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REVIEW

Inflammation-associated microsatellite alterations: Mechanisms and significance in the prognosis of patients with colorectal cancer

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Abstract

Microsatellite alterations within genomic DNA frameshift as a result of defective DNA mismatch repair (MMR). About 15% of sporadic colorectal cancers (CRCs) manifest hypermethylation of the DNA MMR gene *MLH1*, resulting in mono- and di-nucleotide frameshifts to classify it as microsatellite instability-high (MSI-H) and hypermutated, and due to frameshifts at coding microsatellites generating neo-antigens, produce a robust protective immune response that can be enhanced with immune checkpoint blockade. More commonly, approximately 50% of sporadic non-MSI-H CRCs demonstrate frameshifts at di- and tetra-nucleotide microsatellites to classify it as MSI-low/elevated microsatellite alterations at selected tetranucleotide repeats (EMAST) as a result of functional somatic inactivation of the DNA MMR protein MSH3 *via* a nuclear-to-cytosolic displacement. The trigger for MSH3 displacement appears to be inflammation and/or oxidative stress, and unlike MSI-H CRC patients, patients with MSI-L/EMAST CRCs show poor prognosis. These inflammatory-associated microsatellite alterations are a consequence of the local tumor microenvironment, and in theory, if the microenvironment is manipulated to lower inflammation, the microsatellite alterations and MSH3 dysfunction should be corrected. Here we describe the mechanisms and significance of inflammatory-associated microsatellite alterations, and

propose three areas to deeply explore the consequences and prevention of inflammation's effect upon the DNA MMR system.

Key words: Microsatellite instability; Microsatellite stable; Elevated microsatellite alterations at selected tetranucleotide repeats; Colorectal cancer; Mismatch repair; Inflammation; *MSH3*

Core tip: Inflammation can trigger microsatellite stable colorectal cancers (CRCs) to acquire a nuclear-to-cytoplasm displacement of the DNA mismatch repair protein MSH3, rendering the CRC with di- and tetra-nucleotide microsatellite instability (MSI-low/elevated microsatellite alterations at selected tetranucleotide repeats) and modifying the biological behavior of the CRC towards metastasis and poor patient survival. We herein discuss the mechanisms and significance of these induced inflammatory-associated microsatellite alterations, and suggest three content areas to further examine interventions that may modify the observed behavior of these CRCs.

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INTRODUCTION

The Cancer Genome Atlas (TCGA) for colorectal cancers (CRCs) clarified that there are two types of sporadic CRCs - hypermutated and non-hypermutated. Most hypermutated CRCs have a defect in their mismatch repair (MMR) system due to the loss of MLH1 function by promoter silencing of the *MLH1* locus, resulting in high levels of insertion/deletion (I/D) mutations at microsatellite loci (microsatellite instability high: MSI-H)[1]. Most MSI-H CRCs exhibit proximal location, mucinous, undifferentiated histology, abundant $CD8^+$/Th1 T cell infiltrations, and less aggressive clinical behavior, and are susceptible for immune checkpoints blockade[2,3]. Among non-hypermutated CRCs, I/D mutations in microsatellite loci with larger repeat units (di- and tetra-nucleotide repeats) are frequent and have been shown to be caused by tumor cells' exposure to inflammatory tumor-microenvironments[4,5]. In this review, we describe and discuss the penetrance and causes of inflammation-associated microsatellite alterations (IAMAs), and their significance to patients' prognoses in CRC. We also raise "Provocative Questions" whose answers could contribute not only to understand the biology of IAMAs but also to treatment of CRC with IAMAs.

MSI-H, MSI-L AND EMAST IN CRC

Microsatellites or simple sequence repeats are composed of 1-6 nucleotide repeats, occupy 3% of the total human genome, and are located in both coding and non-coding regions[6]. MSI is defined as continuous length changes in simple DNA repeat sequences within microsatellite loci[7]. MSI in CRC was first reported by Aaltonen *et al*[8] and Thibodeau *et al*[9] followed by Ionov *et al*[10] in 1993. It was then shown that a subset of sporadic CRC tumors and tumors from hereditary nonpolyposis colon cancer (HNPCC) exhibit MSI and MMR-defects[11]. Subsequently, germline mutations in *MSH2, MLH1, PMS2 and MSH6* were found in different HNPCC families[12-18] and tumors from these families exhibited MSI[19,20]. A causal relationship between MMR-defect, MSI and cancer susceptibility was shown by knockout mouse studies[21-24]. Genetic complementation studies using tissue cultured MSI-positive CRC cells also confirmed that MSI is caused by MMR-deficiency in human cells[25-27]. It was also shown that MSI exhibited in 10%-15% of sporadic CRC cases was due to transcriptional down-regulation of *MLH1* expression through promoter hyper-methylation[28].

MSI in CRCs was defined at an international workshop meeting sponsored by the National Cancer Institute in 1998[2]. A panel of five microsatellite markers - two markers with mononucleotide repeats and three markers with dinucleotide repeats - were validated to be classified as follows: High-frequency MSI (MSI-H: 2 or more of 5 markers show instability), low-frequency MSI (MSI-L: 1 of 5 markers shows instability), and microsatellite stable (MSS: none of 5 shows instability) CRCs. It was also confirmed that MSI-H in CRC is caused by defective MMR, mainly MSH2 and MLH1, and manifests as sporadic and hereditary forms of CRCs. Both sporadic and inherited MSI-H CRCs have unique clinical and pathological futures compared to MSI-L/MSS sporadic CRCs[2]. At this NIH meeting, the presence of CRCs with MSI-L was appreciated and discussed. However, the etiology of MSI-L and the distinction between MSI-L and MSS CRC remained unclear. Another type of microsatellite alteration, called elevated microsatellite alterations in selected tetra-nucleotide repeats (EMAST), where insertion/deletion mutations in the loci with tri- and/or tetra-nucleotide but not with mono- and/or dinucleotide repeats was recognized as a component of CRC but its etiology and clinic-pathological significance was not determined[2].

Although a consensus on the definition of MSI-L CRC was reached at the NCI meeting, two subsequent studies showed that approximately 80% of non-MSI-H CRC exhibited mutation at < 1 microsatellite locus when a large number of the loci with di-nucleotide repeats were tested for frame-shift mutations, indicating

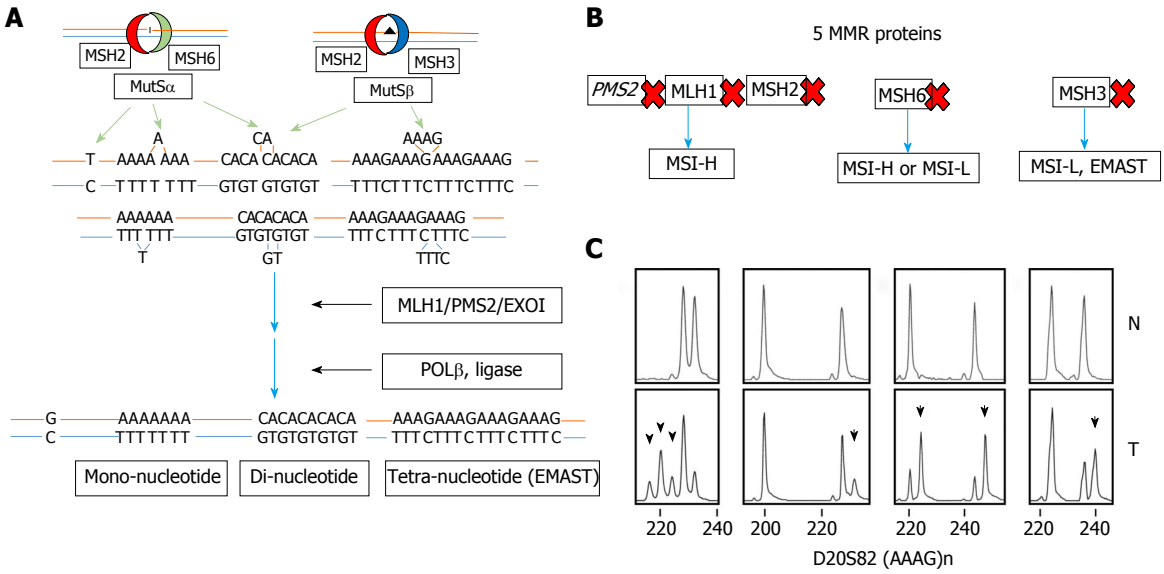

Figure 1 Human DNA mismatch repair. A: Two DNA recognition complexes MutSα, which recognizes insertion-deletion (I/D) loops of 1-2 repeated nucleotides for repair, and MutSβ which recognizes I/D loops of 2 or greater nucleotides for repair, are the key protein complexes of MMR. The MLH1 and PMS2 complex, also known as MutLα, then helps execute the repair with the exonuclease Exo1, polymeraseβ and DNA ligase to fully effect repair; B: Specific efficiency in one of the five DNA MMR proteins yields differing microsatellite instability (MSI) results. Loss of MLH1, MSH2 or PMS2 will yield frameshifts at mono-, di- and tetra-nucleotide microsatellite markers. Loss of MSH6, inactivating MutSα only, will yield mononucleotide mostly but some dinucleotide microsatellite frameshifts, whereas loss of MSH3, inactivating MutSβ, will yield di- and tetranucleotide microsatellite frameshifts, but no mononucleotide microsatellite frameshifts; C: Examples of fragment analysis comparing normal colon tissue (upper panels) with tissue (lower panels) demonstrating frameshifts in the tetranucleotide marker D20S82. MMR: Mismatch repair; MSI: Microsatellite instability; CRC: Colorectal cancer.

that most of CRC is MSI-L, and that the NCI reference panel was inadequate for detection of MSI-L CRC[29,30]. These studies also showed that there were no genetic or clinic-pathological characteristics of tumors to separate MSI-L from MSS CRC. However, both studies observed that the incidence of MSI was non-randomly distributed among non-MSI-H CRC, suggesting that some tumors were more susceptible than others to slippage mutations at microsatellite loci, especially loci with dinucleotide repeats[29,30]. The reason for the observed variation in instability and its pathological significance in patients' prognoses remained unclear.

I/D mutations in loci with selected tetra-nucleotide repeats (EMAST), such as $(AAAG)_n$ or $(ATAG)_n$, have been reported in non-CRCs including non-small cell lung, bladder, ovary, head and neck, skin and kidney cancers[31]. Haugen *et al*[32] first described the frequency of EMAST in CRC, its relationship to MSI-L and its possible cause. They used the five NCI-endorsed MSI markers plus 2 additional markers with dinucleotide repeats to identify MSI-H, MSI-L and MSS CRC. They also used 7 EMAST markers and defined EMAST-positive if one or more of the 7 markers showed ID mutations[32]. They found that EMAST is common in sporadic cases of non-MSI-H CRC (approximately 50%) and is associated with decreased nucleus MSH3 expression in tumor cells. Using MSH3-proficient and -deficient colon cancer cell lines, they also showed evidence that EMAST and low levels of instability at dinucleotide loci repeats - but not with mononucleotide repeats - in non-MSI-H CRC cells are caused by loss of MSH3[32]. Frequent incidence of EMAST in CRCs was confirmed by 2 other studies[33,34]. The genetic cause of EMAST due to the loss of MSH3 was also proven by other studies using tissue cultured human cells[35,36].

BIOCHEMICAL BASIS OF MICROSATELLITE ALTERATIONS

Accumulated evidence supports that MSI-H, MSI-L and EMAST are caused by defects in some components of MMR[37]. When DNA polymerase copies template DNA containing microsatellite loci, it mistakenly adds or deletes a repeat unit in the newly synthesized DNA strand (Figure 1A). The DNA polymerase slippage errors create loops between the two strands, which are recognized and repaired by MMR. *In vitro* experiments using cell extracts and/or purified proteins demonstrate that there are 5 MMR proteins involved in MMR reactions in human cells (Figure 1)[38]. MSH2 plays a major role in recognition of mismatched DNA. MLH1 and PMS2 are the main proteins responsible for down-stream MMR reactions. If MSH2, MLH1 or PMS2 lose their function, slippage errors at microsatellite loci with mono-, di- and tetra-nucleotide repeats are not fixed at all, resulting in MSI-H (Figure 1B). There are 2 pathways for mismatch recognition: (1) MSH2 and MSH6 form a dimer called MutSα that preferentially recognizes mismatched nucleotides and loops containing 1-2 nucleotides; (2) MSH2 and MSH3 form a dimer called MutSβ that recognizes loops containing 2 or more nucleotides generated at di- and tetra-nucleotide repeats, including the EMAST loci (Figure 1A)[39]. Defects in MSH6 result in increased

Table 1 Expression of MSH3 protein within the epithelium of normal colonic mucosa and adenoma of patients with mono- or bi-allelic germline mutation in *MSH3*

Tissue (epithelium)	Monoallelic *MSH3* germline mutation	Bi-allelic *MSH3* germline mutation
Normal colonic mucosa	MSH3 expressed	MSH3 absent
Colon adenoma	Not obtained	MSH3 absent

Extracted from Adam *et al*[42].

missense mutations and in instability at mononucleotide repeats (Figure 1A)[40]. When only MSH3 is disabled, increases in instability at di-, tri- and tetra-nucleotide repeats (EMAST) but not at mononucleotide repeats are observed (Figure 1)[32]. Biochemical data indicates that loops containing 2 nucleotides are preferentially recognized by MutSβ over MutSα[41]. Thus, when loss of MSH3 leaves many loops containing 2 or more nucleotides unrepaired, MutSα may repair some but not all such loops, resulting in low levels of mutation in di-nucleotide repeat loci and high levels of mutation in loci with tetra-nucleotide repeats (EMAST) loci (Figure 1B and C).

MSI-L AND EMAST ARE CAUSED BY MSH3 FUNCTIONAL LOSS IN CRC

The first evidence that loss of MSH3 may result in MSI-L and/or EMAST in CRC was reported by Haugen *et al*[32] in 2008. They used the colon cancer cell line HCT116 that is deficient in MLH1 due to a hemizygous inactivating mutation in exon 9, and is also deficient in MSH3 due to a homozygous frameshift inactivating mutation in exon 7. Thus, this cell line showed the MSI-H phenotype. Introduction of a normal human chromosome 3 carrying a wild-type *MLH1* to HCT116 complemented MLH1-deficiency[25]. The resulting HCT116 with chromosome 3 exhibited stability in loci with mononucleotide repeats but showed low levels of instability at loci with dinucleotide repeats: MSI-L, and high degree of instability at EMAST loci. They further introduced a normal human chromosome 5 carrying wild-type MSH3 into HCT116 + 3 cells. The resulting HCT116 + 3 + 5 cells exhibited complete stability at loci with mono-, dinucleotide repeats and EMAST loci. Finally, they introduced MSH3-shRNA to HCT116 + 3 + 5 cells to knock-down MSH3 and showed that specific knock-down of MSH3 resulted in an MSI-L/EMAST phenotype.

The second evidence that loss of MSH3 results in MSI in loci with di- and tetra-but not mono-nucleotide repeats is from a discovery of two families with bi-allelic MSH3 germ-line mutations, reported by Adam *et al*[42]. Patients with bi-allelic inactivation mutations of the MSH3 locus suffered from a colorectal adenoma polyposis syndrome and early occurrence of multiple adenoma polyps and tumors in other organs. As expected, the expression of MSH3 was null in normal colon and adenoma polyps from these patients (Table 1). MSI assays showed that instability at di-nucleotide repeat loci and EMAST loci, but not loci with mononucleotide repeats, was detected in adenoma polyps but not in normal colon cells from the same patient. This is because adenoma is monoclonal while the normal colon of these patients consists of mixture of cells with MSI at different loci, masking each alteration that occurred in individual colon cells with the exception of germline alleles. However, there is likely dinucleotide and tetranucleotide instability within normal tissues if they were compared to heterozygous *MSH3* germline relatives, or relatives that are homozygous normal for *MSH3* mutation. These results support that MSI-L/EMAST in sporadic CRC is caused by loss of MSH3 function.

EVIDENCE THAT MSI-L/EMAST IN SPORADIC CRC IS INDUCED BY INFLAMMATION THROUGH DISPLACEMENT OF MSH3 FROM NUCLEUS TO CYTOPLASM

While homogeneous loss of nuclear MSH3 can be detected in adenoma polyp with bi-allelic germline *MSH3* mutations, heterogeneous loss of nuclear MSH3 is frequently detected in sporadic CRC exhibiting MSI-L/EMAST (Figure 2A). These results suggest that local loss of MSH3 expression in sporadic MSI-L/EMAST CRC may be not due to genetic loss of MSH3. TCGA data shows that the frequency of *MSH3* somatic mutations in CRC is about 6.6%. This does not explain the high incidence of MSI-L/EMAST (approximately 50%) in CRC. Furthermore, most *MSH3* mutations are frame-shift mutations in exon 7 that are a resulting target from *MLH1* inactivation in sporadic CRC (Table 2)[1].

Lee *et al*[34] found that EMAST CRC is enriched in in the tumor microenvironment of $CD8^+$ T cells compared to non-EMAST CRC, suggesting that some immunological and inflammatory responses are active in EMAST CRC. They also found that EMAST is significantly high in ulcerated tumors. Devaraj *et al*[43] further showed that EMAST-positive rectal tumors are associated with the presence of chronic inflammation. These observations led them to hypothesize that inflammation may somehow affect MSH3 function that induces MSI-L/EMAST.

Tseng-Rogenski *et al*[4,36] demonstrated that several main inflammatory factors, including oxidative stress (hydrogen peroxide), interleukin 6 (IL6) and prostaglandin E2 (PGE2) induce displacement of MSH3 from the nucleus to the cytoplasm in several

Table 2 Comparison of type of mismatch repair gene mutations between sporadic hypermethylated *MLH1* colorectal cancers and *POLE* mutation colorectal cancers from TCGA

MLH1 promoter hypermethylation	22/35 (63%) of hypermutated CRCs	8/22 (36%) with MSH3 frameshift mutation
		1/22 (4.5%) with MSH3 missense/nonsense mutation
		0/22 (0%) with MSH2 mutation
		5/22 (23%) with MSH6 frameshift mutation
		4/22 (18%) with MSH6 missense/nonsense mutation
POLE mutation	13/35 (37%) of hypermutated CRCs	3/13 (23%) with MSH3 frameshift mutation
		2/13 (15%) with MSH3 missense/nonsense mutation
		5/13 (38%) with MSH2 missense/nonsense mutation
		0/13 (0%) with MSH6 frameshift mutation
		7/13 (54%) with MSH6 missense/nonsense mutation

Both types of CRCs are hypermutated, containing hundreds of somatic mutations in genomic DNA. Note that the *MLH1* hypermethylated CRCs demonstrate higher frequency and consistent frameshift mutations in *MSH3* and *MSH6* as compared to *POLE* mutated CRCs, which contain some frameshifts but higher frequency of missense/nonsense mutations in *MSH3*, *MSH2* and *MSH6*. Extracted from: Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. *Nature* 2012; **487**: 333-337. CRCs: Colorectal cancers.

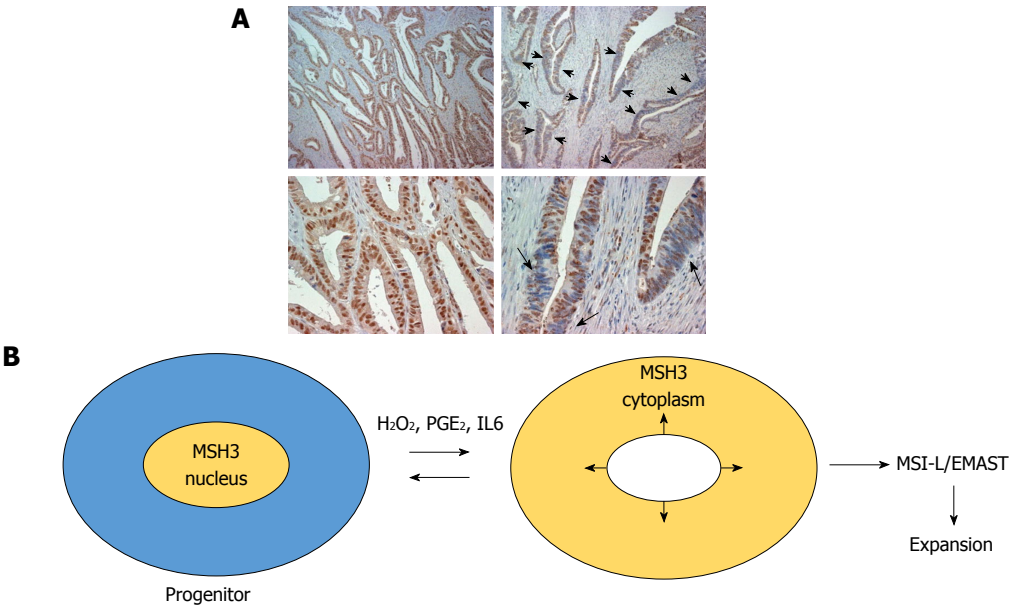

Figure 2 MSH3 expression in sporadic colorectal cancer. A: Immunohistochemistry for MSH3 in sporadic CRC. Arrows show heterogeneous expression of MSH3 in cells and within nuclei in the epithelium; B: Model of MSH3 displacement from the nucleus to the cytosol with inflammatory stimuli to allow accumulation of tetranucleotide frameshift mutations. Progenitor cells could be affected earlier such that subsequent daughter cells amplify the accumulated frameshift mutations. MSI: Microsatellite instability; CRC: Colorectal cancer; EMAST: Elevated microsatellite alterations at selected tetranucleotide repeats.

cancer cell lines. Importantly, other MMR proteins including MLH1, MSH2 and MSH6 do not move from the nucleus to the cytoplasm in response to these stimuli. Repeated treatment of several microsatellite stable colon cancer cell lines with IL6 induced microsatellite instability at EMAST loci. However, other inflammatory cytokines including TNFα, IFNα, IFNβ, and IL1β did not have such an effect. Tseng-Rogenski *et al*[4] also showed that phosphorylation of STAT3 may be required for displacement of MSH3 when induced by IL6. These studies convincingly show that not all, but some, inflammatory factors induce EMAST through loss of MSH3 from the nucleus (Figure 2B).

Evidence that an inflammatory micro-environment induces MSI-L (low levels of MSI at the loci with dinucleotide repeats) has been shown in regenerated colon tissues from ulcerated colitis (UC) patients. The first study, reported by Brentnall *et al*[44], showed for the first time the presence of MSI-L but not MSI-H in colon tissues from UC patients. The second study, by Ozaki *et al*[45], isolated crypts from UC-derived CRC, UC-derived hyperplasia and UC-regenerated colons through laser micro-capture and tested for the presence of microsatellite instability in DNA. Ozaki *et al*[45] detected MSI-L but not MSI-H in some crypts but not in others, regardless of whether they were from cancer or non-cancer tissues. They also showed that MSI was not detected from stroma cells from these UC patients. Each crypt showed a different MSI-profile, indicating that MSI-L occurs independently at the crypt level. Our recent study showed that regenerated colon cells and CRCs from UC patients have a high frequency of MSH3 displacement from the nucleus to the cytoplasm, and demonstrate MSI-L/EMAST[46].

These results further support the role of inflammation in displacement of MSH3-induced MSI-L/EMAST in human tissues including cancers.

PROGNOSTIC VALUE OF MSI-L/EMAST IN CRC

Several studies have examined the impact of MSI-L and/or EMAST genotypes on patient prognoses in CRC. There have been 4 studies evaluating the prognosis values of MSI-L[47-50]. Kohonen-Corish *et al*[47] showed that patients with stage C colon cancers defined as MSI-L by the NCI panel plus one tetra-nucleotide marker (*MYCL1*) showed poor overall survival (OS) compared to patients with MSI-H and/or MSS colon cancers. Similar results were obtained by Wright *et al*[48]. They showed that stage C CRC patients that are positive for MSI-L as defined by the NCI panel, plus an additional 2 markers with mono-nucleotide repeats, 3 with di-nucleotide repeats and the tetra-nucleotide *MYCL1* marker, exhibited poor cancer-specific survival compared to MSS CRC patients[48]. They also observed that most MSI-L CRC exhibited MSI at one di- or tetra-nucleotide but not at mono-nucleotide repeat markers[48]. Lee *et al*[49] examined 3019 CRC cases for MSI using an NCI microsatellite marker panel and evaluated prognoses of those patients. Similar to other studies, they showed that most MSI-L CRC exhibited MSI at dinucleotide repeats, and patients with MSI-L CRCs was associated with poor OS by Cox regression analysis[49]. Although the previous 2 studies suggested that MSI-L may have a significant prognostic value for stage C CRC patients, Lee *et al*[49] did not examine the prognostic significance of MSI-L for cancer-specific survival in their large cohort. In contrast to the above three studies, Azzoni *et al*[50] reported that MSI-L is associated with improved patient survival as compared to MSS CRC. However, the percentage of MSI-H cases in their cohort was unusually high (37%: 68 of 184 cases) compared to other studies (10%-15%), suggesting the presence of some bias in the studied cohort. Lastly, a study reported by Garcia *et al*[51] did not find any association between MSI-L and disease-free survival (DFS) or OS in stage Ⅱ and Ⅲ CRC cohorts.

There are 2 studies examining the relationship between EMAST and OS in CRC; they found no association between the two[33,51]. However, when both MSI-L and EMAST cases were combined, Garcia *et al*[51] found that MSI-L/EMAST was associated with shorter DFS but not OS compared with non-MSI-L/EMAST CRC. In their cohort, MSI-H CRC patients exhibited the highest survival. Thus, the MSI-L/EMAST genotype in CRC may be associated with recurrence and/or metastasis after surgery. There appears to be heterogeneity even among MSI-L/EMAST CRC patients[52,53]. One group of MSI-L/EMAST CRC exhibited loss of heterozygosity (LOH) at chromosome 9p24.2. and the other did not exhibit 9p24.2 LOH. When the prognoses of these two groups were compared, the one with 9p24.2 LOH at stage Ⅲ showed improved survival after surgery and OS in Kaplan-Meier analysis and in multi variate analysis over the one without 9p24.2. LOH at stage Ⅲ[53]. The results also showed that MSI-L/EMAST/9p24.2 LOH is an independent factor that predicts improved OS in stage Ⅱ/Ⅲ CRC. Thus, MSI-L/EMAST may be associated with recurrence, but additional genetic or epigenetic changes may modify the behavior of recurrent tumors[53]. Overall, the data presented so far suggest that MSI-L and/or EMAST could be a biomarker for DFS and/or OS of stage Ⅱ and/or Ⅲ CRC. However, additional studies using a population-based large cohort are needed to confirm the prognostic value of MSI-L and EMAST.

