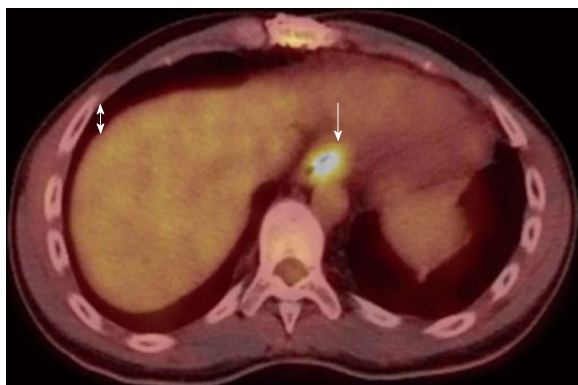


**Figure 2 Biopsy results.** A: Esophageal biopsy, with circled area showing a large endothelial cell with cytomegalovirus inclusions; B: Positive CD20 staining; C: Epstein Barr virus encoded RNA *in situ* hybridization showing positive staining; D: Positive Ki-67 staining in most of the lymphoma cells.

included a bilateral bone marrow biopsy, a positive emission tomography (PET) and a computed tomography (CT) scan of chest, abdomen and pelvis. Bone marrow biopsy was negative for involvement by lymphoma as was the peripheral blood flow cytometric analysis. PET scan showed focal uptake within the region of the GE junction only but no other significant uptake throughout the chest, abdomen, or pelvis, nor was the patient noted to have any paraesophageal or gastric involvement (Figure 3). CT scans showed only subtle prominence at the GE junction. He was discharged with plan to start chemotherapy as an outpatient. Unfortunately, he was re-admit-

ted one week later for severe epigastric pain, nausea and vomiting. Repeat endoscopy was done which showed significantly enlarged GE junction ulcers (Figure 1B). He was then started on treatment with dose adjusted R-EP-OCH (Rituximab, Etoposide, Prednisone, Vincristine, Doxorubicin) for his limited stage esophageal PTLT, DLBCL type. After his first cycle his laboratory values were consistent with tumor lysis syndrome, requiring a dose of Rasburicase. Throughout the inpatient stay, our patient continued to have delayed nausea and vomiting. He had a diagnostic lumbar puncture to rule out central nervous system disease (which was negative) and was





**Figure 3** Above shows a coronal section of positron emission tomography-computed tomography showing increased uptake at the gastroesophageal junction. Green arrow points to the area of increased activity.

also administered intrathecal Methotrexate at the time. A repeat PET/CT scan done to reassess disease after chemotherapy showed interval improvement in patient's lymphoma at the GE junction. Patient was then discharged on prophylactic Dapsone, Valacyclovir and Fluconazole. He has received four cycles of chemotherapy and is currently doing well, and is scheduled to complete six cycles followed by restaging.

## DISCUSSION

In summary, we present an immunosuppressed renal transplant patient with a prior history of GERD and four weeks of progressive retrosternal burning, nausea, vomiting and odynophagia. The differential was broad and included both infectious and non-infectious etiologies. He underwent an EGD with biopsy of GE junction consistent with non-germinal center DLBCL and CMV inclusions. His laboratory findings were remarkable for a normal CBC and liver profile, and elevated LDH. PET/CT imaging was only remarkable for focal uptake at the GE junction. So the ensuing plan was to get 6 cycles of dose adjusted R-EPOCH and intrathecal prophylaxis with methotrexate.

Approximately 85% of PTLDs are B cell in origin and most commonly of the monomorphic DLBCL type (50%) typically occurring after solid organ transplant. The major risk factor in developing PTLD is type, degree, and cumulative length of immunosuppression as well as EBV status<sup>[6]</sup>. PTLDs have a diverse clinical presentation, commonly with extranodal manifestations (approx. 70%) with gastrointestinal tract involvement occurring in up to 30% of patients who usually present with GI complaints (*i.e.*, weight loss, bowel perforation, bleeding, fever, and pain). PTLD with multiple lesions throughout the gastrointestinal tract has been described in a child<sup>[9]</sup>. Specifically, in renal transplant recipients who acquire EBV associated PTLD, the allograft is affected in approximately one-third of all cases<sup>[10]</sup>. In general, data on gastrointestinal lesions in PTLD is limited. In the study by Shitrit *et*

