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EDITORIAL

Clinical relevance of cancer genome sequencing

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Abstract

The arrival of both high-throughput and bench-top next-generation sequencing technologies and sequence enrichment methods has revolutionized our approach to dissecting the genetic basis of cancer. These technologies have been almost invariably employed in whole-genome sequencing (WGS) and whole-exome sequencing (WES) studies. Both WGS and WES approaches have been widely applied to interrogate the somatic mutational landscape of sporadic cancers and identify novel germline mutations underlying familial cancer syndromes. The clinical implications of cancer genome sequencing have become increasingly clear, for example in diagnostics. In this editorial, we present these advances in the context of research discovery and discuss both the clinical relevance of cancer genome sequencing and the challenges associated with the adoption of these genomic technologies in a clinical setting.

Key words: Next-generation sequencing; Exome; Cancer; Diagnostics; Familial cancer syndrome; Somatic mutation

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INTRODUCTION

Sporadic cancers are complex diseases that are caused by the accumulation of somatic mutations that are acquired by the genomes of the cells of the tissue in which the cancer originated[1]. The importance of identifying the "driver" (causal) somatic mutations amongst the much more numerous "passenger" mutations has long been recognized. However, previous cancer genome sequencing studies have also been constrained by technological limitations. Although several early attempts were made to sequence the coding regions of the majority of the consensus coding sequence and/or RefSeq genes in several cancers (*e.g.*, breast and colorectal), these studies were conducted in "brute-force" mode employing traditional low-throughput polymerase chain reaction-Sanger sequencing methods[2,3]. The advent of next-generation sequencing (NGS) technologies has revolutionized the sequencing of cancer genomes, the first example of which employed whole-genome sequencing (WGS) to characterize an acute myeloid leukemia (AML) genome thereby identifying numerous tumor-specific mutations[4]. This study clearly demonstrated the technical feasibility of applying NGS to interrogate the genome-wide somatic mutational spectra of entire cancer genomes in tandem with paired constitutional DNA samples.

In parallel, the development of a variety of exome enrichment methods to selectively capture the entire collection of exons in the human genome has made whole-exome sequencing (WES) technically feasible[5]. Coupling this development to the high-throughput NGS techniques has allowed the exome to be sequenced very

rapidly and in unprecedented detail. In comparison to WGS, WES is more affordable for larger sample sizes and is analytically less challenging since only 1%-2% of the entire genome needs to be sequenced[6-8]. As a result, a larger number of cancer DNA samples have been sequenced by WES than WGS in attempts to identify recurrent mutations (*i.e.*, identical mutations that recur in different samples) and highly mutated genes (genes harboring multiple mutations in a significant proportion of the cancer samples)[9-12]. The other reason for the more widespread adoption of WES has been that the mutations identified within protein coding regions are inherently easier to interpret than those in the non-coding regions, which still remain largely uncharacterized in functional terms. In addition to the advances being made in characterizing the somatic mutational landscape in various cancers, the applications of cancer genome sequencing in a clinical setting have also become increasingly numerous.

SOMATIC MUTATIONS IN SPORADIC CANCERS

WGS and WES have been commonly applied to study the patterns of somatic mutation in a range of different cancers[13,14]. Collectively, these endeavors have generated new insights into the mutational landscape of various cancers, and have resulted in the identification of a large number of recurring mutations as well as many highly mutated genes. For example, in the context of gastrointestinal cancers, WES of 15 gastric adenocarcinomas and their matched normal DNAs succeeded in identifying several frequently mutated genes such as *TP53*, *PIK3CA* and *ARID1A*[15]. In addition, it was found that cell adhesion was the most enriched biological pathway among the frequently mutated genes. More importantly, mutations in three chromatin remodeling genes (*ARID1A*, *MLL3* and *MLL*) were detected in almost half of the gastric cancers examined[15]. In fact, an earlier study which performed WES in 22 gastric cancer samples also identified frequent inactivating mutations in *ARID1A*, which encodes a member of the switch/sucrose non-fermenting chromatin remodeling family. Further, the mutational spectrum for *ARID1A* was found to differ between molecular subtypes of gastric cancer[7]. In similar vein, mutations in multiple chromatin regulator genes such as *ARID1A*, *ARID1B*, *ARID2*, *MLL* and *MLL3* were also found in about half of hepatocellular carcinomas through WGS[16]. The consistent finding of mutations in chromatin remodeling genes in different cancers, which also included renal carcinoma and glioblastoma multiforme, further highlights a close inter-relationship (or possibly a "synergy" interaction effect) between somatic mutations and aberrant epigenetic regulation in the pathogenesis of cancers[8,17-19].

In addition to individual studies, technological advances have made possible large-scale international projects such as the International Cancer Genome Consortium which aims to interrogate the somatic mutational landscape of at least 50 different cancer types and subtypes in thousands of samples, with the eventual aim of integrating these genomic data with both transcriptomic and epigenomic data. NGS technologies are instrumental in generating these "omics datasets"[20]. The concept of an integrative approach for a range of different omics data is not new, but in recent years it has resurfaced and become reinvigorated by technological advancement. The integrative analysis of different omics datasets (providing information in different dimensions, from DNA sequence to the transcriptional and translational levels) is expected to be more informative, and hence ought to provide new and more detailed biological insights, than would be possible using individual datasets[21].

Although most of the cancer genome sequencing studies were not conducted with a view to investigating their applications in a direct clinical context *per se* (but rather to characterize the somatic mutational spectrum in order to understand better the genetic basis and biology of the cancer in question), the data generated are nevertheless important as a means to identify the drug targets as well as potential biomarkers (*e.g.*, single mutations or mutational patterns that could be used for diagnostic and prognostic applications). The potential of driver mutations to shape the future science of cancer taxonomy was recently outlined by Stratton (2011) *i.e.*, the drawing up of a system based on causal mutations rather than the conventional organ-based (*e.g.*, breast, lung or colorectum tissue) classification and TNM-staging system that are widely applied in the clinic[22].

So far, what are the potential implications of cancer genome sequencing for the clinical setting? The application of cancer genome sequencing in diagnostics has been increasingly evident, as demonstrated by two recent studies using WGS[23,24]. WGS has demonstrated both its discovery and confirmatory role in a specific patient characterized by an ambiguous diagnosis or clinical presentation. More specifically, it has been used to determine the genetic aberration in a patient with a diagnosis of AML of unclear subtype[23]. The ambiguity came from the observation that the patient's clinical presentation was consistent with acute promyelocytic leukemia (which is a subtype of AML with a favorable prognosis), but it was contradicted by cytogenetic analysis. The cytogenetic analysis revealed a different subtype associated with a poor prognosis for which bone marrow transplantation in first remission is recommended. The diagnostic and treatment uncertainty was resolved by WGS performed on the original leukemic bone marrow and from a skin biopsy. The WGS analysis detected a novel insertional translocation on chromosome 17 which generated a pathogenic *PML-RARA* gene fusion thereby confirming a diagnosis of acute promyelocytic leukemia. This type of complex rearrangement could not have been made by targeted sequencing (as the genetic etiology was unsuspected), further demonstrating that WGS represents both a discovery and a comprehensive

analytical tool for the entire genome. More importantly, the molecular confirmatory diagnosis carries important clinical implications for the treatment and management of the patient[23]. Similarly, WGS was also employed to resolve the genetic basis of a suspected cancer susceptibility syndrome based upon the early onset of several primary tumors[24]. Further, therapeutic prediction has also benefited from NGS as a powerful discovery tool. For example, a recent study employed a targeted NGS approach to sequence 138 cancer genes in melanomas derived from a patient (before and after relapse) and succeeded in identifying the underlying genetic mutation in the *MEK1* gene responsible for acquired resistance to PLX4032 (vemurafinib) after an initial dramatic response, revealing a novel mechanism of acquired drug resistance[25].

The potential applications of cancer genome sequencing in the clinical arena are promising, but what are the challenges associated with their adoption? As WGS and WES are high-throughput methods which generate huge amounts of data, our ability to perform both the analysis and the interpretation of the data in a clinically relevant time-frame is critical. This challenge is being addressed in the context of a "comprehensive genomic approach" where WGS, WES and transcriptome sequencing are applied to cancer samples to evaluate their clinical utility and feasibility (in terms of technical, time and cost)[26]. In particular, the time required, from biopsy sampling and wet-lab experiments to computational analysis and initial results, was streamlined to just 24 d with the cost of all the sequencing and analysis estimated to be only USD5400. An obvious advantage of this "integrative genomic approach" is that the findings can be cross-validated more efficiently. For example, both WGS and WES detected an amplification event on chromosome 13q spanning the *CDK8* gene in a metastatic colorectal carcinoma; the over-expression of *CDK8* was confirmed by transcriptome sequencing. Although this "comprehensive genomic approach" was shown to be both time- and cost-effective, the handling and interpretation of the huge amount of genomic data remains a key issue. To address this challenge, it was proposed that a multidisciplinary "sequencing tumor board" (which included professionals from multiple disciplines such as clinicians, geneticists, pathologists, biologists, bioinformatic specialists and bioethicists) should take responsibility for the clinical interpretation of the sequencing data obtained from each patient[26].

FAMILIAL CANCER SYNDROMES

In addition to the investigation of somatic mutations in sporadic cancers, cancer genome sequencing has also made significant advances in relation to the study of the germline mutations underlying familial cancer syndromes. The early successes in the identification of causal mutations and genes for familial cancer syndromes (*e.g.*, *RB1* and *APC)* were achieved by painstaking family linkage analysis and positional cloning. However, the genetic causes of many familial cancer syndromes have remained elusive. For example, *CDH1* was the first and only causal gene identified for hereditary diffuse gastric cancer through linkage analysis[27], but germline mutations in this gene account for only a proportion of hereditary diffuse gastric cancer cases. Thus germline mutations in *CDH1* were found in 30%-40% of clinically defined families with hereditary diffuse gastric cancer from different ethnic backgrounds[28,29]. This suggests that an as-yet-to-be identified gene(s) is likely to be responsible for the remaining cases unexplained by *CDH1*. On the other hand, whereas most Lynch syndrome cases can be accounted for by mutations in DNA mismatch repair genes, the genetic basis of familial colorectal cancer type X still remains elusive[30,31]. Similarly, the genetic causes of other familial cancer syndromes, such as familial pancreatic cancer, still remain largely unknown[32,33]. In a fashion similar to that noted with the identification of somatic mutations, cancer genome sequencing provides new opportunities to identify germline mutations for familial cancer syndromes. This is well exemplified by the case of hereditary pheochromocytoma, a rare neural crest cell tumor; by harnessing the latest technological advances, germline mutations in *MAX* were identified in three unrelated individuals with hereditary pheochromocytoma by WES[34]. The segregation of two *MAX* gene variants with hereditary pheochromocytoma was observed in families from whom DNA from affected relatives was available. Further, additional data to support the causative role of the *MAX* variants came from their absence (or non-detection) in more than 750 population-matched control chromosomes. Additional screening for *MAX* mutations in 59 cases lacking germline mutations in known genes for hereditary pheochromocytoma then identified two additional truncating mutations and three missense variants in the gene[34]. Following this discovery, a recent study found that germline mutations in *MAX* are responsible for 1.12% pheochromocytomas or paragangliomas (both are genetically heterogeneous neural crest-derived neoplasms) by sequencing *MAX* in 1694 patients[35].

