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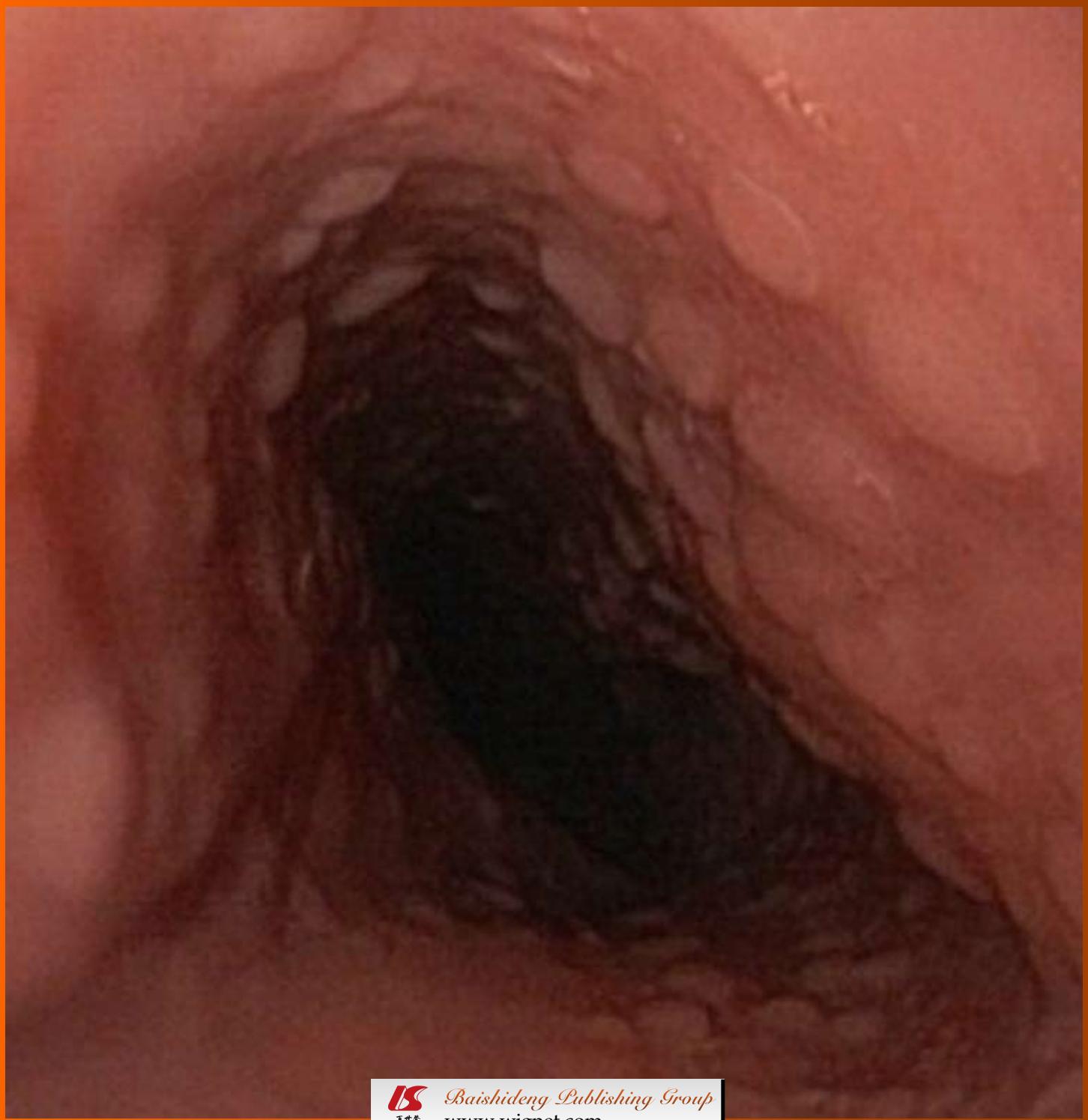

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World Journal of Gastrointestinal Oncology (*World J Gastrointest Oncol*, *WJGO*, online ISSN 1948-5204, DOI: 10.4251) is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJGO covers topics concerning carcinogenesis, tumorigenesis, metastasis, diagnosis, prevention, prognosis, clinical manifestations, nutritional support, molecular mechanisms, and therapy of benign and malignant tumors of the digestive tract. The current columns of *WJGO* include editorial, frontier, diagnostic advances, therapeutics advances, field of vision, mini-reviews, review, topic highlight, medical ethics, original articles, case report, clinical case conference (Clinicopathological conference), and autobiography. Priority publication will be given to articles concerning diagnosis and treatment of gastrointestinal oncology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

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EDITORIAL

Era of universal testing of microsatellite instability in colorectal cancer

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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 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.

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

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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[1]. 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[2-4]. Chromosomal instability (CIN) and microsatellite instability (MSI) constitute the predominant tumorigenic pathways in CRC (Figure 1)[5-8]. CIN is associated with high mutation rates in genes tightly linked to the development of CRC, such as *APC, KRAS, SMAD4, PI3KCA SOX9, ARID1A, FAM123B* and *TP53*, which lead to the development of CIN tumors[5]. 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[9].

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[10-12]. 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[13,14]. A most recent study showed that the prevalence of Lynch syndrome is 3.1% in all CRCs[15]. 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[9,16]. 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[17]. 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[18]. A proficient MMR system enhances replication accuracy 1000-10 000-fold[16]. 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 $(A)_n$ or $(CA)_n$ 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*)[16]. The major *E. coli* MMR proteins include MutS and MutL[19]. 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: MutSα (MSH2-MSH6 heterodimer) and MutSβ (MSH2-MSH3 heterodimer). Eukaryotic MutL activities also function as heterodimeric complexes with MLH1 serving as a common subunit including MutLα (MLH1-PMS2 heterdimer), MutLβ (MLH1-PMS1 heterodimer) and MutLγ (MLH1-MLH3 complex)[20]. Specifically, when a mismatch exists, MSH2 will form a MutSα or MutSβ complex. Both MutSα and MutSβ can then recruit either MutLα, MutLβ, or the MutLγ complex, which in turn will mediate the processes of mismatch recognition and enzymatic repair[9,16,19,20]. 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[11,21]. 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*[10,22,23]. 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[10]. 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[24]. 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[25]. Though frequency of *EpCAM* deletions have been reported in different populations[24-26], 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

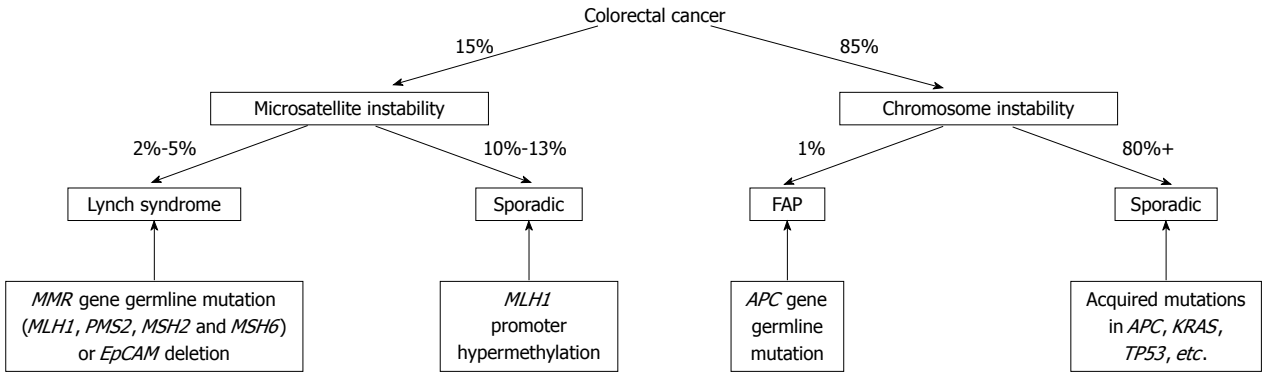

Figure 1 Molecular classification of colorectal cancer. EpCAM: Epithelial cell adhesion molecule; MMR: DNA mismatch repair; FAP: Familial adenomatous polyposis; APC: Adenomatous polyposis coli.

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*[27] 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[22,23].

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[28], and also EpCAM negative immunostaining can be detected even at a precancerous stage[29]. 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)[30]. 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[31]. 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) addended 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[31]. 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[32-34].

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)[35]. 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*[36] 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, sensitive (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[37]. 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[16]. 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[16,22]. 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[38]. In addition, stage Ⅱ MSI-H CRCs achieved similar progression free survival and overall survival with or without 5-flurouracil (5-FU)-based neoadjuvant chemotherapy[39]. 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[40]. 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 extracolonic carcinomas[41].

Historically, diagnosis of Lynch syndrome relied on clinical characteristics of personal and family history of cancer. The Amsterdam criteria[42], later revised to Amsterdam Ⅱ criteria[43] 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[44], and then the revised Bethesda guidelines[31], 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[16,23]. 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[45]. Julié *et al*[46] 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[46]. Morrison *et al*[47] 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[47]. Most recently, Pérez-Carbonell *et al*[48] 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[48]. 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[49-52]. 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[22].

Recent data have shown that testing for MMR expression can be performed on the diagnostic CRC biopsy samples prior to definitive surgery[53], with results comparable to those obtained on the surgical resection specimens[54,55]. 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 immunoexpression 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[56].

ALGORISM 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[10], and both PCR-based and IHC MSI detection are highly sensitive methods for the identification of individuals with defective MMR[21], 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[57]. 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[58]. 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[59]. 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

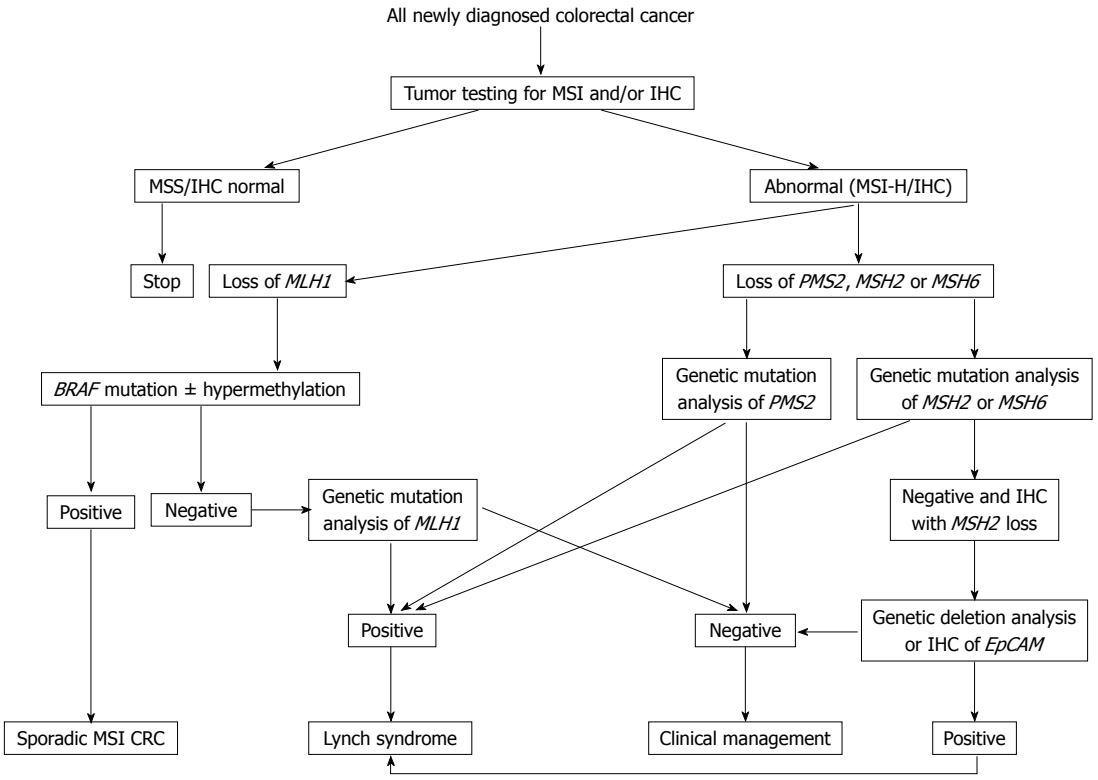

Figure 2 Testing algorithm for Lynch syndrome and sporadic microsatellite instability colorectal cancer. MSI: Microsatellite instability; IHC: Immunohistochemical staining; CRC: Colorectal cancer; *EpCAM*: Epithelial cell adhesion molecule.