*al*<sup>[12]</sup> that included 17 patients with gastrointestinal manifestations, median time of occurrence of PTLD was 36 mo, and in 6 patients, PTLD was diagnosed within 18 mo. In 29%, or 5 out of 17 patients, involvement of organs other than the GI tract were found<sup>[11]</sup>. However, it must be noted that much of this data is extracted from pediatric populations who tend to develop PTLD earlier than adults.

Our patient was identified to have isolated PTLD of the DLBCL type at the gastroesophageal junction. Primary esophageal B cell lymphoma outside of the post-transplant setting has been reported but is also very rare<sup>[13]</sup>. In the most extensive case series on GI involvement, O'Conner *et al*<sup>[14]</sup> reported the findings of 6/14 children who were found to have isolated gastroesophageal PTLD on EGDs after presenting with GI symptoms. The incidence of PTLD in pediatric patients is higher than in the adult transplant population presumably secondary to the population being more frequently EBV naive. O'Conner *et al*<sup>[14]</sup> reviewed the possibility of using EGD as a screening tool for PTLD, which has not been utilized in the adult population.

Our patient was treated with induction R-EPOCH (Rituximab, Etoposide, Vincristine, Cyclophosphamide, and Doxorubicin) chemotherapy, prophylactic intrathecal Methotrexate, and Valganciclovir for his presumptive CMV gastritis. He has responded well to treatment thus far. In general, the treatment for EBV driven PTLD remains a controversial subject, with no SE based treatment or multi-centered trials to support any particular treatment modality. However, a reduction in immunosuppression is the most common management with less efficacy against the monomorphic disease therefore typically necessitating immunochemotherapy with Rituximab.

In our case it is important to comment on his presumptive CMV gastritis. CMV infection has been an identified risk factor in the development of PTLD through the modification of inflammatory cytokines<sup>[15]</sup>. In a group of patients with primary CMV disease, the post-transplant risk of PTLD was found to be 4 to 6 fold higher<sup>[16]</sup>. Also in one small study, children with simultaneous CMV and EBV infection had earlier onset, worse symptoms, and a greater rise in serum creatinine than was seen with primary CMV or EBV disease alone<sup>[17]</sup>. This provides rationale to further study the relationship between CMV and EBV.

From this case we have learned the importance of prompt investigation with EGD in the post-transplant setting, immunocompromised state, or EBV positive state when upper gastrointestinal symptoms occur. Additionally, specific to our case, the fact that the patients' presentation was suspicious for CMV gastritis raises the possibility that his CMV disease predated his PTLD increasing his risk of acquiring PTLD. This reemphasizes the take home message that early screening with EGD in a patient after transplantation has important diagnostic utility.

## COMMENTS

### Case characteristics

It highlights the spectrum of presentation found in an uncommon diagnosis of post transplant lymphoproliferative disorder (PTLD).

### Clinical diagnosis

It allows for a review of the more common clinical manifestations to PTLD with a focus on the workup of such patients.

### Differential diagnosis

It most importantly highlights a unique unreported presentation in an unambiguous case of PTLD.

### Laboratory diagnosis

One grey area was the diagnosis of cytomegalovirus (CMV), although it is certainly not the most salient point to the case.

### Imaging diagnosis

In the patient there was no peripheral blood CMV viremia, which would verify the CMV infection.

### Peer review

The paper is worth being published.

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## Management of afferent loop obstruction from recurrent metastatic pancreatic cancer using a venting gastrojejunostomy

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**Key words:** Afferent loop; Cholangitis; Pancreatic cancer; Biliary obstruction; Gastrojejunostomy

**Core tip:** Complications from recurrent pancreatic cancer can result in afferent loop obstruction. This leads to stasis of the biliary, intestinal and pancreatic secretions. We present here a unique approach to manage afferent loop obstruction caused by recurrent peritoneal metastases from pancreatic cancer. The patient underwent decompression of the afferent limb as well as the biliary tree using a venting gastrojejunostomy to the blind loop. This represents a novel surgical approach for management of this complicated and difficult problem.