In addition to its role in research discovery, cancer genome sequencing has also been used as a diagnostic tool to detect known germline mutations for familial cancer syndromes. Indeed, by leveraging technological advances in genomic sequence enrichment methods and NGS technologies, studies have developed NGS-based diagnostic tests for breast and ovarian cancers and Lynch syndrome. For example, Walsh *et al*[36] designed custom oligonucleotides in solution to capture 21 genes responsible for hereditary breast and ovarian cancers, and the enriched genomic DNA was then subjected to sequencing using an NGS platform. This NGS-based test was evaluated in 20 women diagnosed with breast or ovarian cancer and with a known mutation in one of the genes responsible for inherited predisposition to these cancers. The results were very promising in that all the known

point mutations and small indel mutations (ranging from 1 bp to 19 bp), as well as large genomic duplications and deletions (ranging from 160 bp to 101 013bp), were detected in all the samples. The potential to detect different mutations of various sizes has further demonstrated the technical advantages of NGS-based tests over conventional PCR-Sanger sequencing methods. For example, two different tests were offered separately to detect point mutations and large deletions/amplifications for genetic testing of *BRCA1/2* genes, respectively[36]. Similarly, attempts have also been made to incorporate custom genomic enrichment and NGS methods into the genetic diagnostic testing of Lynch syndrome by capturing every exon in a panel of 22 genes (most of which are known to be associated with hereditary colorectal cancer) followed by NGS[37].

Technological advances have facilitated the accessibility of cancer genome sequencing in the clinical arena. In addition to the custom sequence enrichment methods (*i.e.*, either based on polymerase chain reaction amplification such as Fluidigm and RainDance technologies, or based on target-probe hybridization such as the Agilent and Nimblegen technologies) that allow one to selectively capture the genomic regions of interest, the arrival of several bench-top NGS instruments has not only made the sequencing of a panel of genes highly feasible technically but also cost-effective[5,38,39]. This is an important step towards the development and adoption of NGS-based diagnostic tests in the clinic. The bench-top NGS instruments (Roche 454 Genome Sequencer Junior, Ion Torrent Personal Genome Machine Sequencer and IlluminaMiSeq) have a much lower throughput (ranging from > 10 Mb to > 1 Gb per run) than the conventional high-throughput NGS machines[38,39]. The bench-top NGS instruments are therefore more suitable in terms of their throughput for sequencing panels of genes (as discussed earlier for the panels of genes for breast/ovarian cancers and Lynch syndrome) than WES or WGS. Further, sample indexing (or barcoding) is also available for the bench-top NGS instruments which can further optimize sample throughput and cost-effectiveness by multiplexing up to several tens of samples for sequencing. However, the level of multiplexing is dependent on the sizes of the regions to be sequenced and the throughputs of the instruments being used. Although it remains to be demonstrated in the context of cancer, WES has been widely assessed and shown as a promising diagnostic tool for various Mendelian disorders[40-43]. In addition to diagnosis, WGS has also been applied to optimize patient treatment regimens, although not in the context of cancer. In the context of inherited disease, WGS has been applied to sequence a fraternal twin pair diagnosed with dopa (3,4-dihydroxyphenylalanine)-responsive dystonia (OMIM 128230); germline compound heterozygous mutations were identified in the *SPR* gene encoding sepiapterin reductase. As a result, supplementation of L-dopa therapy with 5-hydroxytryptophan has led to clinical improvements in both twins[44].

PERSPECTIVES AND CONCLUSIONS

NGS technologies have already made a major contribution to characterizing somatic mutations in cancer genomes. This endeavor will be further accelerated by international initiatives such as the International Cancer Genome Consortium. Although the number of studies is currently still very limited, NGS should also be applied to identify germline mutations in those familial cancer syndromes whose genetic causes have not yet been fully characterized. On the other hand, the successful demonstration of the applications of NGS/WGS/WES in a clinical setting such as cancer diagnostics are likely to be just the first examples of how the new technologies will prove their worth; the numbers are expected to increase in the coming year.

So far, the applications of NGS in a clinical setting have been very promising. However, challenges ranging from technical, analytical and interpretational, to the need for a considerable number of well-trained professionals from a range of disciplines in these genomic technologies must be further addressed before the adoption of NGS-based tests in the clinic. The technical challenges include, for example, incomplete capture of the exons in WES and uneven sequencing across the genome which might result in poor sequence coverage in some of the regions and affect both the sensitivity and specificity of variant detection[39]. Having specialists trained in genomic technologies is critical to (1) obtaining fully informed consent from patients in relation to the genomic tests; (2) ensuring accurate and reliable interpretation of the data for clinical decision-making; and (3) counselling the patients on the basis of the results obtained. It is also evident from the Global Cancer Genomics Consortium (GCGC) that the translation of emerging cancer genomics knowledge into clinical applications can only be achieved through the integration of multidisciplinary expertise[45]. The GCGC is an international collaborative platform that brings cancer biologists and cutting edge high-throughput genomics expertise together with medical oncologists and surgical oncologists to address the most important translational questions that are central to cancer research, diagnosis and treatment.

As to test affordability, although the total cost of sequencing for several genomic experiments was only a few thousands of USD per patient, it should be appreciated that this is unlikely to be the final chargeable cost to the patients. The cost of sequencing is currently plummeting and will become ever cheaper in the future with new developments. However, it should be appreciated that hidden costs are likely to be incurred for data storage, interpretation of results and subsequent clinical consultation.

Further, handling of the complex ethical issues such as revealing findings that might be considered incidental to the initial testing (WGS and WES) procedure must also be given serious consideration[46]. Determining what to disclose and what not to disclose to the patients is

likely to be quite challenging *e.g.*, those results which are deemed clinically important *i.e.*, those which could have a direct impact on the patient's care or management, but which are irrelevant to the initial purpose of the diagnostic test (*i.e.*, incidental findings). Have the patients the right to be informed about those results which are/might be clinically important but not actionable *e.g.*, mutations that are considered likely to predispose to certain inherited diseases, although preventive treatments are not yet available? Adequate consultation must also be given to the reporting of results that are of unknown clinical importance. This raises concerns as to whether periodic reanalysis of the WGS/WES data might be needed, which in turn would lead to some practicality issues potentially incurring additional costs. Finally, any results from WGS- and WES-based tests that would affect clinical decision-making must be properly validated or the tests must be performed in a heavily regulated clinical setting according to the College of American Pathologists/Clinical Laboratory Improvement Amendments.

It is widely anticipated that cancer genome sequencing or the NGS-based tests will gradually become more accessible in clinical practice once the associated challenges and ethical issues have been adequately addressed. Irrespective of the challenges that still remain to be overcome, the application of NGS in the clinic appears inevitable.

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REVIEW

Clinical application of microRNA in gastric cancer in Eastern Asian area

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Abstract

Recent research has shown that microRNA (miRNA), which is involved in almost every step of gastric carcinogenesis, has broad prospective application in diagnosis and therapy of gastric carcinoma. Eastern Asia (South Korea, Japan and China) has the highest incidence of gastric cancer in the world. There were 988 000 new cases of gastric cancer worldwide and 736 000 deaths in 2008. Approximately 60% of the cases of gastric cancer are found in East Asia (mainly China). We herein provide a brief review of the clinical applications of miRNA, which include the following aspects: (1) miRNA may serve as a potential new generation of tumor markers; (2) a complete miRNA expression profile is highly specific, can reflect the evolutionary lineage and differentiation of tumors, and be used to carry out diversity analysis; (3) detecting specific miRNA expression in peripheral blood will become a new method for diagnosis of gastric cancer; (4) miRNA can predict prognosis of gastric cancer; (5) miRNA has predictive value in determining chemotherapy and radiotherapy resistance; and (6) miRNA could be a type of innovative drug. Finally, we focus on assessing the value of miRNA from laboratory to clinical application and the challenges it faces in East Asia.

Key words: microRNA; Prognosis; Clinical application; Gastric cancer; Eastern Asia

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INTRODUCTION

Gastric cancer is a leading disease in Eastern Asia (South Korea, Japan and China) (Figure 1). The incidence and mortality of gastric cancer in East Asian areas rank respectively the second and the third among the most common cancers worldwide[1,2]. According to World Health Organization[3] statistics, there were 988 000 new cases of gastric cancer worldwide and 736 000 deaths in 2008. Approximately 60% of the cases are found in East Asia (mainly China). In China, approximately two-thirds of patients develop advanced or metastatic disease, and more than half have recurrent disease after curative surgery. The median survival time for these patients is only 6-9 mo[4-6]. Several reasons restrict the diagnosis and treatment of gastric cancer: (1) limited diagnostic measures for early detection; (2) weak prognostic value of outcome; (3) poor effect of surgery or cytotoxic cell treatment for advanced disease; and (4) lack of biomarkers for targeted therapy. The discovery of microRNA (miRNA) may change the above-mentioned difficulties, and improve the level of diagnosis and treatment of gastric cancer.

miRNAs include 20-24 nucleotides and are a class of noncoding small molecular single chain RNAs, and

have highly conservative, temporal and tissue-specific characteristics[7-9]. Through complete or incomplete base pairing with target gene mRNA, RNA-induced silencing complex degrades mRNA or blocks its translation, and regulates target gene expression at the post-transcriptional level[10]. They exist widely in eukaryotic organisms and regulate cell proliferation, differentiation and apoptosis. Although the tissues of the body appear malignant, specific miRNAs are overexpressed or underexpressed in different tumors and at different stages, which implies a correlation with occurrence and development of tumor and prognosis[11-13]. Further study of the relation of miRNA and gastric cancer could provide new applications in early tumor detection, monitoring, prognosis, gene therapy, and resolving chemotherapy resistance.

miRNA AND CANCER DIAGNOSIS

Specific tumor markers are often ideal screening tools. Existing clinical tumor markers [such as carcinoembryonic antigen (CEA), cancer antigen (CA)19-9, and CA72-4] for gastric cancer lack specificity and sensitivity[14]. miRNA may serve as a potential new generation of tumor markers for the following reasons[15-17]: (1) good tissue specificity - Rosenfeld *et al*[18] have detected unknown sources of miRNA in order to make clear its sources, and its specificity is 90%; (2) expression of miRNA in tumors differs significantly from that in normal tissues; (3) miRNA participates in tumor occurrence and development; (4) expression of miRNA has stage specificity - the same tumors at different stages have different expression profiles[19,20]; and (5) miRNA in fresh tissues, paraffin-embedded tissues, and cells and peripheral blood shows good stability[21].