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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REFERENCES

1 **Siegel R**, Naishadham D, Jemal A. Cancer statistics, 2012. *CA Cancer J Clin* 2012; **62**: 10-29 [PMID: 22237781 DOI: 10.3322/caac.20138]
2 **Thibodeau SN**, Bren G, Schaid D. Microsatellite instability in cancer of the proximal colon. *Science* 1993; **260**: 816-819 [PMID: 8484122]
3 **Ionov Y**, Peinado MA, Malkhosyan S, Shibata D, Perucho M. Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. *Nature* 1993; **363**: 558-561 [PMID: 8505985 DOI: 10.1038/363558a0]
4 **Fishel R**, Lescoe MK, Rao MR, Copeland NG, Jenkins NA, Garber J, Kane M, Kolodner R. The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. *Cell* 1993; **75**: 1027-1038 [PMID: 8252616]
5 **Cancer Genome Atlas Network.** Comprehensive molecular characterization of human colon and rectal cancer. *Nature* 2012; **487**: 330-337 [PMID: 22810696 DOI: 10.1038/nature11252]
6 **Cunningham D**, Atkin W, Lenz HJ, Lynch HT, Minsky B, Nordlinger B, Starling N. Colorectal cancer. *Lancet* 2010; **375**: 1030-1047 [PMID: 20304247 DOI: 10.1016/S0140-6736(10)60353-4]
7 **Ogino S**, Chan AT, Fuchs CS, Giovannucci E. Molecular pathological epidemiology of colorectal neoplasia: an emerging transdisciplinary and interdisciplinary field. *Gut* 2011; **60**: 397-411 [PMID: 21036793 DOI: 10.1136/gut.2010.217182]
8 **Shi C**, Washington K. Molecular testing in colorectal cancer: diagnosis of Lynch syndrome and personalized cancer medicine. *Am J Clin Pathol* 2012; **137**: 847-859 [PMID: 22586043 DOI: 10.1309/AJCPI83DINULUJNI]
9 **Vilar E**, Gruber SB. Microsatellite instability in colorectal cancer-the stable evidence. *Nat Rev Clin Oncol* 2010; **7**: 153-162 [PMID: 20142816 DOI: 10.1038/nrclinonc.2009.237]
10 **Weissman SM**, Burt R, Church J, Erdman S, Hampel H, Holter S, Jasperson K, Kalady MF, Haidle JL, Lynch HT, Palaniappan S, Wise PE, Senter L. Identification of individuals at risk for Lynch syndrome using targeted evaluations and genetic testing: National Society of Genetic Counselors and the Collaborative Group of the Americas on Inherited Colorectal Cancer joint practice guideline. *J Genet Couns* 2012; **21**: 484-493 [PMID: 22167527 DOI: 10.1007/s10897-011-9465-7]
11 **Goodenberger M**, Lindor NM. Lynch syndrome and MYH-associated polyposis: review and testing strategy. *J Clin Gastroenterol* 2011; **45**: 488-500 [PMID: 21325953 DOI: 10.1097/MCG.0b013e318206489c]
12 **Win AK**, Lindor NM, Young JP, Macrae FA, Young GP, Williamson E, Parry S, Goldblatt J, Lipton L, Winship I, Leggett B, Tucker KM, Giles GG, Buchanan DD, Clendenning M, Rosty C, Arnold J, Levine AJ, Haile RW, Gallinger S, Le Marchand L, Newcomb PA, Hopper JL, Jenkins MA. Risks of primary extracolonic cancers following colorectal cancer in lynch syndrome. *J Natl Cancer Inst* 2012; **104**: 1363-1372 [PMID: 22933731 DOI: 10.1093/jnci/djs351]
13 **Ladabaum U**, Ford JM. Lynch syndrome in patients with

colorectal cancer: finding the needle in the haystack. *JAMA* 2012; **308**: 1581-1583 [PMID: 23073955 DOI: 10.1001/jama.2012.14171]

14 **Piñol V**, Castells A, Andreu M, Castellví-Bel S, Alenda C, Llor X, Xicola RM, Rodríguez-Moranta F, Payá A, Jover R, Bessa X. Accuracy of revised Bethesda guidelines, microsatellite instability, and immunohistochemistry for the identification of patients with hereditary nonpolyposis colorectal cancer. *JAMA* 2005; **293**: 1986-1994 [PMID: 15855432 DOI: 10.1001/jama.293.16.1986]

15 **Moreira L**, Balaguer F, Lindor N, de la Chapelle A, Hampel H, Aaltonen LA, Hopper JL, Le Marchand L, Gallinger S, Newcomb PA, Haile R, Thibodeau SN, Gunawardena S, Jenkins MA, Buchanan DD, Potter JD, Baron JA, Ahnen DJ, Moreno V, Andreu M, Ponz de Leon M, Rustgi AK, Castells A. Identification of Lynch syndrome among patients with colorectal cancer. *JAMA* 2012; **308**: 1555-1565 [PMID: 23073952 DOI: 10.1001/jama.2012.13088]

16 **Pino MS**, Chung DC. Microsatellite instability in the management of colorectal cancer. *Expert Rev Gastroenterol Hepatol* 2011; **5**: 385-399 [PMID: 21651356 DOI: 10.1586/egh.11.25]

17 **Loeb LA**. Human cancers express mutator phenotypes: origin, consequences and targeting. *Nat Rev Cancer* 2011; **11**: 450-457 [PMID: 21593786 DOI: 10.1038/nrc3063]

18 **Hsieh P**, Yamane K. DNA mismatch repair: molecular mechanism, cancer, and ageing. *Mech Ageing Dev* 2008; **129**: 391-407 [PMID: 18406444 DOI: 10.1016/j.mad.2008.02.012]

19 **Fukui K**. DNA mismatch repair in eukaryotes and bacteria. *J Nucleic Acids* 2010 Jul 2 [PMID: 20725617 DOI: 10.4061/2010/260512]

20 **Modrich P**. Mechanisms in eukaryotic mismatch repair. *J Biol Chem* 2006; **281**: 30305-30309 [PMID: 16905530 DOI: 10.1074/jbc.R600022200]

21 **Palomaki GE**, McClain MR, Melillo S, Hampel HL, Thibodeau SN. EGAPP supplementary evidence review: DNA testing strategies aimed at reducing morbidity and mortality from Lynch syndrome. *Genet Med* 2009; **11**: 42-65 [PMID: 19125127 DOI: 10.1097/GIM.0b013e31818fa2db]

22 **Klarskov L**, Holck S, Bernstein I, Okkels H, Rambech E, Baldetorp B, Nilbert M. Challenges in the identification of MSH6-associated colorectal cancer: rectal location, less typical histology, and a subset with retained mismatch repair function. *Am J Surg Pathol* 2011; **35**: 1391-1399 [PMID: 21836479 DOI: 10.1097/PAS.0b013e318225c3f0]

23 **Pino MS**, Chung DC. Application of molecular diagnostics for the detection of Lynch syndrome. *Expert Rev Mol Diagn* 2010; **10**: 651-665 [PMID: 20629513 DOI: 10.1586/erm.10.45]

24 **Ligtenberg MJ**, Kuiper RP, Chan TL, Goossens M, Hebeda KM, Voorendt M, Lee TY, Bodmer D, Hoenselaar E, Hendriks-Cornelissen SJ, Tsui WY, Kong CK, Brunner HG, van Kessel AG, Yuen ST, van Krieken JH, Leung SY, Hoogerbrugge N. Heritable somatic methylation and inactivation of MSH2 in families with Lynch syndrome due to deletion of the 3′ exons of TACSTD1. *Nat Genet* 2009; **41**: 112-117 [PMID: 19098912 DOI: 10.1038/ng.283]

25 **Kovacs ME**, Papp J, Szentirmay Z, Otto S, Olah E. Deletions removing the last exon of TACSTD1 constitute a distinct class of mutations predisposing to Lynch syndrome. *Hum Mutat* 2009; **30**: 197-203 [PMID: 19177550 DOI: 10.1002/humu.20942]

26 **Pérez-Cabornero L**, Infante Sanz M, Velasco Sampedro E, Lastra Aras E, Acedo Becares A, Miner Pino C, Durán Domínguez M. Frequency of rearrangements in Lynch syndrome cases associated with MSH2: characterization of a new deletion involving both EpCAM and the 5′ part of MSH2. *Cancer Prev Res* (Phila) 2011; **4**: 1556-1562 [PMID: 21791569 DOI: 10.1158/1940-6207.CAPR-11-0080]

27 **Parsons MT**, Buchanan DD, Thompson B, Young JP, Spurdle AB. Correlation of tumour BRAF mutations and MLH1 methylation with germline mismatch repair (MMR) gene mutation status: a literature review assessing utility of tumour features for MMR variant classification. *J Med Genet* 2012; **49**: 151-157 [PMID: 22368298 DOI: 10.1136/jmedgenet-2011-100714]

28 **Musulen E**, Blanco I, Carrato C, Fernandez-Figueras MT, Pineda M, Capella G, Ariza A. Usefulness of epithelial cell adhesion molecule expression in the algorithmic approach to Lynch syndrome identification. *Hum Pathol* 2013; **44**: 412-416 [PMID: 23026194 DOI: 10.1016/j.humpath.2012.06.006]

29 **Huth C**, Kloor M, Voigt AY, Bozukova G, Evers C, Gaspar H, Tariverdian M, Schirmacher P, von Knebel Doeberitz M, Bläker H. The molecular basis of EpCAM expression loss in Lynch syndrome-associated tumors. *Mod Pathol* 2012; **25**: 911-916 [PMID: 22388758 DOI: 10.1038/modpathol.2012.30]

30 **Boland CR**, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN, Srivastava S. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res* 1998; **58**: 5248-5257 [PMID: 9823339]

31 **Umar A**, Boland CR, Terdiman JP, Syngal S, de la Chapelle A, Rüschoff J, Fishel R, Lindor NM, Burgart LJ, Hamelin R, Hamilton SR, Hiatt RA, Jass J, Lindblom A, Lynch HT, Peltomaki P, Ramsey SD, Rodriguez-Bigas MA, Vasen HF, Hawk ET, Barrett JC, Freedman AN, Srivastava S. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J Natl Cancer Inst* 2004; **96**: 261-268 [PMID: 14970275]

32 **Cicek MS**, Lindor NM, Gallinger S, Bapat B, Hopper JL, Jenkins MA, Young J, Buchanan D, Walsh MD, Le Marchand L, Burnett T, Newcomb PA, Grady WM, Haile RW, Casey G, Plummer SJ, Krumroy LA, Baron JA, Thibodeau SN. Quality assessment and correlation of microsatellite instability and immunohistochemical markers among population- and clinic-based colorectal tumors results from the Colon Cancer Family Registry. *J Mol Diagn* 2011; **13**: 271-281 [PMID: 21497289 DOI: 10.1016/j.jmoldx.2010.12.004]

33 **Bacher JW**, Flanagan LA, Smalley RL, Nassif NA, Burgart LJ, Halberg RB, Megid WM, Thibodeau SN. Development of a fluorescent multiplex assay for detection of MSI-High tumors. *Dis Markers* 2004; **20**: 237-250 [PMID: 15528789]

34 **Agostini M**, Enzo MV, Morandi L, Bedin C, Pizzini S, Mason S, Bertorelle R, Urso E, Mescoli C, Lise M, Pucciarelli S, Nitti D. A ten markers panel provides a more accurate and complete microsatellite instability analysis in mismatch repair-deficient colorectal tumors. *Cancer Biomark* 2010; **6**: 49-61 [PMID: 20164541 DOI: 10.3233/CBM-2009-0118]

35 **Bartley AN**, Luthra R, Saraiya DS, Urbauer DL, Broaddus RR. Identification of cancer patients with Lynch syndrome: clinically significant discordances and problems in tissue-based mismatch repair testing. *Cancer Prev Res* (Phila) 2012; **5**: 320-327 [PMID: 22086678 DOI: 10.1158/1940-6207.CAPR-11-0288]

36 **Rigau V**, Sebbagh N, Olschwang S, Paraf F, Mourra N, Parc Y, Flejou JF. Microsatellite instability in colorectal carcinoma. The comparison of immunohistochemistry and molecular biology suggests a role for hMSH6 [correction of hMLH6] immunostaining. *Arch Pathol Lab Med* 2003; **127**: 694-700 [PMID: 12741892 DOI: 10.1043/1543-2165(2003)127<694:]