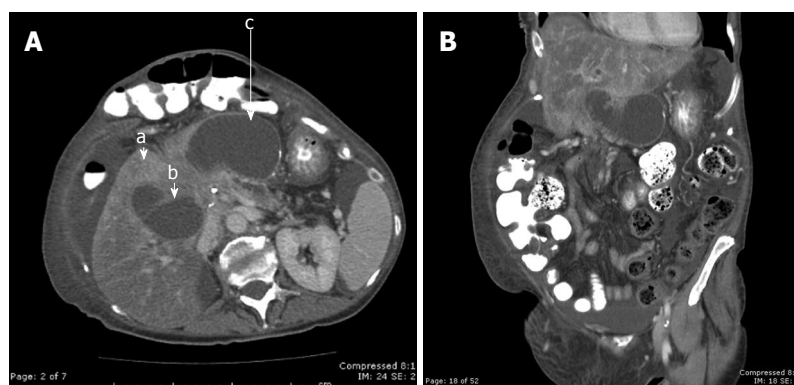
### Abstract

Pancreatic cancer is an aggressive malignancy potentially curable with surgical intervention. Following pancreaticoduodenectomy for suspected pancreatic head malignancy, patients have a high risk for both immediate and delayed problems due to surgical complications and recurrent disease. We report here a patient with pancreatic cancer treated with pancreaticoduodenectomy who developed recurrent disease resulting in obstruction of the afferent limb. The patient developed biliary obstruction and cholangitis at presentation. Her biliary tree failed to dilate which precluded safe percutaneous biliary decompression. During surgical exploration, she was found to have a dilated afferent limb at the level of the transverse mesocolon. The patient underwent decompression of the afferent limb as well as the biliary tree using a venting gastrojejunostomy to the blind loop. This represents a novel surgical approach for management of this complicated and difficult problem.

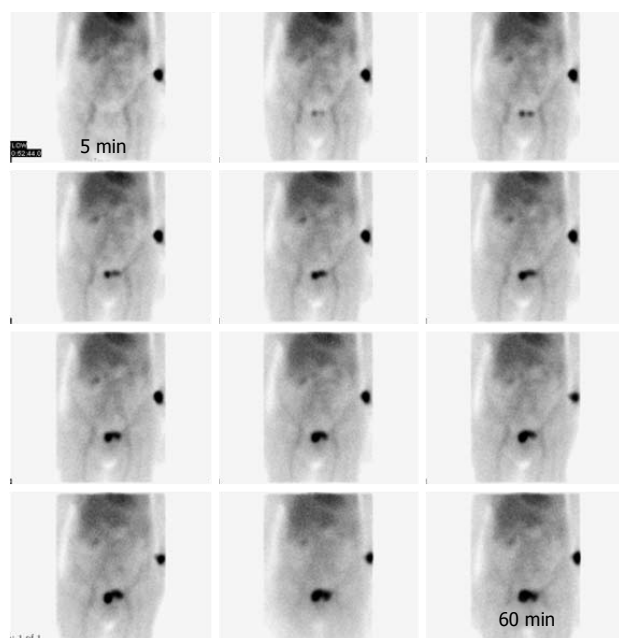
Bakes D, Cain C, King M, Dong XD. Management of afferent loop obstruction from recurrent metastatic pancreatic cancer using a venting gastrojejunostomy. *World J Gastrointest Oncol* 2013; 5(12): 235-239 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v5/i12/235.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v5.i12.235>

### INTRODUCTION

Pancreatic cancer is the fourth leading cause of cancer in the United States<sup>[1]</sup>. Pancreaticoduodenectomy (Whipple procedure) remains a mainstay of treatment for resectable pancreatic head malignancy. Unfortunately, the incidence of recurrent disease remains high with frequent peritoneal metastases<sup>[1-3]</sup>. Complications from recurrent disease range from bowel obstruction, pancreatitis, sepsis, and cholangitis due to biliary obstruction<sup>[2-4]</sup>. A unique type