One concern regarding EMAST is that various studies have not reached a full consensus on the definition of EMAST. As described above, current evidence supports the idea that MSI-L and EMAST in sporadic CRC share the same etiology: both are induced by the absence of nuclear MSH3 in response to exogenous inflammatory factors such as IL6, and oxidative stress[4]. Based on these observations, we propose that EMAST cancer is a non-MSI-H cancer, and MSI at EMAST markers is not caused by loss of other MMR proteins including MLH1, MSH2, PMS2[51,53]. The next question should be whether or not non-EMAST CRC really exits. Similar to MSI-L in CRC[29,30], almost all CRC could be EMAST-positive if a large number of EMAST markers are used. A recent study by Cortes-Ciriano *et al*[54] showed that all non-MSI-H cancers contain various levels of frame-shift mutations in microsatellite loci with mono-, di-, tri- and tetra-nucleotide repeats. Considering that all tumor tissues contain some degree of inflammatory elements, many of those mutations could be induced by the loss of MSH3 triggered by inflammation in the tumor-microenvironment. Furthermore, a study for UC suggested that frequent exposure to inflammation increased the incidence of MSI-L and EMAST[46]. Thus, while the purpose of the MSI assay is primarily to detect MMR-deficient CRC, the purpose of an EMAST assay could be to distinguish CRCs whose precursors were exposed to high levels of inflammation to CRCs whose precursors were exposed to lower levels of inflammation. Therefore, the results of the studies by Kohonen-Corish, Wright, Lee and Garcia could be re-interpreted according to the idea that high levels of inflammatory tumor-microenvironments not only induce MSI-L/EMAST in cancer cells at the primary site but also include some property that promotes recurrence and/or metastasis when they disseminate. Additional studies will be required to determine whether the numbers and kinds of EMAST markers and cut-off levels for determining EMAST-positive/negative used so far are adequate to distinguish CRCs with different prognoses[31].

PROVOCATIVE QUESTIONS

Here, we have raised three questions whose answers can be important for not only clinical but also basic

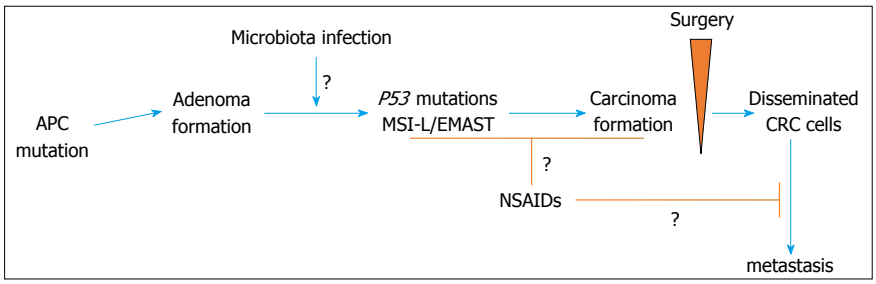

Figure 3 Model of adenoma-to-carcinoma formation in the human colon, with actual and potential sites of interventions to improve survival. MSI: Microsatellite instability; CRC: Colorectal cancer; EMAST: Elevated microsatellite alterations at selected tetranucleotide repeats.

aspects of MSI-L/EMAST in CRC (Figure 3).

Question 1: Does treatment of CRC with non-steroidal anti-inflammatory drugs reduce not only recurrence/metastasis but also the incidence of MSI-L/EMAST?

The idea that inflammation is associated with recurrence and/or metastasis is indirectly supported by observations that an intake of the anti-inflammatory drug, aspirin, may not only prevent adenomas[55] and CRC formations[56], but also prevent recurrence and metastasis of CRC following surgery[57]. Other non-steroidal anti-inflammatory drugs (NSAID) including celecoxib and rofecoxib, specific inhibitors of cyclooxygenase 2 (COX-2), have been shown to reduce the incidence of adenomas[58-60]. But it was also found that COX-2 inhibitors suppressed colorectal tumor growth and metastasis in mouse models[61,62]. Furthermore, Chan *et al*[63] reported that the regular intake of aspirin after curative surgery reduced cancer-specific mortality in a sub-group of CRC cells expressing a high level of COX-2 protein. In addition, CRCs expressing HLA class Ⅰ compared to those not expressing HLA class Ⅰ are susceptible for aspirin treatment after diagnosis[64]. Also, CRCs with *PIK3CA* mutations responded better to aspirin treatment after diagnosis than did CRCs with wild-type *PIK3CA*[65]. However, a recent study by Gray *et al*[66] showed that the efficacy of aspirin on cancer-specific survival, and OS was associated with levels of COX-2 expression but not with mutational status of *PIK3CA* in CRCs. Ng *et al*[67] showed that aspirin and COX-2 inhibitors improved recurrence-free survival, DFS and OS of stage Ⅲ CRC patients who either received fluorouracil (FU) plus leucovorin (LV) or FU plus LV with irinotecan. These studies support the idea that NSAIDs can be used as part of adjuvant therapy for stage Ⅰ-Ⅲ CRC, however, the efficacy of NSAIDs on the recurrence/metastasis of CRC are still under investigation through several randomized controlled trials[57].

Ma *et al*[68] showed that PGE2 and its receptor, the prostaglandin E receptor 2 (EP2), are necessary for colon cancer formation in inflammatory tissue environments. Compared to wild-type mice treated with azoxymethan (AOM) followed by dextran sodium sulfate (DSS), AOM/DSS-treated EP2-knockout and prostaglandin E synthase (*Ptges*)-knockout mice bore a significantly reduced number of colon tumors. They identified neutrophil, probably myeloid-derived suppressor cells (MDSC), and cancer-associated fibroblast (CAF) as the main cell components recruited in tumor-microenvironments, expressing EP2, responding to PEG2, and contributing to tumor formation. These cells form a positive-feedback loop of COX-2-PGE2-EP2-NF-κB-COX-2 cycles, and produce TNF-α and IL6[68]. The presence of MDSC and CAF in the tumor-microenvironment are also significantly associated with stage progression and a poor prognosis for CRC, while activation of Th1 helper and cytotoxic memory T cells play a key role in anti-tumor activities preventing recurrence and/or metastasis in CRC[69,70]. Interestingly, Zelenay *et al*[71] showed that, depending on the level of COX-activity in cancer, the immunological landscape of tumor-microenvironments can be switched between anti-tumor and inflammatory pro-tumor. Therefore, the level of PEG2 and of COX-2 may be major factors in controlling immunological responses to cancer cells, and thereby a patient's prognosis. Regarding the relationship between MSI-L/EMAST and PEG2, we have observed that the exposure of colon cancer cells in tissue cultures to PGE2 triggers movement of MSH3 from the nucleus to the cytoplasm, which may induce MSI-L/EMAST. Therefore, it is reasonable to speculate that MSI-L/EMAST in CRC may be associated with high levels of COX-2 expression in cancer cells and/or in tumor-microenvironments. This could be the reason why patients with MSI-L/EMAST CRCs exhibit a shorter RFS[51,53]. Thus, reduction of PGE2 by NSAIDs may reduce the incidence and recurrence/metastasis of MSI-EMAST. If this is the case, MSI-L/EMAST could be a biomarker for susceptibility to the NSAIDs treatment.

Question 2: Do microbiota play a role in MSI-L/EMAST formation, adenoma/carcinoma transition and recurrence/metastasis?

Lee *et al*[34] discovered that EMAST is less frequent in colorectal adenomas and well-differentiated adenocarcinomas than in moderately differentiated and poorly differentiated adenocarcinomas, suggesting that EMAST is progressively acquired during the histological adenoma-carcinoma sequence, from adenoma to well-differentiated carcinomas to moderately and poorly differentiated carcinomas. Because a key gene alteration responsible for adenoma-carcinoma sequence

in CRC is *p53* mutation[72], MSI-L/EMAST formation may be associated with *p53* mutation. In fact, Ahrendt *et al*[73] reported that EMAST is associated with *p53* mutations in non-small cell lung cancer. Li *et al*[74] observed an association between LOH at *TP53* and EMAST in CRC. Interestingly, *p53* mutations are the most frequently found in inflammatory bowel disease (IBD)-associated CRC among other gene mutations (60%-90%)[75,76]. One half of the *p53* mutations are C:G>T:G transitions, thought to be caused by nitric oxide exposure due to increased inducible nitric oxide synthase expression in IBD[75]. Our preliminary data showed that IBD-associated CRC exhibit a higher frequency of MSI-L/EMAST than do sporadic CRC (unpublished data). Taken together, these results suggest that the inflammatory tissue environment may enhance *p53* mutations and MSI-L/EMAST formation in sporadic adenomas, leading to carcinoma transition. As mentioned earlier, MSI-L/EMAST in stage Ⅱ CRC patients is associated with shorter RFS, suggesting that the inflammatory tumor-environment in primary tumor tissues somehow promotes recurrence or metastasis. These observations lead to the next question: What establishes an inflammatory environment in colorectal adenoma and carcinoma?

Microbiota in the colon and rectum create an inflammatory microenvironment and promote CRC formation[77]. Several bacterial organisms including *Fusobacterium nucleatum* (*F. nucleatum*), Enterotoxigenic *Bacteroides fragilis* (*ETBF*), and colibactin-producing *Escherichia coli* (*E. coli*) are epidemiologically associated with CRC, and have been found to be enriched in CRC[77,78]. The enrichment of *F. nucleatum* was also found in colorectal adenoma relative to non-adenoma or surrounding tissues[79-81]. McCoy *et al*[79] showed that *F. nucleatum* abundance in colorectal adenoma is associated with local inflammatory cytokine gene expression including IL-10 and TNF-α. Kostic *et al*[80] investigated the effect of *F. nucleatum* infection on the development of intestinal tumors in *APC*$^{Min/+}$, IL10$^{-/-}$ and T-bet$^{-/-}$ X Rag2$^{-/-}$ mice. There was an increase in the number of tumors in *APC*$^{Min/+}$ mice. Importantly, infection with *F. nucleatum* accelerated adenocarcinoma formation in the small intestines of *APC*$^{Min/+}$ mice compared to sham-treated control mice. In contrast, infection with *F. nucleatum* did not induce any tumor formation in IL10$^{-/-}$ and T-bet$^{-/-}$ X Rag2$^{-/-}$ mice. These results suggest that the effects of *F. nucleatum* may manifest on existing adenomas, and may stimulate adenoma-carcinoma transition by creating an oxidative stress-rich, carcinogenic environment[80]. It would be interesting to determine whether *F. nucleatum* -induced adenocarcinomas in *APC*$^{Min/+}$ mice gain *p53* mutations. Kostic *et al*[80] further showed that infection of tumor tissues with *F. nucleatum* results in recruitment of MDSCs, tumor-associated macrophages, and dendritic cells in tumor tissues, and modulate the tumor immune micro-environment that promote tumor progression. In addition, they found the up-regulation of genes that are down-stream of NF-κB including *PTGS2 (COX-2), IL6, IL1β*, and *TNF* in both human and mouse CRC infected with *F. nucleatum*[80]. It is tempting to speculate that *F. nucleatum*-induced adenocarcinoma may gain MSI-L/EMAST in response to oxidative stress, PEG2 and/or IL6 that cause displacement of MSH3 from the nucleus to the cytoplasm. Recently, a heavy load of *F. nucleatum* has been associated with MSI-H CRC, proximal colon cancer and a poor prognosis[81-84]. Yu *et al*[85] showed that *F. nucleatum* infection in primary CRC is associated with recurrence after surgery followed by adjuvant chemotherapy. They showed that *F. nucleatum* induces chemo-resistance in infected cells through autophagy[85]. One of the reasons why 5-FU-based adjuvant therapy does not have benefit for a sub-group of MSI-H CRC[86,87] could be partly explained by the infection of *F. nucleatum*[85]. It is also possible that the CpG Island Methylator Phenotype (CIMP) including promoter methylation of the *MLH1* locus could be induced by chronic inflammation due to a heavy load of *F. nucleatum* infection[82]. Considering that infection of *F. nucleatum* is associated with recurrence of CRC after surgery, a group of such CRCs may exhibit MSI-L/EMAST CRC[51,53].

Another bacterium, *ETBF*, is also associated with CRC[88-90] and can target colorectal cells to promote an adenoma and/or adenoma-carcinoma transition in *APC*$^{Min/+}$ mice[91]. *ETBF* produces a metalloprotease toxin called BFT. BFT binds to the surface of colorectal epithelial cells and induces E-cadherin cleavage, resulting in an increase in barrier permeability and inducing an inflammatory micro-environment with Th-17/IL-17 predominance[91,92]. Th-17/IL-17 plays a major role in *ETBF* tumorigenesis because the depletion of CD4$^+$ T cells and blockade of IL-17 inhibited it. IL-17 attracts neutrophils, MDSCs and macrophages, and induces carcinogenic and immunosuppressive factors including nitric oxide, ROS, and Arg1 in mouse models[92]. Colibactin-producing *E. coli* is also associated with CRC[93,94] and initiates inflammation and promotes adenoma formation in *APC*$^{Min/+}$mice[94] and in *APC*$^{Min/+}$, IL10$^{-/-}$ mice[95]. Taken together, infection with all three bacterial organisms, that are found to be associated with CRC, induces an inflammatory environment in adenoma tissue and promotes adenoma and/or a transition from adenoma to carcinoma in mouse models. It would be interesting to determine whether MSI-L/EMAST and *p53* mutations coincide with bacterial-induced transitions to adenoma/carcinoma. Recently, Scott *et al*[96] showed that the efficacy of 5-FU treatment maybe largely influenced by microbiota in the gut.

Question 3: Is MSH3 a component of DNA damage signaling?

The MutSβ hetero-duplex between MSH3 and MSH2 not only functions in MMR but may also play a role

in double strand break (DSB) repair *via* homologous recombination (HR)[97-100]. DNA double strand breaks (DSB) induce cell death if not repaired. Cells have evolved two pathways to re-connect the broken DNA ends: Non-homologous end joining (NHEJ) and homologous recombination (HR). If one of these pathways is disabled when DSB is created, cells use the other pathway for survival. The HR reaction starts with a nuclease-mediated resection of broken DNA ends to be coated by the single stranded (ss) DNA-binding protein, replication protein A (RPA). Then, Ataxia telangiectasia and Rad3-related (ATR) kinase is recruited to the RPA-coated ssDNA *via* an ATR-interacting partner (ATRIP). The topoisomerase Ⅱβ-binding protein 1 (TOPBP1), which is recruited to the DSB site, interacts with ATRIP and activates ATR. Activated ATR phosphorylates CHEK2 that regulate cell cycle progression. TOPBP1 also interacts with polo-like kinase (PLK) that phosphorylate RAD51 for its loading on resected ssDNA[101]. Burdova *et al*[99] showed that recruitment of ATR/ATRIP to RPA-coated ssDNA is mediated by MutSβ which binds to the loop structure formed within the ssDNA. Therefore, MutSβ is required in the early stage of HR-DSB repair and its loss due to an MSH2 or MSH3 defect forces a cell to use NHEJ for survival under the presence of DSBs[98,100]. Thus, when oxidative stress causes DSBs, it may induce elimination of MSH3 from the nucleus, resulting in activation of Ataxia-telangiectasia mutated (ATM)[102] but not ATR, and dependence of NHEJ for survival.

An intriguing question is why and how nuclear MSH3 proteins translocate in response to oxidative stress or exposure to IL6 or PGE2 (Figure 2B)[4,36]. H_2O_2 and oxidative stress causes DSBs, resulting in activation of NF-κB[103,104]. IL6 and PGE2 are mediators that possibly form a loop associated with activation of NF-κB through STAT3[105-107]. IL6 activates STAT3, which directly interacts with the NF-κB family member RELA, contributing to constitutive NF-κB activation[105], and COX2/PGE2 also activates STAT3, leading to NF-κB activation[106]. We found that MSH3 itself is a shuttling protein. It contains a bona fide bipartite nuclear localization signal (NLS) that directs its nuclear import to perform DNA repair (unpublished data). It also contains two functional nuclear export signals (NESs) that allow it to exit the nucleus upon the treatment of a pro-inflammatory cytokine, IL-6 (unpublished data). Among the other main MMR proteins including MSH2, MSH3 is the only MMR protein that shifts into the cytoplasm upon oxidative stress or IL6 treatment, suggesting that MSH3 moves alone or does so with other unknown partner proteins. Recent data indicate that the NF-κB Essential Modulator (NEMO), when used as a bait, can pull down MSH3, suggesting physical interaction between these two proteins[108]. As one of the three components of the IKK complex, NEMO's role in regulating the NF-κB pathway is well documented[104]. It is possible that simultaneous or sequential movement of MSH3, NEMO and ATM in the cell may transmit a DNA damage signal to NF-κB, depending on the degree of DNA damage. Further studies are necessary for clarify these possibilities.

MSI-L/EMAST IS COMMON IN HUMAN CANCERS

Since the discovery of MSI in CRC, MSI-L and EMAST have been examined in cancers from other organs and tissues. MSI-L has been found in stomach[109], cervical[110], pancreatic[111], ovarian[112], skin[113], nerve[114], breast, endometrial[115], liver[116], esophageal[117], eye[118], soft tissue[119], gallbladder[120], head and neck[121], prostate[122], lung[123] and cancers of the urinary tract[124]. EMAST has also been widely detected in other various human cancers[31]. A recent study by Cortes-Ciriano *et al*[54] showed that there are MSI-H prone cancers including colorectal, esophageal, stomach and endometrial cancers, and non-MSI-H prone cancers that include ovarian, kidney, liver, breast, head and neck, cervical, lung, pancreatic, bladder, prostate, skin, adrenal, cortical and thyroid cancers. They also showed that most non-MSI-H cancers exhibit different degrees of MSI at not only loci with mono- but also loci with di-, tri- and tetra-nucleotide repeats, suggesting that inflammation-induced MSH3 replacement from the nucleus to the cytoplasm is probably common in human cancers[54,125,126]. Thus, the answers to the provocative questions raised above may also apply to many human cancers.

CONCLUSION

MSI-L/EMAST is common in human cancers. MSI-L/EMAST is caused by displacement of MSH3 from the nucleus to the cytoplasm in replicating cells triggered by inflammatory stimuli, and can be termed Inflammatory-Associated Microsatellite Alterations (IAMAs). MSI-L/EMAST is associated with recurrence and/or metastasis in CRC patients. MSI-L/EMAST CRC is a heterogeneous group and consists of sub-groups with different genetic changes and prognoses.

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REVIEW

Advance in plasma *SEPT9* gene methylation assay for colorectal cancer early detection

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Abstract

This review article summarizes the research advances of the plasma-based *SEPT9* gene methylation assay for the clinical detection of colorectal cancer and its limitations. Colorectal cancer is a common malignancy with a poor prognosis and a high mortality, for which early detection and diagnosis are particularly crucial for the high-risk groups. Increasing evidence supported that *SEPT9* gene methylation is associated with the pathogenesis of colorectal cancer and that detecting the level of methylation of *SEPT9* in the peripheral blood can be used for screening of colorectal cancer in susceptible populations. In recent years, the data obtained in clinical studies demonstrated that the *SEPT9* gene methylation assay has a good diagnostic performance with regard to both sensitivity and specificity with the advantage of better acceptability, convenience and compliance with serological testing compared with fecal occult blood tests and carcinoembryonic antigen for colorectal cancer (CRC). Furthermore, the combination of multiple methods or markers has become a growing trend for CRC detection and screening. Nevertheless, the clinical availability of the methylated SEPT9 assay is still limited because of the large degree of sample heterogeneity caused by demographic characteristics, pathological features, comorbidities and/or technique selection. Another factor is the cost-effectiveness of colorectal cancer screening strategies that hinders its large-scale application. In addition, improvements in its accuracy in detecting adenomas and premalignant polyps are required.

Key words: Plasma; *SEPT9*; Methylation; Colorectal cancer; Early detection

Core tip: The methylated *SEPT9* gene has been implicated as a biomarker for colorectal cancer associated with the pathogenesis of colorectal cancer (CRC). In this article, we reviewed the literature on the correlation of *SEPT9* gene and colorectal cancer and the theoretical basis of the *SEPT9* gene methylation assay. Then, we focused on the diagnostic performance of the *SEPT9* gene methylation assay for CRC by analyzing the clinical trial studies and compared that assay with other methods. Finally, we discussed the limitations of the *SEPT9* gene methylation assay in clinical application. We hope that this article can provide a comprehensive overview of the progress achieved in the *SEPT9* methylation assay for both the basic and clinical sciences.

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INTRODUCTION

Colorectal cancer (CRC) is one of the most common malignant tumors of the digestive system and results in significant morbidity and mortality. As it is estimated, there were approximately 135430 new cases of colorectal cancer, including men and women, in 2017[1]. The incidence is higher in men than women and markedly increases with age[2]. CRC kills almost 700000 people every year, making it the world's fourth deadliest cancer (after lung, liver and stomach cancers)[3]. As research has shown, the incidence and mortality rates of CRC vary up to 10-fold worldwide, with distinct gradients across human development, pointing towards widening disparities and an increasing burden in countries in transition[4]. In general, its incidence and mortality rates are still rising rapidly in many low-income and middle-income countries.

The initial symptoms of colorectal cancer, however, are atypical, leading to a poor prognosis and high fatality rate. Therefore, screening of CRC in the population is of great significance for its early diagnosis and treatment. Currently, CRC screening approaches are divided into two categories: Invasive and noninvasive methods. The invasive methods, such as colonoscopy, remain the main screening tools due to their very good diagnostic performance, enabling the detection and removal of precancerous lesions[5]. However, it requires thorough bowel preparations. Additionally, discomfort and privacy infringement contribute to poor compliance among patients. Non-invasive screening approaches, which include fecal occult blood tests (FOBT), fecal immunochemical tests (FITs) and carcinoembryonic antigen (CEA), are more easily acceptable. However, their effectiveness may not be guaranteed. Although various guideline-recommended methods are available for CRC detection, patient compliance remains low. The data in 2013 showed that only approximately 57% of eligible adults adhered to the screening recommendations provided by the United States Preventive Services Task Force[6]. Thus, it is very important to develop an efficient approach to enhance patient compliance that can be applied to screening the general population.

Studies[7-9] have shown that the DNA methylation of certain genes is closely related to the development of colorectal cancer. Beggs *et al*[10] verified that methylation changes contribute substantially to the progression from normal mucosa to adenoma and to carcinoma; for instance, GRASP, which encodes the general receptor for phosphoinositide 1-associated scaffold protein, was differentially methylated in colorectal cancer. Aberrant DNA methylation in the genome may contribute to malignant transformation by silencing multiple tumor-suppressor genes. This type of epigenetic alteration is believed to occur early in tumor development and may precede genetic changes[11]. In recent years, *SEPT9* gene methylation has been recognized as a hotspot and is considered to be a specific biomarker of the early stages of colorectal cancer. It may be a reliable indicator for screening CRC among high-risk individuals. This paper reviews the progress in the plasma-based *SEPT9* gene methylation assay for the detection of colorectal cancer.

SEPT9

As we know, there are 14 members (SEPT1-SEPT14) in the SEPT gene family, whose protein products Septins are a series of highly conserved GTP binding protein family. In humans, there are 13 genes, respectively named SEPT1 to SEPT13; the *SEPT9* gene is located on the human chromosome 17q25. 3[12], contains 17 exons, and spans 240 × 10^3 bp. The 5'-end regulatory regions of the *SEPT9* gene have a -C- phosphor -G- site (CpG island), which is the main site of DNA methylation. In mammals, 60%-90% of CpG sites are methylated, and most of the remaining unmethylated residues are clustered in CpG islands within functional gene promoters[13]. It has been shown[12,14,15] that SEPT 9 has 18 distinct transcripts encoding 15 polypeptides, with two transcripts (SEPT9_v4 and v4*) encoding the same polypeptide.

SEPT9 GENE AND COLORECTAL CANCER

In recent years, growing evidence has shown that the *SEPT9* gene is associated with malignant tumors. Peterson *et al*[16] used immunoprecipitation and immunofluorescence studies to analyze SEPT9_i1 and found that it interacts with both α and γ tubulin.

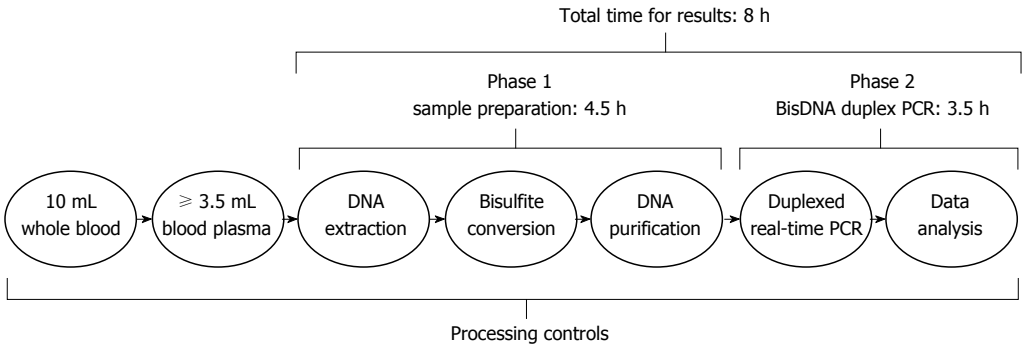

Figure 1 The outline of the Epi proColon work flow. The test consists of the Epi proColon Plasma Quick kit, PCR kit, and Control kit. The total assay time is approximately 8 h. For the Plasma Quick kit, 3.5 mL of plasma was mixed with an equal volume of lysis buffer; after incubating for 10 min, magnetic beads and absolute ethanol were added. After 45 min, impurities were removed from the magnetic beads by centrifugation; the purified DNA was then released from the beads in the elution buffer and treated at 80 ℃ with a solution of ammonium bisulfite for deamination of cytosine[34]. After a series of washing steps, the converted DNA (bisulfite-modified DNA, bisDNA) was captured by magnetic beads. The bisDNA was assayed with the PCR kit on a Duplexed Real-Time PCR device. Finally, methylated SEPT9 and PCR results were recorded by the instrument software. In the whole working flow, the processing controls were included to monitor the execution of the procedure and ensure the validity of the test result and model[34].