37 **Lindor NM**, Burgart LJ, Leontovich O, Goldberg RM, Cunningham JM, Sargent DJ, Walsh-Vockley C, Petersen GM, Walsh MD, Leggett BA, Young JP, Barker MA, Jass JR, Hopper J, Gallinger S, Bapat B, Redston M, Thibodeau SN. Immunohistochemistry versus microsatellite instability testing in phenotyping colorectal tumors. *J Clin Oncol* 2002; **20**: 1043-1048 [PMID: 11844828]

38 **Popat S**, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. *J Clin Oncol* 2005; **23**: 609-618 [PMID: 15659508 DOI: 10.1200/JCO.2005.01.086]

39 **Des Guetz G**, Schischmanoff O, Nicolas P, Perret GY, Morere JF, Uzzan B. Does microsatellite instability predict the efficacy of adjuvant chemotherapy in colorectal cancer? A systematic review with meta-analysis. *Eur J Cancer* 2009; **45**: 1890-1896 [PMID: 19427194 DOI: 10.1016/j.ejca.2009.04.018]

40 **Järvinen HJ**, Renkonen-Sinisalo L, Aktán-Collán K, Peltomäki P, Aaltonen LA, Mecklin JP. Ten years after mutation testing for Lynch syndrome: cancer incidence and outcome in mutation-positive and mutation-negative family members. *J Clin Oncol* 2009; **27**: 4793-4797 [PMID: 19720893 DOI: 10.1200/JCO.2009.23.7784]

41 **Burn J**, Gerdes AM, Macrae F, Mecklin JP, Moeslein G, Olschwang S, Eccles D, Evans DG, Maher ER, Bertario L, Bisgaard ML, Dunlop MG, Ho JW, Hodgson SV, Lindblom A, Lubinski J, Morrison PJ, Murday V, Ramesar R, Side L, Scott RJ, Thomas HJ, Vasen HF, Barker G, Crawford G, Elliott F, Movahedi M, Pylvanainen K, Wijnen JT, Fodde R, Lynch HT, Mathers JC, Bishop DT. Long-term effect of aspirin on cancer risk in carriers of hereditary colorectal cancer: an analysis from the CAPP2 randomised controlled trial. *Lancet* 2011; **378**: 2081-2087 [PMID: 22036019 DOI: 10.1016/S0140-6736(11)61049-0]

42 **Vasen HF**, Mecklin JP, Khan PM, Lynch HT. The International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer (ICG-HNPCC). *Dis Colon Rectum* 1991; **34**: 424-425 [PMID: 2022152]

43 **Vasen HF**, Watson P, Mecklin JP, Lynch HT. New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative group on HNPCC. *Gastroenterology* 1999; **116**: 1453-1456 [PMID: 10348829]

44 **Rodriguez-Bigas MA**, Boland CR, Hamilton SR, Henson DE, Jass JR, Khan PM, Lynch H, Perucho M, Smyrk T, Sobin L, Srivastava S. A National Cancer Institute Workshop on Hereditary Nonpolyposis Colorectal Cancer Syndrome: meeting highlights and Bethesda guidelines. *J Natl Cancer Inst* 1997; **89**: 1758-1762 [PMID: 9392616]

45 **Byfield SA**, Syngal S. Clinical guidelines versus universal molecular testing: are we ready to choose an optimal strategy for Lynch syndrome identification? *Am J Gastroenterol* 2008; **103**: 2837-2840 [PMID: 19032469 DOI: 10.1111/j.1572-0241.2008.02091.x]

46 **Julié C**, Trésallet C, Brouquet A, Vallot C, Zimmermann U, Mitry E, Radvanyi F, Rouleau E, Lidereau R, Coulet F, Olschwang S, Frébourg T, Rougier P, Nordlinger B, Laurent-Puig P, Penna C, Boileau C, Franc B, Muti C, Hofmann-Radvanyi H. Identification in daily practice of patients with Lynch syndrome (hereditary nonpolyposis colorectal cancer): revised Bethesda guidelines-based approach versus molecular screening. *Am J Gastroenterol* 2008; **103**: 2825-235; quiz 2836 [PMID: 18759827 DOI: 10.1111/j.1572-0241.2008.02084.x]

47 **Morrison J**, Bronner M, Leach BH, Downs-Kelly E, Goldblum JR, Liu X. Lynch syndrome screening in newly diagnosed colorectal cancer in general pathology practice: from the revised Bethesda guidelines to a universal approach. *Scand J Gastroenterol* 2011; **46**: 1340-1348 [PMID: 21879804 DOI: 10.3109/00365521.2011.610003]

48 **Pérez-Carbonell L**, Ruiz-Ponte C, Guarinos C, Alenda C, Payá A, Brea A, Egoavil CM, Castillejo A, Barberá VM, Bessa X, Xicola RM, Rodríguez-Soler M, Sánchez-Fortún C, Acame N, Castellví-Bel S, Piñol V, Balaguer F, Bujanda L, De-Castro ML, Llor X, Andreu M, Carracedo A, Soto JL, Castells A, Jover R. Comparison between universal molecular screening for Lynch syndrome and revised Bethesda guidelines in a large population-based cohort of patients with colorectal cancer. *Gut* 2012; **61**: 865-872 [PMID: 21868491 DOI: 10.1136/gutjnl-2011-300041]

49 **Greenson JK**, Huang SC, Herron C, Moreno V, Bonner JD, Tomsho LP, Ben-Izhak O, Cohen HI, Trougouboff P, Bejhar J, Sova Y, Pinchev M, Rennert G, Gruber SB. Pathologic predictors of microsatellite instability in colorectal cancer. *Am J Surg Pathol* 2009; **33**: 126-133 [PMID: 18830122 DOI: 10.1097/PAS.0b013e31817ec2b1]

50 **Halvarsson B**, Anderson H, Domanska K, Lindmark G, Nilbert M. Clinicopathologic factors identify sporadic mismatch repair-defective colon cancers. *Am J Clin Pathol* 2008; **129**: 238-244 [PMID: 18208804 DOI: 10.1309/0PP5GDRTXUDVKAWJ]

51 **Lugli A**, Vlajnic T, Giger O, Karamitopoulou E, Patsouris ES, Peros G, Terracciano LM, Zlobec I. Intratumoral budding as a potential parameter of tumor progression in mismatch repair-proficient and mismatch repair-deficient colorectal cancer patients. *Hum Pathol* 2011; **42**: 1833-1840 [PMID: 21664647 DOI: 10.1016/j.humpath.2011.02.010]

52 **Zlobec I**, Bihl MP, Foerster A, Rufle A, Lugli A. The impact of CpG island methylator phenotype and microsatellite instability on tumour budding in colorectal cancer. *Histopathology* 2012; **61**: 777-787 [PMID: 22803799 DOI: 10.1111/j.1365-2559.2012.04273.x]

53 **Warrier SK**, Trainer AH, Lynch AC, Mitchell C, Hiscock R, Sawyer S, Boussioutas A, Heriot AG. Preoperative diagnosis of Lynch syndrome with DNA mismatch repair immunohistochemistry on a diagnostic biopsy. *Dis Colon Rectum* 2011; **54**: 1480-1487 [PMID: 22067175 DOI: 10.1097/DCR.0b013e318231db1f]

54 **Kumarasinghe AP**, de Boer B, Bateman AC, Kumarasinghe MP. DNA mismatch repair enzyme immunohistochemistry in colorectal cancer: a comparison of biopsy and resection material. *Pathology* 2010; **42**: 414-420 [PMID: 20632816 DOI: 10.3109/00313025.2010.493862]

55 **Shia J**, Stadler Z, Weiser MR, Rentz M, Gonen M, Tang LH, Vakiani E, Katabi N, Xiong X, Markowitz AJ, Shike M, Guillem J, Klimstra DS. Immunohistochemical staining for DNA mismatch repair proteins in intestinal tract carcinoma: how reliable are biopsy samples? *Am J Surg Pathol* 2011; **35**: 447-454 [PMID: 21297438 DOI: 10.1097/PAS.0b013e31820a091d]

56 **Bao F**, Panarelli NC, Rennert H, Sherr DL, Yantiss RK. Neoadjuvant therapy induces loss of MSH6 expression in colorectal carcinoma. *Am J Surg Pathol* 2010; **34**: 1798-1804 [PMID: 21107085 DOI: 10.1097/PAS.0b013e3181f906cc]

57 **Kalloger SE**, Allo G, Mulligan AM, Pollett A, Aronson M, Gallinger S, Torlakovic EE, Clarke BA. Use of mismatch repair immunohistochemistry and microsatellite instability testing: exploring Canadian practices. *Am J Surg Pathol* 2012; **36**: 560-569 [PMID: 22301492 DOI: 10.1097/PAS.0b013e31823f3b28]

58 **Sanchez JA**, Vogel JD, Kalady MF, Bronner MP, Skacel M, Church JM. Identifying Lynch syndrome: we are all responsible. *Dis Colon Rectum* 2008; **51**: 1750-1756 [PMID: 18682882 DOI: 10.1007/s10350-008-9414-1]

59 **Beamer LC**, Grant ML, Espenschied CR, Blazer KR, Hampel HL, Weitzel JN, MacDonald DJ. Reflex immunohistochemistry and microsatellite instability testing of colorectal tumors for Lynch syndrome among US cancer programs and follow-up of abnormal results. *J Clin Oncol* 2012; **30**: 1058-1063 [PMID: 22355048 DOI: 10.1200/JCO.2011.38.4719]

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MINIREVIEW

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.

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

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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 and 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%[1]. 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[2].

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[3]. 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[4].

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[5]. 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[6]. 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[7]. 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[8].

The addition of cetuximab to adjuvant gemcitabine was investigated in an open label, multi-center, phase Ⅱ trial reported by Fensterer *et al*[9]. 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%[10].

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%[11]. 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 a open-label phase Ⅱ trial, RR of rubitecan monotherapy was only 7%, and MST was 3 mo[12]. 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[13].

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[14]. 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[15].

Kwon *et al*[16] 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 finding 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[17].

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[18]. 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[19]. 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[20]. 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[21]. 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%)[22]. 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[23].

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[24]. 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[25]. 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[26].

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[27]. 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 neoadjuvant 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 trails 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 metastices 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 sides 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[28]. 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[29].

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[30]. 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[31]. 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[32].

Problems

Radiotherapy is a treatment option for pancreatic cancer patients who don't have heart, liver, or kidney dysfunctions 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 are 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[32] phosphorus (P) into solid tumors helped to kill tumor cells and reduced the blood flow to the tumor[33].

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[34].

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[35].

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[35].

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 guaidance 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[36].

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 ^{51}Cr labeled pancreatic cells. ^{131}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 antinuclear 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-pShutle, 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[37]. The *IL-2* gene plus interferon-γ can increase the total amount of $CD4^+$, $CD8^+$ 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[38]. MALP-2 is a synthetic lipopeptide that can inhibits 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[39].

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 $CD4^+CD25^+$ 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[40]. GEM can induce the differentiation of $CD14^+$ and $CD11c^+$ DC and improve the therapeutic effect of GEM in combination with other therapies[41]; 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-l molecules and can be recognized by the immune system. Thus, it can be used as tumor cell vaccine[42]; (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) Idiotype 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.