Detection methods of miRNA and miRNA expression in gastric cancer: miRNA is a good tumor marker in clinical application[22,23]. At present, the detection method for miRNA has become mature. By deep sequencing, we can discover unknown miRNAs, and miRNA chips can be used for identification of the differences in miRNAs between the study and control groups. Finally, they can be verified by real-time quantitative polymerase chain reaction (qPCR)[24].

In diagnosis of gastric cancer, a single miRNA is often characterized by poor specificity or sensitivity, but a complete miRNA expression profile is highly specific, can reflect the evolutionary lineage and differentiation of tumors, and be used to carry out diversity analysis[25,26]. Through horizontal comparison of gastric carcinoma with adjacent normal tissues, we have found specific expression of miRNAs in cancer tissues. Further longitudinal comparison at different tumor stages has enabled us to identify the different miRNAs at each stage and to complete final tumor diagnosis and staging[27,28].

We acquired gastric cancer miRNA expression profiles from numerous Chinese and international study groups from 2008 to 2012[29-36] (Table 1). These results differed considerably and lacked stability and consistency

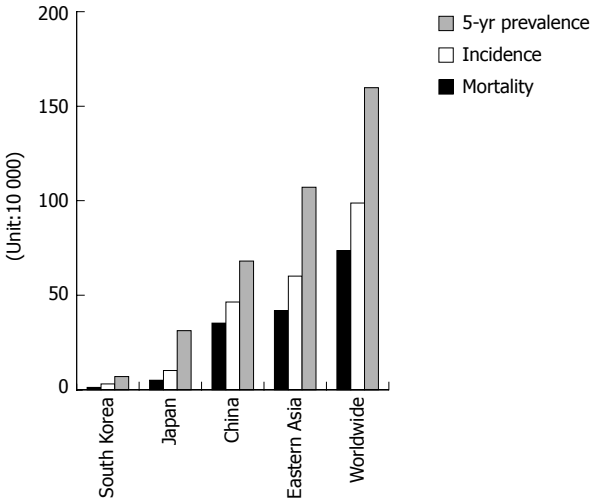

Figure 1 Gastric cancer incidence, mortality and prevalence in South Korea, Japan, China, Eastern Asia, and worldwide in 2008.

for the following reasons: (1) differences in miRNA chips and software; (2) individual differences between races and patients; (3) differences in collected specimen standards; (4) differences in sample size; and (5) miRNA expression profile differences for different cancer types and stages. According to the above expression profiling, we confirmed several reliable miRNAs in the multiple experiments which had 1.5 fold differential expressions between gastric cancer and normal gastric tissues. We are looking forward to having a large sample multi-center study or even international cooperation to compare complete miRNA expression profiling based on different pathological types and stages of gastric cancer. In particular, countries like China, Japan and South Korea should cooperate using the same platform in complete standard miRNA expression profiling of gastric cancer in East Asian populations.

Change in miRNA expression is an early event during the development of gastric tumor[37,38]. Tracking the changes in miRNA expression profiling in the relevant gastric tissues might enable early tumor diagnosis. Traditional methods for the detection of gastric cancer are endoscopy and biopsy. A minimally invasive examination method would be helpful for screening and early detection of cancer in high-risk populations[39]. Detection of tumor in the peripheral blood of patients with specific miRNA expression level has been a research hotspot in recent years, which will perhaps become a new method for diagnosis of gastric cancer[40-42].

Researchers have shown that 90% of plasma miRNA is based on protein-miRNA complex formation. As tumor markers in peripheral blood, miRNAs have the following advantages: (1) miRNAs exist in great volume in peripheral blood[43,44]; (2) miRNAs can resist enzymatic digestion[45-47]; (3) miRNAs have strong resistance to the external environment; and (4) miRNAs show abnormal expression in tumor patients' serum[48].

In the past two years, Japanese and Chinese research-

Table 1 microRNA expression in gastric cancer in 2008-2012

Group	Method	Sample	Upregulated	Downregulated
Katada *et al*[29]	TaqManmiRNA assays + qRT-PCR	42 undifferentiated gastric cancer and controls	miR-34b miR-34c miR-128a	miR-128b, miR-129, miR-148
Guo *et al*[30]	Microarray	3 gastric cancers and adjacent normal tissues	miR-20b, miR-20a, miR-17, miR-106a, miR-18a, miR-21, miR-106b, miR-18b, miR-421, miR-340, miR-19a, miR-658	miR-768-3p, miR-378, miR-31, miR-139-5p, miR-195, miR-497, miR-133b, miR-638, miR-378
Yao *et al*[31]	Microarray + qRT-PCR	10 gastric cancers and adjacent normal tissue	miR-223, miR-106b, miR-147, miR-34a, miR-130b, miR-106a, miR-18a, miR-17, miR-98, miR-616,miR-181a-2, miR-185, miR-1259, miR-601, miR-196a, miR-221, miR-302f, miR-340, miR-337-3p, miR-520c-3p, miR-575 and miR-138	
Luo *et al*[32]	Microarray + qRT-PCR	24 gastric cancers	MiR-26b, miR-30a-5p, miR-212, miR-320, miR-379, miR-518b, miR-409-3b	MiR-9, miR-19b, miR-155, miR-188, miR-197, miR-338, miR-370, miR-383, miR-433, miR-490, miR-503, miR-545, miR-551a, miR-567, miR-575, miR-611, miR-630, miR-649, miR-652
Ueda *et al*[33]	Microarray + qRT-PCR	353 (184 gastric cancers 169 controls)	miR-181d, miR-181a-1, miR-181a-2, miR-181c, miR-181b-1, miR-181b-2, miR-21, miR-25, miR-92-1, miR-92-2, miR-93, miR-17-5p, miR-106a, miR-20b, miR-135a-1, miR-135a-2, miR-425-5p, miR-106b, miR-20a, miR-19b-1, miR-19b-2	miR-148a, miR-148b, miR-375, miR-29b-1, miR-29b-2, miR-29c, miR-152, miR-218-2 miR-451, miR-30d
Tsukamoto *et al*[34]	Microarray (470) + qRT-PCR	22 gastric cancers	miR-18a, miR-106a, miR-17, miR-146a, miR-93, miR-19a, miR-20a, miR-20b, miR-25, miR-15b, miR-425, miR-92a, miR-194, miR-10a, miR-222, miR-7, miR-106b, miR-320a, miR-21, miR-34a, miR-19b, miR-103, miR-215, miR-192, miR-429, miR-27a, miR-223, miR-23a, miR-107, miR-200b, miR-24, miR-15a, miR-16	miR-375, miR-29c, miR-148a, miR-30a-5p, miR-30e-5p, miR-638
Li *et al*[35]	TaqManmiRNA assays + qRT-PCR	30 gastric cancer and controls	miR-223, miR-21, miR-23b, miR-222, miR-25, miR-23a, miR-221, miR-107, miR-103, miR-99a, miR-100, miR-125b, miR-92, miR-146a, miR-214 and miR-191,	let-7a, miR-126, miR-210, miR-181b, miR-197, miR-30aa-5p
Carvalho *et al*[36]	Microarray + qRT-PCR	76 gastric cancers	miR-582-5p, miR-151-5p, miR-296-5p, miR-30b, miR-513-5p, miR-335, miR-576-5p, miR-219-2-3p, miR-331-5p, miR-889, miR-152, miR-992, miR-93, miR-519c, miR-599, miR-520a-5p, miR-631, miR-550, miR-136, miR-22, miR-515-5p, miR-127-3p, miR-374a, miR-181a, miR-192, miR-532-3p, miR-30d, miR-640, miR-425, miR-92b, miR-501-5p, miR-514, miR-576-3p, miR-519e, miR-149, miR-219-1-3p, miR-424, miR-220, miR-96, miR-218-2, miR-649, miR-215, miR-182, miR-122, miR-524-3p, miR-187, miR-526b, miR-770-5p, miR-545, miR-200b, miR-9, miR-141, miR-579, miR-493, miR-137, miR-216a, miR-503, miR-126, miR-23b, miR-99b, miR-101, miR-323-3p, miR-25, miR-92a-1, miR-429	miR-451, miR-502-3p, miR-101 miR-33a, miR-516a-3p/miR-516b

qRT-PCR: Quantitative reverse transcriptase polymerase chain reaction.

ers have investigated miRNA in the peripheral blood of patients with gastric cancer[49-55] (Table 2) and have obtained some positive results. For example, by comparing serum of 61 gastric cancer patients with that of 61 healthy persons, Liu *et al*[49] found that the expression of miR-378 in the gastric cancer group was significantly higher than that in the healthy group. The area under the receiver-operating characteristic curve was 0.861 (95%CI: 0.766-0.928), and sensitivity/specificity was 87.5%/70.7%, respectively. Similarly, after investigating the peripheral serum in 69 gastric cancer patients and 30 healthy volunteers by qRT-PCR, Tsujiura *et al*[55] found that the plasma concentrations of miRNAs (miR-106a and miR-106b) were significantly higher in the patients than in the controls, whereas let-7a concentration was lower in the patients, in which the area under the curve (AUC) for miR-106a and let-7a was 0.879, and sensitivity/specificity was 85.5%/80.0%, respectively. These miRNAs could become ideal tumor markers for gastric cancer. In addition, Liu *et al*[49] observed that the plasma miRNAs (miR-1, miR-20a, miR-27a, miR-34, and miR-423-5P) in the gastric cancer patients had significantly higher expression than in the control group (164 gastric cancer patients *vs* 127 healthy individuals). The AUC was 0.879 (95%CI: 0.822-0.936). It is interesting that, in the same sample, they also compared the AUC values of CEA and CA19-9 which were only 0.503 and 0.600, respectively. The results show that miRNA has some advantages as a tumor marker. We have found that miRNAs have good sensitivity and specificity for gastric cancer and are promising tumor markers. However, at present, some factors still limit their clinical diagnostic applications[56]: (1) relative difficulty of detection (in quality and quantity); (2) lack of a unified testing platform and standardization; (3) plasma miRNA source and release mechanism are not clear; and (4) differences in expression of tissue and peripheral blood miRNA still exist[57]. Thus, searching and identifying specific miRNAs for the diagnosis of gastric cancer is the first task that

Table 2 Expression of miRNA and area under curve, sensitivity and specificity in serum samples of patients with gastric cancer

Group	Sample	MicroRNA	AUC	Method	Sensitivity/specificity (%)
Liu *et al*[49]	61 GC/61 C	miR-378↑	0.861	qRT-PCR	87.5/70.7
Liu *et al*[50]	164 GC/127 C	(miR-1, miR-20a, miR-27a, miR-34,	0.879	Microarray + qRT-PCR	79.3/86.5
		miR-423-5P)↑	0.831		
Konish *et al*[51]	56 GC/30 C	miR-451↑	0.96	Microarray + qRT-PCR	96.0/100
		miR-486↑	0.92		86.0/97.0
Song *et al*[52]	82 GC/82 C	miR-221, miR-744 and miR-376c↑	NA	qRT-PCR	82.4/58.8
Zhou *et al*[53]	90 GC/27 C	miR-106a↑	0.684	Microarray + qRT-PCR	48.2/90.2
		miR-17↑	0.743		51.9/92.7
Wang *et al*[54]	174 GC/39 C	miR-21↑	0.81	Microarray + qRT-PCR	56.7/94.9
Tsujiura *et al*[55]	69 GC/30 C	miR-106b↑	0.72	Microarray + qRT-PCR	NA
		miR-106a↑, let-7a↓	0.879		85.5/80.0

qRT-PCR: Quantitative reverse transcriptase polymerase chain reaction; GC: Gastric cancer, C: Control; AUC: Area under curve; NA: Not available; ↑: Upregulated; ↓: Downregulated.