SEPT9_i1-expressing cells demonstrated dramatic chromosome segregation defects, centrosome amplification and cytokinesis defects, which indicates that SEPT9_i1 increases genomic instability in the process of tumorigenesis through two potential molecular mechanisms: defective chromosome segregation and cytokinesis failure. Additionally, expression of HIF[17], JNK[18] and Rho signaling pathways[19] may also be potential mechanisms of colorectal cancer development in which the *SEPT9* gene is involved.

SEPT9 gene encodes a protein called septin-9, which is part of a group of proteins called septins. Septins are involved in various biological processes such as division of cytoplasm, cell polarization, vesicle transport and membrane reconstruction. The septin-9 protein also appears to act as a tumor suppressor, which means that it regulates cell growth and prevents cells from dividing too fast or in an uncontrolled way[20]. When the methylation occurs at a CpG island, genes with high levels of 5-methylcytosine in their promoter region are transcriptionally silent[21]; DNA methylation gradually accumulates on long-term silenced gene and may result in the inactivation of cancer suppressor genes. Tóth *et al*[22] have found that *SEPT9* mRNA expression decreased from adenoma to dysplasia to carcinoma in the progression of colon neoplastic disease, which presents a strong significant correlation of *SEPT9* methylation with the mRNA's low expression in CRC. Thus, downregulation of *SEPT9* mRNA and the decrease in *SEPT9* expression may account for the pathological progression from benign to malignant lesions in colon tissues.

PLASMA *SEPT9* GENE METHYLATION ASSAY

Methods of the SEPT9 gene methylation assay

Due to epigenetic silencing of the *SEPT9* gene by promoter methylation in plasma, the company Epigenomics AG first studied SEPT9 methylation based on the SEPT9 biomarker available in Europe in 2008[23]. After one year, a commercial kit was finalized and the first generation of the CE-marked Epi proColon real-time PCR kit was launched. This CE-marked IVD (*In vitro* Diagnostic) kit became publicly available in Europe in 2010. Currently, the second generation of the assay is commercially available as the Epi proColon 2.0 assay[24].

In general, The Epi proColon test is an *in vitro* diagnostic PCR method for the qualitative detection of SEPT9 DNA methylation levels in plasma derived from patients' whole blood specimens (Figure 1). To perform the test, approximately 10 mL of whole blood is a source of sufficient plasma for the analysis. The testing cycle performed with the current manual workflow takes approximately 8 h. As a first step, a minimum of 3.5 mL of blood plasma is isolated from the blood sample. Then, the Epi proColon 2.0 test consists of two phases. In Phase Ⅰ, DNA is extracted from the plasma fraction and treated with bisulfite-conversion reagents and purified to obtain highly purified DNA[25]. In Phase Ⅱ, the test detects the hyper-methylated v2 region of the SEPT 9 gene and a region of the ACTB (β-actin) gene as an internal control by duplex real-time PCR[26]. Finally, the Epi proColon 2.0 test only reports qualitative positive and negative results. A positive test is indicative of an increased likelihood for having CRC and a colonoscopy is recommended as a follow-up for diagnostic evaluation.

Diagnostic performance of plasma SEPT9 gene methylation

Increasingly, studies[27-30] are suggesting that the methylation status of SEPT9 is a reliable index for screening CRC. For evaluating its diagnostic performance, we have collected several research results in which the sensitivity and specificity are key indicators. Table 1

Table 1 Sensitivity and specificity of the *SEPT9* gene methylation assay for colorectal cancer detection

Publications	Number of cases	Sensitivity	Specificity	Algorithm	Assay used	Ref.
Tóth *et al* (2012)	184 (92 CRC, 92 no evidence of disease)	95.6% (95%CI: 89.2%-98.8%)	84.8% (95%CI: 75.8%-91.4%)	1/3	Epi proColon 2.0	[27]
		79.3% (95%CI: 69.6%-87.1%)	98.9% (95%CI: 94.1%-100%)	2/3		
Church *et al* (2014)	1516 (53 CRC, 1457 without CRC)	48.2% (95%CI: 32.%-63.6%)	91.5% (95%CI: 89.7%-93.1%)	1/3	Epi proColon 1.0	[31]
Potter *et al* (2014)	1544 (44 CRC, 1500 non-CRC)	68.0% (95%CI: 53%-80%)	80.0% (95%CI: 78%-82%)	-	Epi proColon 1.0	[34]
Su *et al* (2014)	234 (172 CRC, 62 controls)	88.4%	93.5%	-	MSP-DHPLC	[28]
Johnson *et al* (2014)	301 (101 CRC, 200 non-CRC)	73.3% (95%CI: 63.9%-80.9%)	81.5% (95%CI: 75.5%-86.3%)	-	Epi proColon 1.0	[32]
Jin *et al* (2014)	476 (135 CRC, 341 non-CRC)	74.8% (95%CI: 67.0%-81.6%)	87.4% (95%CI: 83.5%-90.6%)	2/3	Epi proColon 2.0	[29]
Ørntoft *et al* (2015)	300 (150 CRC, 150 controls)	73.0% (95%CI: 64%-80%)	82.0% (95%CI: 75%-88%)	1/3	Epi proColon 1.0	[33]
Sharif *et al* (2016)	90 (45 CRC, 45 controls)	84.4%	99.0%	-	MS-HRM assay	[52]
Wu *et al* (2016)	1031 (291 CRC, 740 non-CRC)	73.0%	97.5%	-	Epi proColon 2.0	[30]
		76.6% (95%CI: 71.3%-81.4%)	95.9%	-	New SEPT9 assay	
Nian *et al* (2016)	25 studies, 9927 samples (2975 (CRC, 6952 non-CRC)	71.0% (95%CI: 67%-75%)	92.0% (95%CI: 89%-94%)	2/3	Epi proColon 2.0	[35]

CRC: Colorectal cancer.

shows the data from clinical trials using the *SEPT9* gene methylation assay published since 2012.

From the table, it can be seen that the plasma *SEPT9* gene methylation assay exhibited a high overall sensitivity and specificity for CRC detection. Moreover, with the improved method used in the subsequent studies, especially after the application of the second-generation SEPT9 methylation assay (Epi proColon 2.0, Epigenomics AG, Germany), the detection sensitivity increased from approximately 48.2%-73.3%[31-34] to approximately 71.0%-95.6%[27,29,30,35], while the specificity improved from 80.0%-91.5% to 84.8%-98.9%. Meanwhile, Wu *et al*[30] reported that the new SEPT9 assay, with enhanced technical simplicity and a lower cost, presented a sensitivity of 76.6% and a specificity of 95.9%, which not only did not differ in performance compared with Epi proColon 2.0 but also reduced the complexity of the testing process and appeared to be a simpler, cheaper, more efficient, convenient, and user-friendly alternative for CRC screening. Additionally, methylation of SEPT9 detected by MSP-DHPLC (methylation-specific polymerase chain reaction (PCR)-denaturing high-performance liquid chromatography)[28] shows that the sensitivity and specificity are as high as 88.4% and 93.5%, respectively, which also appears to be a useful biomarker in a clinical laboratory setting. Tóth *et al*[36] measured the positive predictive value and negative predictive value, which reached up to 93.8% (30/32) and 84.6% (22/26), respectively, supporting the reliability of this assay for CRC detection. Nian *et al*[35] also estimated an area under the curve (AUC) of 0.88 and diagnostic odds ratio of 27 (95%CI: 18-42) using a bivariate mixed effect model. Furthermore, Ørntoft *et al*[33] found that the clinical sensitivity for CRC stages Ⅰ-Ⅳ was 37%, 91%, 77%, and 89%, respectively. In comparison, Jin *et al*[29] described that methylated SEPT9 was positive in 66.7% of stage Ⅰ (12/18), 82.6% of stage Ⅱ (19/23), 84.1% of stage Ⅲ (37/44), and 100% of stage Ⅳ (5/5) cases in 90 cases of CRC whose stages were identified based on the surgically resected specimens. The results indicate that advanced stage CRCs are more easily detected by SEPT9 methylation than the early stage. Although the sensitivity and specificity reported in Table 1 come from different studies, leading to the variation in the ability to detect CRC, these results are still comparable because the majority of studies used Epi proColon products as the commercialized tests, and multiple PCR reactions are performed in all of these studies, which determine the final test result.

As for the test performance of other non-invasive CRC detection approaches, according to retrospective case control studies[27,31,37,38], the FOBT identifies individuals with CRC with a sensitivity between 33% and 79% and a specificity between 87% and 98%. Another recent case control study by Tóth *et al*[27] showed that the FOBT was positive in 29.4% (5/17) of NED (no evidence of disease) and 68.2% (15/22) of CRC and that elevated CEA levels were detected in 14.8% (4/27) of NED and 51.8% (14/27) of CRC. Both the FOBT and CEA showed a lower sensitivity and specificity than SEPT9 (95.6% and 84.8%). In addition, Lee *et al*[39] reported that the sensitivity was as high as 79% (95%CI: 69%-86%) for FIT for CRC with a specificity of 94% (95%CI: 92%-95%) by meta-analysis, which is at the same level as SEPT9[27]. Johnson *et al*[32] obtained estimates of 68.0% (95%CI: 58.2-76.5%) for the sensitivity and 97.4% (95%CI: 94.1%-98.9%) for the specificity of FIT, and drew the

conclusion that the sensitivity of the Epi proColon test was statistically comparable to FIT by analyzing the paired samples. A study by Song *et al*[40] also showed that the SEPT9 assay exhibited significantly higher sensitivity than the FIT test (75.6% *vs* 67.1%, $P < 0.05$) in pooled data of the symptomatic population. In general, compared with these other CRC detection tests, the *SEPT9* gene methylation assay shows a good diagnostic performance in both sensitivity and specificity with the advantage of better acceptability and compliance of serological testing.

Hence, the promoter hyper-methylation analysis of plasma SEPT9 DNA has the potential to serve as a non-invasive screening method for the identification of specific biomarkers, enabling early detection of CRC in a large population. This approach holds promise for increased accuracy, safety, affordability, and patient compliance[41].

Combined detection of the SEPT9 assay with other colorectal cancer detection tests

The combination of multiple methods or markers has become an increasing trend in CRC detection and screening. A recent study conducted by Wu *et al*[30] demonstrated that the combination of SEPT9 + FIT had a high sensitivity for CRC detection (94.4%), and the sensitivity of combined examination of SEPT9 + FIT + CEA was 97.2% (76.6%, SEPT9 alone). Another study[42] found that the sensitivity of joint examination of SEPT9 and FIT in CRC diagnosis was 97.8% (80.0%, SEPT9 alone) and that the specificity was 52.9%, whereas the advanced adenoma diagnosis was 67.6% (10.8%, SEPT9 alone) and 47.4%, respectively, which suggested that the combination of the SEPT9 and FIT assays not only significantly enhanced the sensitivity for CRC detection but also increased the positive detection rate for advanced adenoma. In the study of Yu *et al*[43], it was seen that the under-ROC curve area of SEPT9 with CEA and FOBT for CRC detection reached 0.935. Furthermore, other than the tests mentioned above, SEPT9 may be combined with other existing biomarkers for CRC detection, such as glycoprotein markers or other methylation markers[12]. A study published by Tänzer *et al*[44] demonstrated the combined analysis of methylation status of SEPT9 and ALX4 to be highly significant in the detection of colorectal polyps with a sensitivity and specificity reaching 71% and 95%, respectively, indicating the potential use of the combined methods in detecting advanced precancerous colorectal lesions. However, further studies are still required to evaluate the effect of combined biomarker assays on CRC detection and screening.

Limitations of the SEPT9 methylation assay

Although the plasma-based SEPT9 methylation assay performs well with regard to both sensitivity and specificity, its clinical availability is still limited. As we can see in Table 1, there is a large degree of heterogeneity among studies, which may be due to many causes, especially the impacts of non-tumor-related factors on DNA methylation, such as aging, sex, race, hormone levels, dietary factors[45], lifestyle factors (smoking and alcohol consumption)[46], and other environmental exposure factors. Song *et al*[47] found a high PDR (positive detection rate) of SEPT9 methylation in normal subjects and cancer patients over 60 years, which may reflect increased *SEPT9* gene methylation levels with age. Additionally, the increased false negative rate of the SEPT9 assay is associated with diabetes, arthritis and arteriosclerosis ($P < 0.05$)[33], which can explain why the diagnostic performance of the SEPT9 assay varies compared to previous retrospective case-control studies. Nevertheless, not enough is known to approximate the effect of demographic characteristics, pathological features and/or comorbidities on the results of the SEPT9 methylation assay. Moreover, using a 2/3 algorithm test has a high true negative rate, although its sensitivity was higher with a 1/3 algorithm test[35]. On account of the capability of excluding non-cancer samples and avoiding the rate of misdiagnosis, the 2/3 algorithm is recommended for CRC detection. Therefore, the technique and method selection could also affect the laboratory results and lead to heterogeneity. Further studies should pay more attention to examining the variation in diagnostic accuracy and validating potential confounding factors affecting DNA methylation status, in the design of future experimental studies. These non-neoplastic factors should be taken into consideration when evaluating DNA methylation to avoid the influence those caused on the testing results.

The cost-effectiveness is another limitation that limited large-scale application of the SEPT9 methylation assay. It was reported[48] that the methylated SEPT9-based strategies were not a cost-saving with the costs of $8400 to $11500 per quality-adjusted life-year gained in comparison with established screening strategies including FOBT, FIT, and colonoscopy. The current cost of the methylated SEPT9 test in Europe is approximately 150 Euros, considerably more than fecal tests[31]. In brief, FIT dominated methylated SEPT9 and was preferred among all of the alternatives[49,50]. Even so, the biomarker for colorectal cancer screening still offers potential benefits over current methods, but in order to realize its full potential, the plasma-based assay will need to be acceptable to clinicians and patients compared to current technologies and the medical environment. As the emerging SEPT9 methylation assay becomes available clinically, the decision over whether to adopt it will require weighing its costs, utilization and longitudinal adherence against the alternative of putting efforts into improving current screening strategies. At the population level, methylated SEPT9 yielded incremental benefit at acceptable costs when it increased the fraction of the population screened more than it was substituted for other strategies[48]. Thus, screening costs, utilization,

adherence, and follow-up are the influential determinants of the cost-effectiveness of colorectal cancer screening strategies.

Moreover, the capability of the *SEPT9* gene methylation assay for detecting adenomas, which is the most common precancerous lesion of CRC, is limited. For early stage CRC (Stage Ⅰ), polyps or adenomas, methylated SEPT9 alone presented quite low sensitivity with approximately 35%[25], 20%[51] and 11.2%[31], respectively, indicating that this biomarker may be far from sufficient and effective at screening asymptomatic CRC patients, despite the diagnostic value of detecting advanced stage CRCs (Ⅲ-Ⅳ). With the transformation of the medical pattern, the focus of hygiene work is switching to prevention rather than curing. Thus, the detection of precancerous or early stage colorectal cancer is very crucial for the health workers to identify high-risk groups and to provide an accurate early diagnosis. Still, this assay faces significant challenges nowadays when introduced for detecting early pre-invasive pathological changes, such as adenomas and premalignant polyps. On the one hand, there is plenty of room for improvement in the method of the methylated SEPT9 assay itself, such as amelioration of DNA isolation or enhancement of PCR efficiency. On the other hand, the combination of the SEPT9 assay with other markers in CRC detection is at its initial stage, in spite of the detection rate increasing to 37%[44] by applying an additional methylation marker like ALX4, but further research is still needed to evaluate the effect of joint detection and to explore its possibility, for the sake of improving the sensitivity for detection of early cancers and advanced adenomas. More studies on early-stage CRC are expected in the future.

FUTURE PERSPECTIVES

Taken together, the use of the plasma-based methylated biomarker *SEPT9* gene should be the alternative approach for CRC screening due to greater diagnostic performance, convenience, and compliance in comparison with non-serological methods. The methylated SEPT9 assay showed relatively high pooled sensitivity, whereas it was also affected by many factors, leading to the high level of heterogeneity. Future clinical diagnostic studies of methylation in blood should consider the impacts of these factors, especially non-neoplastic factors (*e.g.*, aging, sex, lifestyle, coexistent disease, methodology) on diagnostic accuracy. Moreover, the cost of the SEPT9 methylation assay is still much higher than the FOBT and FIT. And further investigation of early CRC is still required, as a result of its sensitivity for the asymptomatic population in the screening setting still not being satisfactory, but improvements in accuracy can be expected as the diagnostic technology evolves.

In the future, deciphering epigenetic information including DNA methylation and applying it to the selection of appropriate detection methods and the development of relevant therapy is likely to transform the diagnosis and treatment of colorectal cancer, consequently decreasing mortality.

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MINIREVIEWS

Vitamin D in esophageal cancer: Is there a role for chemoprevention?

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Abstract

Vitamin D has emerged as a promising anti-cancer agent due to its diverse biological effects on tumor differentiation, apoptosis and suppression of cellular proliferation. Current evidence suggests a protective role of vitamin D in colon cancer. The effect of vitamin D on esophageal cancer remains controversial. Multiple studies investigated the association between vitamin D and esophageal cancer, employing different modes of assessment of vitamin D status such as serum 25-hydroxyvitamin D levels, vitamin D dietary intake or exposure to ultraviolet B (UVB) radiation. Genetic variations of the vitamin D receptor (VDR) gene and VDR expression in esophageal specimens have also been investigated. Ecological studies evaluating exposure to UVB radiation yielded an inverse correlation with esophageal cancer. When vitamin D dietary intake was assessed, direct association with esophageal cancer was observed. However, circulating 25-hydroxyvitamin D concentrations showed inconsistent results. In this review article, we present a detailed summary of the current data on the effects of vitamin D on various histological subtypes of esophageal cancer and their precursor lesions. Well-powered prospective studies with accurate measurement of vitamin D status are needed before chemoprevention with vitamin D is recommended, as current evidence does not support a chemopreventive role of vitamin D against esophageal cancer. Future studies looking at the incidence of esophageal cancer in patients with pre-cancerous lesions (Barrett's esophagus and squamous cell dysplasia) receiving vitamin D supplementation are needed.

Key words: Vitamin D; Vitamin D receptor; Esophageal adenocarcinoma; Esophageal squamous cell carcinoma; Genetic polymorphism

Core tip: Vitamin D has emerged as a promising anti-cancer agent due to its diverse biological effects on tumor differentiation, apoptosis and suppression of cellular proliferation. Ecological studies evaluating exposure to ultraviolet B radiation yielded an inverse correlation with esophageal cancer. When vitamin D dietary intake was assessed, direct association with esophageal cancer was observed. However, circulating 25-hydroxyvitamin D concentrations showed inconsistent results.

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INTRODUCTION

« Sol est remediorum maximum » (The sun is the best remedy)--Pliny, the Elder.

This remark, attributed to Pliny, exemplifies the healing properties of sunlight known since ancient times[1]. The fact that most of the beneficial effects of sunlight are mediated by vitamin D came to light by experimental studies on Rickets in the 1930s[1,2]. Epidemiologic research in the 1980s showed that incidence and death rates for certain cancers were lower among individuals with higher exposure to sunlight[3]. Researchers hypothesized that variation in vitamin D levels might account for this association. Since then, laboratory studies elucidated several antineoplastic properties of vitamin D such as its role in promoting cellular differentiation, decreasing cancer cell growth, stimulating apoptosis, and inhibiting angiogenesis[4,5].

Vitamin D appears to have a protective role in colorectal and breast cancers but confirmatory data for cancers of other organs such as prostate or esophagus remains lacking[6-9]. Esophageal cancer is a major public health concern due to increasing incidence and poor survival rates after diagnosis. Numerous studies investigated the association between vitamin D status and esophageal cancer with inconsistent results.

The aim of this review is to present the available scientific evidence for the role of vitamin D in esophageal squamous cell cancer (ESCC), esophageal adenocarcinoma (EAC) and their precursor lesions-squamous cell dysplasia and Barrett's esophagus (BE) respectively.

LITERATURE SEARCH

A PubMed search of all studies published in English from 2006 to 2016 was performed. Medical subject headings (MeSH terms) used were "vitamin D", "calcitriol", "vitamin D receptor", "sun", "sunlight", "esophageal neoplasm", "esophageal adenocarcinoma", "Barrett's esophagus", and "esophageal squamous cell carcinoma". References of relevant articles were also reviewed and selected.

VITAMIN D METABOLISM AND ANTI-CANCER PROPERTIES

The two main sources of vitamin D are diet and solar radiation. Provitamin D in the skin is converted to previtamin D by ultraviolet B (UVB) radiation, which is then converted to vitamin D3 (cholecalciferol) through isomerization. Vitamin D3 is hydroxylated in the liver to form 25-hydroxycholecalciferol [25(OH)D_3]. Another hydroxylation reaction occurs in the kidneys, where 25(OH)D_3 is converted to the biologically active form 1α,25(OH)$_2D_3$ (calcitriol), involved in bone and calcium metabolism[4]. Calcitriol also regulates its own catabolic cascade: it induces the expression of the CYP24A1 gene, which encodes the 24-hydoxylase enzyme. The latter converts 25(OH)D_3 and 1α,25(OH)$_2D_3$ to the less active metabolites 24,25(OH)D_3 and 1α,24,25(OH)$_3D_3$ respectively. This is the rate-limiting step of vitamin D catabolism[4].

Calcitriol, thought to be the metabolite involved in the anticancer properties of vitamin D, binds to the vitamin D receptor (VDR). The calcitriol-VDR complex binds to the retinoid X receptor (RXR), forming the heterodimer VDR-RXR, which translocates to the nucleus and binds to the vitamin D response element (VDRE) on a particular gene, with subsequent transcription and translation of various proteins, including the ones involved in the vitamin D anti-carcinogenic properties, *i.e.*, anti-proliferation, apoptosis, differentiation, and angiogenesis inhibition[4,5] (Figure 1). Calcitriol inhibits proliferation by inducing cell cycle arrest at the G0/G1 phase. Cyclins and cyclin-dependent kinase inhibitors regulate cell cycle progression and induce G1 cell-cycle arrest. Interestingly, cyclin-dependent kinase inhibitor 1A contains a VDRE, which accounts for the anti-proliferative effects of vitamin D[4,10]. Apoptosis is another key mechanism in inhibiting carcinogenesis. Calcitriol induces the expression of pro-apoptotic proteins and activates caspase, a cysteine protease that mediates apoptosis. In addition to its apoptotic and anti-proliferative effects, vitamin D inhibits angiogenesis. In prostate cancer, vitamin D interrupts signaling of an angiogenic factor, interleukin 8, leading to decreased endothelial cell migration and possibly metastasis[4].

Osteopontin and E-cadherin are two proteins induced by vitamin D with antagonistic growth regulatory activity. While osteopontin promotes cellular invasion[11], E-cadherin suppresses cell growth by inhibiting the transcriptional activity of β-catenin, a protein that induces genes involved in promoting cell growth and proliferation[12]. In colon adenocarcinoma, for instance, E-cadherin is preserved as opposed to low osteopontin levels[13]. Subsequently, high levels of calcitriol would

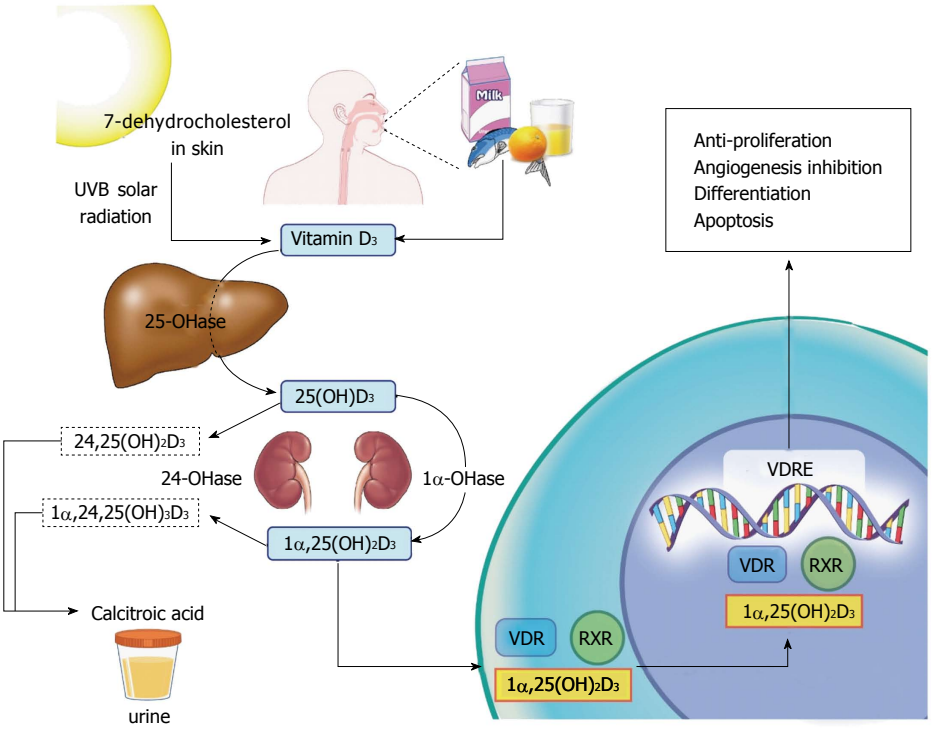

Figure 1 Vitamin D metabolism and anti-cancer properties. UVB: Ultraviolet B radiation; VDR: Vitamin D receptor; RXR: Retinoid X receptor; VDRE: Vitamin D response element; 25(OH)ase: 25-hydroxylase; 24(OH)ase: 24-hydroxylase; 1α (OH)ase: 1α-hydroxylase; 25(OH)D3: 25-hydroxyvitamin D; 1α,25(OH)2D3: 1α,25-dihydroxyvitamin D; 1α,24,25(OH)2D3: 1α,24,25-trihydroxyvitamin D; 24,25(OH)2D3: 24,25- dihydroxyvitamin D.

lead to further E-cadherin-induced tumor suppression with low osteopontin levels, and subsequent cell growth inhibition[14].