REFERENCES

1 **Saif MW**. Controversies in the adjuvant treatment of pancreatic adenocarcinoma. *JOP* 2007; **8**: 545-552 [PMID: 17873458]

2 **Pliarchopoulou K**, Pectasides D. Pancreatic cancer: current and future treatment strategies. *Cancer Treat Rev* 2009; **35**: 431-436 [PMID: 19328630 DOI: 10.1016/j.ctrv.2009.02.005]

3 **Heinemann V**, Quietzsch D, Gieseler F, Gonnermann M, Schönekäs H, Rost A, Neuhaus H, Haag C, Clemens M, Heinrich B, Vehling-Kaiser U, Fuchs M, Fleckenstein D, Gesierich W, Uthgenannt D, Einsele H, Holstege A, Hinke A, Schalhorn A, Wilkowski R. Randomized phase III trial of gemcitabine plus cisplatin compared with gemcitabine alone in advanced pancreatic cancer. *J Clin Oncol* 2006; **24**: 3946-3952 [PMID: 16921047 DOI: 10.1200/JCO.2005.05.1490]

4 **Stathopoulos GC**, Syrigos K, Polyzos A, Fountzilas G, Rigatos SK, Ziras N, Potamiannou A, Tsiakopoulos I, Androulakis N, Aravantinos G, Athanasiadis A, Papakotoulas P, Georgoulias V. Front-line treatment of inoperable or metastatic pancreatic cancer with gemcitabine and capecitabine: an intergroup, multicenter, phase II study. *Ann Oncol* 2004; **15**: 224-229 [PMID: 14760113 DOI: 10.1093/annonc/mdh065]

5 **Boeck S**, Wilkowski R, Bruns CJ, Issels RD, Schulz C, Moosmann N, Laessig D, Haas M, Golf A, Heinemann V. Oral capecitabine in gemcitabine-pretreated patients with advanced pancreatic cancer. *Oncology* 2007; **73**: 221-227 [PMID: 18424886 DOI: 10.1159/000127413]

6 **Xiong HQ**, Varadhachary GR, Blais JC, Hess KR, Abbruzzese JL, Wolff RA. Phase 2 trial of oxaliplatin plus capecitabine (XELOX) as second-line therapy for patients with advanced pancreatic cancer. *Cancer* 2008; **113**: 2046-2052 [PMID: 18756532]

7 **Blaya M,** Lopes GL, Roman E, Ahn E, Macintyre J, Quesada J, Levi J, Walker G, Green M, Rocha Lima CM. Phase II trial of capecitabine and docetaxel as second line therapy for locally advanced and metastatic pancreatic cancer. *J Clin Oncol* 2007; **25** (18S): abstract 15029

8 **Gelibter A,** Milella M, Malaguti P, De Marco S, Ruggeri E, Carlini P, Pino M, Nuzzo C, Sternberg CN, Cognetti F. Pilot study of capecitabine combined with celecoxib (CapCel) as second-line treatment for advanced pancreatic (P) and biliary tree (BT) cancer. *J Clin Oncol* 2006; **24**(18S): abstract 14055

9 **Fensterer H,** Schade-Brittinger C, Mueller H, Schupfner B, Fass J, Lindig U, Settmacher U, Ebert MP, Kornmann M, Hofheinz R, Endlicher E, Brendel C, Barth PJ, Bartsch DK, Gress TM, Arbeitsgemeinschaft Internistische Onkologie (AIO); Department of Gastroenterology, Philipps-University Marburg, Marburg, Germany; Koordinierungszentrum für Klinische Studien, Philipps-University Marburg, Marburg, Germany; Institute of Medical Informatics, Biometry and Epidemiology, Muenchen, Germany; Klinik für Allgemein- und Viszeralchirurgie, Klinikum Kassel, Kassel, Germany; Abt. Hämatologie und Int. Onkologie, University Jena, Jena, Germany; Department of Surgery, University of Jena, Jena, Germany; Medical Department II, University Hospital Mannheim, Mannheim, Germany; Department of General, Visceral, and Transplantation Surgery, University of Ulm, Ulm, Germany; Day Treatment Centre at the Interdisciplinary Tumour Centre Mannheim, Universitätsmedizin Mannheim, Mannheim, Germany; University Clinic Regensburg, Regensburg, Germany; Department of Hematology, Oncology and Immunology, Philipps-University Marburg, Marburg, Germany; Institute of Pathology, University of Münster, Münster, Germany; Department of Surgery, University Marburg, Marburg, Germany. Multicenter phase II trial to investigate safety and efficacy of an adjuvant therapy with gemcitabine and cetuximab in patients with R0 or R1 resected pancreatic cancer. *J Clin Onclol* 2011; **29** (Suppl): abstract 4039

10 **Ueno H**, Okusaka T, Ikeda M, Ishiguro Y, Morizane C, Matsubara J, Furuse J, Ishii H, Nagase M, Nakachi K. A phase I study of combination chemotherapy with gemcitabine and oral S-1 for advanced pancreatic cancer. *Oncology* 2005; **69**: 421-427 [PMID: 16319514 DOI: 10.1159/000089997]

11 **Yi SY**, Park YS, Kim HS, Jun HJ, Kim KH, Chang MH, Park MJ, Uhm JE, Lee J, Park SH, Park JO, Lee JK, Lee KT, Lim HY, Kang WK. Irinotecan monotherapy as second-line treatment in advanced pancreatic cancer. *Cancer Chemother Pharmacol* 2009; **63**: 1141-1145 [PMID: 18839175 DOI: 10.1007/s00280-008-0839-y]

12 **Burris HA**, Rivkin S, Reynolds R, Harris J, Wax A, Gerstein H, Mettinger KL, Staddon A. Phase II trial of oral rubitecan in previously treated pancreatic cancer patients. *Oncologist* 2005; **10**: 183-190 [PMID: 15793221 DOI: 10.1634/theoncologist.10-3-183]

13 **Boeck S**, Weigang-Köhler K, Fuchs M, Kettner E, Quietzsch D, Trojan J, Stötzer O, Zeuzem S, Lordick F, Köhne CH, Kröning H, Steinmetz T, Depenbrock H, Heinemann V. Second-line chemotherapy with pemetrexed after gemcitabine failure in patients with advanced pancreatic cancer: a multicenter phase II trial. *Ann Oncol* 2007; **18**: 745-751 [PMID: 17229775 DOI: 10.1093/annonc/mdl463]

14 **Herman JM**, Swartz MJ, Hsu CC, Winter J, Pawlik TM, Sugar E, Robinson R, Laheru DA, Jaffee E, Hruban RH, Campbell KA, Wolfgang CL, Asrari F, Donehower R, Hidalgo M, Diaz LA, Yeo C, Cameron JL, Schulick RD, Abrams R. Analysis of fluorouracil-based adjuvant chemotherapy and radiation after pancreaticoduodenectomy for ductal adenocarcinoma of the pancreas: results of a large, prospectively collected database at the Johns Hopkins Hospital. *J Clin Oncol* 2008; **26**: 3503-3510 [PMID: 18640931 DOI: 10.1200/JCO.2007.15.8469]

15 **Neoptolemos JP**, Stocken DD, Bassi C, Ghaneh P, Cunningham D, Goldstein D, Padbury R, Moore MJ, Gallinger S, Mariette C, Wente MN, Izbicki JR, Friess H, Lerch MM, Dervenis C, Oláh A, Butturini G, Doi R, Lind PA, Smith D, Valle JW, Palmer DH, Buckels JA, Thompson J, McKay CJ, Rawcliffe CL, Büchler MW. Adjuvant chemotherapy with fluorouracil plus folinic acid vs gemcitabine following pancreatic cancer resection: a randomized controlled trial. *JAMA* 2010; **304**: 1073-1081 [PMID: 20823433 DOI: 10.1001/jama.2010.1275]

16 **Kwon JH,** Chie EK, Kim JH, Im S, Oh D, Kim Y, Han S, Lee K, Jang J, Kim T, Kim JS, Han HS, Kim S, Yoon YB, Bang Y, Ha SW; Department of Internal Medicine, Seoul National University College of Medicine, Seoul National University Hospital, Seoul, South Korea; Department of Radiation Oncology, Seoul National University College of Medicine, Seoul National University Hospital, Seoul, South Korea; Department of Internal Medicine, Seoul National University College of Medicine, Seoul National Unversity Bundang Hospital, Gyeonggi-do, South Korea; Seoul National University Hospital, Seoul, South Korea; Department of Internal Medicine, Seoul National University College of Medicine, Seoul National University Bundang Hospital, Seongnam, South Korea; Department of Internal Medicine, Seoul National University Bundang Hospital, Seongnam-si, Gyeonggi-do, Republic of Korea, Gyeonggi-do, South Korea; Department of Surgery, Seoul National University College of Medicine, Seoul National University Hospital, Seoul, South Korea; Department of Radiation Oncology, Seoul National University College of Medicine, Seoul National University Bundang Hospital, Seongnam, South Korea; Department of Surgery, Seoul National University College of Medicine, Seoul National University Bundang Hospital, Bundang, South Korea; Seoul National University College of Medicine, Seoul, South Korea. Phase II trial of postoperative adjuvant gemcitabine and cisplatin chemotherapy followed by chemoradiation with gemcitabine in patients with resected pancreatic cancer. *J Clin Onclol* 2011; **29** (Suppl): abstract 4094

17 **Hartwig W**, Schneider L, Diener MK, Bergmann F, Büchler MW, Werner J. Preoperative tissue diagnosis for tumours of the pancreas. *Br J Surg* 2009; **96**: 5-20 [PMID: 19016272 DOI: 10.1002/bjs.6407]

18 **Burtness BA,** Powell M, Berlin J, Liles D, Chapman A, Mitchell E, Benson AB, Eastern Cooperative Oncology Group. Phase II trial of irinotecan/docetaxel for advanced pancreatic cancer with randomization between irinotecan/docetaxel and irinotecan/docetaxel plus C225, a monoclonal antibody to the epidermal growth factor receptor (EGF-r) : Eastern Cooperative Oncology. *J Clin Oncol* 2007; **25** (18S): Abstract 4519

19 **Cascinu S**, Berardi R, Labianca R, Siena S, Falcone A, Aitini E, Barni S, Di Costanzo F, Dapretto E, Tonini G, Pierantoni C, Artale S, Rota S, Floriani I, Scartozzi M, Zaniboni A. Cetuximab plus gemcitabine and cisplatin compared with gemcitabine and cisplatin alone in patients with advanced