Table 3 Gastric cancer with potential predictive role of miRNAs

	Potential predictive role of miRNA	
	MiRNAs of high expression	MiRNAs of low expression
Short survival time	miRNA-20b, miRNA-150[29], miRNA-142-5p[61], miRNA-375, miRNA-214[62]	miRNA-451, let7g[1], miRNA-433[1][33], miRNA-125-5p[3][63]
Lymph node metastasis	miRNA-27a[29], miRNA-650[64]	miRNA-126[65], miRNA-146a[66], miRNA-148[67], miRNA-218[68], miRNA-335[69], miRNA-429[70]
Relapse	miRNA-375[61], miRNA-451[2], miRNA-199-3p[2], miRNA-195[2][71]	miRNA-142-5p[61]
Advanced gastric cancer	miRNA-221[4][72]	miRNA-126[65], miRNA-148a[67], miRNA-218[68]
Invasion, metastasis	miRNA-223[35], miRNA-148a[73], miRNA-107[74]	miRNA-610[75], miRNA-200b[5][76], miRNA-7[77]

[1]The two miRNAs also indicate short survival and lymph node metastasis, and deeper invasion; [2]The three miRNAs also indicate short survival and recurrence, especially miRNA-451; [3]The miRNA also indicates short survival and hepatic metastases, deeper invasion, and tumor enlargement; [4]The miRNA also indicates lymph node metastasis and deeper invasion, and advanced gastric cancer; [5]The miRNA also indicates lymph node metastasis and deeper invasion, and the tumor is enlarged.

must be undertaken. Establishment of a suitable standard testing system for clinical application, including quality control, and diagnostic threshold determination are still issues that require some work. There is a high incidence of gastric cancer in East Asian countries. High-risk populations could be screened by miRNAs, which should be able to increase the detection rate of early gastric cancer and improve the effects of treatment.

miRNA AND PROGNOSIS PREDICTION

Predicting patient survival time, disease progression, prognostic outcome or response to treatment is challenging. Because of the stability and specificity of expression in tissues and circulation, miRNA may be regarded as a forecasting tool for disease outcomes. A lot of the literature suggests that miRNAs have a close relationship with survival time of gastric cancer patients, disease stage, tumor recurrence, and lymph node metastasis. Li *et al*[58] have shown that a seven-miRNA signature (miR-10b, miR-21, miR-223, miR-338, let-7a, miR-30a-5p, and miR-126) is an independent predictor of overall survival [hazard ratio (HR) = 3.046; P = 0.015] and relapse-free survival (HR = 3.337; P = 0.012). It can predict the prognosis of the patient in relation to tumor stage, cytological subtypes, and Lauren classification[59,60]. In gastric cancer, many Chinese and other research teams have discovered a number of miRNAs that play a role as a predictor. For example, high expression of miRNA-20b, miRNA-150, miRNA-142-5p[61], miRNA-375 and miRNA-214[62] and low expression of miRNA-451, let7g, miRNA-433 and miRNA-125-5p[63] are associated with short survival time. High levels of miRNA-27a and miRNA-650[64] and low levels of miRNA-126[65], miRNA-146a[66], miRNA-148[67], miRNA-218[68], miRNA-335[69] and miRNA-429[70] indicate lymph node metastasis. Patients with overexpression of miRNA-375, miRNA-451, miRNA-199-3p and miRNA-195[71] and decreased expression of miRNA-142-5p are more likely to relapse. High levels of miRNA-221[72] and decreased levels of miRNA-126, miRNA-148a and miRNA-218 indicate advanced gastric cancer. High expression of miRNA-223, miRNA-148a[73] and miRNA-107[74] and reduced expression of miRNA-610[75], miRNA-200b[76] and miRNA-7[77] were associated with invasion and metastasis (Table 3). Therefore, many potential predictors have proved useful for judging the prognosis of gastric cancer patients and are the basis for targeted therapy. However, researching miRNAs as prognostic factors involves small sample sets, high volume of work in validation, and research of independent cohorts,

Table 4 Expression of miRNA and prediction of the effect of chemotherapy and radiotherapy

	Upregulated	Downregulated
Chemosensitivity	(let-7g, miR-342, miR-16, miR-181, miR-1, miR-34)[1][81]	
Chemoresistance	(miR-518f, miR-520a, miR-520d, miR-519e, miR-363, miR-517)[1][81]	(miR-196a, miR-200family, miR-338, miR-126, miR-31, miR-98, let-7g, miR-7)[2][82] miR-15b, miR-16[83]
Radiosensitivity	miR-451[85]	
Radioresistance	miR-221/222[97]	

[1]Drugs were cisplatin and 5-fluorouracil; [2]Drug was hydroxy camptothecin.

all of which are required before assays for miRNAs can be used clinically.

miRNAs AND CHEMOTHERAPY AND RADIOTHERAPY

Resistance to chemotherapy and radiotherapy is a major obstacle to improving the survival of the patients with gastric cancer[78-80]. We can predict the occurrence of resistance to chemotherapy and radiotherapy[81-83] (Table 4) through detecting the miRNA expression profile of the patients. Through investigating drug resistance to cisplatin and 5-fluorouracil in 90 patients with gastric cancer and comparing patients' miRNA expression before and after chemotherapy, Kim *et al*[81] found that high expression of let-7g, miR-342, miR-16, miR-181, miR-1 and miR-34 indicated sensitivity to chemotherapy, and high expression of miR-518f, miR-520a, miR-520d, miR-519e, miR-363 and miR-517 indicated resistance to chemotherapy. By predicting miRNAs, we used a new method for choosing chemotherapy regimen and monitoring its effects, and even reversing the chemotherapy resistance through transfecting specific pre-miRNA. miRNA-15b and miRNA-16 are downregulated severely in the multidrug resistant gastric carcinoma cell line SGC7901/VCR. By improving miRNA-15b and miRNA-16 expression levels, sensitivity to vincristine was enhanced. Chen *et al*[84] transfected miRNA-200c into SGC7901/DDP gastric cancer cells, which increased sensitivity to DDP, 5-fluorouracil, paclitaxel and doxorubicin. The same situation occurred in radiotherapy on gastric cancer by transfection into AS-miRNA-221/222, which down-regulated the miRNA-221/222 expression in gastric cancer cell line SGC7901. Zhang *et al* found that the survival rate of cancer cell was significantly lower than that in the control group. Radiosensitivity was promoted through 0-6 Gy irradiation. In addition, Bandres *et al*[85] transfected cancer cells with pre-miRNA-451, which improved expression of miRNA-451 in AGS gastric cancer cells. Under 0-4 Gy irradiation, the effect of treatment was significantly better than that in the control group.

miRNAs AND TREATMENT

miRNAs are regulatory factors for gene expression and act as a control center in the process of tumor development[86,87]. miRNAs can modulate protein expression and affect multiple information pathways[88]. miRNAs will be more effective than coding genes as a biological treatment of tumor target molecules. The basic strategy of current treatment based on miRNAs is to adopt gene knockout to inhibit or downregulate the expression level of oncogene miRNAs. On the contrary, for anti-oncogenes, we used the method of gene knock-in to introduce foreign miRNAs, increase the expression level, and achieve the purpose of tumor treatment. The following strategies were used: administration of small molecule drugs for inhibiting miRNA, *e.g.*, anti-miRNA oligonucleotides (AMOs) following base pairing rules, competitively blocked the miRNA with target gene interaction[89], such as locked nucleic acid[90-91]; miRNA sponges, *e.g.*, the adsorption with miRNA could not combine with the natural target[92]; and miR-Mask[93], miRNA inhibitors and so on. miRNA expression is often increased by using viruses as a carrier to introduce a specific miRNA or miRNA mimics and finally upregulate miRNA and inhibit the tumor[94].

A number of Eastern Asian researchers followed the above principle of miRNA-mediated treatment and achieved good results *in vitro* and in animal experiments. For example, miRNA-221/222 is upregulated in gastric cancer cell line SGC7901. By transfecting AS-miR-221/222 2000 into cancer cells with liposomes, miRNA-221/222 is knocked out. This inhibits gastric cancer cell growth and invasion. Their target molecule is PTEN[95]. MiR-516-3p has been transfected into the gastric scirrhous carcinoma cell line 44AS3 with liposomes, was significantly overexpressed, and finally inhibited cancer cell growth, invasion and metastasis[96]. Similar results were obtained in a nude mouse transplantation model of human gastric cancer. Zhang *et al*[97] through AMOs, knocked down the originally high expression of miRNA-21 and caused the proliferation of gastric cancer cells to slow and apoptosis to increase visibly. In addition, Ji *et al*[98] have added a miRNA-34 analog to *p53* mutated gastric cancer cell lines to restore its function and upregulate its expression, which inhibited cell growth and maintained them at phase G1.

Many studies based on treatment of gastric cancer by miRNA have shown good results. In particular, Chinese, Japanese and South Korean researchers have attempted this. However, we still have several major obstacles to overcome. First, the multi-targeting nature of miRNAs brings the risk of unconscious off-target effects. Second, the expression of target genes may often be regulated by

multiple miRNAs, which could greatly reduce the effect of treatment based on a specific miRNA. Finally, we still lack good specificity and an efficient miRNA delivery system for treatment[99,100].

CONCLUSION

miRNAs are involved in almost all stages of gastric carcinogenesis, and may have broad applications in early diagnosis of gastric carcinoma, prognosis, detection of radiotherapy and chemotherapy efficacy, and be a new target for treatment. However, studies based on the clinical application of miRNAs for gastric cancer still lack reliable and exact data from large multi-center studies. In recent years, miRNAs have been a focus of biomedical research. New miRNAs have been discovered and research techniques constantly updated. It will be a great challenge to integrate new data and establish standard procedures. For diagnosis, we need unified standards and testing platforms. For treatment, we need better-designed small-molecule drugs based on a well detailed and more accurate medication carrier without toxic side effects. We look forward to further studies of miRNAs improving their clinical applications for the diagnosis and treatment of gastric cancer. East Asia, as an area with a high incidence of gastric cancer, should undertake more studies for the application of miRNAs in gastric cancer.

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ORIGINAL ARTICLE

Measurement of calprotectin in ascitic fluid to identify elevated polymorphonuclear cell count

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Abstract

AIM: To evaluate the diagnostic capability of calprotectin in ascitic fluid for detecting a polymorphonuclear (PMN) cell count > 250/μL ascites.