From an immunologic perspective, multiple cells are known to be involved in EAC and its precursor lesions, BE and reflux esophagitis: In addition to the dendritic cells and CD4 T cells, signaling pathways involved include NFk-B, Wnt and Hegdehog pathways. Immunologically, the role of vitamin D in esophageal cancer remains inconclusive and unclear. For instance, vitamin D was shown to inhibit the Hedgehog signaling cascade which is overexpressed in BE. Similarly dendritic cells, increased in BE and EAC, are maintained in an immature form by vitamin D. On the other hand, BE is characterized by a Th2-predominant response and data suggests that 1α,25-hydroxyvitamin D promotes the Th2 response. In addition, vitamin D was shown to increase interleukin-4 cytokine production, which has been implicated in BE. In view of these multiple contradictory effects on neoplastic progression, the role of vitamin D in esophageal cancer needs to be evaluated[15].

MODES OF ASSESSMENT OF VITAMIN D STATUS

Serum concentration of vitamin D seems to be the most accurate indicator of a patient's vitamin D status and is usually monitored to treat vitamin D deficiencies. More than 50 vitamin D metabolites have been identified over the past years but only two gained particular attention: 1α,25(OH)2D3 and 25(OH)D3. While 1α,25(OH)2D3's half-life is around 4 h and levels are widely dependent on an individual's calcium needs, 25(OH)D3 has a half-life of around 3 wk, reflecting more accurately a patient's vitamin D stores, and therefore widely accepted as an indicator of an individual's vitamin D status[16]. The normal levels are considered to be 10-68 ng/mL (24.9-169.5 nmol/L) with different cut-offs in various assays and laboratories[17]. Sunlight is a major contributor to vitamin D status. Many studies attempted to validate different UVB exposure questionnaires and found correlations ranging between 0.16 and 0.4 for vitamin D serum concentration and reported UVB exposure[18-21]. Correlations noted were not strong however, raising the hypothesis that sun exposure alone does not explain serum vitamin D levels[19]. Multiple studies also used dietary vitamin D intake as a surrogate of vitamin D status and showed a good correlation between dietary vitamin D intake and serum vitamin D levels. This correlation could be stronger for instance, in wintertime, when exposure to UVB radiation is reduced[19].

Taking vitamin D dietary intake, lifetime UVB exposure and vitamin D serum concentrations into account seems to be the most accurate method to assess an individual's vitamin D status[19]. As a matter of fact, Giovannucci *et al*[22] built a predictor score to assess long-term vitamin D status using multiple determining factors of vitamin D exposure including dietary and

Table 1 Studies investigating correlations between vitamin D and esophageal squamous cell dysplasia and carcinoma

Ref.	Study design/location	Vitamin D exposure/status/genetics studies	Statistical correlation
Abnet *et al*[27]	Cross-sectional study China	25-hydroxyvitamin D serum level	RR = 1.86, 95%CI: 1.35-2.62
Chen *et al*[28]	Prospective study China	25-hydroxyvitamin D serum level	ESCC in men: HR = 1.77, 95%CI: 1.16-2.70
Lipworth *et al*[29]	Case-control study Italy	Vitamin D dietary intake	ESCC: OR = 0.58, 95%CI: 0.39-0.86
Tran *et al*[30]	Case-control study Australia	Ultraviolet B radiation	ESCC: No association
Wang *et al*[24]	Case-control study China	Genetic polymorphisms	ESCC: No association

ESCC: Esophageal squamous cell carcinoma; OR: Odds ratio; RR: Relative risk; HR: Hazard ratio; CI: Confidence interval.

supplementary vitamin D intake, geographic residence, race, physical activity and body mass index[22].

On another note, two genome wide association studies of vitamin D levels have been conducted and common genetic variants of genes involved in vitamin D metabolism pathways were identified[23,24]. Subsequently, multiple single nucleotide polymorphisms (SNPs) were investigated in an attempt to find correlations with esophageal cancer[24,25].

ESOPHAGEAL CANCER AND VITAMIN D

Esophageal cancer encompasses two histological subtypes: ESCC and EAC, which differ epidemiologically, by risk factors and outcomes. ESCC is the most common esophageal cancer worldwide with an increased incidence in developing countries. Esophageal squamous dysplasia is the histologic precursor of ESCC. Developed countries witness a higher prevalence of EAC[26], which is commonly related to chronic acid reflux exposure, with BE being the main risk factor for EAC. Potential associations of vitamin D have been investigated in both histological subtypes of esophageal cancer as presented below.

Esophageal squamous cell dysplasia

Only one study based on the Linxian population in China investigated the role of vitamin D in esophageal squamous cell dysplasia and found a linear association between vitamin D levels and development of squamous dysplasia: 230 out of 724 patients had esophageal squamous dysplasia. Patients diagnosed with esophageal squamous dysplasia had higher median levels of 25(OH)D$_3$ levels compared to controls (36.5 nmol/L *vs* 31.5 nmol/L, $P = 0.0004$)[27].

Esophageal squamous cell carcinoma

Three studies evaluated vitamin D status and ESCC with diverging results depending on mode of assessment of Vitamin D status[28-30] (Table 1). While one study in China concluded a direct correlation between ESCC and measured serum 25(OH)D$_3$ concentrations[28], another study conducted in Italy noted an inverse association between increased dietary vitamin D intake and ESCC[29]. The third one, done in Australia, found no association between ESCC and lifetime UVB radiation exposure[30].

The study from China was population-based and included 2018 participants, out of which 545 developed ESCC, with an overall trend towards higher concentrations in serum 25(OH)D$_3$ in those who developed cancers. Multivariate analysis demonstrated increased risk with higher 25(OH)D$_3$ values (4th quartile hazard ratio (HR): 1.30, 95%CI: 0.97-1.73, $P = 0.013$). When stratified by gender, ESCC risk remained increased in men with higher vitamin D levels (4th quartile HR = 1.77, 95%CI: 1.16-2.70, $P = 0.003$) but not in women. These conclusions could not be extrapolated to other populations due to overall low vitamin D levels and high rate of exposure to polycyclic aromatic hydrocarbons in this study population, with the latter factor placing them at higher risk for neoplasia[28]. It is worthwhile noting however, that pre-neoplastic lesions with squamous cell dysplasia were also found to have an E-cadherin/osteopontin disequilibrium, with E-cadherin suppression and osteopontin up-regulation leading to increased risk of cell growth, proliferation and subsequently malignant transformation with higher calcitriol levels[14].

The study from Italy was a case-control study with 304 patients and investigated the association between dietary vitamin D intake over the prior two years and ESCC[29]. In ESCC patients, an inverse relationship was noted between vitamin D intake and esophageal neoplasia. The highest tertile corresponded to > 3.5 μg/d with a risk reduction of around 40% compared to lowest tertile (< 2.51 μg/d).

The last case-control study from Australia assessed UVB exposure and prevalence of ESCC. No relationship was observed between lifetime UVB radiation and ESCC (OR = 0.94, 95%CI: 0.82-1.09) in contrast to EAC and esophago-gastric junction adenocarcinoma[30].

An association between SNPs in the genes involved in vitamin D pathway and ESCC was also evaluated: Wang *et al*[24] investigated 12 SNPs in four genes known to be part of the vitamin D pathway: vitamin D binding protein, 7-dehydrocholesterol reductase, 25-hydroxylase and 24-hydroxylase or CYP24A1.

SNPs related to vitamin D levels were not found to be associated with ESCC risk.

The rate-limiting step of vitamin D synthesis was also investigated in regards to ESCC. In one study of 42 patients with esophageal cancer of which 39 had ESCC, CYP24 gene expression was assessed by semi-quantitative RT-PCR assay. Cases with lower CYP24 expression (n = 25) had significantly higher survival rate compared to patients with increased CYP24 expression (n = 17, P < 0.05), making of CYP24 a "candidate oncogene" that might serve as a biomarker of increased ESCC risk[31].

Barrett's esophagus

Vitamin D dietary intake and supplementation have been studied with regards to BE. An Irish study evaluated the association between vitamin D intake assessed *via* food questionnaires, among patients with BE (n = 224), reflux esophagitis (n = 230) and EAC (n = 227), compared to 260 healthy controls[32]. Vitamin D intake was not found to be associated with reflux esophagitis or BE. After adjusting for reflux symptoms however, a positive correlation emerged between patients with BE and the highest tertile of dairy products intake (⩾ 493.2 g/d) (OR = 1.94, 95%CI: 1.01-3.71). This could imply that patients are consuming dairy products to treat their symptoms, rather than an actual association with BE, as proposed by the authors[32]. In a clinical trial studying the effect of vitamin D supplementation on BE, 3 of the first 10 evaluable patients had BE with high-grade dysplasia. After 2 wk of vitamin D supplementation (50000 units weekly), 2 out of 3 patients with BE had regression to low-grade dysplasia on pathology, suggesting a potential benefit of vitamin D in BE[33].

Three studies assessed VDR expression in BE[25,34,35]. Trowbridge *et al*[34] compared VDR expression in normal esophagus, BE and normal gastric tissue, by immunofluorescent staining. No VDR expression was detected in normal squamous mucosa in contrast to normal gastric mucosa and BE mucosa. This suggests a restriction of VDR expression to columnar epithelium and glandular structures, as well as potential chemopreventive effects of vitamin D in patients with BE. Those findings were reproducible in a Dutch study where VDR mRNA had a 2-fold higher expression in BE epithelium compared to squamous epithelium[25]. In another study comprising 37 patients with BE and 107 with EAC, VDR expression was found to be increased in both BE (95%) and EAC (79%), but significantly higher in BE[35]. This implies that VDR might be involved early on in EAC development.

Esophageal adenocarcinoma

To date, the studies that examined the association between vitamin D status and EAC showed inconsistent results[30,32,36-38]. Several of these studies were either population-based or ecologic studies with lack of information on 25(OH)D_3 levels either before or after EAC diagnosis, and therefore relied on various other measures of vitamin D status such as sunlight exposure or dietary vitamin D intake.

The studies that examined the association between vitamin D and EAC are summarized in Table 2. Only 2 studies evaluated the association of serum 25(OH)D_3 concentrations and EAC. Abnet *et al*[36], in a nested case-control study, examined the relationship between upper gastrointestinal cancers and circulating serum 25(OH)D_3 levels. No significant association was noted with EAC when comparing patients with highest and lowest categories of 25(OH)D_3 levels (50-75 nmol/L *vs* < 25 nmol/L, OR = 1.63, 95%CI: 0.25-2.12)[36]. Another US-based study also did not show any association between 25(OH)D_3 levels and incidence or prevalence of EAC among patients with BE[38]. Giovannucci *et al*[22] used a predicted 25(OH)D_3 level derived by modeling various factors that can affect vitamin D status such as UVB, dietary vitamin d intake, supplementation, skin pigmentation and body mass index. A 25nmol/L increment in predicted vitamin D resulted in 17% reduction in total cancer incidence and 29% reduction in cancer mortality. However, the study did not mention the rates of EAC in particular, although there was an inverse association with esophageal cancer incidence (RR = 0.37, 95%CI: 0.17-0.80)[22].

Data from animal models have shown that dietary vitamin D is associated with tumor inhibition and reduction of tumor growth, especially in colorectal cancer and breast cancer[39-41]. However, the epidemiologic studies for EAC have been contradictory[32,37,42]. In fact, in an Irish study, patients with the highest tertile of vitamin D intake had increased risk of EAC compared to the lowest tertile (OR = 1.99, 95%CI: 1.03-3.86)[42]. In another population-based study in the US, no association was found between vitamin D intake and EAC (OR = 1.10, 95%CI: 0.86-1.40)[37]. Similar results were found in a meta-analysis that concluded that higher intake of vitamin D results in a non-significant increase in the risk of EAC (OR = 1.45, 95%CI: 0.65-2.24)[5]. The current evidence hence fails to establish a relationship between vitamin D intake and EAC.

The other significant contributor of vitamin D status is sunlight exposure. To date only one study examined UVB exposure as a risk factor for EAC[30]. Patients with EAC were 41% less likely to have high levels of lifetime ambient UVB radiation compared to population controls (OR = 0.59, 95%CI: 0.35-0.99). Although the study did not check serum vitamin D levels to establish the diagnosis of vitamin D deficiency, the study results were adjusted for several potential confounders such as body mass index, reflux symptoms, education, smoking, alcohol and Helicobacter pylori infection, following which the inverse association remained between UVB and EAC. The same inverse association was seen between number of nevi, which is a

Table 2 Studies investigating correlations between vitamin D and Barrett's esophagus or esophageal adenocarcinoma

Ref.	Study design/location	Vitamin D exposure/status/ genetics studies	Statistical correlation	Other
Tran *et al*[30]	Case-control study Australia	Cumulative ambient ultraviolet B radiation	EAC risk: OR = 0.59, 95%CI: 0.35-0.99 EAC risk for every 107 J/m^2 increase in radiation: OR = 0.82, 95%CI: 0.72-0.93	
Mulholland *et al*[32]	Case-control study Ireland	Vitamin D dietary intake *via* food questionnaire	EAC risk: OR = 1.99, 95%CI: 1.03-3.86 BE risk: no association	
Mayne *et al*[37]	Case-control study United States	Vitamin D dietary intake	EAC: no association	
Thota *et al*[38]	Retrospective study of a prospectively collected database United States	25-hydroxyvitamin D serum levels	EAC: no association BE with HGD: no association	
Abnet *et al*[36]	Nested case-control study United States, Finland, China	25-hydroxyvitamin D serum levels	EAC: no association	
Trowbridge *et al*[43]	Retrospective study United States	Vitamin D receptor expression	Not assessed	VDR expression decreased with tumor dedifferentiation VDR expression lower in neoadjuvant therapy responders
Trowbridge *et al*[34]	Retrospective study United States	Vitamin D receptor expression	Not assessed	VDR expression increased in Barrett's esophagus
Zhou *et al*[35]	Descriptive United States	Vitamin D receptor expression	Not assessed	VDR expressed in 95% of BE (35/37) VDR expressed in 78% of EAC (86/109)
Janmaat *et al*[25]	Cohort study Netherlands	Vitamin D receptor polymorphisms	EAC: 2 GT copies: OR = 0.50, 95%CI: 0.27-0.96 BE: 2 GT copies: OR = 0.46, 95%CI: 0.26-0.80	VDR expression is 2 fold higher in BE as compared to normal esophagus
Chang *et al*[45]	Case- control study Ireland	Vitamin D receptor polymorphisms	EAC: rs2238139 TT: OR 0.26, 95% CI: 0.07-0.93 EAC: rs2107301 TT: OR = 0.19, 95%CI: 0.06-0.67	
Zgaga *et al*[5]	Meta-analysis United States	Ultraviolet B radiation Vitamin D intake Vitamin D serum levels	Vitamin D level and overall esophageal cancer: OR = 1.39, 95%CI: 1.03-1.74 Vitamin D intake and EAC: OR = 1.45; 95%CI: 0.65-2.24	

EAC: Esophageal adenocarcinoma; BE: Barrett's esophagus; HGD: High-grade dysplasia; VDR: Vitamin D receptor; OR: Odds ratio; CI: Confidence interval.

surrogate marker of sun exposure, and EAC, further supporting the hypothesis of sun exposure and tumor inhibition[30].

In an attempt to find biomarkers predicting the malignant potential of an esophageal lesion, response to treatment and prognosis, investigators have evaluated the genetics involved in the vitamin D pathway in regards to EAC. The focus has mainly been on the VDR expression in different tissues as well as SNPs of some of the genes in the vitamin D signaling pathway.

Trowbridge *et al*[43] looked at VDR expression using immunofluorescence in 15 biopsy specimens of patients with EAC. Greater average mean fluorescence, a reflection of higher VDR expression, was observed for moderately and well-differentiated tumors (111.7) compared to poorly differentiated tumors (98.7), which highlights the anti-carcinogenic properties of vitamin D through VDR, particularly differentiation. This was also established in colon adenocarcinoma where decreased VDR expression was noted with progressive de-differentiation[44].

Apart from assessing VDR expression level, VDR polymorphisms in EAC have also been investigated. Vitamin D exerts many of its biological effects by binding to VDR and VDR gene polymorphisms may alter mRNA stability and transcriptional activity.

In an Irish population-based case-control study, 224 cases of EAC were identified and 256 controls were selected[45]. Variants in the VDR gene were explored and TT homozygotes at rs2238139 and rs2107301 SNPs seemed to have a reduced risk of EAC compared to individual with CC alleles at those sites (OR = 0.26, 95%CI: 0.007, 0.93 and OR = 0.19, 95%CI: 0.06-0.67, respectively). However when permutation analyses were done, there was no significant association between EAC and VDR polymorphisms[45]. A later study identified two SNPs of the VDR gene associated with reduced risk of reflux esophagitis, BE and EAC[25]. Patients with the rs1989969 T/rs2238135

G haplotype had a lower risk for reflux esophagitis (OR = 0.48, 95%CI: 0.28-0.81), BE (OR 0.46, 95%CI: 0.26-0.80) as well as EAC (OR = 0.50, 95%CI: 0.27-0.96). Both of these haplotypes appear to be associated with reduced VDR expression. The authors studied the mechanism by which those SNPs work and discovered that the rs1989969 T allele lead to the appearance of a GATA-1 transcription factor binding site, which is known to be a negative transcriptional regulator. This haplotype could be exerting its direct biological effects on the rate of reflux esophagitis with a subsequent decreased rates of BE and EAC[25]. Those findings could have significant clinical implications in terms of identifying patients who would benefit from vitamin D chemoprevention.

CONCLUSION

In summary, data continues to be inconsistent and firm conclusions regarding the chemopreventive role of vitamin D in esophageal cancer cannot be made. While vitamin D studies struggle with measuring the combined influences of dietary vitamin D intake and sunlight, vitamin D serum levels are a single point measure in time, and levels are known to change throughout the year. As a matter of fact, while an inverse association exists between UVB radiation and EAC, this was not observed with vitamin D intake. Serum 25(OH) D_3 levels appear to be associated with higher risk of ESCC especially in Chinese population. No association was noted however between vitamin D serum levels and EAC. Studies have been population-specific making it difficult to apply findings to other populations. Multiple genetic studies provided new grounds for future investigations such as SNPs leading to the appearance of transcription sites with known negative regulatory roles. VDR expression is increased in BE as compared to EAC or normal squamous epithelium, making of VDR a potential biomarker in selecting those who could benefit from vitamin D as a chemopreventive agent. Well-powered prospective studies with accurate measurement of vitamin D status are needed before chemoprevention with vitamin D is recommended, as current evidence does not support a chemopreventive role of vitamin D against esophageal cancer. Future studies looking at the incidence of esophageal cancer in patients with pre-cancerous lesions (BE and squamous cell dysplasia) receiving vitamin D supplementation are needed.

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ORIGINAL ARTICLE

Retrospective Study

Impact of duration of adjuvant chemotherapy in radically resected patients with T4bN1-3M0/TxN3bM0 gastric cancer

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Abstract

AIM

To provide evidence regarding the postoperative treatment of patients with T4bN1-3M0/TxN3bM0 gastric cancer, for which guidelines have not been established.

METHODS

Patients who had undergone curative resection between 1996 and 2014 with a pathological stage of T4bN1-3M0/TxN3bM0 for gastric cancer were retrospectively analyzed; staging was based on the 7th edition of the American Joint Committee on Cancer staging system. The clinicopathological characteristics, administration of adjuvant chemotherapy, and patterns of recurrence were studied. Univariate and multivariate analyses of prognostic factors were conducted. The chemotherapeutic agents mainly included fluorouropyrimidine, platinum and taxanes, used as monotherapy, doublet, or triplet regimens. Patterns of first recurrence were categorized as locoregional

recurrence, peritoneal dissemination, or distant metastasis.

RESULTS

The 5-year overall survival (OS) of the whole group (n = 176) was 16.8%, and the median OS was 25.7 mo (95%CI: 20.9-30.5). Lymphovascular invasion and a node positive rate (NPR) $\geqslant$ 0.8 were associated with a poor prognosis (P = 0.01 and P = 0.048, respectively). One hundred forty-seven (83.5%) of the 176 patients eventually experienced recurrence; the most common pattern of the first recurrence was distant metastasis. The prognosis was best for patients with locoregional recurrence and worst for those with peritoneal dissemination. Twelve (6.8%) of the 176 patients did not receive adjuvant chemotherapy, while 164 (93.2%) patients received adjuvant chemotherapy. Combined chemotherapy, including doublet and triplet regimens, was associated with a better prognosis than monotherapy, with no significant difference in 5-year OS (17.5% *vs* 0%, P = 0.613). The triplet regimen showed no significant survival benefit compared with the doublet regimen for 5-year OS (18.5% *vs* 17.4%, P = 0.661). Thirty-nine (22.1%) patients received adjuvant chemotherapy for longer than six months; the median OS in patients who received adjuvant chemotherapy for longer than six months was 40.2 mo (95%CI: 30.6-48.2), significantly longer than the 21.6 mo (95%CI: 19.1-24.0) in patients who received adjuvant chemotherapy for less than six months (P = 0.001).

CONCLUSION

Patients with T4bN1-3M0/TxN3bM0 gastric cancer showed a poor prognosis and a high risk of distant metastasis. Adjuvant chemotherapy for longer than six months improved outcomes for them.

Key words: Gastric cancer; T4bN1-3M0/TxN3bM0; Recurrence; Distant metastasis; Adjuvant chemotherapy

Core tip: Patients with T4bN1-3M0/TxN3bM0 gastric cancer have a poor prognosis after curative resection. Due to limited evidence and a lack of guidelines for clinical practice, T4bN1-3M0/TxN3bM0 gastric cancer remains a challenging clinical problem. Our retrospective study is complementary to large-scale phase Ⅲ prospective trials and showed that the most common pattern of first recurrence for this population is distant metastasis and that prolonged adjuvant chemotherapy may improve patient outcomes. This finding will need to be confirmed by future prospective randomized controlled studies to improve the outcomes for patients with T4bN1-3M0/TxN3bM0 gastric cancer.

Wang QW, Zhang XT, Lu M, Shen L. Impact of duration of adjuvant chemotherapy in radically resected patients with T4bN1-3M0/TxN3bM0 gastric cancer. *World J Gastrointest Oncol* 2018; 10(1): 31-39 Available from: URL: http://www.wjgnet.com/1948-5204/full/v10/i1/31.htm DOI: http://dx.doi.org/10.4251/wjgo.v10.i1.31

INTRODUCTION

Nearly one million new cases of gastric cancer (GC) were diagnosed in 2012, making it the fifth most common malignancy worldwide[1]. Geographically, GC is most common in East Asian countries including China, Japan and Korea (45% in China). In contrast to the situation in Japan and Korea, GC in China is often detected at a locally advanced or advanced stage. Complete resection with a D2 lymphadenectomy remains the cornerstone of curative treatment; however, more than half of resectable GC patients develop recurrence despite achieving an R0 resection[2].

Efforts to reduce the risk of recurrence and improve survival have focused on perioperative treatment. Postoperative adjuvant chemotherapy in GC is primarily supported by two large randomized phase Ⅲ studies: The Japanese ACTS-GC[3] (Adjuvant Chemotherapy Trial of TS-1 for Gastric Cancer) and the Asian CLASSIC[4] (Capecitabine and Oxaliplatin Adjuvant Study in Stomach Cancer) trials. Both of these trials showed a survival benefit after D2 gastrectomy compared with surgery alone. A recent study, SAMIT[5] (Japanese Stomach Cancer Adjuvant Multi-Institutional Trial), compared additional chemotherapy with single-agent fluoropyrimidine but failed to show a survival benefit. However, GC patients who were resectable at the most advanced stage (T4bN1-3M0/TxN3bM0, mostly ⅢC) were not included in the CLASSIC trial; moreover, this patient population made up only 5% of the sample in the ACTS-GC study and 10% in the SAMIT study. Considering that R0 resection of the primary cancer had barely been achieved due to the locally advanced stage, these patients were at the highest risk for disease recurrence and were more likely to benefit from adjuvant chemotherapy. Due to the limited evidence as well as the difficulties in therapeutic management, T4bN1-3M0/TxN3bM0 gastric cancer remains a challenging problem in clinical practice.

A Korean retrospective study[6] that focused on stage Ⅳ [T4N1-3M0/T1-4N3M0, American Joint Committee on Cancer (AJCC) 6th edition[7]] GC patients, who were equivalent to the T4bN1-3M0/TxN3bM0 (AJCC 7th edition[8]) patients in the current study, showed that patients who received adjuvant chemotherapy exhibited a survival benefit compared with patients who received surgery alone. However, the Korean study did not discuss the appropriate adjuvant therapy modality, which remains undefined for T4bN1-3M0/TxN3bM0 GC patients.

In view of the limited evidence regarding T4bN1-

3M0/TxN3bM0 GC, the difficulty of R0 resection, and the high risk of disease recurrence in this population, the aim of this retrospective study was to discuss the appropriate adjuvant therapy modality for patients with the most locally advanced GC.

MATERIALS AND METHODS

Patients

A total of 326 consecutive patients with primary GC with a pathological stage of T4bN1-3M0/TxN3bM0 based on the AJCC (7th edition) staging system who underwent potentially curative resection (R0) between October 1996 and December 2014 were identified in the database of Peking University Cancer Hospital. Of these patients, 18 had a distant metastasis that was detected before surgery, 48 had distant metastasis or peritoneal seeding (including positive peritoneal cytology) identified during the operation, 26 were given preoperative chemotherapy, 21 had a positive resection margin, 37 had recurrence within one month after surgery, and 176 with T4bN1-3M0/TxN3bM0 disease were available for analysis (Figure 1). All patients had histologically confirmed gastric or gastroesophageal junction adenocarcinoma.

Treatment and recurrence

A total of 145 (82.4%) patients had metastasis in sixteen or more regional lymph nodes with a median number of 20 metastatic lymph nodes (range: 0-70) and a median node positive rate (NPR) of 0.60 (range: 0.0-1.0). D2 lymph node dissection, according to the NCCN Clinical Practice Guidelines in Oncology-Gastric Cancer (Version 1.2017), was performed in 136 (77.3%) patients, and the median number of dissected lymph nodes was 33 (range: 2-108); 49 (27.8%) patients showed invasion of the adjacent structures and underwent a gastrectomy with bloc resection of the involved structures. A total of 132 (75%) patients underwent resection at a single institution in the Peking University Cancer Hospital.