pancreatic cancer: a randomised, multicentre, phase II trial. *Lancet Oncol* 2008; **9**: 39-44 [PMID: 18077217 DOI: 10.1016/S1470-2045(07)70383-2]
20 **Engelhardt K**, Riley C, Cooke L, Mahadevan D. Monoclonal antibody therapies targeting pancreatic ductal adenocarcinoma. *Curr Drug Discov Technol* 2006; **3**: 231-243 [PMID: 17430101]
21 **Kulke MH**, Blaszkowsky LS, Ryan DP, Clark JW, Meyerhardt JA, Zhu AX, Enzinger PC, Kwak EL, Muzikansky A, Lawrence C, Fuchs CS. Capecitabine plus erlotinib in gemcitabine-refractory advanced pancreatic cancer. *J Clin Oncol* 2007; **25**: 4787-4792 [PMID: 17947726 DOI: 10.1200/JCO.2007.11.8521]
22 **Kindler HL**, Friberg G, Singh DA, Locker G, Nattam S, Kozloff M, Taber DA, Karrison T, Dachman A, Stadler WM, Vokes EE. Phase II trial of bevacizumab plus gemcitabine in patients with advanced pancreatic cancer. *J Clin Oncol* 2005; **23**: 8033-8040 [PMID: 16258101 DOI: 10.1200/JCO.2005.01.9661]
23 **Yokoi K**, Sasaki T, Bucana CD, Fan D, Baker CH, Kitadai Y, Kuwai T, Abbruzzese JL, Fidler IJ. Simultaneous inhibition of EGFR, VEGFR, and platelet-derived growth factor receptor signaling combined with gemcitabine produces therapy of human pancreatic carcinoma and prolongs survival in an orthotopic nude mouse model. *Cancer Res* 2005; **65**: 10371-10380 [PMID: 16288027 DOI: 10.1158/0008-5472.CAN-05-1698]
24 **Saif MW**. Anti-angiogenesis therapy in pancreatic carcinoma. *JOP* 2006; **7**: 163-173 [PMID: 16525200]
25 **Ferrari V**, Valcamonico F, Amoroso V, Simoncini E, Vassalli L, Marpicati P, Rangoni G, Grisanti S, Tiberio GA, Nodari F, Strina C, Marini G. Gemcitabine plus celecoxib (GECO) in advanced pancreatic cancer: a phase II trial. *Cancer Chemother Pharmacol* 2006; **57**: 185-190 [PMID: 16151811 DOI: 10.1007/s00280-005-0028-1]
26 **Mesa RA**. Tipifarnib: farnesyl transferase inhibition at a crossroads. *Expert Rev Anticancer Ther* 2006; **6**: 313-319 [PMID: 16503848 DOI: 10.1586/14737140.6.3.313]
27 **Ikeda O**, Tamura Y, Nakasone Y, Shiraishi S, Kawanaka K, Tomiguchi S, Yamashita Y, Takamori H, Kanemitsu K, Baba H. Comparison of intrahepatic and pancreatic perfusion on fusion images using a combined SPECT/CT system and assessment of efficacy of combined continuous arterial infusion and systemic chemotherapy in advanced pancreatic carcinoma. *Cardiovasc Intervent Radiol* 2007; **30**: 912-921 [PMID: 17710478 DOI: 10.1007/s00270-007-9134-2]
28 **Farrel J.** Predictive markers for gemcitabine sensitivity in pancreatic cancer. The 2008 ASCO Gastrointestinal cancer Symposium; Jan 25–27, 2008; Orlando, FL
29 **Hoyer M**, Roed H, Sengelov L, Traberg A, Ohlhuis L, Pedersen J, Nellemann H, Kiil Berthelsen A, Eberholst F, Engelholm SA, von der Maase H. Phase-II study on stereotactic radiotherapy of locally advanced pancreatic carcinoma. *Radiother Oncol* 2005; **76**: 48-53 [PMID: 15990186 DOI: 10.1016/j.radonc.2004.12.022]
30 **Bouchard M**, Amos RA, Briere TM, Beddar S, Crane CH. Dose escalation with proton or photon radiation treatment for pancreatic cancer. *Radiother Oncol* 2009; **92**: 238-243 [PMID: 19454367 DOI: 10.1016/j.radonc.2009.04.015]
31 **Hong TS**, Craft DL, Carlsson F, Bortfeld TR. Multicriteria optimization in intensity-modulated radiation therapy treatment planning for locally advanced cancer of the pancreatic head. *Int J Radiat Oncol Biol Phys* 2008; **72**: 1208-1214 [PMID: 18954714 DOI: 10.1016/j.ijrobp.2008.07.015]
32 **Zhang LP**, Nie Q, Kang JB, Wang B, Cai CL, Li JG, Qi WJ. [Efficacy of whole body gamma-knife radiotherapy combined with thermochemotherapy on locally advanced pancreatic cancer]. *Ai Zheng* 2008; **27**: 1204-1207 [PMID: 19000454]
33 **Zhang K**, Loong SL, Connor S, Yu SW, Tan SY, Ng RT, Lee KM, Canham L, Chow PK. Complete tumor response following intratumoral 32P BioSilicon on human hepatocellular and pancreatic carcinoma xenografts in nude mice. *Clin Cancer Res* 2005; **11**: 7532-7537 [PMID: 16243828 DOI: 10.1158/1078-0432.CCR-05-0400]
34 **Gan SI**, Thompson CC, Lauwers GY, Bounds BC, Brugge WR. Ethanol lavage of pancreatic cystic lesions: initial pilot study. *Gastrointest Endosc* 2005; **61**: 746-752 [PMID: 15855986]
35 **Ashida R**, Chang KJ. Interventional EUS for the treatment of pancreatic cancer. *J Hepatobiliary Pancreat Surg* 2009; **16**: 592-597 [PMID: 19547908 DOI: 10.1007/s00534-009-0129-z]
36 **Wolfsen HC**. Photodynamic therapy for pancreatic cancer: let's get serious. *Gastrointest Endosc* 2008; **67**: 961-963 [PMID: 18440386 DOI: 10.1016/j.gie.2007.11.012]
37 **Angelini C**, Bovo G, Museli P, Mussi C, Crippa S, Caprotti R, Uggeri F. Preoperative interleukin-2 immunotherapy in pancreatic cancer: preliminary results. *Hepatogastroenterology* 2006; **53**: 141-144 [PMID: 16506394]
38 **Gjertsen MK**, Saeterdal I, Saebøe-Larssen S, Gaudernack G. HLA-A3 restricted mutant ras specific cytotoxic T-lymphocytes induced by vaccination with T-helper epitopes. *J Mol Med* (Berl) 2003; **81**: 43-50 [PMID: 12545248 DOI: 10.1007/s00109-002-0390-y]
39 **Schneider C**, Schmidt T, Ziske C, Tiemann K, Lee KM, Uhlinsky V, Behrens P, Sauerbruch T, Schmidt-Wolf IG, Mühlradt PF, Schmidt J, Märten A. Tumour suppression induced by the macrophage activating lipopeptide MALP-2 in an ultrasound guided pancreatic carcinoma mouse model. *Gut* 2004; **53**: 355-361 [PMID: 14960515]
40 **Liu QL**, Wang YS, Wang JX. Effect of insulin on functional status of cord blood-derived dendritic cells and on dendritic cell-induced CTL cytotoxicity against pancreatic cancer cell lines. *Hepatobiliary Pancreat Dis Int* 2009; **8**: 529-534 [PMID: 19822498]
41 **Soeda A**, Morita-Hoshi Y, Makiyama H, Morizane C, Ueno H, Ikeda M, Okusaka T, Yamagata S, Takahashi N, Hyodo I, Takaue Y, Heike Y. Regular dose of gemcitabine induces an increase in CD14+ monocytes and CD11c+ dendritic cells in patients with advanced pancreatic cancer. *Jpn J Clin Oncol* 2009; **39**: 797-806 [PMID: 19797418 DOI: 10.1093/jjco/hyp112]
42 **Dudeja V**, Mujumdar N, Phillips P, Chugh R, Borja-Cacho D, Dawra RK, Vickers SM, Saluja AK. Heat shock protein 70 inhibits apoptosis in cancer cells through simultaneous and independent mechanisms. *Gastroenterology* 2009; **136**: 1772-1782 [PMID: 19208367 DOI: 10.1053/j.gastro.2009.01.070]

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CASE REPORT

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

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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. 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.

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

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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[1-10], population-based case control and cohort studies[11-18] 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[19] 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[19]. 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, cholilithiasis, 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 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).

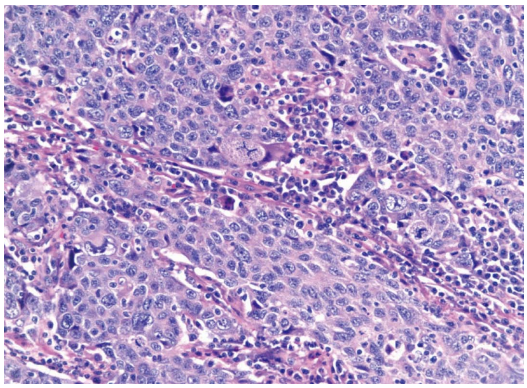

Figure 1 Tumor cells are large with numerous mitotic figures consistent with poorly differentiated carcinoma of the gallbladder.

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[2,20-23]. In contrast cholangiocarcinoma in CD is less frequent. Primary GC in CD is even more infrequent with only 2 reported cases in the literature[24,25]. 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[24,25] 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[14]. 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

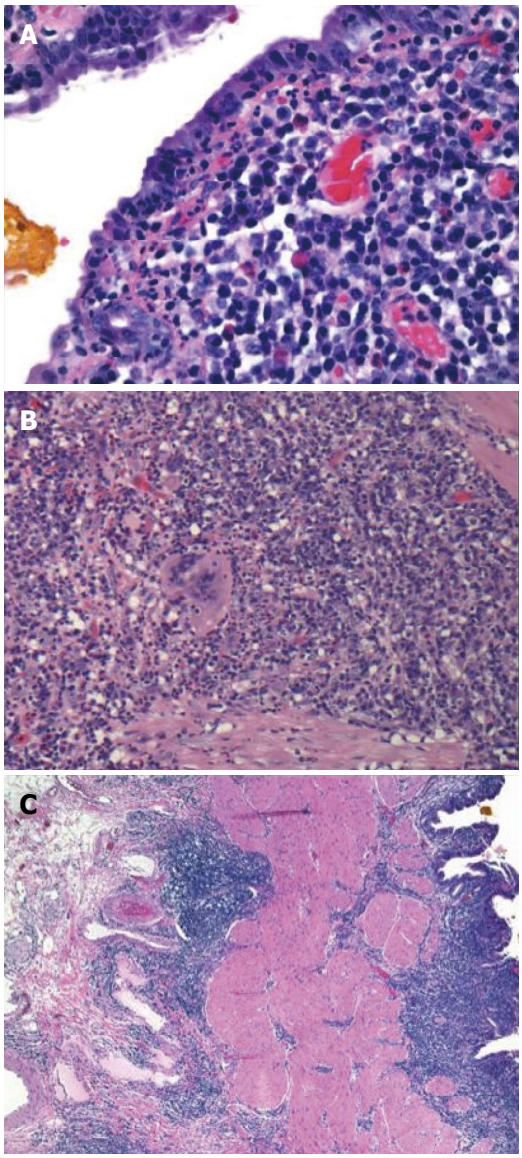

Figure 2 Crohn's disease involving the gallbladder. A: Superficial acute inflammation and numerous plasma cells; B: Non necrotizing granuloma in the gallbladder wall; C: Transmural inflammation of the gallbladder wall.

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[26], 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[25] and one in the setting of concomitant PSC[24].

Persistent inflammation is thought to promote carcinogenesis by causing DNA damage, activating tissue reparative proliferation, and by creating a local environment that is enriched with cytokines and other growth factors for autonomous proliferation and escape from apoptosis[27]. 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[28]. 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[29-34]. Common pathological features include transmural inflammation, granulomatous change, and lymphoid aggregation[29-34]. Similarly, our case demonstrated chronic transmural inflammation and granuloma formation. Common clinical characteristics include initial presentation with acute cholecystitis, as demonstrated in 4 cases[29-31,33] and ileal involvement occurred in 5 cases[29-32,34]. Of note, patients with ileal disease have a 10 fold increase in the incidence of cholelithiasis due to the disruption in bile salt metabolism[35]. 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[36,37]. However 2 of the 7 total cases, including ours, occurred in the absence of gallstone formation[31]. Alternatively, ileal disease may result in disturbance in the microbiome and colonization of the terminal ileum with anaerobic bacteria[38]. This results in deconjugation of bile acids to products that have an irritating effect on the mucosa of the GB resulting in inflammation[39]. 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[40-48]. 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)[49] indicating that estrogens and IGF-1 coordinately regulate cholangiocarcinoma growth and apoptosis[49]. 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[50]. This protracted exposure allows environmental carcinogens to then cause malignant transformation[43]. 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[51,52] but in other studies a positive association between OC use and extrahepatic bile cancer was found[53,54]. 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[55]. The same recommendations should be extended to patients with chronic immunosuppressive states and chronic inflammation, such as IBD.

REFERENCES

1 **Klingenberg-Noftz RD**, Homann N, Bos I, Bruch HP, Ludwig D. Simultaneous detection of synchronous colonic and biliary carcinoma by abdominal ultrasonography in two patients with ulcerative colitis. *Dig Dis Sci* 2004; **49**: 1922-1929 [PMID: 15628726 DOI: 10.1007/s10620-004-9593-0]

2 **Choi PM**, Nugent FW, Zelig MP, Munson JL, Schoetz DJ. Cholangiocarcinoma and Crohn's disease. *Dig Dis Sci* 1994; **39**: 667-670 [PMID: 8131708 DOI: 10.1007/BF02088359]

3 **Chong VF**, Heng A. Cholangiocarcinoma complicating ulcerative colitis. *Ann Acad Med Singapore* 1993; **22**: 798-801 [PMID: 8267364]

4 **Haworth AC**, Manley PN, Groll A, Pace R. Bile duct carcinoma and biliary tract dysplasia in chronic ulcerative colitis. *Arch Pathol Lab Med* 1989; **113**: 434-436 [PMID: 2705876]

5 **Van Steenbergen W**, Fevery J, Vandenbrande P, Desmet V, Ponette E, Kerremans R, de Groote J. Ulcerative colitis, primary sclerosing cholangitis, bile duct carcinoma, and generalized sarcoidosis. Report of a unique association. *J Clin Gastroenterol* 1987; **9**: 574-579 [PMID: 2824599 DOI: 10.1097/00004836-198710000-00020]

6 **Berman MD**, Falchuk KR, Trey C. Carcinoma of the biliary tree complicating Crohn's disease. *Dig Dis Sci* 1980; **25**: 795-797 [PMID: 7428587 DOI: 10.1007/BF01345302]

7 **Wee A**, Ludwig J, Coffey RJ, LaRusso NF, Wiesner RH. Hepatobiliary carcinoma associated with primary sclerosing cholangitis and chronic ulcerative colitis. *Hum Pathol* 1985; **16**: 719-726 [PMID: 4007848 DOI: 10.1016/S0046-8177(85)80158-1]

8 **Krause JR**, Ayuyang HQ, Ellis LD. Occurrence of three cases of carcinoma in individuals with Crohn's disease treated with metronidazole. *Am J Gastroenterol* 1985; **80**: 978-982 [PMID: 3000170]