METHODS: In this prospective observational study, a total of 130 ascites samples were analysed from 71 consecutive patients referred for paracentesis. Total and differential leukocyte cell counts were determined manually with a Neubauer chamber and gentian-violet stain. Calprotectin was measured in 1 mL ascetic fluid by enzyme-linked immunosorbent assay (ELISA) and a point-of-care (POC) lateral flow assay with the Quantum Blue® Reader (Bühlmann Laboratories). All measurements were carried out in a central laboratory by senior personnel blinded to patient history. A PMN count > 250/μL was the primary endpoint of the study. The diagnostic value of ascitic calprotectin measurement was assessed by comparing to the final diagnosis of each patient that had been adjudicated by investigators blinded to calprotectin values.

RESULTS: The PMN count was > 250/μL in 19 samples (14.6%) from 15 patients (21.1%) and varied widely among the study population (range 10-19 800/mL and 1-17 820/mL, respectively). Spontaneous bacterial peritonitis (SBP) was the final diagnosis in four patients (5.6%). All patients with PMN ≤ 250/μL had negative bacterial culture. PMN count was elevated in five patients with peritoneal carcinomatosis, three with lymphoma, one with neuroendocrine carcinoma, and two with secondary peritonitis due to abdominal perforation. PMN cell counts correlated with ascitic calprotectin values (Spearman's rho; r = 0.457 for ELISA, r = 0.473 for POC). A considerable range of ascitic calprotectin concentrations was detected by ELISA [median 0.43 μg/mL, interquartile range (IQR) 0.23-1.23 (range 0.10-14.93)] and POC [median 0.38 μg/mL, IQR 0.38-0.56 (range 0.38-13.31)]. Ascitic calprotectin levels were higher in samples with PMN > 250/μL, by both ELISA [median (IQR) 2.48 μg/mL (1.61-3.65) *vs* 0.10 μg/mL (0.10-0.36), P < 0.001] and POC [2.78 μg/mL (2.05-5.37) *vs* 0.38 μg/mL (0.38-0.41), P < 0.001]. The area under the receiver operating characteristics curve for identifying an elevated PMN count was 0.977 (95%CI: 0.933 to 0.995) for ELISA and 0.982 (95%CI: 0.942 to 0.997) for POC (P = 0.246 *vs* ELISA). Using the optimal cut-off value for ELISA (0.63 μg/mL), ascitic calprotectin had 94.8% sensitivity, 89.2% specificity, positive and negative likelihood ratios of 8.76 and 0.06 respectively, positive and negative predictive values of 60.0% and 99.0% respectively, and 90.0% overall accuracy. Using the optimal cut-off value for POC (0.51 μg/mL), the respective values were 100.0%, 84.7%, 6.53, 0.00, 52.8%, 100% and 87.7%. Correlation be-

tween ELISA and POC was excellent (r = 0.873, P < 0.001). The mean ± SD of the difference was -0.11 ± 0.48 μg/mL with limits of agreement of + 0.8 μg/mL (95%CI: 0.69 to 0.98) and -1.1 μg/mL (95%CI: -1.19 to -0.91).

CONCLUSION: Ascitic calprotectin reliably predicts PMN count > 250/μL, which may prove useful in the diagnosis of SBP, especially with a readily available bedside testing device.

Key words: Calprotectin; Ascites; Liver cirrhosis; Spontaneous bacterial peritonitis; Polymorphonuclear cells

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INTRODUCTION

Liver cirrhosis is the clinical end-stage of different entities of chronic liver disease when patients suffer from substantial mortality and morbidity, both of which are positively correlated with disease severity[1,2]. Ascites is the most common complication, and around 60% of patients with compensated cirrhosis develop ascites within 10 years of disease onset[3]. Spontaneous bacterial peritonitis (SBP) is an important cause of morbidity and mortality in cirrhotic patients with ascites. SBP is estimated to affect 10%-30% of cirrhotic patients hospitalised with ascites, and mortality in this group approaches 30%[4,5]. Many of these patients are asymptomatic, and it is therefore recommended that all patients with ascites undergo paracentesis at the time of admission to confirm the SBP status[5]. Although SBP is less prevalent in an outpatient setting, it is reasonable to also evaluate the ascitic fluid of outpatients because of the high mortality associated with SBP.

The diagnosis of SBP is based upon the polymorphonuclear (PMN) leukocyte cell count exceeding 250/μL in ascitic fluid[6,7]. Currently, differential cell count is usually performed by a manual method using light microscopy and counting chambers. However, the diagnosis is often delayed when laboratory personnel are not readily available or in the private practice setting where specimens are sent to an offsite laboratory. This is a major drawback, as rapid diagnosis of SBP and immediate initiation of antibiotic treatment is of paramount importance. Alternative methods using automated PMN counting[8,9], reagent strips (urine dipsticks)[10-26], or ascitic lactoferrin[27] have been developed; unfortunately, their diagnostic accuracies are limited and their use is dependent upon availability of laboratory personnel and reagents/components from the commercial source. Therefore, an accurate and convenient method of rapid diagnosis of SBP remains an unmet clinical need.

Calprotectin, a calcium and zinc-binding protein, is detected almost exclusively in neutrophils[28], and its presence in body fluids is proportional to the influx of neutrophils[29-33]. However, only one study to date has investigated calprotectin levels in ascites and found higher concentrations in patients with malignant disease than in those with non-malignant disease[34]. In contrast, faecal calprotectin is a well-established marker of inflammation and is used to monitor inflammatory bowel disease[35]. A rapid bedside test has been developed to measure calprotectin in faeces; systematic comparison with the established enzyme-linked immunosorbent assay (ELISA) technique showed good correlation between the two tests' results[36] and the rapid bedside test has been suggested as an equally valuable tool for diagnosing inflammatory bowel disease[37]. It is possible that such a rapid bedside test may be useful for measuring calprotectin in ascitic fluid to indicate PNM levels and SBP status, however the diagnostic accuracy of such a measurement in ascitic fluid is unknown.

This study was designed to test our hypothesis that calprotectin in ascitic fluid could be useful as a surrogate PMN marker for identifying SBP patients (> 250/μL PNM). To this end, we measured calprotectin in ascites of consecutive patients referred for paracentesis using a rapid bedside test and compared the results to those from the traditional ELISA.

MATERIALS AND METHODS

Setting and participants

In this prospective observational study, we recruited patients with ascites referred for paracentesis to the Department of Gastroenterology and Hepatology at the University Hospital Basel, and to the Department of Gastroenterology, Hepatology and Clinical Nutrition at the Cantonal Hospital Liestal in Switzerland. All patients with ascites were eligible for study enrolment, irrespective of the aetiology of ascites. The decision to perform paracentesis was based on clinical findings evaluated by the referring physician who was otherwise not involved in the study. Exclusion criteria were age < 18 years and recent abdominal surgery (< 3 mo). Standardised patient history, clinical symptoms, and demographic data were obtained from all participants. The study was carried out in accordance with the principles of the Declaration of Helsinki and with pre-approval from the local Ethic Committees of both study sites. All patients provided written informed consent prior to participation in any protocol-specific procedures.

Endpoint

The diagnostic value of ascitic calprotectin measurement was assessed in comparison to the adjudicated final diagnosis. A PMN count > 250/μL was the primary endpoint of the study.

Adjudication of the final diagnosis

One month after study participation, the final diagnosis (SBP) and the aetiology of ascites were independently adjudicated in a blinded fashion by two board-certified gastroenterologists not involved in the patients' care. Their final assessment was based upon available medical records, including PMN count and the results of all diagnostic investigations, as well as the patient's response to treatment. Current recommendations were followed[6,7]. The two physicians designated the aetiology of ascites by choosing one or more of the following diagnoses from a standardized list: liver cirrhosis (alcoholic, chronic hepatitis, non-alcoholic steatohepatitis, hemochromatosis, primary biliary cirrhosis, others to be specified), hepatocellular carcinoma, cholangiocellular carcinoma, liver metastasis, peritoneal carcinomatosis, right heart failure, nephrotic syndrome, and others to be specified. If more than one cause of ascites was identified, the leading disorder responsible for the current episode was established and recorded. Any disagreements in the final diagnosis of a given study participant were resolved by consensus with a third clinician who was considered an expert in the field and recruited to independently review and adjudicate the cases.

Paracentesis

Paracentesis was performed under aseptic conditions with the patient in the supine position and the puncture site in the left or right lower quadrant. Prior to needle insertion, ultrasound was performed to visualise the intra-abdominal structures. No study participant suffered complications related to the abdominal puncture procedure. All samples for diagnostic testing were immediately collected at the bedside and processed by laboratory personnel without further delay. Specifically, aliquots of approximately 1mL ascites were centrifuged for 15 min at 500 × *g*. The supernatant phase was transferred to a fresh tube and stored at -20 ℃ until analysis by ELISA or POC, which occurred within 72 h.

Blood samples were also obtained at this time. The ascites samples were used to measure total cell count, PMN count, calprotectin, albumin, total protein, glucose, and lactate dehydrogenase. In addition, two 10 mL aliquots of ascites were subjected to bacterial culture (bottle method) respectively. The serum-ascites albumin gradient (SAAG) was calculated as the difference of albumin in serum and albumin in ascites.

Differential cell count and cytopathology

All laboratory analyses were performed in the Central Laboratories BL (Schönenbuch, Switzerland) by senior laboratory personnel blinded to patient history and calprotectin levels. Total and differential leukocyte cell counts were determined by the manual method using a Neubauer chamber and gentian-violet staining (Leukotic®; bioanalytic GmbH, Freiburg, Germany). The Central Laboratories BL is accredited according to ISO/IEC 17 025 and ISO 15 189 standards. For all study participants who underwent repeated paracentesis, the cytopathological analysis was performed at least once.

Laboratory-based quantitative calprotectin measurement

Ascitic calprotectin in ascites was assayed using a commercially-available ELISA (Bühlmann Laboratories AG, Schönenbuch, Switzerland) and following the manufacturer's instructions. Briefly, 10 μL aliquots of the supernatant samples were diluted 1:50 in incubation buffer and 100 μL was applied to a microtiter plate coated with a monoclonal capture antibody highly specific for the calprotectin heterodimeric and polymeric complexes. After incubation, washing and further incubation with a detection antibody conjugated to horseradish peroxidase, the tetramethylbenzidine chromogenic substrate was added. The reaction was terminated by a stop solution and the absorbance (optical density at 450 nm) was measured by spectrophotometry. The measuring range of the test was 0.2-12 μg calprotectin/mL ascites with an intra- and interassay coefficient of 4.7% and 11.3%, respectively.

Point-of-care quantitative calprotectin measurement

The Quantum Blue® quantitative calprotectin lateral flow assay (Bühlmann Laboratories AG) was used for the point-of-care (POC) measurement of ascitic calprotectin. The Quantum Blue® reader is currently marketed for 2500 USD ($), and the test cartridges cost 20 USD per sample and analysis. Aliquots of 60 μL 1:10 diluted ascites samples (20 μL ascites in 180 μL extraction buffer) were pipetted respectively onto the sample loading port of the test cartridge. After a 12 min incubation, the test cartridge was quantitatively read by the Quantum Blue® Reader. The measurement range of the lateral flow test was 0.38-3.8 μg calprotectin/mL ascites, with an interassay coefficient of 15.6%. Specimens with concentrations above this measurement range were further diluted with extraction buffer.