Adjuvant chemotherapy was administered to 164 (93.2%) patients after curative resection. The chemotherapy regimens included monotherapy (capecitabine/S1/5-FU, n = 10), doublet chemotherapy (FOLFOX, n = 33; XELOX, n = 34; SOX, n = 39; capecitabine/S1+cisplatin, n = 9; paclitaxel+ capecitabine, n = 15; paclitaxel+ cisplatin/oxaliplatin, n = 4) and triplet chemotherapy (based on 5-FU including cisplatin, oxaliplatin, epirubicin, paclitaxel, docetaxel, etoposide, and mitomycin, n = 20); 12 patients did not receive adjuvant chemotherapy. Fourteen patients received intra- or postoperative intraperitoneal perfusion of cisplatin/paclitaxel/5-FU, and four patients received postoperative chemoradiotherapy. All adverse events were assessed using the National Cancer Institute Common Toxicity Criteria (NCI-CTC), version 2.0. Dose modifications were made for patients who experienced hematologic or non-hematologic toxicity.

Disease recurrence was determined by radiologic or histological examination; the sites of recurrence were documented separately and included anastomotic sites, regional lymph nodes, peritoneum, ovary, adrenal gland, liver, lung, bone, extra-abdominal lymph nodes, and Virchow's lymph nodes. Based on these sites, the patterns of the first recurrence were categorized as locoregional recurrence (anastomotic sites and regional lymph nodes), peritoneal dissemination (ovary and the peritoneum), or distant metastasis (the liver, lung, bone, Virchow's lymph nodes, extra-abdominal lymph nodes, and adrenal gland).

Follow-up evaluation

Patients were followed every 3 mo for the first 2 years and then at 6-mo intervals until the fifth year. Regular follow-up evaluations consisted of a physical examination, routine laboratory tests, abdominal computed tomography (CT) scan, endoscopy, and chest X-ray.

Statistical analysis

The statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS) software, version 21.0. Disease-free survival (DFS) was defined as the time from surgery until the recurrence of GC or death from any cause. Overall survival (OS) was defined as the time from surgery until death from any cause. Continuous variables were transformed to dichotomous variables in the survival analysis. χ^2 tests were used to compare clinicopathological characteristics between groups. Variables known to have prognostic value were selected in the final multivariable Cox proportional hazards model. Kaplan-Meier curves for disease-free survival and OS were compared using a log-rank test. A P-value of < 0.05 was considered statistically significant.

RESULTS

Patient characteristics

Our study included a group of 176 patients with metastasis in sixteen or more regional lymph nodes (TxN3bM0) or invasion of adjacent structures (T4bN1-3M0) in whom achieving R0 resection was difficult and who were assumed to be at high risk for recurrence. All patients, including 131 females and 45 males aged 25-81 years (56.4 ± 11.1 years), had histologically confirmed gastric or gastroesophageal junction adenocarcinoma; most had poorly differentiated adenocarcinoma. Of the 176 patients, 156 (88.6%) were classified as stage ⅢC based on the AJCC TNM Staging Classification for Carcinoma of the Stomach (7th ed, 2010). The clinicopathological characteristics of the patients are listed in Table 1.

Survival and prognostic factors

Based on the follow-up data updated on July 31, 2015, the median follow-up time for the 176 patients was

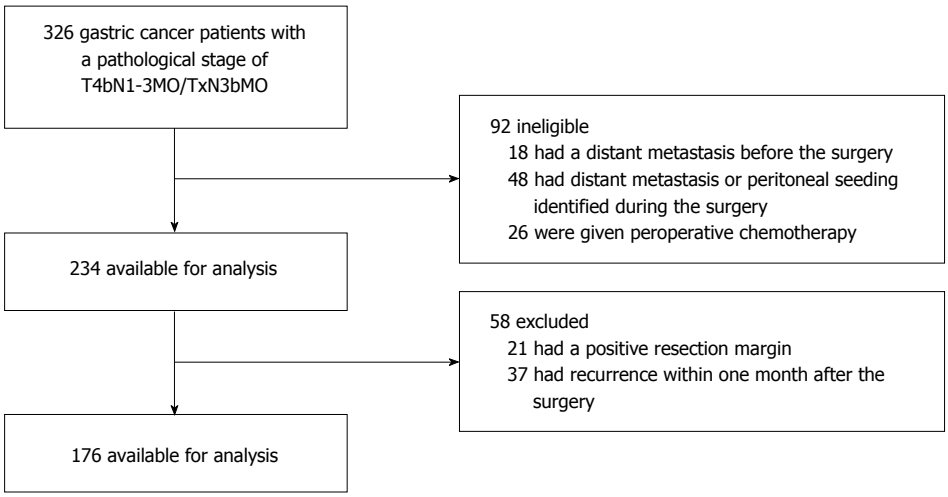

Figure 1 Study flow diagram.

Table 1 Relationship between clinicopathological characteristics and prognosis of T4bN1-3M0/TxN3bM0 gastric cancer patients

Clinicopathological characteristics	All patients (n = 176)		5-yr OS (%)	P value
	n	%		
Sex				
Male	131	74.4%	17.4%	0.702
Female	45	25.6%	15.8%	
Age (yr)				
⩾ 60	68	38.6%	21.8%	0.799
< 60	108	61.4%	13.5%	
Tumor location				
Upper third	43	24.4%	17.7%	0.614
Middle third	56	31.8%	19.6%	
Lower third	62	35.2%	19.6%	
Total	15	8.5%	0.0%	
Tumor grade (differentiation)				
Moderate	15	8.5%	19.3%	0.241
Poor	161	91.5%	16.5%	
Lymphovascular invasion				
Yes	139	79.0%	10.3%	0.010
No	37	21.0%	30.6%	
No. of positive LNs				
0	4	2.3%	37.5%	0.174
1-6	17	9.7%	31.2%	
7-15	10	5.7%	0.0%	
⩾ 16	145	82.4%	15.8%	
No. of dissected LNs				
⩾ 30	106	60.2%	20.6%	0.326
< 30	70	39.8%	11.6%	
Positive LN ratio				
⩾ 0.8	34	19.3%	6.2%	0.048
< 0.8	142	80.7%	20.5%	
Pathologic T stage[1]				
T2	5	2.8%	40.0%	0.420
T3	20	11.4%	30.6%	
T4a	102	58.0%	12.6%	
T4b	49	27.8%	21.2%	
Stage[1]				
ⅢA	5	2.8%	40.0%	0.237
ⅢB	15	8.5%	35.9%	
ⅢC	156	88.6%	14.0%	

[1]Recorded based on the American Joint Committee on Cancer (AJCC) TNM Staging Classification for Carcinoma of the Stomach (7th edition, 2010). LN: Lymph node; OS: Overall survival.

Table 2 Multivariate analysis of the prognostic factors for overall survival of T4bN1-3M0/TxN3bM0 gastric cancer patients

Clinicopathological characteristics	P value	Odds ratio	95%CI	
			Lower	Upper
Lymphovascular invasion	0.01	1.80	1.15	2.8
Node positive rate	0.14	1.36	0.90	2.1
Stage	0.49	0.71	0.34	1.5

LN: Lymph node.

47.4 mo (range: 2-202 mo). By the end of the follow-up period, 123 patients had died, 37 patients were alive, and 16 patients (9.1%) had been lost to follow-up.

The 5-year OS of the group was 16.8%; the median OS was 25.7 mo (95%CI: 20.9-30.5). The 3-year DFS of the whole group was 9.8%, while the median DFS was 11.7 mo (95%CI: 10.0-13.4). The univariate analysis showed that lymphovascular invasion and NPR ⩾ 0.8 were associated with a poor prognosis (P = 0.01 and P = 0.048, respectively), while stage ⅢC was not significantly associated with a poor prognosis according to the Kaplan-Meier method (P = 0.237, Table 1).

In the multivariate analysis, lymphovascular invasion was an independent prognostic factor (P = 0.01, HR: 1.8, 95%CI: 1.15-2.8) for OS in T4bN1-3M0/TxN3bM0 GC patients (Table 2).

Patterns of recurrence

During the follow-up period, 147 (83.5%) of the 176 patients with T4bN1-3M0/TxN3bM0 GC experienced recurrence; the first recurrence was localized to a single site in 78.9% of patients, two sites in 13.6% of patients, and three or more sites in 6.8% of patients. As shown in Table 3, the most common pattern of first recurrence was distant metastasis (45.6%), followed by peritoneal dissemination (25.9%) and locoregional recurrence (22.5%). Nine patients (6.1%) who

Table 3 Overall survival according to patterns of recurrence in T4bN1-3M0/TxN3bM0 gastric cancer patients after curative resection

Recurrent sites	Recurrent patients (n = 147)		Median OS (mo)	5-yr OS (%)	P value
	n	%			
Locoregional	33	22.5%	33.9	28.0%	0.001
Peritoneal	38	25.9%	16.0	0.0%	
Distant	67	45.6%	21.3	14.7%	

Table 4 Overall survival of patients with T4bN1-3M0/TxN3bM0 gastric cancer according to distant site of metastasis

Distant metastasis site	Recurrent patients (n = 147)		Median OS (mo)	5-yr OS (%)
	n	%		
Liver	26	17.7%	18.3	15.5%
Lung and pleura	12	8.2%	16.8	0.0%
Bone	10	6.8%	30.7	29.2%

experienced combined patterns of recurrence were excluded from the survival analysis. The prognosis was best for patients with locoregional recurrence and worst for those who had peritoneal dissemination. Figure 2 presents the OS for each group. The 5-year OS rates were 28.0%, 0% and 14.7% for locoregional recurrence, peritoneal dissemination and distant metastasis, respectively, which showed statistically significant differences (P = 0.001).

We further analyzed OS according to the most distant metastatic sites; the most frequent site of distant metastasis was the liver, followed by the lung (including malignant pleural effusion), bone, and other distant sites. Eight of ten patients had bone metastases as the first recurrence site without liver or lung metastases. The median OS for patients with bone metastasis from GC was 30.7 mo, while that for patients with other metastatic sites was 21.9 mo (P = 0.35). The median OS for patients with lung metastasis was significantly shorter than that for patients with other metastatic sites (16.8 mo *vs* 22.4 mo, P = 0.04) (Table 4). The results showed that patients with bone metastasis had a better prognosis, whereas patients with lung and pleura metastasis had a worse prognosis than those with other metastatic sites.

Adjuvant chemotherapy

During the follow-up period after curative resection, 12 patients did not receive adjuvant chemotherapy because of their poor condition or rejection of chemotherapy; 164 (93.2%) of the 176 patients received at least one cycle of adjuvant chemotherapy. Combined chemotherapy, including doublet and triple regimens, was associated with a better prognosis than monotherapy but with no significant difference in 5-year OS (0% in the monotherapy group and 17.5% in the combined chemotherapy group, P = 0.613). Triple adjuvant chemotherapy showed no significant survival benefit over the doublet regimen (P = 0.449). The 5-year OS rates were 0%, 17.4%, and 18.5% for the monotherapy, doublet chemotherapy and triple chemotherapy groups, respectively (P = 0.661); the 3-year DFS rates were 0%, 5.3%, and 5.3%, respectively (P = 0.583, Table 5). The patient characteristics, except for age, were similar in the three groups; approximately 60.0% of patients in the monotherapy group, 40.7% in the doublet group, and 28.0% in the triplet group were older than 60 years (P = 0.202).

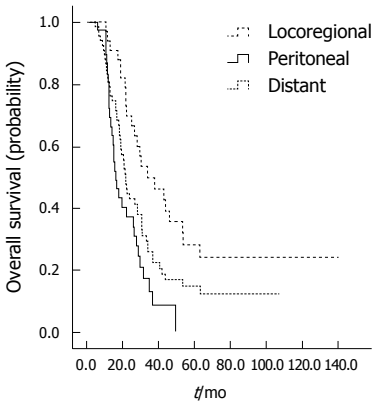

Figure 2 Overall survival of patients with T4bN1-3M0/TxN3bM0 gastric cancer after curative resection according to the patterns of recurrence.

In our study, various chemotherapeutic agents, including platinum-, taxane-, epirubicin-based regimens, did not show any significant differences in survival benefit (data not shown).

The median number of cycles of adjuvant chemotherapy was six, and the median time of adjuvant chemotherapy was 4.2 mo. Thirty-nine (22.1%) of the 176 patients received adjuvant chemotherapy for longer than six mo, as shown in Table 5. A longer duration of adjuvant chemotherapy was significantly associated with a better prognosis; the median OS was prolonged to 40.2 mo (95%CI: 30.6-48.2) in patients given adjuvant chemotherapy for longer than six months, compared with 21.6 mo (95%CI: 19.1-24.0) in patients given adjuvant chemotherapy for less than six months (P = 0.001). The median DFS was 23.2 mo (95%CI: 21.5-24.9) in patients given adjuvant chemotherapy for longer than six months, compared with 9.9 mo (95%CI: 7.6-12.3) in patients receiving adjuvant chemotherapy for less than six months (P = 0.0001) (Table 5, Figure 3). The patient characteristics were similar between the two groups.

Treatment compliance, modifications and adverse events

Of the 164 patients who received adjuvant chemotherapy, only 39 patients continued the treatment for over six months. The most common reasons for withdrawal of treatment included the refusal of the patients to continue treatment due to inadequate

Table 5 Relationship between adjuvant treatment and the prognosis of T4bN1-3M0/TxN3bM0 gastric cancer patients

Treatment		*n*	Median DFS (mo)	3-yr DFS (%)	*P* value	Median OS (mo)	5-yr OS (%)	*P* value
Adjuvant chemotherapy	Yes	164	12.3	10.4%	0.000	25.7	16.1%	0.532
	No	12	2.8	0.0%		18.7	22.2%	
Chemotherapy	Mono-therapy	10	6.7	0.0%	0.583	20.3	0.0%	0.661
Regimen	Doublet	134	12.0	5.3%		26.3	17.4%	
	Triple	20	13.0	5.3%		29.7	18.5%	
Adjuvant chemotherapy time	⩾ 6 mo	39	23.2	20.2%	0.000	40.2	25.0%	0.001
	< 6 mo	125	9.9	7.3%		21.6	13.4%	

DFS: Disease-free survival.

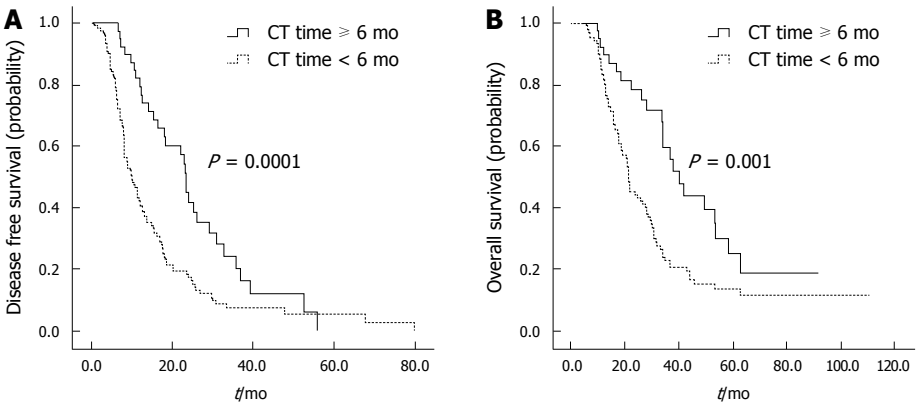

Figure 3 Kaplan-Meier curves of disease-free survival (A) and overall survival (B) for T4bN1-3M0/TxN3bM0 gastric cancer patients after curative gastrectomy according to the duration of adjuvant chemotherapy. *P* value by log-rank test. A: Disease-free survival (DFS): 23.2 mo *vs* 9.9 mo, *P* = 0.0001; B: Overall survival: 40.2 mo *vs* 21.6 mo, *P* = 0.001. CT: Chemotherapy.

social support (32%), adverse events (28%), the detection of relapse or metastasis (14.6%), or other factors (25.4%). A total of 114 patients (69.5%) required dose modifications or chemotherapy delays, including 24/39 (61.5%) in the chemotherapy ⩾ 6 mo group and 90/125 (72.0%) in the chemotherapy < 6 mo group. Of the 154 patients who received doublet or triplet regimens, 20 patients (13.0%) switched to monotherapy because of adverse events or upon their request.

Adverse events, including hematologic and non-hematologic toxic effects, were analyzed. The most frequent grade 3 or 4 adverse events were neutropenia (20.3%), nausea and vomiting (7.3%), anorexia (6.7%), and diarrhea (3.7%). Overall, 44 patients (26.8%) developed grade 3 or 4 toxicities (data not shown).

DISCUSSION

The aim of this retrospective study was to provide evidence for clinical treatment of T4bN1-3M0/TxN3bM0 GC patients after curative resection. This population is at the most advanced stage of GC at which resection is possible; therefore, R0 resection is difficult, and the risk of recurrence is high. Currently, controversy exists regarding whether prolonging the duration of adjuvant chemotherapy, intensifying adjuvant chemotherapy, or undergoing preoperative chemotherapy will improve the prognosis for these patients. More efforts to explore appropriate adjuvant therapy modalities are necessary for clinical practice.

Despite undergoing standardized adjuvant chemotherapy followed by curative resection performed by experienced surgeons in our high-volume GC centers, patients with T4bN1-3M0/TxN3bM0 GC had a high risk of recurrence and a poor prognosis. The 5-year OS of the entire group was 16.8%, which is significantly lower than that of patients with stage Ⅲ disease, ranging between 40%-70% in most phase 3 trials[3,9]. Patients at stage ⅢC accounted for 88.6% of our study population; the 5-year OS for these patients was far lower than that of patients with stage ⅢC GC reported in another study (14.0% *vs* 30.2%)[10]. Moreover, a Korean study[6] showed that the 5-year OS rate of the patients who received adjuvant chemotherapy with T4bN1-3M0/TxN3bM0 GC was 39.6%; only 61.7% of these patients experienced recurrence[11]. However, the 5-year OS of patients in our study who received adjuvant chemotherapy for longer than 6 mo was only 25%, and 147 (83.5%) of the 176 patients experienced recurrence.

Several factors may be responsible for the poor prognosis of patients in our study. First, new diagnostic

modalities such as endoscopic ultrasound (EUS), positron emission tomography/computed tomography (PET/CT), magnetic resonance imaging (MRI), and laparoscopic staging, were not used for preoperative staging of patients treated during the early part of the study, which may have reduced the accuracy of staging and led to the advanced gastric cancer be treated as resectable gastric cancer improperly[12-14]. Therefore, patients included in this study may be mixed with advanced patients actually, and these errors can be avoided using new staging approach. Second, the risk of non-regional lymph node metastases is increased in patients with N3b, although all tumors with T4bN1-3M0/TxN3bM0 are staged regardless of the M1 category; additionally, without appropriate clinical information, surgical pathologists may be unaware that particular lymph node metastases are already distant metastases and they may be classified as N3b instead of M1. Third, Korean and Japanese surgeons have performed more D2+ lymphadenectomies, total gastrectomies, multivisceral resections, and Billroth Ⅱ digestive tract reconstructions than their Chinese counterparts; indeed, the OS of Korean patients was longer than that of Chinese patients, especially for those with stage Ⅲ disease[15]. Fourth, 39 patients in our study underwent limited lymph node dissections, whereas only 4 patients received postoperative chemoradiotherapy, as the INT 0116 study established postoperative chemoradiotherapy as a standard of care for patients who undergo < D2 dissections[16]. These facts reflect the medical status in China and contribute to a new understanding of T4bN1-3M0/TxN3bM0 patients, who mostly belong to stage ⅢC, while they are distinct from conventional stage ⅢC GC patients with regard to the biological behavior and prognosis of the disease.

In our study, the most common pattern of first recurrence was distant metastasis; sites of distant metastasis and locoregional recurrence accounted for 45.6% and 22.5%, respectively, of patients with T4bN1-3M0/TxN3bM0 recurrent GC. Patients with locoregional recurrence showed a better prognosis than patients with distant metastasis, suggesting that systemic therapy, rather than local therapy, was more likely to benefit patients with T4bN1-3M0/TxN3bM0 GC. According to the results of the ACTS-GC and CLASSIC trials[3,9], adjuvant chemotherapy with one year of S1 or 6 mo of the XELOX regimen after a D2 gastrectomy was confirmed to be the standard adjuvant treatment for locally advanced gastric cancer. Without definitive data favoring combined therapy over monotherapy, especially in GC patients with the most advanced stage of T4bN1-3M0/TxN3bM0, it remains unclear whether an intensified or longer duration of adjuvant chemotherapy provides an additional benefit.

In our study, triple adjuvant chemotherapy showed no significant survival benefit compared with a doublet regimen. Recently, the SAMIT study and the ITACA-S study, both of which compared poly-chemotherapy *vs* monotherapy, failed to show any benefit for patients in an adjuvant setting[5,17]. Intensifying adjuvant chemotherapy is almost considered too difficult to provide additional benefit. It is of note that patients who received adjuvant chemotherapy for longer than six months in our study benefited significantly from the treatment, with the median OS prolonged to 40.2 mo. In contrast, the median OS was 21.6 mo for patients who received chemotherapy for less than six months. It is therefore suggested that prolonged adjuvant chemotherapy may improve the outcomes for patients at a high risk of distant recurrence. However, only 22.1% of the patients completed all six months of chemotherapy, which may be explained by the frailty of GC patients after surgery, along with the toxicity of adjuvant poly-chemotherapy. In this case, active dose modification based on the adverse events of chemotherapy should to be performed to ensure adequate chemotherapy time and additional benefit from the treatment.

While preoperative chemotherapy may theoretically be superior to postoperative chemotherapy for several reasons[18-20], preoperative chemotherapy has been widely used for patients with T4bN1-3M0/TxN3bM0 GC in clinical practice. However, whether perioperative or postoperative chemotherapy is more beneficial for T4bN1-3M0/TxN3bM0 patients lacks data supported by prospective studies; the ongoing RESOLVE study (NCT01534546) to compare perioperative chemotherapy of SOX *vs* SOX/XELOX as postoperative chemotherapy in locally advanced gastric cancer with D2 dissection may provide additional evidence. Moreover, patients in arm C of the RESOLVE study will receive 8 cycles of perioperative SOX followed by 3 cycles of S-1 monotherapy, which may provide evidence for prolonged adjuvant chemotherapy.

Based on the classification and statistical analysis, 26 patients with T4b disease were excluded from our study because they had a positive resection margin, which indicates that at least one-third of T4b patients according to preoperative staging failed to eventually undergo R0 resection. Preoperative chemoradiotherapy (CRT) may increase resectability and improve the outcomes of T4b patients. The role of CRT continues to be evaluated in many ongoing clinical trials worldwide, such as the Trial of Preoperative Therapy for Gastric and Esophagogastric Junction Adenocarcinoma (TOPGEAR, NCT01924819) and the ARTIST-Ⅱ trial in patients with lymph node-positive GC after D2 gastrectomy.

Due to the small sample sizes and the heterogeneity of therapy administered over a long period, the results in this study have been mixed and biased. Although this study was conducted based on retrospective data, we think that the bias may be reduced by the fact that the surgeries were performed in our high-volume GC centers and patients had access to good medical care. Indeed, this study is the largest retrospective analysis of the effect of adjuvant therapy

on patients with T4bN1-3M0/TxN3bM0 GC; the results reflect the current medical situation for the treatment of gastric cancer in China and are complementary to those of large-scale phase Ⅲ prospective trials.

Undoubtedly, along with an in-depth understanding of molecular and gene profiling, personalized precision medicine as well as adjuvant and perioperative multimodal therapies[21] will be crucial for improving the outcomes of conventional adjuvant chemotherapeutic treatments in the future.

In conclusion, patients with T4bN1-3M0/TxN3bM0 gastric cancer showed a poor prognosis, with the most common pattern of first recurrence being distant metastasis rather than locoregional recurrence. Adjuvant chemotherapy for longer than six months may improve the outcomes of this patient group. However, a prospective randomized controlled study will be required to confirm these findings and to improve the outcomes for patients with T4bN1-3M0/TxN3bM0 gastric cancer.

ARTICLE HIGHLIGHTS

Research background

In view of the limited evidence regarding T4bN1-3M0/TxN3bM0 GC, as well as the difficulty of achieving R0 resection and the high risk of disease recurrence, this retrospective study is complementary to large-scale phase Ⅲ prospective trials and may provide implications for clinical practice.

Research motivation

The population targeted in our study is difficult to treat with no accepted standard of care. This study is the largest retrospective analysis of the effect of adjuvant therapy on patients with T4bN1-3M0/TxN3bM0 GC. Furthermore, our study explored the patterns of recurrence and their relationships to the prognosis of these patients.

Research objectives

To provide evidence regarding the postoperative treatment of patients with T4bN1-3M0/TxN3bM0 gastric cancer, for which guidelines have not been established.

Research methods

Patients who had undergone curative resection between 1996 and 2014 with a pathological stage of T4bN1-3M0/TxN3bM0 for gastric cancer were retrospectively analyzed; staging was based on the 7th edition of the American Joint Committee on Cancer staging system. The clinicopathological characteristics, administration of adjuvant chemotherapy, and patterns of recurrence were studied. Univariate and multivariate analyses of prognostic factors were conducted. The chemotherapeutic agents mainly included fluorouropyrimidine, platinum and taxanes, used as monotherapy, doublet, or triplet regimens. Patterns of first recurrence were categorized as locoregional recurrence, peritoneal dissemination, or distant metastasis.