9 **Williams SM**, Harned RK. Bile duct carcinoma associated with chronic ulcerative colitis. *Dis Colon Rectum* 1981; **24**: 42-44 [PMID: 7472101 DOI: 10.1007/BF02603450]

10 **Akwari OE**, Van Heerden JA, Foulk WT, Baggenstoss AH. Cancer of the bile ducts associated with ulcerative colitis. *Ann Surg* 1975; **181**: 303-309 [PMID: 165791 DOI: 10.1097/00000658-197503000-00010]

11 **Mellemkjaer L**, Olsen JH, Frisch M, Johansen C, Gridley G, McLaughlin JK. Cancer in patients with ulcerative colitis. *Int J Cancer* 1995; **60**: 330-333 [PMID: 7829239 DOI: 10.1002/ijc.2910600309]

12 **Broomé U**, Glaumann H, Hellers G, Nilsson B, Sörstad J, Hultcrantz R. Liver disease in ulcerative colitis: an epidemiological and follow up study in the county of Stockholm. *Gut* 1994; **35**: 84-89 [PMID: 8307457 DOI: 10.1136/gut.35.1.84]

13 **Shaib YH**, El-Serag HB, Davila JA, Morgan R, McGlynn KA. Risk factors of intrahepatic cholangiocarcinoma in the United States: a case-control study. *Gastroenterology* 2005; **128**: 620-626 [PMID: 15765398 DOI: 10.1053/j.gastro.2004.12.048]

14 **Mellemkjaer L**, Johansen C, Gridley G, Linet MS, Kjaer SK, Olsen JH. Crohn's disease and cancer risk (Denmark). *Cancer Causes Control* 2000; **11**: 145-150 [PMID: 10710198 DOI: 10.1023/A:1008988215904]

15 **Broomé U**, Löfberg R, Veress B, Eriksson LS. Primary sclerosing cholangitis and ulcerative colitis: evidence for increased neoplastic potential. *Hepatology* 1995; **22**: 1404-1408 [PMID: 7590655]

16 **Persson PG**, Karlén P, Bernell O, Leijonmarck CE, Broström O, Ahlbom A, Hellers G. Crohn's disease and cancer: a population-based cohort study. *Gastroenterology* 1994; **107**: 1675-1679 [PMID: 7958678]

17 **Ekbom A**, Helmick C, Zack M, Adami HO. Extracolonic malignancies in inflammatory bowel disease. *Cancer* 1991; **67**: 2015-2019 [PMID: 2004319 DOI: 10.1002/1097-0142(19910401)67: 7<2015: : AID-CNCR2820670731>3.0.CO; 2-R]

18 **Welzel TM**, Mellemkjaer L, Gloria G, Sakoda LC, Hsing AW, El Ghormli L, Olsen JH, McGlynn KA. Risk factors for intrahepatic cholangiocarcinoma in a low-risk population: a nationwide case-control study. *Int J Cancer* 2007; **120**: 638-641 [PMID: 17109384 DOI: 10.1002/ijc.22283]

19 **Erichsen R**, Jepsen P, Vilstrup H, Ekbom A, Sørensen HT. Incidence and prognosis of cholangiocarcinoma in Danish patients with and without inflammatory bowel disease: a national cohort study, 1978-2003. *Eur J Epidemiol* 2009; **24**: 513-520 [PMID: 19557525 DOI: 10.1007/s10654-009-9365-4]

20 **Mir-Madjlessi SH**, Farmer RG, Sivak MV. Bile duct carcinoma in patients with ulcerative colitis. Relationship to sclerosing cholangitis: report of six cases and review of the literature. *Dig Dis Sci* 1987; **32**: 145-154 [PMID: 3542446 DOI: 10.1007/BF01297102]

21 **Ritchie JK**, Allan RN, Macartney J, Thompson H, Hawley PR, Cooke WT. Biliary tract carcinoma associated with ulcerative colitis. *Q J Med* 1974; **43**: 263-279 [PMID: 4850750]

22 **Akwari OE**, van Heerden JA, Adson MA, Foulk WT, Baggenstoss AH. Bile duct carcinoma associated with ulcerative colitis. *Rev Surg* 1976; **33**: 289-293 [PMID: 940978]

23 **Schrumpf EFO.** The frequency of cholangiocarcinoma (CC) in primary sclerosing cholangitis. *J Hepatol* 1989; **9:** 83 [DOI: 10.1016/0168-8278(89)90342-5]

24 **Joffe N**, Antonioli DA. Primary carcinoma of the gallbladder associated with chronic inflammatory bowel disease. *Clin Radiol* 1981; **32**: 319-324 [PMID: 6263537 DOI: 10.1016/S0009-9260(81)80053-0]

25 **Ogawa H**, Funayama Y, Naito H, Fukushima K, Shibata C, Matsuno S, Sasaki I. Gallbladder carcinoma complicating Crohn's disease. *Am J Gastroenterol* 2001; **96**: 263-264 [PMID: 11197277 DOI: 10.1111/j.1572-0241.2001.03499.x]

26 **Sheth S**, Bedford A, Chopra S. Primary gallbladder cancer: recognition of risk factors and the role of prophylactic cholecystectomy. *Am J Gastroenterol* 2000; **95**: 1402-1410 [PMID: 10894571 DOI: 10.1111/j.1572-0241.2000.02070.x]

27 **Wise C**, Pilanthananond M, Perry BF, Alpini G, McNeal M, Glaser SS. Mechanisms of biliary carcinogenesis and growth. *World J Gastroenterol* 2008; **14**: 2986-2989 [PMID: 18494047 DOI: 10.3748/wjg.14.2986]

28 **Lin J**, Shen B, Lee HJ, Goldblum JR. Histopathological characterization of cholecystectomy specimens in patients with inflammatory bowel disease. *J Crohns Colitis* 2012; **6**: 895-899 [PMID: 22397872 DOI: 10.1016/j.crohns.2012.02.002]

29 **McClure J**, Banerjee SS, Schofield PS. Crohn's disease of the gall bladder. *J Clin Pathol* 1984; **37**: 516-518 [PMID: 6725598 DOI: 10.1136/jcp.37.5.516]

30 **Post AB**, van Stolk R, Broughan TA, Tuthill RJ. Crohn's disease of the gallbladder. *J Clin Gastroenterol* 1993; **16**: 139-142 [PMID: 8463617 DOI: 10.1097/00004836-199303000-00012]

31 **Rettally CA**, Treviño HH, Molina K, Kane SV. Crohn's disease involving the gallbladder. case report and review of the literature. *Am J Gastroenterol* 2003; **98**: 509-511 [PMID: 12591085]

32 **Andoh A**, Endo Y, Kushima R, Hata K, Tsujikawa T, Sasaki M, Mekata E, Tani T, Fujiyama Y. A case of Crohn's disease involving the gallbladder. *World J Gastroenterol* 2006; **12**: 977-978 [PMID: 16521232]

33 **Sharma AK**, Nawroz IM, Evgenikos N, Daniel T. Isolated involvement of the gallbladder by Crohn's disease manifesting as acute cholecystitis. *J Gastrointest Surg* 2005; **9**: 357-359 [PMID: 15749596 DOI: 10.1016/j.gassur.2004.08.012]

34 **Bouteloup C**, Guédon C, François A, Hemet J, Lerebours E. [Gallbladder involvement in Crohn disease]. *Gastroenterol Clin Biol* 1997; **21**: 153-155 [PMID: 9161482]

35 **Lapidus A**, Bångstad M, Aström M, Muhrbeck O. The prevalence of gallstone disease in a defined cohort of patients with Crohn's disease. *Am J Gastroenterol* 1999; **94**: 1261-1266 [PMID: 10235204 DOI: 10.1111/j.1572-0241.1999.01076.x]

36 **Kurchin A**, Ray JE, Bluth EI, Merritt CR, Gathright JB, Pehrsson BF, Ferrari BT. Cholelithiasis in ileostomy patients. *Dis Colon Rectum* 1984; **27**: 585-588 [PMID: 6468196 DOI: 10.1007/BF02553842]

37 **Vantrappen G**, Ghoos Y, Rutgeerts P, Janssens J. Bile acid studies in uncomplicated Crohn's disease. *Gut* 1977; **18**: 730-735 [PMID: 604194 DOI: 10.1136/gut.18.9.730]

38 **Van de Merwe JP**, Schröder AM, Wensinck F, Hazenberg MP. The obligate anaerobic faecal flora of patients with Crohn's disease and their first-degree relatives. *Scand J Gastroenterol* 1988; **23**: 1125-1131 [PMID: 3247591 DOI: 10.3109/00365528809090179]

39 **Heaton KW**. Disturbances of bile acid metabolism in intestinal disease. *Clin Gastroenterol* 1977; **6**: 69-89 [PMID: 330053]

40 **Sadoon S**, Hodgett S. Unusual cause of itching in a pregnancy (cholangiocarcinoma). *J Obstet Gynaecol* 2008; **28**: 230-231 [PMID: 18393029 DOI: 10.1080/01443610801916296]

41 **Purtilo DT**, Clark JV, Williams R. Primary hepatic malignancy in pregnant women. *Am J Obstet Gynecol* 1975; **121**: 41-44 [PMID: 46711]

42 **Marasinghe JP**, Karunananda SA, Angulo P. Cholangiocarcinoma in pregnancy: a case report. *J Obstet Gynaecol Res* 2008; **34**: 635-637 [PMID: 18840169 DOI: 10.1111/j.1447-0756.2008.00810.x]

43 **Liao WS,** Wang TE, Lin SC, Chang KM, Yang TL, Yang YC, Chen YJ, Yung YY. Chloangiocarcinoma in pregnancy - A case report. *Neikexue Zhi* 2002; 13: 286-292

44 **Devoe LD**, Moossa AR, Levin B. Pregnancy complicated by extrahepatic biliary tract carcinoma. A case report. *J Reprod Med* 1983; **28**: 153-155 [PMID: 6300394]

45 **Zelissen PM**, van Hattum J. [A young woman with a liver tumor and hypercalcemia]. *Ned Tijdschr Geneeskd* 1986; **130**: 1705-1707 [PMID: 3020447]

46 **Sophia S,** Girling JC. Deranged liver function tests in pregnancy: the importance of postnatal follow-up. *Obstet Med* 2009; **2:** 32-33 [DOI: 10.1258/om.2008.080036]

47 **Ismael G**, de Azambuja E, Awada A. Molecular profiling of a tumor of unknown origin. *N Engl J Med* 2006; **355**: 1071-1072 [PMID: 16957161 DOI: 10.1056/NEJMc061533]

48 **DePalma F,** Henry P, Hahm A, Lee T, Elfant A. Cholangiocarcinoma during pregnancy diagnosed by ERCP with cholangioscopy (Abstract). Available from: URL: http://universe.gi.org/contentlist.asp?page=31&eventtype=51

49 **Alvaro D**, Barbaro B, Franchitto A, Onori P, Glaser SS, Alpini G, Francis H, Marucci L, Sterpetti P, Ginanni-Corradini S, Onetti Muda A, Dostal DE, De Santis A, Attili AF, Benedetti A, Gaudio E. Estrogens and insulin-like growth factor 1 modulate neoplastic cell growth in human cholangiocarcinoma. *Am J Pathol* 2006; **169**: 877-888 [PMID: 16936263 DOI: 10.2353/ajpath.2006.050464]

50 **Venniyoor A**. Cholesterol gallstones and cancer of gallbladder (CAGB): molecular links. *Med Hypotheses* 2008; **70**: 646-653 [PMID: 17855001 DOI: 10.1016/j.mehy.2007.06.040]

51 Combined oral contraceptives and gallbladder cancer. The WHO Collaborative Study of Neoplasia and Steroid Contraceptives. *Int J Epidemiol* 1989; **18**: 309-314 [PMID: 2767843 DOI: 10.1093/ije/18.2.309]

52 **Moerman CJ**, Berns MP, Bueno de Mesquita HB, Runia S. Reproductive history and cancer of the biliary tract in women. *Int J Cancer* 1994; **57**: 146-153 [PMID: 8157350 DOI: 10.1002/ijc.2910570203]

53 **Altaee MY**, Johnson PJ, Farrant JM, Williams R. Etiologic and clinical characteristics of peripheral and hilar cholangiocarcinoma. *Cancer* 1991; **68**: 2051-2055 [PMID: 1717133 DOI: 10.1002/1097-0142(19911101)68: 9<2051: : AID-CNCR2820680934>3.0.CO; 2-M]

54 **Yen S**, Hsieh CC, MacMahon B. Extrahepatic bile duct cancer and smoking, beverage consumption, past medical history, and oral-contraceptive use. *Cancer* 1987; **59**: 2112-2116 [PMID: 3567872 DOI: 10.1002/1097-0142(19870615)59: 12<2112: : AID-CNCR2820591226>3.0.CO; 2-H]

55 **Chapman R**, Fevery J, Kalloo A, Nagorney DM, Boberg KM, Shneider B, Gores GJ. Diagnosis and management of primary sclerosing cholangitis. *Hepatology* 2010; **51**: 660-678 [PMID: 20101749 DOI: 10.1002/hep.23294]

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CASE REPORT

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.