In addition, a random subgroup of samples (n = 17) was immediately measured by POC, without first performing the centrifugation step of processing. These results were compared to the results from the POC measurements obtained in the laboratory setting after processing and storage.

Statistical analysis

All statistical analyses were performed using the SPSS software package, version 19.0 (SPSS Inc., Chicago, IL, United States). A P-value of less than 0.05 indicated statistical significance. Intergroup comparisons were made using the Mann-Whitney U test and the χ^2 test where appropriate. Correlations between numerical data were determined with the Spearman's rank correlation coefficient. All hypothesis testing was two-tailed. The Bland-Altman plot was used to assess agreement between ELISA test results and POC test results, in which the differences between the results of the two tests for each individual patient were plotted against the corresponding mean of the two readings. The mean and SD of the differences and the limits of agreement, defined as the mean ± 2 SD of the difference (95%CI), were calculated. Analysis of the receiver operator characteristics (ROC) and calculation of the area under the curve (AUC) were used to evaluate the capability of calprotectin to identify a PMN count >

Table 1 Baseline characteristics of patients with liver cirrhosis (*n* = 54)

Aetiology of liver cirrhosis	
Alcoholic	36 (66.6)
Viral hepatitis	7 (13.0)
Alcoholic + viral hepatitis	5 (9.3)
Non alcoholic steatohepatitis	1 (1.9)
Others	5 (9.3)
Child-turcotte-pugh class	
Child A	0
Child B	29 (53.7)
Child C	25 (46.3)
MELD score	12.2 (10.0-16.0)

Others included 2 patients with autoimmune hepatitis, 2 patients with primary biliary cirrhosis and 1 patient with primary sclerosing cholangitis. The child-turcotte-pugh classification contains five variables, including serum levels of bilirubin and albumin, prothrombin time, ascites, and encephalopathy. Child A: 5-6 points; Child B: 7-9 points; Child C: 10-12 points. The model for end-stage liver disease (MELD) score: 9.57 Ln [serum level of creatinine (mg/dL)] + 3.78 Ln [serum bilirubin (mg/dL)] + 11.2 Ln (international normalised ratio for prothrombin time) + 6.43. Data are presented as number of patients (%) or medians (25th to 75th percentiles).

250/μL. The ROC analysis identified the cut-off points for maximal diagnostic capability. The test characteristics of sensitivity, specificity, positive and negative likelihood ratios (LR+ and LR-), and positive and negative predictive values (NPV) were determined. Overall accuracy of the test was calculated according to the following formula: [(true positive test results + true negative test results)/total population]. As this study was exploratory in design, no formal power calculations were carried out.

RESULTS

Patient characteristics

A total of 136 samples from 75 patients were prospectively collected from October 2010 to January 2012. Among these, 130 samples were included in the final analysis, representing 71 patients (94.7% of the total; 40 males and 31 females) with a median age of 64 years (IQR 55-71 years). Sixty-three of the patients (88.7%) had been referred for diagnostic paracentesis. Twenty-four of the patients (33.8%) underwent the procedure more than once (median 3, range 2-12).

The majority of patients (54, 76.1%) suffered from liver cirrhosis (Table 1). A total of 11 patients (15.5%) had malignant ascites, which included three ovarian, two lymphomas, two breast, one stomach, one colorectal, one pancreatic cancer, and one neuroendocrine carcinoma. Of those 11 patients, two also had liver cirrhosis. Additionally, three patients with ascites also had heart failure and five patients with ascites also had portal hypertension from metastatic liver disease (but no malignant cells were present in the ascites). No intervention-related complications occurred after paracentesis.

Ascitic fluid cell count

Total cell count and PMN cell count at presentation varied widely among the study population (range 10-19 800/mL and 1-17 820/mL, respectively). PMN count > 250/mL was detected in 19 samples (14.6%) from 15 patients (21.1%). Among the study population, SBP was the final diagnosis for four patients (5.6%) and only one of these four had positive ascitic bacterial cultures (*Streptococcus pneumoniae*). All bacterial cultures from patients with PMN ≤ 250/mL were negative. Additionally, PMN count was elevated in five patients with peritoneal carcinomatosis (two with ovarian cancer, and one each with gastric, colorectal and pancreatic cancer), in three patients with lymphoma, in one patient with neuroendocrine carcinoma, and in two patients with secondary peritonitis due to an abdominal perforation. All patients with SBP received antibiotic treatment and recovered well. None of the patients died. Table 2 details the findings from ascitic fluid analysis.

Table 2 Ascitic fluid analysis

Variable	PMN count > 250/μL (*n* = 19)	PMN count ≤ 250/μL (*n* = 111)
Total cell count	1300.0 (350.0-19 800.0)	250.0 (10.0-1970.0)
PMN count	553.0 (277.0-17 820.0)	21.0 (1.0-212.0)
Albumin, g/L	13.0 (4.8-16.8)	7.0 (3.0-10.0)
Protein, g/L	22.0 (9.3-37.5)	12.0 (8.0-20.0)
LDH, U/L	117.0 (100.8-138.5)	55.0 (42.0-81.0)
Glucose, mmol/L	7.6 (6.2-9.7)	7.0 (6.2-8.2)

Values are given as medians (range) for total cell count and polymorphonuclear cell count (PMN) count and median (25th to 75th percentiles) for all other values. LDH: Lactate dehydrogenase.

Calprotectin measurement in ascitic fluid

The ascitic calprotectin concentrations ranged considerably in the ELISA [median 0.43 μg/mL, IQR 0.23-1.23 (range 0.10-14.93)] and POC test [median 0.38 μg/mL, IQR 0.38-0.562 (range 0.38-13.31)]. However, the calprotectin values measured by the laboratory-based ELISA and the POC test correlated well with the PMN count (r = 0.476, P < 0.001 and r = 0.473, P < 0.001, respectively), and the correlation between the two tests was excellent (r = 0.873, P < 0.001). The degree of agreement between the measurements of ascitic calprotectin from the ELISA and the POC test is illustrated in Figure 1. The mean ± SD of the difference was -0.11 ± 0.48 μg/mL, with limits of agreement of +0.8 μg/mL (95%CI: 0.69 to 0.98) and -1.1 μg/mL (95%CI: -1.19 to -0.91).

Comparative analysis of the POC detection of ascitic calprotectin levels in samples measured at the bedside (unprocessed and processed after centrifugation) and in the lab (after centrifugation) showed that the calprotectin measurements correlated well. For unprocessed samples, r = 0.831 (P < 0.001), and for processed samples, r = 0.656 (P = 0.004).

Diagnostic value of ascitic calprotectin

Ascitic calprotectin levels were higher in samples (n = 19) with PMN > 250/μL, both when measured by ELISA [median (IQR) 2.48 μg/mL (1.61-3.65) *vs* 0.10 μg/mL (0.10-0.36), P < 0.001] and the POC test [median 2.78

Table 3 Test characteristics of ascitic calprotectin to identify > 250 polymorphonuclear leukocytes per mL ascites

	AUC (95%CI)	Best cut-off (μg/mL)	Sens (%)	Spec (%)	LR+	LR-	PPV (%)	NPV (%)	Accuracy (%)
ELISA	0.977 (0.933-0.995)	0.63	94.8	89.2	8.76	0.06	60.0	99.0	90.0
POC	0.982 (0.942-0.997)	0.51	100	84.7	6.53	0.00	52.8	100	87.7

Area under the receiver operating characteristics curve (AUC) with corresponding sensitivity (Sens), specificity (Spec), positive and negative likelihood ratio (LR+, LR-), and negative and positive predictive values (NPV, PPV) for ascitic calprotectin to identify polymorphonuclear > 250/μL. Overall accuracy was calculated using the following formula: (true positive test results + true negative test results)/total population. ELISA: Enzyme-linked immunosorbent; POC: Point-of-care.

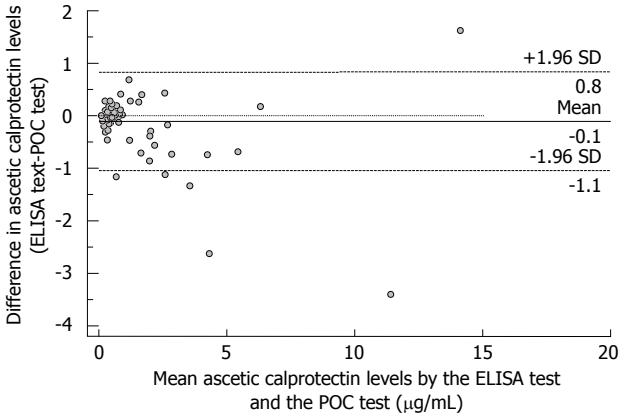

Figure 1 Measurement of ascitic calprotectin with the enzyme-linked immunosorbent test and the point-of-care test (Bland Altman plot). The differences between the results of the enzyme-linked immunosorbent (ELISA) and point-of-care (POC) tests in each patient are plotted against the mean of the two measurements, showing the limits of agreement, defined as the mean ± 2 SD of the difference.

μg/mL (2.05-5.37) *vs* 0.38 μg/mL (0.38-0.41), $P < 0.001$] (Figure 2). Evaluation of the ascitic calprotectin measurement as a diagnostic test to identify patients with PMN count > 250/μL yielded an AUC of 0.977 (95%CI: 0.933-0.995) for the ELISA and an AUC of 0.982 (95%CI: 0.942-0.997) for the POC test. Furthermore, the two tests did not show significantly different diagnostic capacity ($P = 0.246$ *vs* ELISA) (Figure 3).

Using the optimal cut-off value from the ROC of ELISA (0.63 μg/mL), ascitic calprotectin yielded a sensitivity of 95%, a specificity of 89.2%, and an accuracy of 90.0% (Table 3). To identify all patients with PMN count > 250/μL and to obtain 100% test sensitivity, a slightly lower cut-off value (0.44 μg/mL) is necessary. However, use of this lower value is accompanied by lower specificity (82.9%) and lower LR+ (5.84).

Analysis of the POC test characteristics revealed a nearly identical profile to the ELISA characteristics (Table 3). The optimal cut-off value for POC (0.51 μg/mL) yielded a sensitivity of 100% and a specificity of 84.7%, with 6.53 LR+ and 0.0 LR-. The overall accuracy of the POC test was 87.7% (Figure 4).

Patients with false positive test results had PMN counts between 3 and 212 (median 70.0, IQR 35.0-127.5) for the ELISA, and between 3 and 197 (median 45.0, IQR 16.0-100.0) for the POC test.