**Figure 1** Computed tomography horizontal image. A: Dilated jejunal Roux limb in the right upper quadrant. No evidence of biliary dilatation was evident of the computed tomography scan. Furthermore, the liver parenchyma showed evidence of hepatic steatosis. There is also the presence of a large incisional hernia in the anterior abdominal wall. Arrow a points to an intrahepatic metastasis from cancer. Arrow b points to the dilated Roux limb prior to the obstruction at the level of the mesentery of the colon. Arrow c points to the proximal portion of the dilated Roux limb that was close to the stomach used for the venting gastrojejunostomy; B: The close proximity of the stomach to the dilated Roux limb.



**Figure 2** Hepatobiliary iminodiacetic acid scan showing delayed uptake of the liver up to 60 min after administration of radiotracer. The cause of the delayed uptake likely reflects long term liver dysfunction and likely contributed to the inability of the biliary tree to dilate in a prompt fashion.

of obstruction occurs at the retrocolic jejunal limb of the afferent loop which can lead to stasis of the biliary, intestinal and pancreatic secretions<sup>[3-6]</sup>. We present here a case of afferent loop obstruction caused by recurrent peritoneal metastases from pancreatic cancer.

## CASE REPORT

The patient was a 70-year-old Caucasian female who first presented with painless jaundice leading to the diagnosis of pancreatic cancer. The patient underwent a pancreaticoduodenectomy at an outside institution for management of her condition followed by adjuvant chemotherapy and radiation. The patient required another operation a year later due to an episode of small bowel

obstruction, complicated with postoperative development of a small enterocutaneous fistula along with a large ventral hernia. She then presented to our hospital 2 years from her pancreaticoduodenectomy procedure with new onset abdominal pain along with jaundice. The patient had evidence of cholangitis with fevers, chills, leukocytosis of 21700/mm<sup>3</sup> and a bilirubin level of 16.0 mg/dL. Preoperative imaging, including a computed tomographic scan, showed severely dilated afferent limb in the right upper quadrant (Figure 1). A cholescintigraphy (DISIDA) scan showed poor visualization of the liver even 2.5 h after isotope injection suggesting intrinsic liver dysfunction (Figure 2). Her case was complicated by the lack of significant biliary dilatation precluding the use of interventional radiology techniques to percutaneously manage her cholangitis. Therefore, the patient underwent surgical exploration for management of her afferent loop obstruction. Intraoperative findings showed the obstruction to be secondary to a tight stricture at the jejunal limb as it traversed the mesentery of the colon. Biopsy of the area confirmed peritoneal recurrence from pancreatic cancer. The presence of the small enterocutaneous fistula at the level of mid-jejunum precluded the use of another small bowel loop for Roux-en-Y reconstruction. Because of the proximity of the stomach to the dilated afferent limb, we employed a primary anastomosis between the dilated afferent limb and the lesser curvature of the stomach as a venting gastrojejunostomy. The patient also had primary repair of her incisional hernia without the use of a mesh. She recovered uneventfully from surgery and was discharged from the hospital to rehabilitation center following 10 d. Her bilirubin level slowly improved and tapered off to a level of 4.1 mg/dL about 35 d postoperatively. The patient eventually succumbed to her metastatic pancreatic cancer 4 mo later.

## DISCUSSION

Complications from advanced pancreatic cancer remain common. After pancreaticoduodenectomy for resect-