Key words: Ganglioneuromatosis; Gastrointestinal poliposis; Phosphatase and tensin homolog; Cowden síndrome; Hamartoma

Herranz Bachiller MT, Barrio Andrés J, Pons F, Alcaide Suárez N, Ruiz-Zorrilla R, Sancho del Val L, Lorenzo Pelayo S, De la Serna Higuera C, Atienza Sánchez R, Perez Miranda M. Diffuse intestinal ganglioneuromatosis an uncommon manifestation of Cowden syndrome. *World J Gastrointest Oncol* 2013; 5(2): 34-37 Available from: URL: http://www.wjgnet.com/1948-5204/full/v5/i2/34.htm DOI: http://dx.doi.org/10.4251/wjgo.v5.i2.34

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[1]. 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[2]. 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[3]. 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[4]. 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 was 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).

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 (≥ 97% ile)
Endometrial cancer
Minor criteria
Other structural thyroid lesions (*e.g.*, adenoma, multinodular goiter)
Mental retardation (*i.e.*, intelligence quotient ≤ 75)
Gastrointestinal hamartomas
Fibrocystic disease of the breast
Lipomas
Fibromas
Genitourinary tumours (*e.g.*, 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

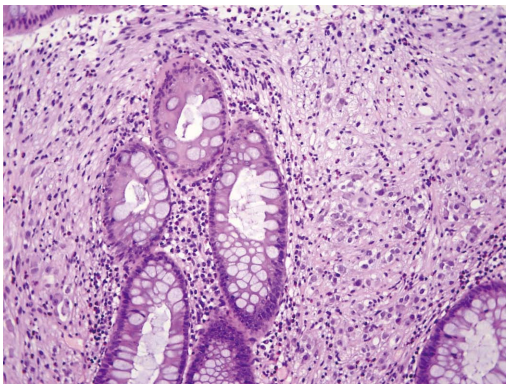

Figure 1 Pathological study revealed diffuse intestinal ganglioneuromatosis.

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)[5,6], which could be involved as a regulator of multiple processes of cell proliferation, 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 Lhermite-Duclos disease, as well as autism syndromes associated with macrocephalia[6].

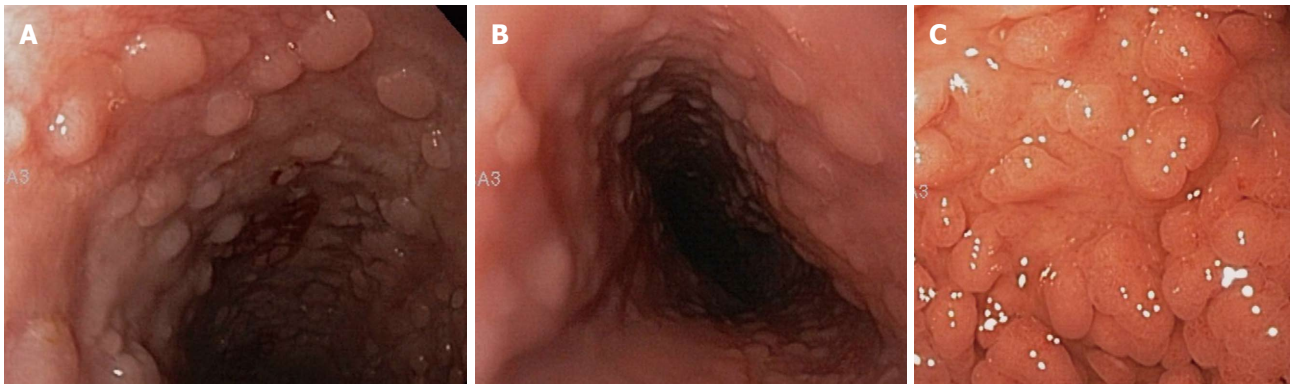

Figure 2 A gastroscopy was subsequently performed showing esophagic (A, B) and gastric polyposis (C).

The prevalence of Cowden syndrome has been estimated at 1/200 000-250 000 inhabitants in a German series published in 1999[7], 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[5].

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[4]. 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[4]. 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[3,8]. 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[6]. In the previously mentioned series of 127 patients[4], 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[9] to the strictest which recommend starting at 15 years and monitoring every 1-2 years[10], 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[4].

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.

REFERENCES

1 **Chambonnière ML**, Porcheron J, Scoazec JY, Audigier JC, Mosnier JF. [Intestinal ganglioneuromatosis diagnosed in adult patients]. *Gastroenterol Clin Biol* 2003; **27**: 219-224 [PMID: 12658132]

2 **Chan OT**, Haghighi P. Hamartomatous polyps of the colon: ganglioneuromatous, stromal, and lipomatous. *Arch Pathol Lab Med* 2006; **130**: 1561-1566 [PMID: 17090203 DOI: 10.1043/1543-2165(2006)130[1561: HPOTCG]2.0.CO; 2]

3 **Kay PS**, Soetikno RM, Mindelzun R, Young HS. Diffuse esophageal glycogenic acanthosis: an endoscopic marker of Cowden's disease. *Am J Gastroenterol* 1997; **92**: 1038-1040 [PMID: 9177527]

4 **Heald B**, Mester J, Rybicki L, Orloff MS, Burke CA, Eng C. Frequent gastrointestinal polyps and colorectal adenocar-

cinomas in a prospective series of PTEN mutation carriers. *Gastroenterology* 2010; **139**: 1927-1933 [PMID: 20600018 DOI: 10.1053/j.gastro.2010.06.061]

5 **Farooq A**, Walker LJ, Bowling J, Audisio RA. Cowden syndrome. *Cancer Treat Rev* 2010; **36**: 577-583 [PMID: 20580873 DOI: 10.1016/j.ctrv.2010.04.002]

6 **Pilarski R**. Cowden syndrome: a critical review of the clinical literature. *J Genet Couns* 2009; **18**: 13-27 [PMID: 18972196 DOI: 10.1007/s109897-008-9187-7]

7 **Nelen MR**, Kremer H, Konings IB, Schoute F, van Essen AJ, Koch R, Woods CG, Fryns JP, Hamel B, Hoefsloot LH, Peeters EA, Padberg GW. Novel PTEN mutations in patients with Cowden disease: absence of clear genotype-phenotype correlations. *Eur J Hum Genet* 1999; **7**: 267-273 [PMID: 10234502 DOI: 10.1038/sj.ejhg.5200289]

8 **Umemura K**, Takagi S, Ishigaki Y, Iwabuchi M, Kuroki S, Kinouchi Y, Shimosegawa T. Gastrointestinal polyposis with esophageal polyposis is useful for early diagnosis of Cowden's disease. *World J Gastroenterol* 2008; **14**: 5755-5759 [PMID: 18837096 DOI: 10.3748/wjg.14.5755]

9 **Riegert-Johnson DL**, Gleeson FC, Roberts M, Tholen K, Youngborg L, Bullock M, Boardman LA. Cancer and Lhermitte-Duclos disease are common in Cowden syndrome patients. *Hered Cancer Clin Pract* 2010; **8**: 6 [PMID: 20565722 DOI: 10.1186/1897-4287-8-6]

10 **Schreibman IR**, Baker M, Amos C, McGarrity TJ. The hamartomatous polyposis syndromes: a clinical and molecular review. *Am J Gastroenterol* 2005; **100**: 476-490 [PMID: 15667510]

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CASE REPORT

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.

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[1]. 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[2]. Therefore, only palliative treatment, such as, transarterial chemoembolization (TACE) or systemic treatment, is available for patients with advanced HCC[2].

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[3]. The SHARP trial reported that sorafenib has significant survival benefit in advanced HCC, and that is the only effective systemic agent[4]. 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[5-8].

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

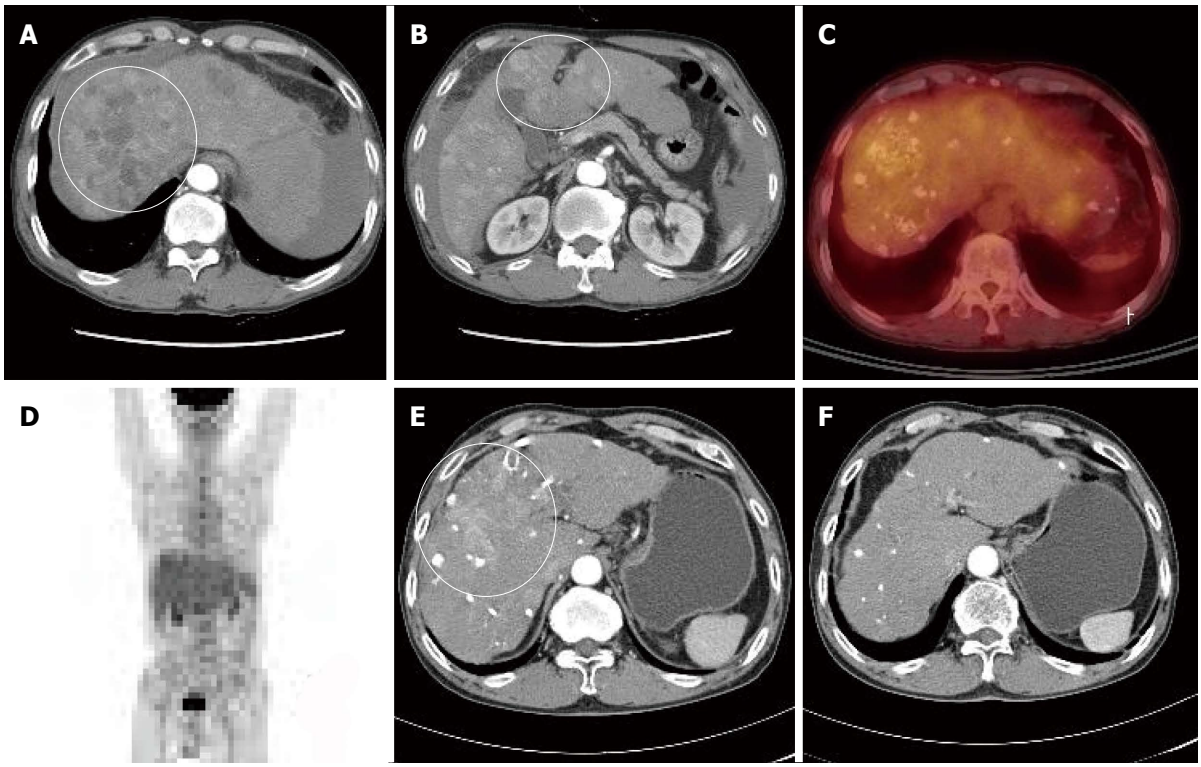

Figure 1 Computed tomography of the patient. A, B: Computed tomography (CT) scan taken at the time of hepatocellular carcinoma (HCC) diagnosis showing intrahepatic multinodular HCCs (white circles); C, D: Positron emission tomography CT scan showing multiple lipiodolized masses and abnormal fluorine-18 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-