The ELISA and POC test had similar diagnostic capability for identifying PMN > 250/μL in the subgroup of patients with liver cirrhosis (95 samples from 54 patients; ELISA AUC 0.987, and POC test AUC 0.982). In addition, when the ascites samples were analysed according to the SAAG > 11g/L (115 samples from 62 patients), the AUCs of ascitic calprotectin were 0.983 for the ELISA and 0.988 for the POC test (data not shown).

DISCUSSION

This prospective study evaluated the diagnostic utility of measuring calprotectin in ascites to identify ascitic PMN counts > 250/μL in patients referred for paracentesis, and provides the following new information: Patients with an elevated PMN count (> 250/μL) had higher ascitic calprotectin levels than those with normal cell counts; this finding indicates that ascitic calprotectin levels correlate well and reliably with PMN count. It is clinically significant that calprotectin levels in ascitic patients can identify elevated PMN counts using both laboratory-based ELISA and bedside POC testing. Indeed, ascitic calprotectin may serve as a surrogate marker for PMN count and would be amenable to routine SBP screening, especially when measured by a bedside test.

Ascites is commonly found in patients with liver cirrhosis and may promote bacterial translocation, enhancing the risk of SBP[3]. SBP in outpatients is rare, but when it occurs it often requires hospitalisation to manage to disease course[4,5]. In our study, four of 71 patients were diagnosed with SBP (5.6%). In general, SBP symptoms are nonspecific and current guidelines recommend paracentesis be performed in all patients with ascites to rule out abdominal infection[6,7]. The diagnosis of SBP in patients with liver cirrhosis is based on a PMN count of > 250/μL in ascitic fluid, with or without positive bacterial cultures[5-7]. This cut-off is recognized as more sensitive than other criteria (PMN > 500/μL; white blood cell count > 500/μL)[38,39] for identifying SBP[40]. SBP diagnosis based solely on bacterial culture is considered unreliable, since up to 60% of patients with increased PMN count are reported as culture-negative[41,42]. In our study, all patients with culture-positive abdominal infection, including both SBP and secondary peritonitis patients, had elevated PMN counts. In the four SBP patients, the bacterial cultures were positive for only one (25.0%).

Our study measured calprotectin in ascitic fluid in 130 unselected samples from 71 consecutive patients. Ascitic calprotectin levels correlated well and reliably with PMN counts, and the samples with PMN > 250/μL also had higher ascitic calprotectin levels than the samples with

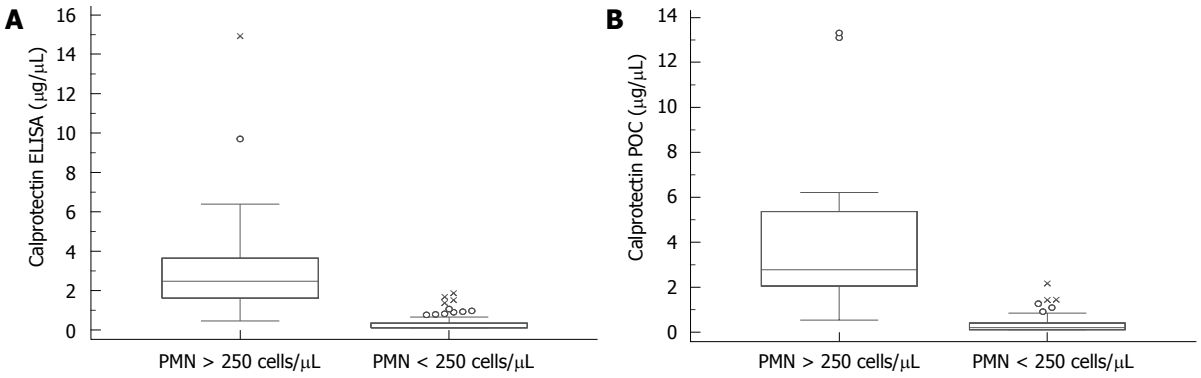

Figure 2 Ascitic calprotectin values in patients with normal and elevated ascitic polymorphonuclear cell count. Box-and-whisker plot representing the median, 25th to 75th percentiles, minimum/maximum values, and outliers outside 1.5 times (circle) and 3 times (cross) the interquartile range of ascitic calprotectin values, measured with the enzyme-linked immunosorbent (ELISA) test (A) and the point-of-care (POC) test (B). PMN: Polymorphonuclear.

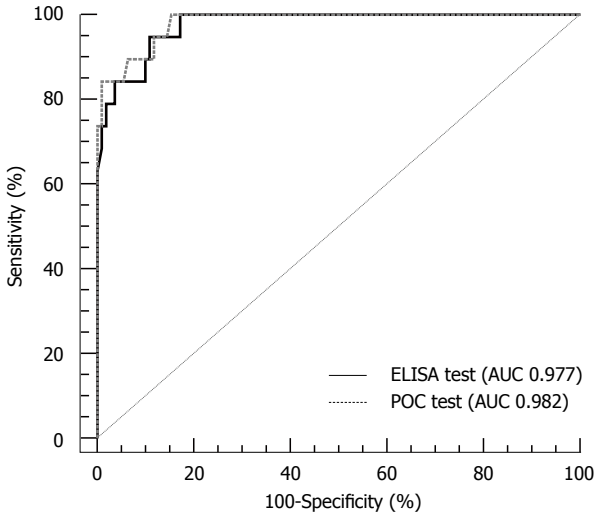

Figure 3 Receiver operating characteristics analysis of the enzyme-linked immunosorbent test and the point-of-care test to identify elevated ascitic polymorphonuclear cell count. The area under the receiver operating characteristics curve (AUC) for ascitic calprotectin for identifying a polymorphonuclear count > 250/μL. No differences between the two tests (P = 0.155) were detected. The diagonal line represents no discrimination. POC: Point-of-care; ELISA: Enzyme-linked immunosorbent.

PMN ⩽ 250/μL. Both the ELISA and the POC test accurately measured ascitic calprotectin, and the correlation between the two tests was excellent with high sensitivity (95% and 100%, respectively) and high specificity (89% and 85%, respectively) at the optimal cut-off points (from ROC analysis). In a diagnostic test that is used to screen for a specific disease, it is preferred to test all patients at risk, especially when potentially life-threatening complications may occur. In screening tests, high sensitivity is therefore favoured over high specificity. In our study, the NPVs of calprotectin testing in ascites were excellent (99% for the ELISA and 100% for the POC test). Notably, these results suggest that no patient with elevated PMN count would have been missed by the bedside test.

In daily clinical practice, PMN count is often not readily available and clinicians frequently rely on total cell count when initiating empiric antibiotic treatment[43]. It has been suggested that a total cell count < 1000/μL (obtained from automated cell counting procedures) is unlikely to signify SBP, having a NPV of 96%[44]. In our study, using such a criteria would have misclassified five patients (26.3%) with elevated PMN counts. Moreover, the use of total cell count in combination with ascitic calprotectin measurement did not increase the diagnostic accuracy of calprotectin testing, as calculated by ROC analysis (data not shown).

To avoid diagnostic delay, it has been proposed that automated PMN counting should replace the laborious and time-consuming manual cell counting technique[8,9]. Studies have demonstrated that automated blood cell counts correlate well with manual ascitic leukocyte differential counts[45]. However, despite the potential benefit of automated cell counters in clinical practice, widespread use of this technology is limited by the cost of the sophisticated laboratory equipment and requirement for trained operators; this is a particular challenge for practitioners' office settings and small clinics without in-house laboratories.

The use of reagent strips (urine dipsticks) for PNM counting (by colorimetric detection of leukocyte esterase activity) has also been evaluated as a rapid SBP diagnostic tool[45,46]. A number of these studies have reported sensitivities between 85% and 100% and specificities between 90% and 100%[10-25]. However, these results came from mostly single-centre studies with small numbers of SBP cases. The only large, multicentre study reported in the literature produced very different results; in particular, using 2123 paracenteses, the sensitivity was only 45% for identifying PMN > 250/μL in cirrhotic patients[26]. Although specificity was still high, it was concluded that urinary dipstick testing lacks sufficient accuracy for diagnosing SBP. The risk of false negative results seemed to be especially problematic in patients with lower PMN counts[46]. These results dampened the initial enthusiasm for reagent strips, and currently this method is not recommended for rapid diagnosis of SBP[45].

Only one study in the literature, to date, has provided data on calprotectin measurement in ascites[35]. In that study, Homann *et al*[34] compared ascites from patients with malignant disease to ascites from patients with non-

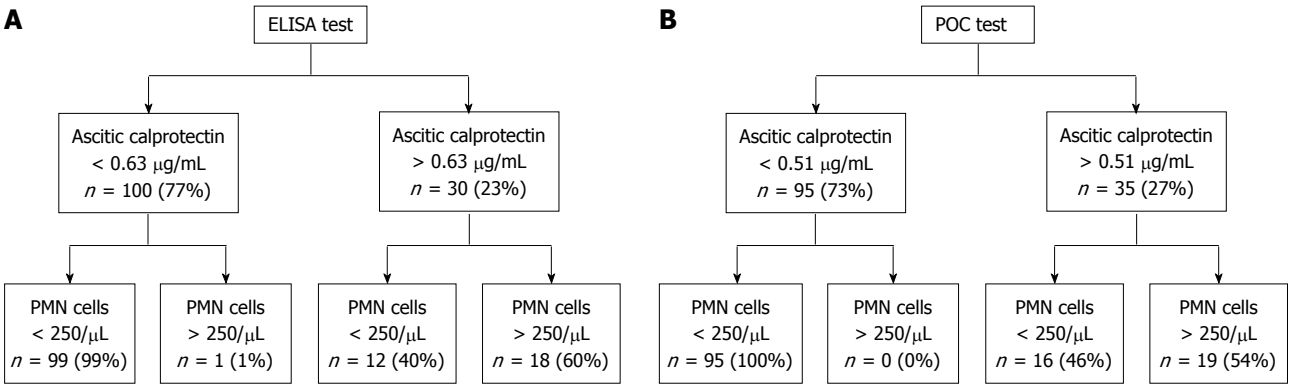

Figure 4 Diagnostic accuracy. For the enzyme-linked immunosorbent (ELISA) test, the overall test accuracy of ascitic calprotectin was 87% when 0.63 μg/mL was used as the best cut-off value (A) and was 84% for the point-of-care (POC) test when 0.51 μg/mL was used as best cut-off (B). PMN: Polymorphonuclear.

malignant disease. Higher ascitic calprotectin levels were found in the malignant patients and shown to correlate with increased mortality in patients with decompensated liver cirrhosis. However, the authors did not investigate the diagnostic potential of ascitic calprotectin. More recently, Parsi *et al*[27] measured ascitic lactoferrin (an iron-binding protein also found in PMNs) in cirrhotic patients with ascites and investigated its potential for identifying SBP. The lactoferrin measurements correctly identified PMN counts > 250/μL in 22 of 218 samples (10.1%), yielding a sensitivity of 95.5% and specificity of 97.0%. However, the quantitative assay (ELISA) used in that study is not commercially available, and to date no bedside test, qualitative or quantitative, exists for lactoferrin.