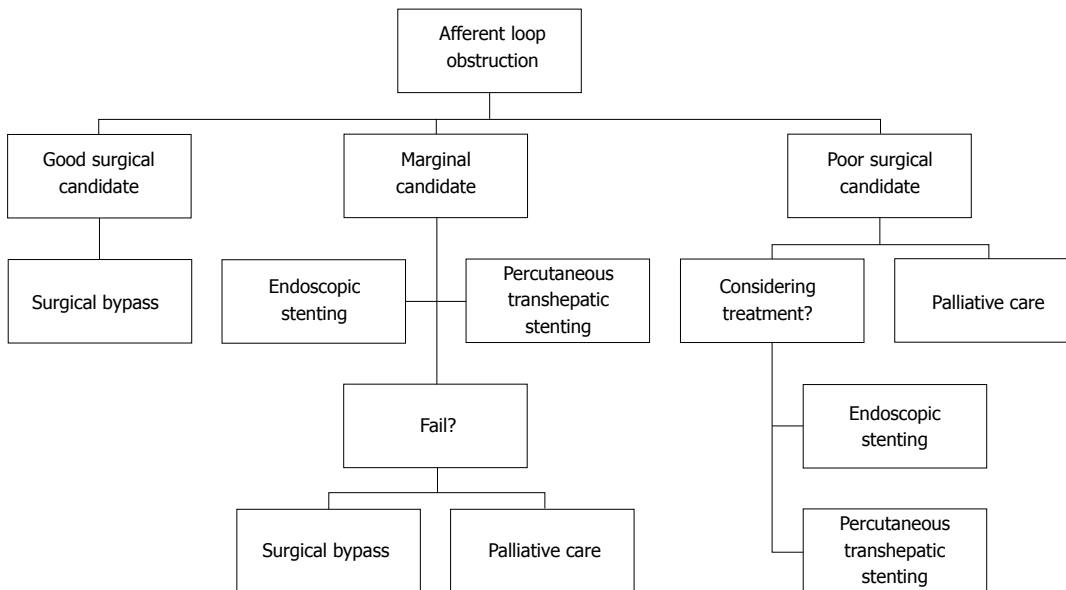


Figure 3 Management algorithm for afferent loop obstruction following pancreaticoduodenectomy.

able pancreatic cancer, patients present with substantial morbidity following chemotherapy and radiation along with the effects of recurrent pancreatic cancer<sup>[1,2]</sup>. Jacobs *et al*<sup>[3]</sup> noted that even long term survivors of pancreatic cancer develop multiple complications including infection, diabetes, depression, bowel obstruction and other malignancies<sup>[4]</sup>. Although not well described until more recently, the incidence of afferent loop syndrome developing in a cohort of patients after pancreaticoduodenectomy may be as high as 13%, as described by Pannala *et al*<sup>[5]</sup> from Virginia Mason Medical Center. The occurrence of this uncommon condition has been well described in the literature following gastrectomy with Bill-*roth* II reconstruction, with an incidence of 0.5%-2%<sup>[6]</sup>.

Clinical and radiographic presentations associated with this condition have been separated into acute and chronic afferent loop obstruction<sup>[6-11]</sup>. Acute and chronic afferent loop syndromes differ by the timing of presentation with the acute afferent loop syndrome more often in the postoperative period and the chronic type presenting years later<sup>[6-11]</sup>. The first case of afferent loop syndrome was described by McNealy *et al*<sup>[12]</sup> in 1942 as a cause of early postoperative duodenal stump leak. Roux *et al*<sup>[12]</sup> eventually coined the terms afferent loop syndrome in 1950.

Pancreaticoduodenectomy employs a roux limb of jejunum for reconstruction of the pancreatic and biliary drainage. Since the reconstruction following a pancreaticoduodenectomy employs an afferent limb for drainage of the bile, pancreatic and proximal intestinal secretions, it is functionally similar and also frequently termed afferent loop obstruction in the literature when obstructed<sup>[11]</sup>. However, the etiology, clinical presentation, and management options can vary from those following gastric surgeries.

The presentation of a roux limb obstruction is associated with high rates of morbidity and mortality<sup>[7,8]</sup>.

Patients are frequently malnourished due to long standing history of underlying malignancy. The most common presenting symptom following pancreaticoduodenectomy is cholangitis at 50% followed by nausea and abdominal pain<sup>[5]</sup>. The presentation is different from those following gastric surgeries, which is exemplified by bilious vomiting for chronic afferent loop obstruction. Complicating the presentation is that patients with pancreatic cancer frequently have a poor long term prognosis which frequently leads to a minimalist approach for management of these patients.