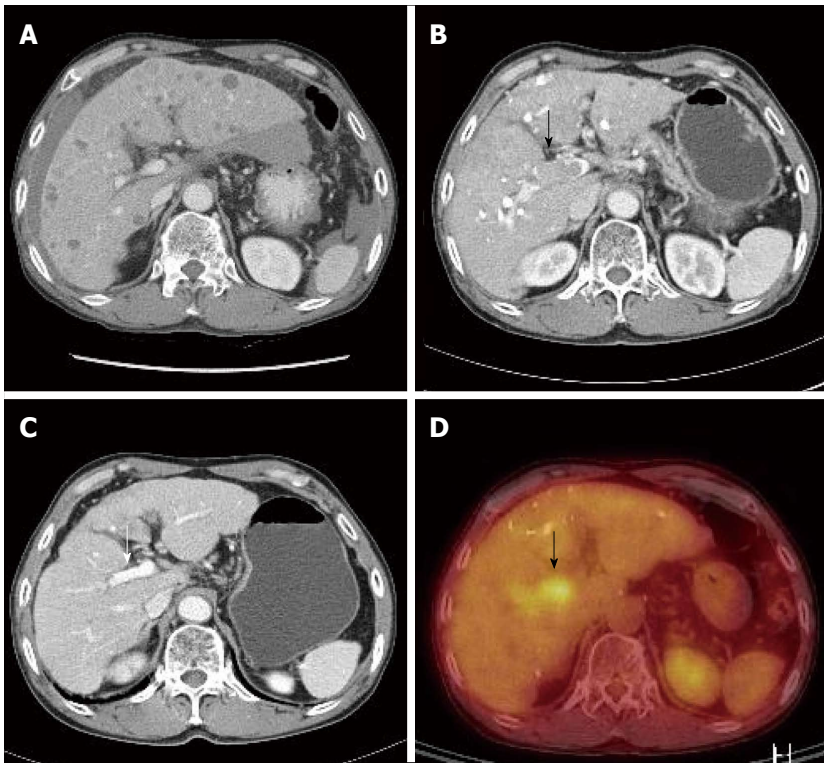

Figure 2 The diagnosis of portal vein tumor. A, B, D: Portal vein tumor (A), which was not evident at diagnosis, was detected by liver dynamic computed tomography (CT) (B, black arrow) and positron emission tomography-CT (D, black arrow) after four sessions of transarterial chemoembolization; C: Portal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

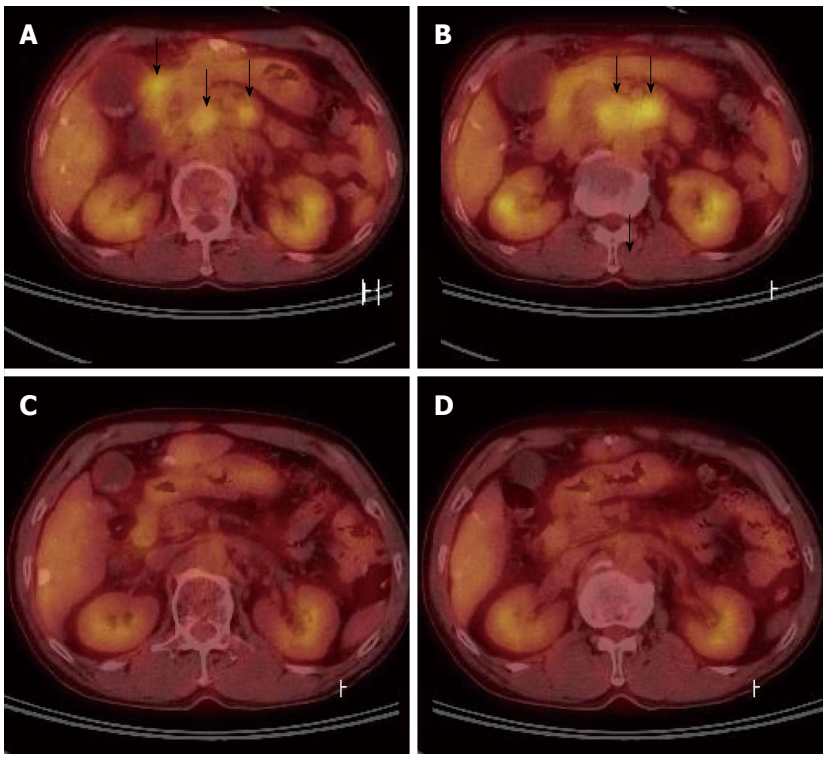

Figure 3 Positron emission tomography computed tomography. A, B: After four sessions of transarterial chemoembolization, significant multiple abdominal lymphadenopathy was detected by positron emission tomography (PET)-computed tomography (CT) (black arrows); C, D: Fluorine-18 2-fluoro-2-deoxy-*D*-glucose 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[3], 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-β. 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[9,10].

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[3]. 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[4]. Based on the findings of these studies, sorafenib is now used in HCC patients with BLCL 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[5-8]. In our case, PVT and intraabdominal lymphadenopathy were also improved by administrating 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[11,12]. A variety of treatment modalities, such as, TACI, chemotherapy (5-fluorouracil, cisplatin, *etc.*), and radiotherapy have been attempted in cases with PVTT, but all have been found to be ineffective[13]. 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[14]. 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[15,16]. 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[13]. The previous studies have reported the combined radiotherapy and TACE in advanced HCC with PVTT[17-19], and there are cases that report the effectiveness of radiation therapy in HCC that accompanies abdominal lymph node metastasis[20]. 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[21]. 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[22]. However, HFSR is considered an indirect effect of sorafenib. Epidermal keratinocytes synthesize PDGF-α and PDGF-β, which activate dermal capillaries, fibroblasts, and PDGRF in eccrine glands[22]. 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[22].

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 recommands 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.

REFERENCES

1 **Parkin DM**, Bray F, Ferlay J, Pisani P. Estimating the world cancer burden: Globocan 2000. *Int J Cancer* 2001; **94**: 153-156 [PMID: 11668491]

2 **Llovet JM**, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003; **362**: 1907-1917 [PMID: 14667750 DOI: 10.1016/

S0140-6736(03)14964-1]
3 **Llovet JM**, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378-390 [PMID: 18650514 DOI: 10.1056/NEJMoa0708857]
4 **Cheng AL**, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, Luo R, Feng J, Ye S, Yang TS, Xu J, Sun Y, Liang H, Liu J, Wang J, Tak WY, Pan H, Burock K, Zou J, Voliotis D, Guan Z. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 2009; **10**: 25-34 [PMID: 19095497 DOI: 10.1016/S1470-2045(08)70285-7]
5 **Inuzuka T**, Nishikawa H, Sekikawa A, Takeda H, Henmi S, Sakamoto A, Saito S, Kita R, Kimura T, Osaki Y, Kudo M. Complete response of advanced hepatocellular carcinoma with multiple lung metastases treated with sorafenib: a case report. *Oncology* 2011; **81** Suppl 1: 152-157 [PMID: 22212950 DOI: 10.1159/000333279]
6 **Wang SX**, Byrnes A, Verma S, Pancoast JR, Rixe O. Complete remission of unresectable hepatocellular carcinoma treated with reduced dose of sorafenib: a case report. *Target Oncol* 2010; **5**: 59-63 [PMID: 20309643 DOI: 10.1007/s11523-010-0133-x]
7 **Sacco R**, Bargellini I, Gianluigi G, Bertini M, Bozzi E, Altomare E, Battaglia V, Romano A, Bertoni M, Capria A, Bresci G, Bartolozzi C. Complete response for advanced liver cancer during sorafenib therapy: case report. *BMC Gastroenterol* 2011; **11**: 4 [PMID: 21241463 DOI: 10.1186/1471-230X-11-4]
8 **Chelis L**, Ntinos N, Souftas V, Deftereos S, Xenidis N, Chamalidou E, Maltezos E, Kakolyris S. Complete response after sorafenib therapy for hepatocellular carcinoma in an HIV-HBV co infected patient: Possible synergy with HAART ? A case report. *Med Oncol* 2011; **28** Suppl 1: S165-S168 [PMID: 20809183 DOI: 10.1007/s12032-010-9669-y]
9 **Semela D**, Dufour JF. Angiogenesis and hepatocellular carcinoma. *J Hepatol* 2004; **41**: 864-880 [PMID: 15519663 DOI: 10.1016/j.jhep.2004.09.006]
10 **Liu L**, Cao Y, Chen C, Zhang X, McNabola A, Wilkie D, Wilhelm S, Lynch M, Carter C. Sorafenib blocks the RAF/MEK/ERK pathway, inhibits tumor angiogenesis, and induces tumor cell apoptosis in hepatocellular carcinoma model PLC/PRF/5. *Cancer Res* 2006; **66**: 11851-11858 [PMID: 17178882 DOI: 10.1158/0008-5472.CAN-06-1377]
11 **Llovet JM**, Bustamante J, Castells A, Vilana R, Ayuso Mdel C, Sala M, Brú C, Rodés J, Bruix J. Natural history of untreated nonsurgical hepatocellular carcinoma: rationale for the design and evaluation of therapeutic trials. *Hepatology* 1999; **29**: 62-67 [PMID: 9862851 DOI: 10.1002/hep.510290145]
12 **Villa E**, Moles A, Ferretti I, Buttafoco P, Grottola A, Del Buono M, De Santis M, Manenti F. Natural history of inoperable hepatocellular carcinoma: estrogen receptors' status in the tumor is the strongest prognostic factor for survival. *Hepatology* 2000; **32**: 233-238 [PMID: 10915729 DOI: 10.1053/jhep.2000.9603]
13 **Minagawa M**, Makuuchi M. Treatment of hepatocellular carcinoma accompanied by portal vein tumor thrombus. *World J Gastroenterol* 2006; **12**: 7561-7567 [PMID: 17171782]
14 **Li Q**, Xu B, Fu L, Hao XS. Correlation of four vascular specific growth factors with carcinogenesis and portal vein tumor thrombus formation in human hepatocellular carcinoma. *J Exp Clin Cancer Res* 2006; **25**: 403-409 [PMID: 17167981]
15 **Novi M**, Lauritano EC, Piscaglia AC, Barbaro B, Zocco MA, Pompili M, Gasbarrini A. Portal vein tumor thrombosis revascularization during sorafenib treatment for hepatocellular carcinoma. *Am J Gastroenterol* 2009; **104**: 1852-1854 [PMID: 19574982 DOI: 10.1038/ajg.2009.140]
16 **Yang MY**, Jeong SW, Kim DK, Kim SG, Jang JY, Kim YS, Lee JS, Kim BS, Kim JH, Kim YJ. Treatment of hepatocellular carcinoma with portal vein thrombosis by sorafenib combined with hepatic arterial infusion chemotherapy. *Gut Liver* 2010; **4**: 423-427 [PMID: 20981227 DOI: 10.5009/gnl.2010.4.3.423]
17 **Tazawa J**, Maeda M, Sakai Y, Yamane M, Ohbayashi H, Kakinuma S, Miyasaka Y, Nagayama K, Enomoto N, Sato C. Radiation therapy in combination with transcatheter arterial chemoembolization for hepatocellular carcinoma with extensive portal vein involvement. *J Gastroenterol Hepatol* 2001; **16**: 660-665 [PMID: 11422619]
18 **Ishikura S**, Ogino T, Furuse J, Satake M, Baba S, Kawashima M, Nihei K, Ito Y, Maru Y, Ikeda H. Radiotherapy after transcatheter arterial chemoembolization for patients with hepatocellular carcinoma and portal vein tumor thrombus. *Am J Clin Oncol* 2002; **25**: 189-193 [PMID: 11943901]
19 **Park MS**, Kim SU, Park JY, Kim do Y, Ahn SH, Han KH, Chon CY, Seong J. Combination treatment of localized concurrent chemoradiation therapy and transarterial chemoembolization in locally advanced hepatocellular carcinoma with intrahepatic metastasis. *Cancer Chemother Pharmacol* 2013; **71**: 165-173 [PMID: 23079897 DOI: 10.1007/s00280-012-1993-9]
20 **Park YJ**, Lim do H, Paik SW, Koh KC, Lee JH, Choi MS, Yoo BC, Nam HR, Oh DR, Park W, Ahn YC, Huh SJ. Radiation therapy for abdominal lymph node metastasis from hepatocellular carcinoma. *J Gastroenterol* 2006; **41**: 1099-1106 [PMID: 17160521 DOI: 10.1007/s00535-006-1895-x]
21 **Grünwald V**, Heinzer H, Fiedler W. Managing side effects of angiogenesis inhibitors in renal cell carcinoma. *Onkologie* 2007; **30**: 519-524 [PMID: 17890892 DOI: 10.1159/0000107721]
22 **Lacouture ME**, Wu S, Robert C, Atkins MB, Kong HH, Guitart J, Garbe C, Hauschild A, Puzanov I, Alexandrescu DT, Anderson RT, Wood L, Dutcher JP. Evolving strategies for the management of hand-foot skin reaction associated with the multitargeted kinase inhibitors sorafenib and sunitinib. *Oncologist* 2008; **13**: 1001-1011 [PMID: 18779536 DOI: 10.1634/theoncologist.2008-0131]

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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

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

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:10.1097/00003086-200208000-00026]

No volume or issue

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

Books

Personal author(s)

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

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

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12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

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

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

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

Patent (list all authors)

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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