Research results

The 5-year overall survival (OS) of the whole group (n = 176) was 16.8%, and the median OS was 25.7 mo (95%CI: 20.9-30.5). Lymphovascular invasion and a node positive rate (NPR) $\geqslant$ 0.8 were associated with a poor prognosis (P = 0.01 and P = 0.048, respectively). One hundred forty-seven (83.5%) of the 176 patients eventually experienced recurrence; the most common pattern of the first recurrence was distant metastasis. The prognosis was best for patients with locoregional recurrence and worst for those with peritoneal dissemination. Twelve (6.8%) of the 176 patients did not receive adjuvant chemotherapy, while 164 (93.2%) patients received adjuvant chemotherapy. Combined chemotherapy, including doublet and triplet regimens, was associated with a better prognosis than monotherapy, with no significant difference in 5-year OS (17.5% *vs* 0%, P = 0.613). The triplet regimen showed no significant survival benefit compared with the doublet regimen for 5-year OS (18.5% *vs* 17.4%, P = 0.661). Thirty-nine (22.1%) patients received adjuvant chemotherapy for longer than six months; the median OS in patients who received adjuvant chemotherapy for longer than six months was 40.2 mo (95%CI: 30.6-48.2), significantly longer than the 21.6 mo (95%CI: 19.1-24.0) in patients who received adjuvant chemotherapy for less than six months (P = 0.001).

Research conclusions

Patients with T4bN1-3M0/TxN3bM0 gastric cancer showed a poor prognosis, with the most common pattern of first recurrence being distant metastasis rather than locoregional recurrence. Adjuvant chemotherapy for longer than six months may improve the outcomes of this patient group.

Research perspectives

To date, few retrospective studies have analyzed the survival and prognosis factors for T4bN1-3M0/TxN3bM0 GC patients; however, due to the small sample sizes and different treatment regimens, the results have been mixed. No meta-analyses have been conducted on this topic. However, a prospective randomized controlled study will be required to confirm these findings and to improve the outcomes for patients with T4bN1-3M0/TxN3bM0 gastric cancer.

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ORIGINAL ARTICLE

Clinical Practice Study

Neoadjuvant hyperfractionated accelerated radiotherapy plus concomitant 5-fluorouracil infusion in locally advanced rectal cancer: A phase Ⅱ study

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Abstract

AIM

To evaluate the efficacy and tolerability of neoadjuvant hyperfractionated accelerated radiotherapy (HART)

and concurrent chemotherapy in patients with locally advanced infraperitoneal rectal cancer.

METHODS

A total of 30 patients with histopathologically confirmed T2-3/N0+ infraperitoneal adenocarcinoma of rectum cancer patients received preoperative 42 Gy/1.5 Gy/18 days/bid radiotherapy and continuous infusion of 5-fluorouracil (325 mg/m^2). All patients were operated 4-8 wk after neoadjuvant concomitant therapy.

RESULTS

In the early phase of treatment, 6 patients had grade Ⅲ-Ⅳ gastrointestinal toxicity, 2 patients had grade Ⅲ-Ⅳ hematologic toxicity, and 1 patient had grade Ⅴ toxicity due to postoperative sepsis during chemotherapy. Only 1 patient had radiotherapy-related late side effects, *i.e.*, grade Ⅳ tenesmus. Complete pathological response was achieved in 6 patients (21%), while near-complete pathological response was obtained in 9 (31%). After a median follow-up period of 60 mo, the local tumor control rate was 96.6%. In 13 patients, distant metastasis occurred. Disease-free survival rates at 2 and 5 years were 63.3% and 53%, and corresponding overall survival rates were 70% and 53.1%, respectively.

CONCLUSION

Although it has excellent local control and complete pathological response rates, neoadjuvant HART concurrent chemotherapy appears to not be a feasible treatment regimen in locally advanced rectal cancer, having high perioperative complication and intolerable side effects. Effects of reduced 5-fluorouracil dose or omission of chemotherapy with the aim of reducing toxicity may be examined in further studies.

Key words: Hyperfractionated accelerated radiotherapy; Rectal cancer; Neoadjuvant chemoradiotherapy

Core tip: This study includes a first phase Ⅱ study evaluating neoadjuvant hyperfractionated accelerated radiotherapy plus concomitant infusional 5-fluorouracil (5-FU) chemotherapy in locally advanced rectal cancer (not resectable cancer). This regimen may allow clinicians to design other neoadjuvant hyperfractionated accelerated radiotherapies. This study showed excellent local control but high rate of perioperative complications. Decreasing or modifying the 5-FU dose could provide better local control.

Gural Z, Saglam S, Yucel S, Kaytan-Saglam E, Asoglu O, Ordu C, Acun H, Sharifov R, Onder S, Kizir A, Oral EN. Neoadjuvant hyperfractionated accelerated radiotherapy plus concomitant 5-fluorouracil infusion in locally advanced rectal cancer: A phase Ⅱ study. *World J Gastrointest Oncol* 2018; 10(1): 40-47 Available from: URL: http://www.wjgnet.com/1948-5204/full/v10/i1/40.htm DOI: http://dx.doi.org/10.4251/wjgo.v10.i1.40

INTRODUCTION

Rectal cancer is associated with a high incidence of local recurrence and distant metastasis[1,2]. In randomized studies, local-regional recurrence despite mesorectal resection has been reported to occur in 15% to 30% of the patients undergoing surgery alone[3-8]. In this regard, addition of preoperative and postoperative treatments to surgery have been shown to significantly improve local recurrence and survival rates[9-13], leading to standard administration of such treatments. Currently, preoperative chemoradiation (CRT) is the preferred treatment regimen in these patients, owing to low local recurrence rates and higher chance of sphincter-sparing surgery; although, studies comparing preoperative and postoperative CRT are relatively limited.

Besides conventional radiotherapy (RT) consisting of 45-50 Gy/1.8-2 Gy/5-6 wk, hypofractionated and hyperfractionated accelerated RT (HART; 42 Gy/1.5 Gy/18 d) are also used. HART reduces the risk of repopulation in tumor cells by shortening the treatment time and increases the repair capacity of normal tissues after sublethal damage through the reduction of the fraction dose. Thus, a survival advantage is provided in favor of normal cells, since tumor cells exhibit a poor repair mechanism[14]. In this background, a fractionated HART scheme was examined in this study.

Therefore, this study was carried out to observe the early and late effects of HART regimen in combination with neoadjuvant chemotherapy in patients diagnosed with locally advanced rectal cancer.

MATERIALS AND METHODS

Patient selection

Previously untreated patients with histologically confirmed adenocarcinoma of the rectum (mid and distal ⩽ 12 cm from the anal verge) were included in the study at Istanbul University Oncology Institute. Patient inclusion criteria were as follows: presence of resectable tumor; Karnofksy performance score ⩾ 80; adequate bone marrow reserve (hemoglobin > 11 g/dL, white blood cell > 3500 mL, platelet count > 100000 mL), normal kidney and liver function tests (creatinine < 1.3 mg/dL, alanine aminotransferase and aspartate aminotransferase < 80 U/L), and ⩽ 70 years of age. Patients who had received pelvic RT previously and patients with clinically detected distant metastases were excluded from the study. Clinical staging prior to treatment was accomplished based on physical examination, tumor markers (carcinoembryonic antigen, CA19-9), complete blood count and biochemistry tests, positron emission-computed tomography, pelvic-diffusion magnetic resonance imaging (MRI), and endorectal ultrasound. This prospective study was approved by the local ethics committee. A written informed consent was obtained from all patients prior to treatment.

Table 1 Patient characteristics

Characteristic	*n* = 30
Sex, M/F	19/11
Age, median (range)	53 (30-70)
Tumor location, distance from anal verge	
≤ 5 cm	19 (63)
> 5 cm	11 (37)
Clinical TN stage	
T2N2	1 (3)
T3N0	2 (7)
T3N1	15 (50)
T3N2	12 (40)
Tumor differentiation	
Well	10 (33)
Moderate	10 (33)
Poor	4 (14)
Mucinous	3 (10)
Signet ring cell	3 (10)

Unless otherwise stated, data are presented as *n* (%). M: Male; F: Female.

Preoperative CRT

All patients received preoperative HART (42 Gy/1.5 Gy/18 d/bid) and concurrent continuous infusion of 5-fluorouracil (5-FU; 325 mg/m^2) and were hospitalized during treatments to observe the possible acute side effects.

Prior to RT planning, computed tomography was performed in prone position with belly board, with a 0.5 cm slice thickness for all patients. Gross tumor volume and clinical target volume were estimated by the radiologist and radiation oncologist. Patients were treated with a 3-D conformal RT technique, through posterior and lateral fields using a linear accelerator (18 MV) and with an isodose of 95% of planned target volume. RT regimen was defined by a fraction dose of 150 cGy/fr given 2 times/d, 5 d/wk, with a minimum 8 h between fractions. Total dose was 4200 cGy and total treatment duration was 18 d.

Port or subclavian catheter was used to give 5-FU in the form of a continuous infusion during the entire treatment. The daily dose of 5-FU that was given to patients was 325 mg/m^2[15]. Surgery was performed 4-8 wk after the completion of CRT.

Low anterior or abdominoperineal resection (total mesorectal excision) was performed depending on the location of the tumor and response rate. Four cycles of 5-FU (400 mg/m^2, D1-5, q 28 d) plus folinic acid (20 mg/m^2, D1-5, q 28 d) were administered postoperatively.

Assessment of efficacy and side effects

The primary endpoint was pathological response rate after CRT, and secondary endpoints included the local control rate, surgical margin positivity, survival and toxicity. Patients were assessed for toxicity during CRT on a daily basis. During the period between the end of CRT and surgery, patient assessments for side effects were performed weekly. Acute radiation toxicity criteria of the Radiation Therapy Oncology Group and the European Organization for Research and Treatment of Cancer (EORTC) were used for side effect assessments[16]. Pathologic response and staging were defined according to the Dworak regression scoring system[17] and TNM staging system[18], as described by the American Joint Committee on Cancer.

Statistical analysis

Statistical analyses were performed with SPSS 16.0 (SPSS Inc., Chicago, IL, United States) statistical software. Survival was calculated using the Kaplan-Meier method.

RESULTS

Thirty patients (19 males and 11 females) who were diagnosed with locally advanced rectum cancer between October 2007 and March 2009 were included. The median age was 53 years (range: 30-70 years). Patient characteristics are summarized in Table 1. There were only 2 patients with T3N0 disease, and one of them had positive circumferential margins in staging MRI.

Pathological findings

Surgery was performed in all subjects except for one, who was found to have metastases during the early period after the CRT. Surgery was performed at week 4 in 15 patients and between weeks 6 and 8 in 13 patients. Twelve patients (41%) underwent sphincter-sparing surgery. According to the Dworak total regression scoring system, 6 of 29 (21%) patients who underwent surgery had grade Ⅳ (total) regression, and 9 patients (31%) had grade Ⅲ (near total) regression. Corresponding figures for grade Ⅱ, Ⅰ and 0 regression were 11 patients (38%), 2 patients (7%) and 1 patient (3%), respectively.

Positive margins were found in 2 patients (6.6%). In 14 patients, mesorectal fascia invasion was detected in staging MRI and only 2 of those patients had positive radial surgical margin. Comparison of ypT and cT yielded a down-staging rate of 59%. Clinical and pathological tumor stages are shown in Table 2. The median number of lymph nodes that were excised was 25 (2-58), respectively. No pathologic lymph nodes were present in 19 (63%) patients. With regard to N stage, 20 (69%) patients were found to have down-staging.

Local control and survival

One (3.3%) patient had local recurrence while distant metastases were found in 13 (43.3%) patients during a median follow up of 60 mo (5-78 mo). None of the patients with T3N0 disease had local recurrence. Overall, 14 patients (46.6%) died during the study period. The causes of death were systemic metastasis (13 patients) and chemotherapy-related toxicity (1 patient). Median time to progression was 59 mo (2-78

Table 2 Clinical (cT2) and pathological (ypT) tumor stages

	cT2	cT3	Total
ypT0	-	6 (20.6)	6 (20.6)
ypT1	-	3 (10.3)	3 (10.3)
ypT2	-	8 (27.5)	6 (20.6)
ypT3	1	11 (37.9)	12 (41.3)
Total	1	28	29

Data are presented as *n* (%).

Table 3 Surgical complications

Timing of the complication	
Perioperative	6 (20.6)[1]
Early postoperative	4 (13.7)[2]
Late postoperative	2 (6.8)[3]

[1]Bladder-urethra injury (*n* = 4), rectum perforation (*n* = 1), necrosis due to proctotectomy (*n* = 1); [2]Acute renal failure (*n* = 3), perirectal abscess (*n* = 1); [3]Colovaginal fistula (*n* = 1), perirectal abscess (*n* = 1). Data are presented as *n* (%).

mo). The 2- and 5-year disease-free survival (DFS) rates were 63% and 53%, while the 2- and 5-year overall survival (OS) rates were 70% and 53.1%, respectively. The patients with complete or near-complete pathological response were compared to patients with less favorable group for survival. We found no significant difference in either group for DFS (P = 0.63) and OS (P = 0.32).

Toxicity and complications

Early side effects of preoperative CRT: The highest frequency of side effects occurred at weeks 3-4. During the acute phase 6 (20%) patients developed grade Ⅲ-Ⅳ gastrointestinal system toxicity (3 grade Ⅲ tenesmus/diarrhea and 3 grade Ⅳ tenesmus and diarrhea), and 2 (6.7%) patients developed grade Ⅲ-Ⅳ hematopoietic system toxicity (1 grade Ⅲ leucopenia and 1 grade Ⅳ neutropenia). There were no interruptions in RT due to toxicity, while in 4 patients chemotherapy was interrupted for 1 wk. Perianal abscess formation was observed in 3 patients before the planned date of surgery. One patient experienced spontaneous perforation at the tumor zone prior to surgery.

Perioperative complications: One patient had spontaneous perforation of the colon before surgery. Surgery was complicated in 4 patients with urethra-bladder injury, and in 1 patient with rectal perforation. Temporary nephrostomy tube was inserted in 3 patients. One patient developed incontinence and impotence due to nerve damage caused by bladder injury. Total proctotectomy procedure was performed in 1 patient due to sudden onset of ischemia during mesorectal resection. Perirectal abscesses developed in 2 patients. Surgical complications are shown in Table 3.

Postoperative chemotherapy: Sixteen (53%) patients underwent adjuvant chemotherapy. Chemotherapy was not given to 13 patients with pathologic complete response after surgery or who had preoperative grade Ⅳ toxicity due to CRT. Grade Ⅴ toxicity (sepsis) was seen in only 1 patient after three cycles of chemotherapy. Adjuvant treatment was terminated prematurely in 2 patients due to grade Ⅳ hematologic toxicity.

DISCUSSION

Despite the continuous search for effective multidisciplinary treatment protocols, patients diagnosed with rectum cancer remain a high-risk population for local and distant recurrence. This study provided encouraging results with neoadjuvant HART plus chemotherapy.

A variety of preoperative RT regimens is used in patients with rectum cancer, and conventional RT (45-50 Gy/5 wk) represents the standard regimen for preoperative concurrent CRT. While a statistically significant advantage in terms of local recurrence rates was reported in 14 previous studies examining this regimen, a survival advantage could be shown in only 2 studies for preoperative RT[9,19]. In these studies, patients with early stage disease (I) and no requirement for preoperative CRT represented the majority of the participants. In a Polish study comparing short-term preoperative RT and conventional CRT, a statistically significant superiority of CRT was observed in terms of complete response rates (P < 0.0001); however, no difference was found in local control and survival[20]. In a randomized study from France comparing preoperative RT and CRT, better pathologic complete response rate (11.4% *vs* 3.6%, P < 0.0001) and reduced local recurrence (8% *vs* 16.5%, P < 0.051) were observed in the CRT arm[10]. In the similarly designed EORTC 22921 study, lower local recurrence was demonstrated in the CRT arm (P < 0.001)[21].

Several phase Ⅱ studies administrating HART alone or with concurrent chemotherapy have also been performed[22-28]. In the HART study by Bouzourene *et al*[29] none of the patients had complete response and 8% of the patients had local remission. In another study by Voelter *et al*[23] examining HART and CT, the reported positive circumferential resection margin was 21% and local control was 100%. In our study, radial surgical margin positivity was 7%, and after a median follow-up of 60-mo the local control rate was 97%. Local recurrence was seen in only 1 patient preoperatively staged as T3N1 and the radial surgical margin was pathologically positive in this patient. In contrast with a phase Ⅱ study by Marsh *et al*[26], where 17 patients receiving preoperative capecitabine and HART had a complete response of 18%, the complete response rate was 21% (grade Ⅳ) and the

Table 4 Studies investigating hyperfractionated accelerated radiotherapy regimen for locally advanced rectal cancer

Study	Number of patients	Design	Follow-up (mo)	Total RT dose	Intervals (wk)	Concomitant chemotherapy	pCR[1]	Local control	Down-staging
Coucke *et al*[24] 2006	250	Prospective	39 mo	41.6 Gy/1.6 Gy	1 wk	None	1.20%	91.70%	38%
Ceelen *et al*[22] 2007	50 *vs* 91	Prospective	67 mo *vs* 28 mo	41.6 Gy/1.6 Gy *vs* 45 Gy/1.8 Gy	13 d *vs* 6 wk	None *vs* 5-FU bolus chemotherapy	4% *vs* 18%	94% *vs* 95.6%	30% *vs* 51%
Voelter *et al*[23] 2006	33	Prospective	104 mo	41.6 Gy/1.6 Gy	1wk	CPT-11	NA	100%	33%
Brooks *et al*[42] 2006	20	Prospective	31 mo	25 Gy/1.67 Gy (CHART)	1 wk	None	NA	95%	NA
Widder *et al*[43] 2005	184	Prospective	43 mo	25 Gy/2.5 Gy	1 wk	None	NA	97.90%	NA
Bouzourene *et al*[29] 2003	104	Prospective	40 mo	41.6 Gy/1.6 Gy	1 wk	None	0%	92.30%	43%
Marsh *et al*[26] 2010	17	Prospective	NA	50.4-55.2 Gy/1.2 Gy	4-6 wk	Capesitabine 825 mg/m^2-twice per day	18.80%	NA	81.25%
The present study	30	Prospective	60 mo	42 Gy/1.5 Gy	6-8 wk	5-FU (325 mg/m^2) continuous infusion	21%	96.70%	59%

[1]Pathological complete response; NA: Not available; RT: Radiotherapy; pCR: Pathological complete response.

Table 5 Biological equivalent doses[44]

	Tumor control/acute normal tissue complication probability		Late normal tissue complication probability
	Bed (Gy) (α/β = 10 Gy)		Bed (Gy) (α/β = 3 Gy)
Regimen	No time correction	With time correction	
25 Gy/5 fr/5 d (d = 5 Gy)	37.5	37.5	66.7
50 Gy/25 fr/33 d (d = 2 Gy)	60.0	44.4	83.4
42 Gy/28 fr/18 d (d = 1.5 Gy)	48.3	41.7	63.0

Equation 1: Linear quadratic based isoeffect, basic formula without time correction, BED = nd (1+d/α/β), where *n* = number of fractions, d = dose (Gy) per fraction, α/β = the LQ quotient, Equation 2: Time-corrected LQ- formula, BED = nd (1+d/α/β)-γ/α (T- Tk), where γ/α = repair rate (set to 0.6 Gy/d), T = overall treatment time and Tk = proliferation delay (set to 7 d, or maximally T).

near-complete response rate was 31% (grade Ⅲ) among our participants. Studies with HART regimen are shown in Table 4.

The primary aim of this study was to search for possible therapeutic strategies that may help increase the rate of pathological tumor response and to decrease late side effects. In the regimen examined herein, decreased fraction size and shortened total treatment duration were hypothesized to result in decreased late and early side effects, respectively. Treatment duration and doses were different from those administered in conventional RT schemes. Therefore, a biological effective dose formula was used for dose calculations instead of the given dose, according to a time-corrected linear quadratic model[30,31]. Biological equivalent doses are shown in Table 5.

In this study combining HART and concurrent chemotherapy, 8 patients developed (26.6%) CRT-related grade Ⅲ-Ⅳ toxicity. Although there was an increase in acute reactions, these effects were generally tolerable and RT was completed without interruption in all patients. In 4 patients, chemotherapy was interrupted shortly due to chemotherapy-related acute side effects. Toxicity was increased as a result of combined use of chemotherapy and RT regimen together with a higher chemotherapy dose as compared to conventional chemotherapy. The highest incidence of side effects was observed at weeks 3 and 4, which correspond to the development of acute mucosal side effects.

In addition, there is some literature data available on early side effects in rectum cancer patients treated with neoadjuvant conventional CRT. For example, in the EORTC 22921 study, grade Ⅲ-Ⅳ toxicity occurred in 14% of the patients[21]. In that study, the probable cause of increased side effects was the total treatment duration and impaired tissue repair as a consequence of shorter intervals between fractions of the chosen HART regimen. In a retrospective study where neoadjuvant CRT and HART alone were compared, no grade Ⅲ-Ⅳ toxicity was reported in the HART arm of the study[22]. In the Phase Ⅱ 93-01 study, patients were treated with neoadjuvant HART with no significant increase in acute side effects[32]. In another phase Ⅱ study with preoperative HART and concurrent irinotecan (CPT-11), addition of chemotherapy was associated with an increase in grade Ⅲ-Ⅳ toxicity[23],

while the most common grade Ⅲ-Ⅳ side effects observed in this study included diarrhea (24%) and infection (9%). In that phase Ⅱ study, early side effects were more frequent than in our study. Probably, reduced incidence of diarrhea in this study could be explained on the basis of sparing the bowel volume out of the RT field.

Bowel perforation occurring in 2 of our patients raises the question of whether a period of 4 wk allows adequate time with normal tissue recovery following an intensive therapy regimen with neoadjuvant HART and concurrent chemotherapy. 5-FU is known to affect the repair mechanism in intestinal cells[33] and the 5-FU dose used in this study might have played a role in the development of perforation in 2 of our patients.

The ideal duration between neoadjuvant therapy and surgery remains a source of debate. The objective of early surgery following short-term RT is to reduce or prevent long-term side effects. However, delayed surgery has been reported to result in increased rates of tumor regression and pathological complete response. In randomized studies utilizing short-term preoperative RT, the time between RT and surgery is relatively short[19,34], posing some challenges in the interpretation of the effects of the timing of surgery following RT. Early and delayed surgery were compared in the Stockholm Ⅲ study where local control, DFS and OS were found to be similar in between three arms[35]. In the randomized Istanbul R-01 study examining the ideal timing for surgery after preoperative CRT, no significant associations were observed between the time-to-surgery and regression rates or local control rates. Surgical margin seems to be the most important factor for local recurrence[36].

In our study, no surgery-related deaths occurred (0/29). In a phase Ⅱ study utilizing HART and concurrent CPT-11, the postoperative complication rate was 27%, similar to other neoadjuvant CRT studies[23]. Operative complications were recorded in 7% of the cases in this study. Occurrence of late toxicity only in 1 patient suggests that the strategy of utilizing HART to reduce late toxicity may prove to be successful. While no late side effects were observed in the 91-10 study with preoperative HART[37], in another study comparing conventional CRT with HART alone, late side effects were more frequently observed in the HART arm[22].

In this study, the ability of the HART regimen to achieve a higher tumor regression rate due to decreasing tumor repopulation was examined. In this regard, complete and near-complete response was achieved in 21% and 31% of the participants, respectively. In a previous study comparing HART alone *vs* conventional CRT regimens, lower complete response rates observed in the HART arm underscores the additive effect of chemotherapy[22]. Similarly, in the French and EORTC studies comparing conventional RT and CRT, the reported pathological complete response rates in the CRT arm were 11.4% and 14%, respectively[38,39]. In our study, HART with concurrent chemotherapy was found to achieve complete or near-complete tumor regression in 52% of the patients. Preoperative HART scheme appeared to be capable of increasing tumor response and local control rates, but no difference was found for OS in phase Ⅱ studies[22]. This study showed no survival benefit despite a high pathological response rate. A study by Petrelli *et al*[36,40] and randomized Istanbul R-01 study did not find any correlation between pathological complete response rate and survival.

Circumferential (lateral) margin positivity was found in 2 patients, whereas only 1 patient showed local recurrence during a median follow-up period of 60 mo. Thirteen patients had distant metastases. Extensive hepatic metastases were found in early phase in 3 patients who died due to systemic disease.

In conclusion, earlier studies have proven the feasibility of HART treatment in terms of early and late side effects in this patient population. As in our study, improved local control rates and tumor regression may be achieved with HART but with higher toxicity. Toxicity could be reduced by giving chronomodulated concomitant capecitabine in Brunch Study[41]. A plausible option would be to reduce the dose of 5-FU to reduce toxicity.

ARTICLE HIGHLIGHTS

Research background

Currently, preoperative chemoradiation (CRT) is the preferred treatment regimen in locally advanced rectal cancer patients, owing to low local recurrence rates and higher chance of sphincter-sparing surgery. Besides conventional radiotherapy consisting of 45-50 Gy/1.8-2 Gy/5-6 wk, other radiotherapy schemes are also used. The hyperfractionated accelerated radiotherapy (HART) scheme reduces the risk of repopulation in tumor cells by shortening the treatment time and increases the repair capacity of normal tissues. In this background, a HART scheme and the combination of infusional 5-fluorouracil (5-FU) was examined in this study to augment the pathological complete response.

Research motivation

Local recurrence is still a substantial problem for locally advanced rectal cancers. Investigating tolerability and the effect of different radiotherapy schemes on local control other than conventional and hypofractionated radiotherapy can be a solution.

Research objectives

This study was mainly designed to observe the early and late effects of HART regimen in combination with neoadjuvant chemotherapy in patients diagnosed with locally advanced rectal cancer. The primary aim of this study was to search for possible therapeutic strategies that may help increase the rate of pathological tumor response and to decrease late side effects.

Research methods

Previously untreated locally advanced rectal cancer patients with histological confirmation were included in the study. The patients were clinically staged according to positron emission-computed tomography and pelvic-diffusion magnetic resonance imaging. All patients received preoperative HART (42 Gy/1.5 Gy/18 d/bid) and concurrent continuous infusion of 5-FU (325 mg/m^2) and were hospitalized during treatments to observe the possible acute side effects. Total mesorectal excision was performed 4-8 wk after the completion of chemoradiotherapy. Four cycles of 5-FU (400 mg/m^2, D1-5, q 28 d) plus folinic acid (20 mg/m^2, D1-5, q 28 d) were administered postoperatively. The primary

endpoint was pathological response rate after CRT, and secondary endpoints included the local control rate, surgical margin positivity, survival and toxicity.