Prior to the introduction of endoscopic and percutaneous techniques, initial management options for afferent loop obstruction were first centered on surgical approaches. However, surgical approaches are limited due to the location of the Roux limb, which is through the mesentery of the colon. Resection of the obstructed afferent loop is a poor choice because of the necessity for reconstruction of both the pancreatic and biliary anastomoses. In addition, an *in situ* jejuno-jejunal bypass at the obstructed site is not feasible because of the vascular anatomy. Therefore, the most common approaches described in the literature involved the use of another jejunal Roux limb to decompress the dilated afferent loop<sup>[7,8,13]</sup>. Recently, advances in endoscopic and percutaneous techniques have been described in the literature to offer options for management of these patients<sup>[14-19]</sup>. More commonly, biliary decompression by interventional radiology manages to treat the acute cholangitis seen in these patients<sup>[15-17]</sup>. In addition, successful endoscopic stenting with dual stents of the stenotic segment avoids external drains and its associated long term problems<sup>[17,19]</sup>. Even transgastric drainage endoscopically has been reported recently<sup>[18]</sup>. Unfortunately, long term survival is usually unaffected regardless of the approach employed for salvage of this situation. As shown in Figure 3, we provided an algorithm for the management of

these patients based on their medical co-morbidities.

The case presented here is a long term complication following pancreaticoduodenectomy, frequently seen for recurrent disease<sup>[5]</sup>. In the case described here, our patient presented several unique challenges precluding the use of conservative and traditional surgical approaches. Our patient lacked biliary dilatation on the CT imaging which precluded the use of interventional radiology as a means for biliary decompression. Furthermore, the presence of enterocutaneous fistula, albeit small, made another Roux limb not feasible during surgery due to the lost of additional length of small bowel following resection and reconstruction. Our approach for performing a venting gastrojejunostomy was an idea developing following preoperative imaging review and surgical planning. The dilated jejunal Roux limb was in close proximity of the stomach making the anastomosis rather straight forward. Our goal of extending the life of our patient was also accomplished without significant post-operative morbidity. The patient lived approximately 4 mo postoperatively, which is consistent with the historically described outcome for this disease.

In summary, recurrent pancreatic cancer is associated with a poor prognosis. Afferent loop syndrome is a known complication from recurrent pancreatic cancer, with the classic radiographic presentations of a dilated small bowel loop and clinical evidence of cholangitis due to failure of biliary excretion. Early recognition of this serious condition, due to the ascending cholangitis, can lead to prompt management. Management options are evolving with improved endoscopic techniques and percutaneous options. However, surgery remains a viable option in appropriate candidates to establish a lasting decompression of the dilated Roux limb.

## COMMENTS

### Case characteristics

Afferent loop syndrome is a known complication from recurrent pancreatic cancer, with the classic radiographic presentations of a dilated small bowel loop and clinical evidence of cholangitis due to failure of biliary excretion.

### Clinical diagnosis

A unique type of biliary obstruction following pancreaticoduodenectomy occurs at the retrocolic jejunal limb of the afferent loop, leading to stasis of the biliary, intestinal and pancreatic secretions.

### Differential diagnosis

Complications from recurrent pancreatic cancer range from bowel obstruction, pancreatitis, sepsis, and cholangitis due to biliary obstruction.

### Laboratory diagnosis

Patients typically have evidence of cholangitis with fevers, chills, leukocytosis and hyperbilirubinemia.

### Imaging diagnosis

Radiographic imaging using a computed tomographic scan typically shows a severely dilated afferent limb in the right upper quadrant.

### Treatment

Management of afferent loop syndrome has traditionally relied on surgical approaches using another jejunal Roux limb. However, recent advances in endoscopic and percutaneous techniques have been described in the literature to offer other options for management of these patients.

### Experiences and lessons

Early recognition of afferent loop syndrome can lead to prompt management

using endoscopic, percutaneous, or surgical techniques to decompress the dilated Roux limb.

### Peer review

The manuscript is well-written, concise and easy to comprehend. Venting Gastrojejunostomy for recurrent pancreatic cancer has not been described before.

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