Research results

Thirty patients were included between October 2007 and March 2009. The median age was 53 years. Most of the patients clinically staged as T3N+ disease (90%). Surgery was performed at week 4 in half of the patients. Twelve patients (41%) underwent sphincter-sparing surgery. The Dworak total regression scoring system was used to evaluate pathological response, and grade Ⅳ (total) regression was found in 6 of 29 (21%) patients; nine patients (31%) had grade Ⅲ (near total) regression. Positive margins were found in 2 patients (6.6%). One (3.3%) patient had local recurrence during a median follow-up of 60 mo. The 5-year disease-free survival rate was 53%, while the 5-year overall survival rate was 53.1%. There were no interruptions in RT due to toxicity, while in 4 patients chemotherapy was interrupted for 1 wk. Sixteen (53%) patients underwent adjuvant chemotherapy.

Research conclusions

Improved local control rates and tumor regression may be achieved with HART but with higher acute toxicity. Toxicity could be reduced by giving chronomodulated concomitant chemotherapy or reducing the dose of 5-FU. Surgery timing has no effect on survival but still should be considered because of increased acute side effects due to HART fractionation. Besides an increased pathological response rate, this study showed no survival benefit.

Research perspectives

Different HART schemes can be examined with concomitant chemotherapy in the future studies. Because of the high incidence of acute toxicity, fraction dose and chemotherapy doses should be designed properly for new studies.

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META-ANALYSIS

Comparison between laparoscopic and open surgery for large gastrointestinal stromal tumors: A meta-analysis

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Abstract

AIM

To investigate whether laparoscopic surgery is as safe and feasible as open resection for patients with larger gastrointestinal stromal tumors (GISTs) ($\geqslant$ 5 cm).

METHODS

A systematic search of PubMed, EMBASE, Web of Science and the Cochrane Library database was performed. Relevant studies of laparoscopic and open surgery for GISTs of > 5 cm published before December 2016 were identified from these databases. The quality of the studies was assessed by the Newcastle-Ottawa Quality Assessment Scale. The tumor size, operation time, blood loss, postoperative hospital stay, complication rate, and disease-free survival rate were assessed. The software Stata (version 12.0) was used for the meta-analysis.

RESULTS

Five clinical trials comprising 209 patients with GISTs of similar larger sizes were evaluated. The pooled analysis of 100 patients in the laparoscopic resection group and 109 patients in the open resection group demonstrated that laparoscopic surgery was significantly associated with a shorter postoperative hospital stay (P < 0.001)

and less blood loss (P = 0.002). Moreover, there were no statistically significant differences in the operation time (P = 0.38), postoperative complication rate (P = 0.88), or disease-free survival rate (P = 0.20) between two groups.

CONCLUSION

Our findings revealed that for patients with large GISTs of comparable sizes, laparoscopic surgery did not significantly influence the operation factors or clinical outcomes compared with open surgery. This suggests that laparoscopic resection is as acceptable as open surgery for treatment of large gastric GISTs.

Key words: Laparoscopic resection; Open resection; Gastrointestinal stromal tumor; Meta-analysis; Clinical outcome

Core tip: Whether laparoscopic resection is also effective and feasible for treatment of larger gastric gastrointestinal stromal tumors (GISTs) (> 5 cm) remains unknown. This meta-analysis collected up-to-date clinical data of comparison of laparoscopic and open resection for larger gastric GISTs (> 5 cm). Our results showed that laparoscopic resection is an upgraded minimal invasive technique with a shorter postoperative hospital stay and less intraoperative blood loss compared with open surgery in treating patients with larger GISTs.

Cui JX, Gao YH, Xi HQ, Cai AZ, Zhang KC, Li JY, Wei B, Chen L. Comparison between laparoscopic and open surgery for large gastrointestinal stromal tumors: A meta-analysis. *World J Gastrointest Oncol* 2018; 10(1): 48-55 Available from: URL: http://www.wjgnet.com/1948-5204/full/v10/i1/48.htm DOI: http://dx.doi.org/10.4251/wjgo.v10.i1.48

INTRODUCTION

Gastrointestinal stromal tumors (GISTs) are the most common gastrointestinal sarcomas. They usually arise from the interstitial cells of Cajal and regulate gastrointestinal motility[1,2]. GISTs are often characterized by cellular markers such as CD117 (a receptor tyrosine kinase protein also known as tyrosine-protein kinase Kit). The stomach is the most prevalent location of GISTs, and the proximal stomach is involved in about two-thirds of suffering patients[3]. It is well accepted that the malignant potential of GISTs depends on the tumor size, cell mitotic rate, and tumor location[4].

Although substantial advances have been made in the targeted therapies for these tumors, surgical resection is still the most important component in the treatment of primary GISTs with no evidence of metastasis. Because wide margins (> 5 cm) and lymph node dissection are not necessary in the surgical management of GISTs[5], laparoscopic surgery seems to be more suitable for resection of these tumors. Various types of laparoscopic procedures for GISTs have been performed in a few specialized centers, including wedge resection of the stomach, intragastric tumor resection, and combined endoscopic–laparoscopic resection, *etc*. However, during laparoscopic surgery, these tumors must be handled with great care because rupture of their capsule confers a near 100% risk of recurrence.

Several studies and meta-analyses have shown that laparoscopic resection for gastric GISTs is as safe and efficacious as open surgery; additionally, laparoscopy is associated with less blood loss, less morbidity, and quicker recovery[6-8]. The long-term survival of patients with GISTs mainly depends on the tumor progression, and laparoscopic surgery does not increase the risk of tumor relapse and metastasis. The clinical practice guidelines for the management of GISTs released by the National Comprehensive Cancer Network and the Japanese Study Group on GIST note that laparoscopic surgical resection is the preferred therapy for relatively small GISTs with a diameter of < 5 cm[9].

However, most cohort studies have focused on laparoscopic surgery for relatively smaller tumors; few have been designed for evaluation of larger GISTs (> 5 cm)[10-14]. Although the size limit was not clearly stated, the practice guideline of the European Society for Medical Oncology recommends application of laparoscopic procedures in patients with large GISTs[15]. However, the complex surgical skills and long learning curve associated with laparoscopic surgery might prevent its application to larger GISTs to some extent[16]. Therefore, the feasibility and safety of laparoscopic surgery for GISTs of > 5 cm remains unclear. Additionally, whether 5 cm is the most appropriate cutoff for performance of minimally invasive procedures in patients with larger GISTs remains controversial. This meta-analysis was performed to assess the short- and long-term results of patients with larger gastric GISTs (> 5 cm) undergoing laparoscopic surgery.

MATERIALS AND METHODS

Literature search

Systematic electronic searches of PubMed, EMBASE, the Cochrane Library, the Clinical Trials Database, Web of Science, and Google Scholar were performed to identify relevant articles published up to 30 December 2016, utilizing the following search terms: "gastrointestinal stromal tumor," "GIST," "laparoscopic," "laparoscopy," "open resection," "gastrectomy," and "stomach". Citations and references of identified studies were also reviewed for additional literature and trials. The language of the publications was limited to English.

Table 1 Main characteristics of enrolled trials

Ref.	Region	Year	Study design	Study period	Sample size		Tumor size (cm)		CS	Follow-up (mo)
					LAP	Open	LAP	Open		
Kim *et al*[10]	South Korea	2012	OCS (R)	1998-2011	24	14	6.1 ± 1.3	7.2 ± 1.7	0	49.3 (8.4-164.4)
Lin *et al*[11]	China	2014	OCS (R)	2007-2012	23	23	7.2 ± 1.6	7.3 ± 1.5	1	34.0 (6-78)
Hsiao *et al*[12]	Taiwan	2015	OCS (P)	2002-2012	18	37	6.1 ± 1.0	6.0 ± 0.9	0	43.2 (16.8-133.2)
Takahashi *et al*[13]	Japan	2015	OCS (R)	1995-2011	12	15	7.5 ± 1.9	5.5 ± 0.73	3	63 (7-154)
Khoo *et al*[14]	Japan	2016	OCS (R)	2002-2015	23	36	NA	NA	1	45

OCS: Observational clinical study; R: Retrospective study; P: Prospective study; NA: Not available; CS: Convention surgery.

Study selection

The inclusion criteria were as follows: (1) The studies involved patients with gastric GISTs larger than 5 cm; (2) The specific interventions were laparoscopic and open surgical resection; (3) The clinical outcomes were the operation time, intraoperative blood loss, conversion rate, length of hospital stay, adverse events, and long-term outcomes (overall survival, disease-specific survival, or recurrence rate); (4) Controlled studies (randomized controlled trials, cohort studies, and case-control studies) were included for the pooled analysis. However, case reports and case series were included for the systematic review; and (5) The informative data and full text of the articles were available.

The exclusion criteria were as follows: (1) The patients had GISTs that were located outside of the stomach or complicated with mixed disease; (2) Duplicate publications; (3) the size of the GIST was not specifically stated; (4) The article was a case report or review; and (5) The publication was in a language other than English.

Data extraction and management

Two reviewers independently screened the titles and abstracts of the publications. Once deemed acceptable, the whole manuscripts were obtained and screened. Controversial issues were resolved by discussion or referred to a third reviewer. Another two reviewers independently extracted the data using a unified form and resolved any discrepancies through discussion. The variables of interest included the author, study period, number of patients, tumor size, operation time, blood loss, length of postoperative hospital stay, complication rate, and long-term outcome (namely disease-free survival). In addition, if the original studies included the median, range, and size of a sample, we estimated the mean and variance using the methods described by Hozo *et al*[12].

The quality of the included papers was assessed using the Newcastle-Ottawa Quality Assessment Scale[17]. This scale ranges from 0 to 9 points; studies with a score of $\geqslant$ 6 were considered methodologically sound.

Statistical analysis

The meta-analysis was performed using weighted mean differences (WMDs) for continuous variables, odds ratios for dichotomous variables, and hazard ratios for time-to-event variables. Statistical heterogeneity was assessed by performing χ^2 tests and calculating the Higgins I^2 statistic, and a value of P < 0.10 or I^2 > 50%, indicated statistical significance. A fixed-effects model was generally employed. If the heterogeneity was statistically significant, a random-effects model was adopted. Publication bias was evaluated by Begg's test. A P value of < 0.05 was considered significant. Statistical analyses were performed using Stata software (version 12.0; StataCorp, College Station, TX, United States).

RESULTS

Enrolled studies and quality assessment

No eligible randomized controlled trials were identified, but 5 nonrandomized trials were analyzed (209 patients with GISTs of similar size). Overall, 100 patients underwent laparoscopic resection and 100 underwent open resection. A flow chart of the search strategy is illustrated in Figure 1. The main characteristics and quality assessment results of the included studies are shown in Tables 1 and 2, respectively.

Tumor size

Four studies reported no statistically significant differences in tumor size between the laparoscopy and open group, while Kim *et al*[10] reported that the tumor size in the open group was significantly larger than that in the laparoscopy group. Additionally, in the pooled data from a fixed-effects model with no significant heterogeneity (I^2 = 53.3%, P = 0.073) (Table 3), no significant difference was identified in the total analysis [WMD = -0.038 cm, 95% confidence interval (95%CI): -0.699 to 0.362, P = 0.632] (Figure 2).

Operative factors

All enrolled studies provided data for analysis of the operation time. The results showed no significant difference between the two groups (WMD = 7.17 min, 95%CI: -56.02 to 70.36, P = 0.824) (Figure 3A). Because obvious heterogeneity was detected (I^2 = 92.9%, P = 0.000) (Table 3), a random-effects model was employed.

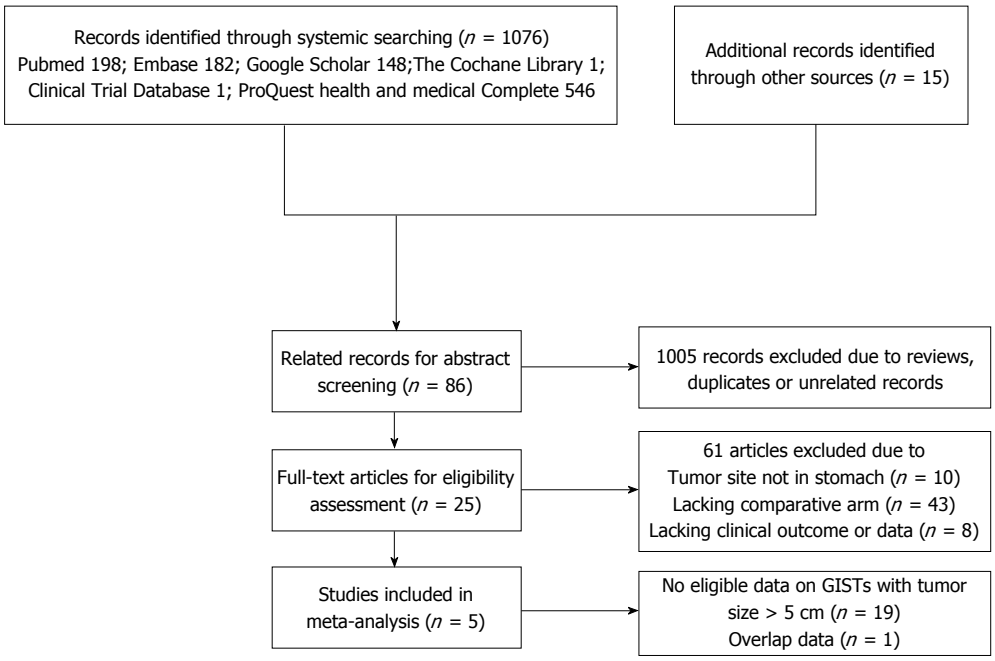

Figure 1 Flow chart of study selection process. GISTs: Gastrointestinal stromal tumors.

Table 2 Newcastle-Ottawa Scale Assessment of enrolled studies

Ref.	Selection (0-4)				Comparability		Outcome (0-3)			Total
	REC	Snec	AE	OINP	SCB	SCA	AO	FU	AFC	
Kim *et al*[10]	1	1	1	1	1	0	1	1	1	8
Lin *et al*[11]	1	1	1	1	1	1	1	1	0	8
Hsiao *et al*[12]	1	1	1	1	1	0	1	1	1	8
Takahashi *et al*[13]	1	1	1	0	1	0	1	1	1	7
Khoo *et al*[14]	1	1	1	1	1	1	1	1	1	9

REC: Representativeness of the exposed cohort; SNEC: Selection of the no exposed cohort; AE: Ascertainment of exposure; OINP: Outcome of interest not presented in the start of study; SCB: Study controls for basic characteristics; SCA: Study controls for additional factor; AO: Assessment of outcome; FU: Follow-up; AFC: Adequacy of follow up.

Table 3 Summary results of meta-analysis of clinical outcomes

Outcomes	No. of studies	Effect value	95%CI of effect	Heterogeneity	
				I^2 (%)	*P* value
Tumor size	4	WMD = -0.0.38	-0.699 to 0.362	53.3	0.073
Operation time	5	WMD = 7.17 min	-56.02 to 70.36	92.9	0.000
Blood loss	4	WMD = -47.47 mL	-93.20 to -1.73	63.2	0.043
Postoperative complications	5	OR = 0.93	0.34 to 2.50	0.0	0.858
Postoperative stay	5	WMD = -2.81 d	-3.68 to -1.94	38.7	0.163
Progression-free survival	5	HR = 0.64	0.35 to 1.19	0.0	0.553

WMD: Weighted mean differences.

Four studies reported data regarding intraoperative blood loss; Lin *et al*[11] reported that laparoscopic surgery was associated with less blood loss. The heterogeneity between the studies was significant (I^2 = 63.2%, *P* = 0.043); therefore, the analysis was performed with a random-effects model. In the pooled data, a significant difference was found among these three groups (WMD = -47.47 mL, 95%CI: -93.20 to -1.73 mL, *P* = 0.042) (Figure 3B).

Among all enrolled studies, five patients in the laparoscopy group reportedly underwent conversion to open surgery. One conversion resulted from the surgeons' initial learning curve for laparoscopy, one was due to dense adhesion to liver, and the other three occurred because of failure to secure the tumor in the visual field of the laparoscope.

Short-term outcomes

All five studies reported postoperative complications. The pooled data revealed no significant difference

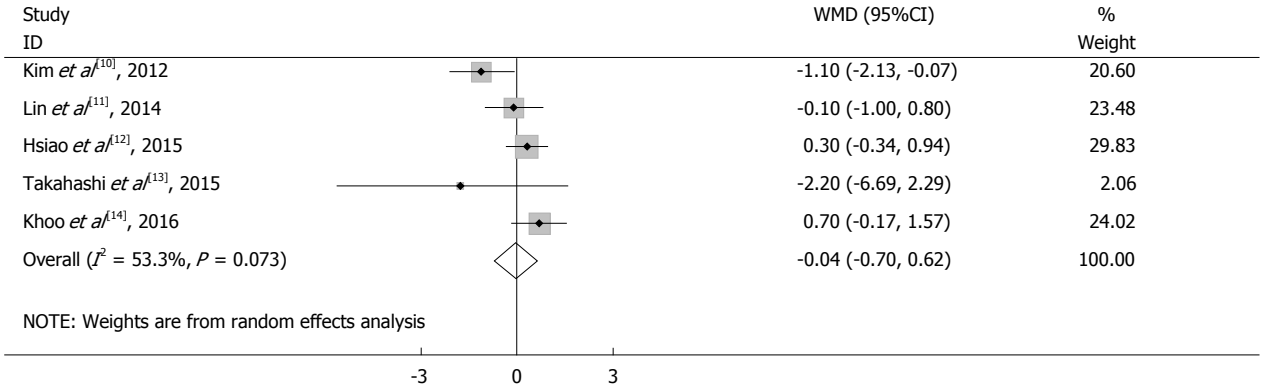

Figure 2 Meta-analysis of tumor size in laparoscopic surgery and open surgery groups.

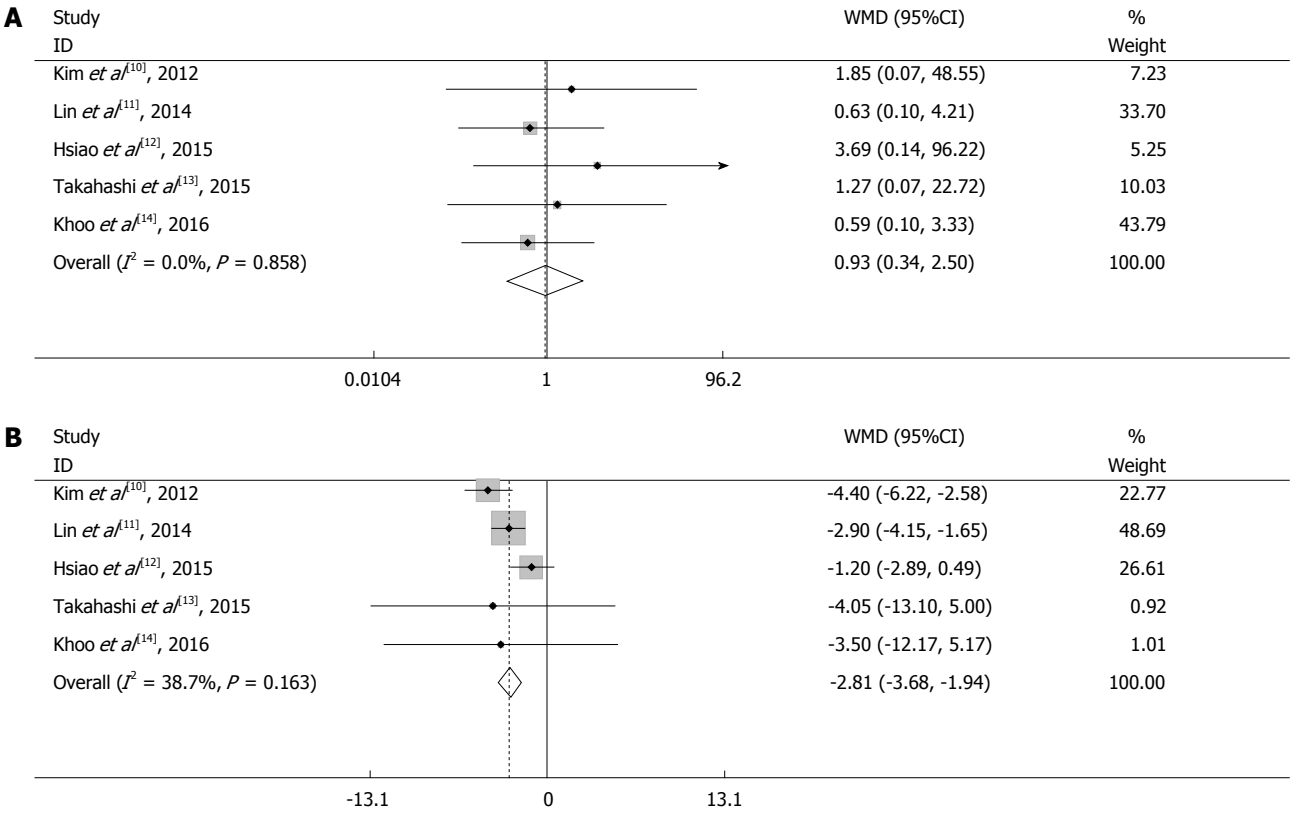

Figure 3 Meta-analysis of operative factors in laparoscopic surgery and open surgery group. A: Pooled analysis of operation time; B: Pooled analysis of blood loss.

between the two groups (odds ratio = 0.93, 95%CI: 0.34 to 2.50, P = 0.88) (Figure 4A). A fixed-effects model was used because of the lack of significant heterogeneity (I^2 = 0.0%, P = 0.858).

Five studies reported data regarding the postoperative hospital stay. A fixed-effects model was employed because of insignificant heterogeneity (I^2 = 38.7%, P = 0.163). The postoperative hospital stay was significantly shorter in the laparoscopy than open group (WMD = -2.81 d, 95%CI: -3.68 to -1.94, P < 0.001) (Figure 4B).

Long-term outcomes

All eligible studies reported the progression-free survival of patients. Figure 5 shows a forest plot of disease-free survival and the results of the meta-analysis. No significant difference was observed in patients with larger GISTs who underwent laparoscopic *vs* open surgery (hazard ratio = 0.64, 95%CI: 0.35 to 1.19, P = 0.157). No obvious heterogeneity was observed in this study; therefore, a fixed-effects model was applied in the survival meta-analysis (I^2 = 0.0%, P = 0.553) (Figure 5).

Publication bias

Publication bias was evaluated based on the postoperative hospital stay using Begg's and Egger's tests. No publication bias was identified in the five studies (Begg's test, P = 0.773; Egger's test, P = 0.825) (Figure 6).

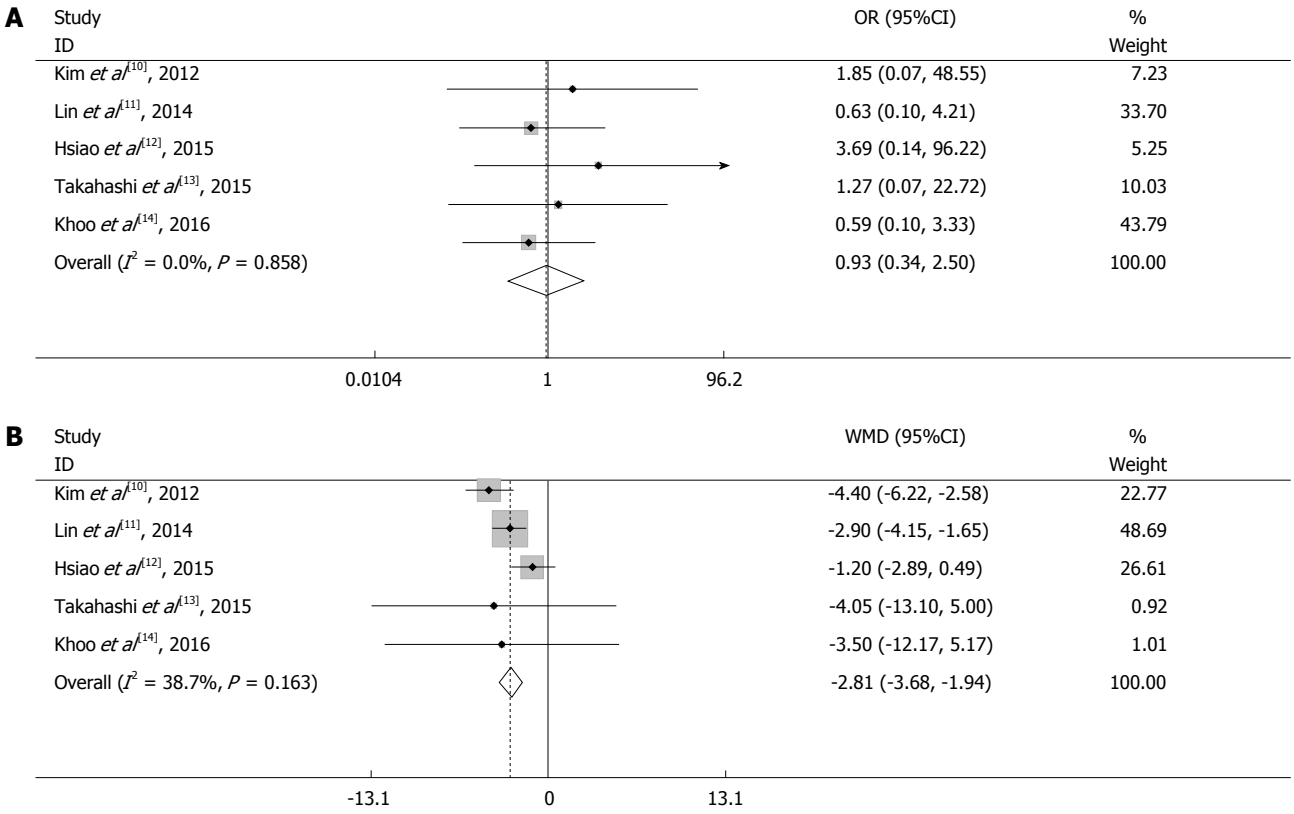

Figure 4 Meta-analysis of short-term outcomes in laparoscopic surgery and open surgery groups. A: Pooled analysis of postoperative complications; B: Pooled analysis of postoperative hospital stay.

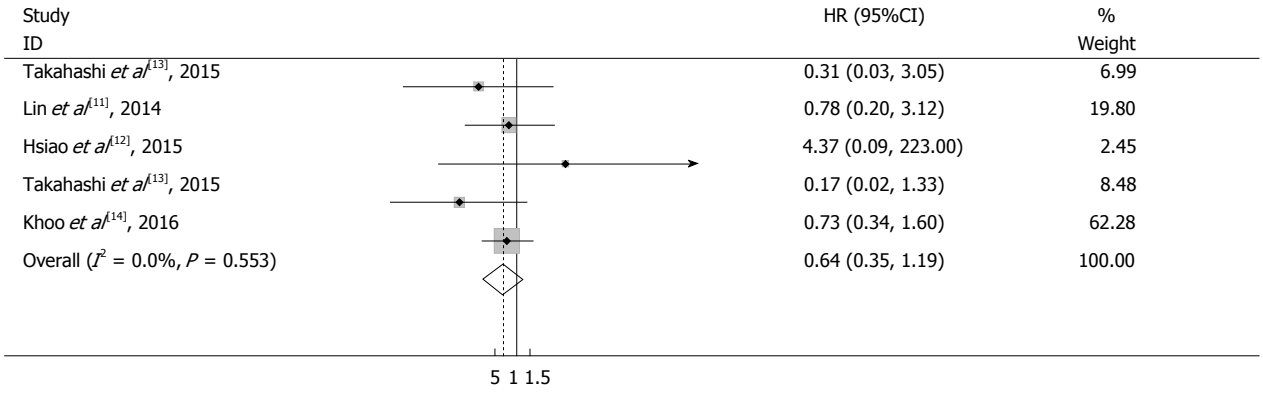

Figure 5 Meta-analysis of progression-free survival in laparoscopic surgery and open surgery groups.

DISCUSSION

Recent studies have suggested that the prognosis of GISTs is mainly based on the tumor size and histological features rather than achievement of wide resection margins[18]. Therefore, laparoscopic resection is more frequently performed for treatment of patients with GISTs using the advances currently being made in surgical techniques.

Although randomized controlled trials are the first choice for high-quality meta-analyses, we failed to enroll any randomized controlled trials in this study. There are several obstacles to design and perform randomized controlled trials, such as ethical issues and organization difficulty[19]. Finally, five nonrandomized controlled studies (one prospective and four retrospective) were enrolled; all were assessed according to the Newcastle-Ottawa Quality Assessment Scale and scored > 6, ensuring their high quality.

Our pooled analysis demonstrated faster recovery and less blood loss in the laparoscopy than open surgery group. Less trauma caused by laparoscopic surgical intervention, only a mild acute inflammatory response, and earlier postoperative activities are considered to contribute to the shorter postoperative hospital stay. Although the blood loss volume might have varied according to the different methods used among the studies, the results of our work indicate

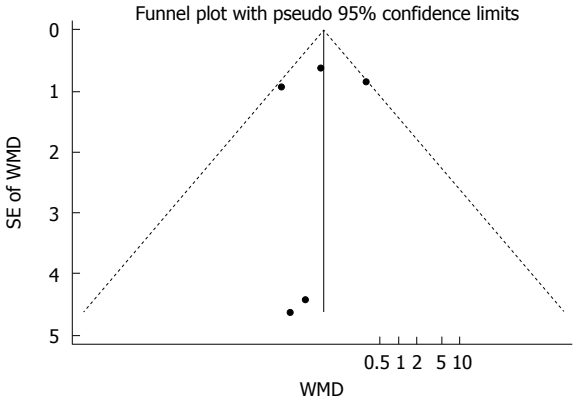

Figure 6 Funnel plot of postoperative hospital stay in laparoscopic surgery and open surgery groups. WMD: Weighted mean difference.

that laparoscopic surgery might reduce patients' surgical trauma to some extent. Furthermore, there was no difference in the postoperative complications between the two groups, adding to the safety of laparoscopic surgery in patients with larger GISTs.

Our review also indicated that laparoscopic resection for larger GISTs is feasible with a conversion rate of 5%, which is similar to other laparoscopic procedures such as laparoscopic gastrectomy[20,21]. The oncological outcome is one of the most concerning problems that prevents application of laparoscopy to the surgical treatment of larger GISTs[22]. Our results showed no difference in the disease-free survival of patients with larger GISTs who underwent laparoscopy *vs* open surgery (hazard ratio = 0.643, 95%CI: 0.349 to 1.185, $P = 0.157$), suggesting that the performance of a laparoscopic procedure does not profoundly influence the oncological outcome compared with open surgery.

Several limitations in our study should be addressed. First, the limited number of patients might affect the reliability of the results (209 patients across 5 studies). Second, most of the patients' tumor sizes ranged from 5 to 10 cm; therefore, the results might not be suitable for patients with GISTs of > 10 cm. Third, treatment of larger GISTs in laparoscopic surgery requires greater surgical skill to prevent tumor rupture and gain adequate resection margins. Therefore, the inclusion of single-center studies with various levels of surgical techniques might have contributed to the bias of our meta-analysis. Finally, the use of different risk classifications and drug therapies within the groups might have also contributed to the bias of recurrence or progression-free survival[23].

In conclusion, this meta-analysis has demonstrated that laparoscopic surgery is as safe and feasible as open surgery for resection of larger GISTs (> 5 cm, mainly 5-10 cm). Moreover, laparoscopic surgery might offer the advantage of faster recovery and less trauma over open surgery in patients with GISTs. More multicenter randomized controlled clinical trials are needed to clarify and confirm the role of laparoscopic surgery in patients with larger GISTs.

ARTICLE HIGHLIGHTS

Research background

Laparoscopic resection of relatively small gastric gastrointestinal stromal tumors (GISTs) is currently well-accepted and has been proven as safe and feasible as traditional open surgery. However, whether laparoscopic resection is also effective and feasible for treatment of larger gastric GISTs (> 5 cm) remains unknown.

Research motivation

The authors aimed to explore whether laparoscopic resection is also effective and feasible for treatment of larger gastric GISTs (> 5 cm), just as the same situation in smaller GISTs.

Research objectives

Laparoscopic resection for small GISTs is now well-accepted. However, whether laparoscopic surgery is as safe and feasible as open resection for patients with larger GISTs (⩾ 5 cm) remains controversial.

Research methods

A systematic search of PubMed, EMBASE, Web of Science and the Cochrane Library database was performed. Relevant studies of laparoscopic and open surgery for GISTs of > 5 cm published before December 2016 were identified from these databases. The meta-analysis was performed using Stata (version 12.0) applying weighted mean differences for continuous variables, odds ratios for dichotomous variables, and hazard ratios for time-to-event variables.

Research results

In terms of operative and oncological factors, our research demonstrated that laparoscopic surgery was significantly associated with a shorter postoperative hospital stay ($P < 0.001$) and less blood loss ($P = 0.002$) in resecting larger GISTs. Moreover, there were no statistically significant differences in the operation time ($P = 0.38$), postoperative complication rate ($P = 0.88$), or disease-free survival rate ($P = 0.20$) between two groups.

Research conclusion

This research stands as the first meta-analysis focusing on this specific type of GISTs. The meta-analysis has demonstrated that laparoscopic surgery is as safe and feasible as open surgery for resection of larger GISTs (> 5 cm, mainly 5-10 cm). Moreover, laparoscopic surgery might offer the advantage of faster recovery and less trauma over open surgery in patients with GISTs.

Research perspectives

Laparoscopic resection is as acceptable as open surgery for treatment of large gastric GISTs.

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CASE REPORT

Leptomeningeal metastases originated from esophagogastric junction/gastric cancer: A brief report of two cases

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Author contributions: Kountourakis P designed the case reports presentation; Kountourakis P, Papamichael D and Andreopoulos D participated in manuscript preparation, revision, patient's investigation and treatment; Haralambous H provided the CT-MRI images; Michael M provided the cytology images; Nakos G provided the pathology images; Lazaridou S, Fotiou E and Vassiliou V participated in patients' investigation and treatment.

Informed consent statement: Patients are deceased and verbal consents were obtained at the time of their hospitalization.

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Abstract

Leptomeningeal carcinomatosis is a very rare manifestation in patients diagnosed with esophagogastric junction and gastric cancer. Its prognosis is ominous and therapy outcomes are disappointing. Herein, we present two patients; one initially diagnosed with gastric cancer and leptomeningeal carcinomatosis but no other evidence of metastatic disease and the other one initially diagnosed with esophagogastric junction cancer, who recurred solitary with leptomeningeal seedings several years after the initial diagnosis and treatment. Furthermore, a thorough and short review of the literature is carried out.

Key words: Esophagogastric junction cancer; Gastric cancer; Leptomeningeal carcinomatosis; Prognosis; Investigation; Therapy

Core tip: Leptomeningeal carcinomatosis (LMC) is related with ominous prognosis and the median survival varies between a few weeks to months. Even LMC is extremely rare in patients diagnosed with esophagogastric junction and gastric cancer, physicians should be alerted when neurological symptoms occurred, are persistent and could not be explained. A single diagnosis test procedure itself is not absolutely sensitive and the investigation algorithm may comprise a gadolinium enhanced brain magnetic resonance imaging and cerebrospinal fluid cytology tests.

Kountourakis P, Papamichael D, Haralambous H, Michael M, Nakos G, Lazaridou S, Fotiou E, Vassiliou V, Andreopoulos D. Leptomeningeal metastases originated from esophagogastric junction/gastric cancer: A brief report of two cases. *World J Gastrointest Oncol* 2018; 10(1): 56-61 Available from: URL: http://www.wjgnet.com/1948-5204/full/v10/i1/56.htm DOI: http://dx.doi.org/10.4251/wjgo.v10.i1.56

INTRODUCTION

Esophagogastric junction (EGJ) and gastric cancer (GC) constitute a major health issue worldwide and are often diagnosed in advanced stage with dismal prognosis[1]. Leptomeningeal carcinomatosis (LMC) is defined as cancerous infiltration of the arachnoid membrane and the pia mater with devastating prognosis. Several routes of spread to meninges have been suggested; direct infiltration from bone metastases, *via* arteries and lymphatics, perineural and perivascular spaces, retrograde flow of Batson's venous plexus[2]. Among cancer patients, it is most often related to breast and lung tumors, melanoma, leukemia and lymphoma[3]. Only a few cases of EGJC and GC patients have been reported with LMC as an upfront disease manifestation. Most of the cases are presented several months or years after the initial diagnosis with synchronous diffuse disease spread. It is reported a 0.16%-0.69% of GC cases with LMC diagnosis, meanwhile it is clinically diagnosed in 2%-4% of all cancer patients[4,5]. Herein, we present two patients with LMC and primary site diagnosis of EGJ and GC, respectively (Table 1).

CASE REPORT

Case 1

A 64-year-old female was referred to our Centre in September 2015. Her disease symptoms started almost 3 wk before with severe episodes of headache, dizziness visual and hearing loss (mainly right). From her past medical and family history nothing important to be mentioned. Initially, she was investigated by a computed tomography (CT) brain scan (Figure 1A) with no evidence of suspicious findings and pain killers were administrated with no benefit. Afterwards, steroids were administrated empirically with initial improvement of symptoms for a few days. Due to deterioration, further investigation by brain magnetic resonance imaging (MRI) revealed diffuse meningeal enhancement (Figure 1B). It was extended also into the internal auditory canal and optic nerves sheaths more into the right side. CT chest, abdomen, pelvis scans revealed only thickness in the area of gastric cardia and further investigation by an upper GI endoscopy confirmed the diagnosis of a poorly differentiated gastric adenocarcinoma with signet ring features (Figure 2). Cerebrospinal fluid cytology (CSF) confirmed the diagnosis of LMC (Figure 3). Full blood count and biochemistry tests were within normal values and tumor markers' evaluation revealed CEA = 33.3 ng/mL. Her clinical status deteriorated rapidly, was in coma, and the other day of IT with Methotrexate (MTX, 12.5 mg) the patient died, approximately within 4 wk after the initial onset of disease symptoms.

Table 1 Characteristics of patients diagnosed with leptomeningeal carcinomatosis

Characteristics	Case 1	Case 2
Age (yr)	64	57
Sex	Female	Male
Primary neoplasm	Stomach	EGJ
Histology	Adenocarcinoma gr Ⅲ, signet ring cells	Adenocarcinoma gr Ⅲ, signet ring cells
Disease status	Initial diagnosis	Recurrence
Systemic disease	None other than LMC	None other than LMC
Main neurological symptoms	Headache, dizziness, visual and hearing loss	Headache, dysphasia, temporary left side paresis
CSF cytology	Positive	Positive
Brain imaging studies	CT: No findings MRI: Positive	CT: No findings MRI: Positive

EGJ: Esophagogastric junction; LMC: Leptomeningeal carcinomatosis; CSF: Cerebrospinal fluid; CT: Computed tomography; MRI: Magnetic resonance imaging.

Case 2

A 51-year-old man was diagnosed with an EGJ poorly differentiated adenocarcinoma (cT3N+, M0) in July 2009. He was therefore commenced on peri-operative chemotherapy with epirubicin, cisplatin and capecitabine regimen and on 02/11/2009 he was operated (Ivor-Lewis gastrectomy, ypT2N1, R0, gr Ⅲ adenocarcinoma with signet ring features, Figure 4). He remained disease-free until September 2015 when he experienced neurological symptoms such as dysphasia, headaches and temporary left side paresis. Initially, investigations by CT brain (Figure 5A), chest, abdomen, pelvis scans revealed no evidence of disease. Subsequently, a brain MRI scan revealed findings of LMC (Figure 5B). A CSF cytology investigation confirmed the diagnosis consistent with GC origin (Figure 6), meanwhile a

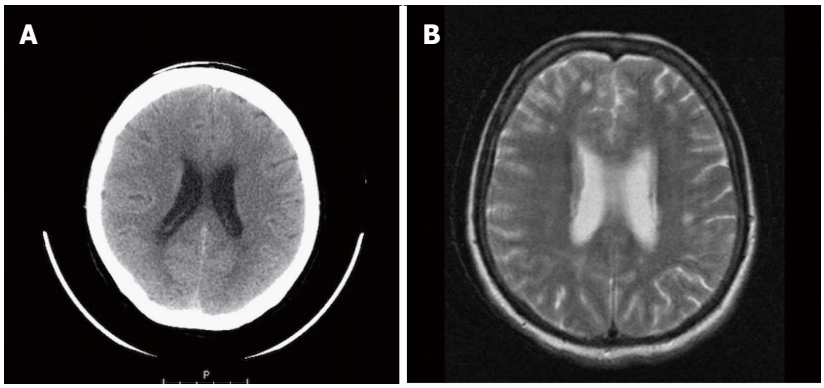

Figure 1 Patient No. 1. A: Computed tomography brain did not reveal brain lesions; B: Magnetic resonance imaging brain showed findings consistent with leptomeningeal carcinomatosis.

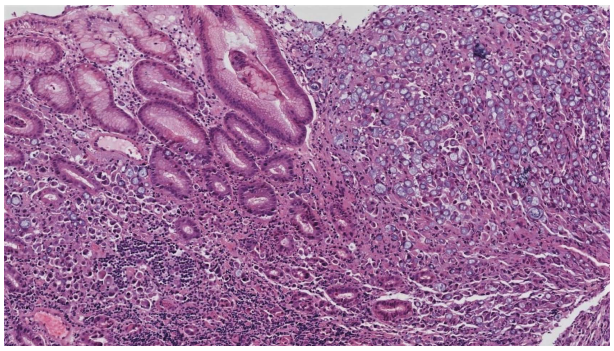

Figure 2 Patient No. 1. Diffuse infiltration of gastric mucosa from a poorly differentiated poorly cohesive gastric adenocarcinoma (including mixed adenocarcinoma with > 50% signet ring cells features (HE 100 ×).

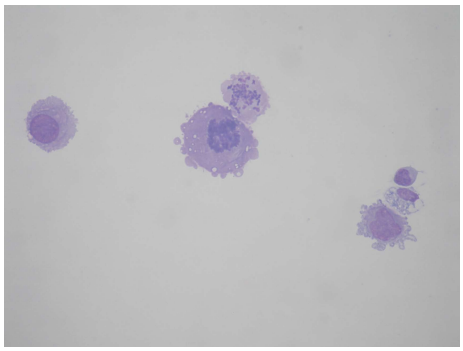

Figure 3 Patient No. 1. Four atypical cells, one lymphocyte and one macrophages next to the lymphocyte. Atypical cells are isolated, two of those show mitotic activity. The size of atypical cells and lymphocyte could be compared (Hemacolor 40 ×).

flow cytometric test confirmed the presence of a non hematopoietic cell population. Full blood count and biochemistry tests were within normal values and tumor markers' evaluation revealed CEA = 6.3 ng/mL, CA 19-9 = 98.1 μg/mL. He was treated with intrathecal MTX (12.5 mg) twice-weekly with relatively good clinical response initially. After three weeks of treatment he was put on "maintenance" application once weekly, but unfortunately his performance status was rapidly deteriorated after a couple of

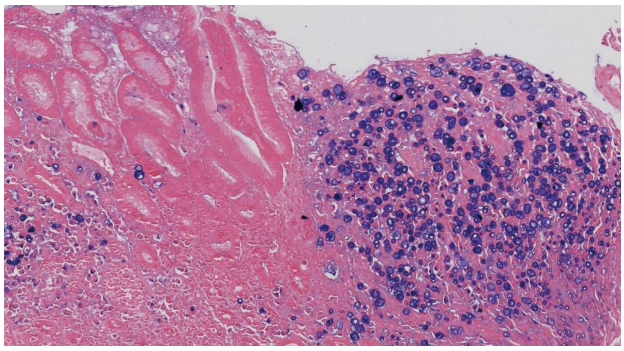

Figure 4 Patient No. 2. Alcian blue highlights difference in mucin production between cancer cells (blue) and normal gastric tissue (no presence), helping us also determine about the extent of the infiltration (Alcian blue 200 ×).

weeks. Therefore, his treatment was changed to MTX, Cytosine Arabinoside (40 mg) and Dexamethasone (4 mg). He received 3 and 5 applications with good clinical response, in October and in December 2009, respectively. Afterwards, the CT chest-abdomen-pelvis re-staging scans revealed no clear evidence of local recurrence or metastatic disease, meanwhile the MRI brain performed revealed slight improvement of meningeal enhancement. In July 2016, his performance status deteriorated with severe episodes of headaches, dizziness, dysphasia, visual and hearing loss and consciousness deduction. He was re-challenged with IT but with modest improvement and died in September 2016.

DISCUSSION

Leptomeningeal carcinomatosis is often presented with non specific clinical symptoms like headache, nausea and vomiting. Supratentorial involvement could cause altered mental and personality status, dysphasia and seizures. When infratentorial lesions occurred, they are mainly presented with cranial nerve palsies and related symptoms[6,7]. The gadolinium enhanced MRI and CSF are the main investigation procedures. The sensitivity and specificity of the brain MRI are

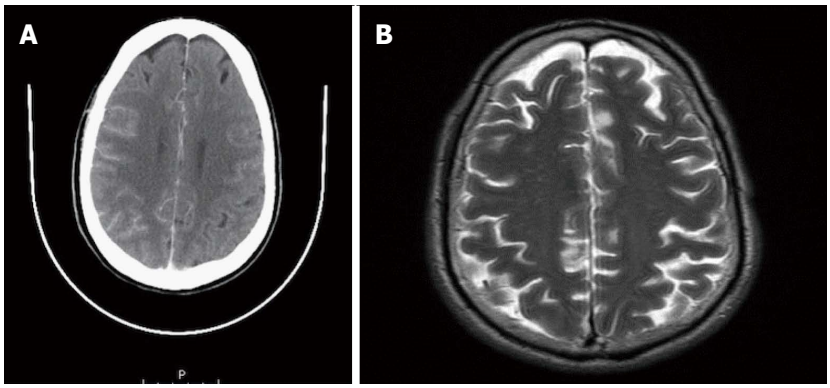

Figure 5 Patient No. 2. A: Computed tomography brain did not reveal brain lesions; B: Magnetic resonance imaging brain showed findings consistent with leptomeningeal carcinomatosis.

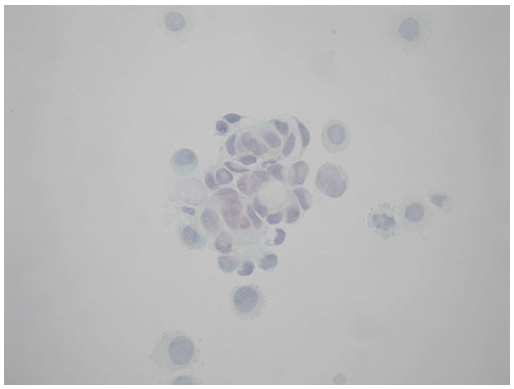

Figure 6 Patient No. 2. An irregular cluster of atypical cells. The cells show intermediate size, degeneration changes, indistinct cytoplasmic borders and moderate size of nuclei. Nuclear/cytoplasmic ratio is increased (Papanicolaou 40 ×).

66%-76% and 75%-77%, respectively, but could be almost two times higher than those of the CT[8-10]. Approximately in 67% of cases imaging studies reveal findings such as focal or diffuse abnormal meningeal enhancement and nodules detection[11]. Moreover, meningeal enhancement is suggestive but does not confirm the diagnosis. Infection or inflammatory causes, intracranial hypertension but even a lumbar puncture procedure before MRI scan could induce diffuse meningeal enhancement for a period of weeks to months and give false positive results[12]. After first sampling of CSF examination the possibility of a positive result is approximately 54%. This raises to 91% after multiple cytology investigations[6]. On the contrary, a negative cytology investigation after three lumbar punctures does not rule out the diagnosis of LMC in the context of other positive tests, like an MRI with characteristic findings.

Reviewing the literature, it is obvious that this is a rare manifestation. In a retrospective analysis of medical records from Memorial Sloan-Kettering Cancer Center of consecutive 90 patients with LMC treated from 1975 to 1981, none was diagnosed with EGC/GC[6]. From MD Anderson cancer Center, between 1985 to 2001 in more than 1500 EGC/GC patients, eight cases with leptomeningeal seedings were identified[13]. Furthermore, in a Korean retrospective multicenter analysis conducted between 1995 to 2007, 54 patients were identified with LMC from a total 22154 GC patients[14].

There is no doubt that LMC is related with ominous prognosis. Treatment goals initially focus to improvement of neurological symptoms and quality of life and secondarily to prolongation of survival. In the meantime, median survival varies between a few weeks to months. Current treatment approaches may be IT, systemic therapy and or cranio-spinal radiation therapy (RT) but the results of these approaches are disappointing and a lot of times confusing and conflicting. There are no robust data from multicenter prospective studies to support the superiority of IT *vs* best supportive care. A study compared MTX/ Ara-C/hydrocortisone combination *vs* single agent MTX. Primary sites were the lung (n = 33), breast (n = 13) and stomach (n = 5). Superiority was revealed for the combination arm for median overall survival (18.6 *vs* 10.4 wk, P = 0.02) and cytology negative conversion (38.5% *vs* 13 %, P = 0.03), respectively[15].

Moreover, a prospective study provided no benefit of IT added to systemic treatment and RT. The first group consisted of 54 patients treated with RT, IT and systemic therapy *vs* 50 patients treated with RT and systemic chemotherapy. There was no differences in median survival (4 mo) and long term survivors[16]. It should be underlined, that in both groups approximately 60% of patients were diagnosed with breast cancer, 15% with lung cancer and the other 25% with various other types of cancer (the percentage of EGJC/GC cases is not clarified, if any). It should be also stated that the aforementioned studies reviewed, reflect various solid tumors with highly variable prognosis, including breast cancer which has a more indolent history, and the regimens of IT chemotherapy and RT administrated are not distinguished based on the various types of solid tumors.

In addition, a multicenter retrospective analysis of patients with GC and LMC revealed no evidence of an additional effect of cranio-spinal RT to IT[14]. Due to the fact that in most cases blood brain barrier is destroyed and LMC is related with highly permeable blood vessels in vascularized tumors, it could be also speculated that systemic therapy may be effective. An experimental model supports this hypothesis[17].

In conclusion, even LMC is rare in patients diagnosed with EGJC and GC, physicians should be alerted when neurological symptoms occurred, are persistent and could not be explained. Furthermore, a single diagnosis test procedure itself is not absolutely sensitive and the investigation algorithm should comprise a gadolinium enhanced brain MRI and repeated CSF cytology tests. Unfortunately, disease prognosis is dismal and newly developed targeted drugs with improved CNS penetration and better outcomes remains a priority.

ARTICLE HIGHLIGHTS

Case characteristics

Two patients are presented. Both of them were presented with severe neurological symptoms and their further investigation revealed the initial diagnosis and the recurrence of gastric cancer (GC)/esophagogastric junction cancer (EGJC), respectively.

Clinical diagnosis

Two cases are presented. One initially diagnosed with GC and leptomeningeal carcinomatosis (LMC) but no other evidence of metastatic disease and the other one initially diagnosed with EGJC, who recurred solitary with leptomeningeal seedings several years after the initial diagnosis and treatment.

Differential diagnosis

Meningeal enhancement is suggestive but does not confirm the diagnosis. Infection or inflammatory causes, intracranial hypertension but even a lumbar puncture procedure before magnetic resonance imaging (MRI) scan could induce diffuse meningeal enhancement give false positive results.

Laboratory diagnosis

Elevation of CEA levels were reported in both patients and mild elevation of CA19-9 was reported in patient with EGJC recurrence.

Imaging diagnosis

Brain MRI images revealed diffuse meningeal enhancement consistent with LMC.

Pathological diagnosis

CSF cytology confirmed the diagnosis of LMC in both patients.

Treatment

Chemotherapy, IT therapy.

Related reports

Only a few cases of EGJC and GC patients have been reported with LMC as an upfront disease manifestation or as solitary disease recurrence.

Term explanation

EGJC/GC are diseases with high malignant potential.

Experiences and lessons

Even LMC is extremely rare in patients diagnosed with EGJC and GC, physicians should be alerted when neurological symptoms occurred, are persistent and could not be explained.

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