The results from our current study confirm the findings reported by Parsi *et al*[27]. Specifically, we show that measurement of calprotectin, a leukocyte-specific protein, may serve as a surrogate marker for the PMN count in ascitic fluid. A particular strength of our study is the quantitative measurement of calprotectin by two methods, a laboratory-based ELISA and a commercially available bedside test. Rapid bedside measurement is advantageous for hospitalised patients, as it supports early antibiotic intervention and limits unnecessary treatments. It will also benefit the outpatient setting by providing on-site testing, since samples are otherwise required to be transported to an offsite laboratory. The POC test that we used can accomplish quantitative measurement of ascitic calprotectin within minutes, and this feature is expected to minimize the problems associated with diagnostic delay that clinicians currently face. Additionally, the cost of POC testing may be less than the other methods, such as contracting with the offsite laboratories.

There are several limitations to the current study that merit consideration. First, the prevalence of SBP in our study cohort was lower than expected from the literature. Second, we included all patients with ascites, irrespective of the aetiology, and it may be that our results cannot be generalised to all patients with liver cirrhosis. Third, this was an exploratory study that aimed to establish the concept of measuring a PNM-related inflammatory protein, rather than PNM cells themselves, as an indicator of elevated cell count in ascites; therefore, no formal sample size calculation was performed. Finally, our sample size was small and larger studies are needed to evaluate this test in different clinical settings and to establish a reliable cut-off for ascitic calprotectin for optimal identification of PMN counts > 250/μL.

In conclusion, we have demonstrated that measurement of calprotectin in ascitic fluid correlates well with the PMN count and reliably predicts levels > 250/μL. Additionally, we showed that an elevated PMN count could easily be measured by a POC test device which may enable a treating physician to obtain useful bedside measurements, especially those practicing in settings with limited equipment and/or technical personnel. Further studies are warranted to define a clinically useful cut-off for the diagnosis of SBP in cirrhotic patients with ascites.

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COMMENTS

Background

Ascites is the most common complication of patients with cirrhosis, and around 60% of patients will develop ascites within 10 years of disease commencement. Spontaneous bacterial peritonitis (SBP) is an important cause of morbidity and mortality in these patients. SBP is estimated to affect 10%-30% of hospitalised patients with ascites, and it is recommended that all patients with ascites undergo paracentesis at the time of admission to assess SBP status and initiate timely therapy.

Research frontiers

The diagnosis of SBP is based on a polymorphonuclear leukocyte (PMN) cell count > 250/μL in the ascitic fluid. Currently, differential cell count is usually performed manually using light microscopy and counting chambers. This procedure is time consuming, and diagnosis may be further delayed when laboratory personnel are not readily available. Several other methods to diagnose SBP (automated cell counting and urine dipstick-based screening for leukocytes) have proven unreliable in clinical practice and are inferior to the manual method. In this study, authors investigated the potential of calprotectin, a neutrophilic

protein and established marker of intestinal inflammation, to screen for SBP when measured in ascites.

Innovations and breakthroughs

To date, only one study in the public literature has measured calprotectin in ascites, and the conclusion was that higher concentrations of calprotectin exist in malignant disease conditions as compared to non-malignant conditions. However, diagnostic accuracy was not assessed and calprotectin was measured using a laboratory-dependent enzyme-linked immunosorbent assay (ELISA). In this study, authors have demonstrated that measurement of calprotectin in ascitic fluid correlates well with PMN count and reliably indicates PNM levels > 250/μL. Additionally, we showed that an elevated PMN count could be easily measured within minutes using a point-of-care (POC) bedside test, suggesting its potential as a rapid diagnostic approach for SBP.

Applications

The rapid diagnosis of SBP and immediate start of antibiotic treatment is of paramount importance as mortality estimates approach 30%. A particular strength of this study is the quantitative measurement of ascitic calprotectin by two methods: a laboratory-based ELISA and a commercially available POC test device. Rapid bedside measurement is advantageous for hospitalised patients, since it facilitates timely antibiotic therapy and minimizes unnecessary treatments. However, it may be especially beneficial to an outpatient setting, where samples are otherwise required to be transported to an offsite laboratory for testing. The POC test device that we used allows quantitative measurement of ascitic calprotectin within minutes and is likely to minimize the diagnostic delay that clinicians currently face.

Terminology

Calprotectin, a calcium and zinc-binding protein, is found almost exclusively in neutrophils. The presence of calprotectin in body fluids is proportional to the influx of neutrophils during inflammation.

Peer review

This article discusses a new method for the diagnosis of SBP. This is a well-designed and methodologically correct exploratory study.

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ORIGINAL ARTICLE

Efficacy of cap-assisted endoscopy for routine examining the ampulla of Vater

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Abstract

AIM: To determine the efficacy of a cap-assisted endoscopy (CAE) to completely visualize the ampulla of Vater (AV) in patients failed by conventional endoscopy.

METHODS: A prospective study was conducted on 120 patients > 20 years of ages who visited the Health Promotion Center of Chungbuk National University Hospital for conscious sedation esophagogastroduodenoscopy (EGD) as a screening test from July to October, 2011. First, forward-viewing endoscopy was performed with reasonable effort using a push and pull method. We considered complete visualization of the AV when we could observe the entire AV including the orifice clearly, and reported the observation as complete or incomplete (partial or not found at all). Second, in cases of complete failure of the observation, an additional AV examination was conducted by attaching a short cap (D-201-10704, Olympus Medical Systems, Tokyo, Japan) to the tip of a forward-viewing endoscope. Third, if the second method failed, we replaced the short cap with a long cap (MH-593, Olympus Medical Systems) and performed a re-examination of the AV.

RESULTS: Conventional endoscopy achieved complete visualization of the AV in 97 of the 120 patients (80.8%) but was not achieved in 23 patients (19.2%). Age (mean ± SD) and gender [male (%)] were not significantly different between the complete observation and the incomplete observation groups. Additional short CAE was performed in patients in whom we could not completely visualize the AV. This group included 13 patients (10.9%) with partial observation of the AV and 10 (8.3%) in which the AV was not found. Short CAE permitted a complete observation of the AV in 21 of the 23 patients (91.3%). Patients in whom visualization of the AV failed with short CAE had satisfactory outcomes by replacing the short cap with a long cap. The additional time for CAE took an average of 141 ± 88 s. There were no complications and no significant mucosal trauma.

CONCLUSION: CAE is safe to use as a salvage method to achieve complete visualization of the AV when a regular EGD examination fails.

Key words: Ampulla of Vater; Conventional endoscopy; Cap-assisted endoscopy; Screening test; Complete observation

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INTRODUCTION

It is recommended to visualize the second portion of the duodenum including the ampulla of Vater (AV) during a standard esophagogastroduodenoscopy (EGD) procedure[1,2]. Adequate visualization of the AV is important for early detection of periampullary or pancreaticobiliary diseases[3,4].

EGD textbooks and guidelines have emphasized complete visualization of the AV[1,2], but at the same time indicated that complete visualization of the AV is difficult due to the anatomical characteristics of the second portion of the duodenum, including the tangential angle, the periampullary diverticulum, and loop formation in the scope[5]. Thus, a side-viewing endoscope has been recommended for complete visualization of the AV in patients with a suspected AV lesion or in whom the AV cannot be observed completely. However, a side-viewing endoscope is not always available in an ordinary endoscopy suite[6]. So clinicians should refer the patient for side-viewing endoscopy. In the case of South Korea, 4.2 million EGDs being performed as a screening test per year. Among them, 84% were performed in clinics that not equipped with side-viewing endoscope[6]. And, although tertiary referral center were equipped with side-viewing endoscope, the Health Promotion Center for screening test were separated from Hospital Endoscopy Center for inpatient. So many endoscopists for screening test are not familiar with side-viewing endoscope for endoscopic retrograde cholangiopancreatography (ERCP). Therefore an additional examination using a side-viewing endoscope is expensive, time-consuming and difficult.

Cap-assisted endoscopy (CAE) has been used widely to facilitate detection of polyps[7-10], improve the success rate of cecal intubation[11,12], and to facilitate inspection of lesions situated in blind areas of the colon[13-15]. We have found that the complete visualization rate of the AV in patients who are referred for incomplete visualization of the AV can be improved by attaching a transparent cap. Therefore, we conducted a prospective observational study to investigate the complete visualization rate of the AV during routine EGD and to evaluate the efficacy and safety of endoscopic examination using a transparent cap to completely visualize the AV in patients in whom this procedure failed with a conventional endoscope.

MATERIALS AND METHODS

Patients

A prospective study was conducted on 120 patients > 20 years of ages who visited the Health Promotion Center of Chungbuk National University Hospital for conscious sedation EGD as a screening test from July to October, 2011. The following patients were excluded from the study: (1) those with poor general condition who had an American Society of Anesthesiology classification ⩾ 3; (2) those who received previous upper gastrointestinal tract surgery or ERCP; and (3) patients with severe comorbidities. All patients provided written informed consent to participate in the study. This study was reviewed and approved by the Institutional Review Board of Chungbuk National University Hospital (D-2011-06-006).

Procedure

All examinations were conducted with the patient lying on their left side, and midazolam was administered as a sedative and pain medication. Approximately 30-40 mg of propofol was also administered depending on patient weight[16]. Additional administration of propofol was used when deemed necessary, according to procedure time. Heart rate and oxygen saturation were checked in real time. Demographic data, procedure time, visualization of the AV, and complications were recorded. All examinations were carried out with a forward-viewing endoscope (GIF 230; Olympus Optical Co, Ltd, Tokyo, Japan) by a well-qualified endoscopist (Han JH), who has conducted more than 1500 EGDs including 400 ERCPs annually. Two types of transparent caps were used: disposable distal attachments; "soft and short" cap (D-201-10704, outer diameter: 11.35 mm, length from distal end of endoscope: 4 mm. Olympus Medical Systems) and a "hard and long" cap (MH-593, outer diameter: 12.9 mm, length from distal end of endoscope: 11 mm; Olympus Medical Systems) (Figure 1).

First, forward-viewing endoscopy was performed with reasonable effort using a push and pull method. We considered complete visualization of the AV when we could observe the entire AV including the orifice clearly, and reported the observation as complete or incomplete (partial or not found at all). Second, in cases of complete failure of the observation, an additional AV examination was conducted by attaching a short cap to the tip of a forward-viewing endoscope. Third, if the second method failed, we replaced the short cap with a long cap and performed a re-examination of the AV.

Statistical analysis

Descriptive statistical analyses were performed using the SPSS software, version 12.0 (SPSS Inc, Chicago, IL, United States), and frequency, percentage, mean, and range were used for descriptive analyses. A P value < 0.05 was considered statistically significant.

RESULTS

Patients' characteristics and outcomes of conventional endoscopy

A total of 181 patients were examined, 61 were excluded. 120 patients were enrolled and agreed to participate in the study by signing an informed consent (Figure 2). Conventional endoscopy achieved complete visualization of the AV in 97 of the 120 patients (80.8%) but was not achieved in 23 patients (19.2%) (Figure 3). The reasons

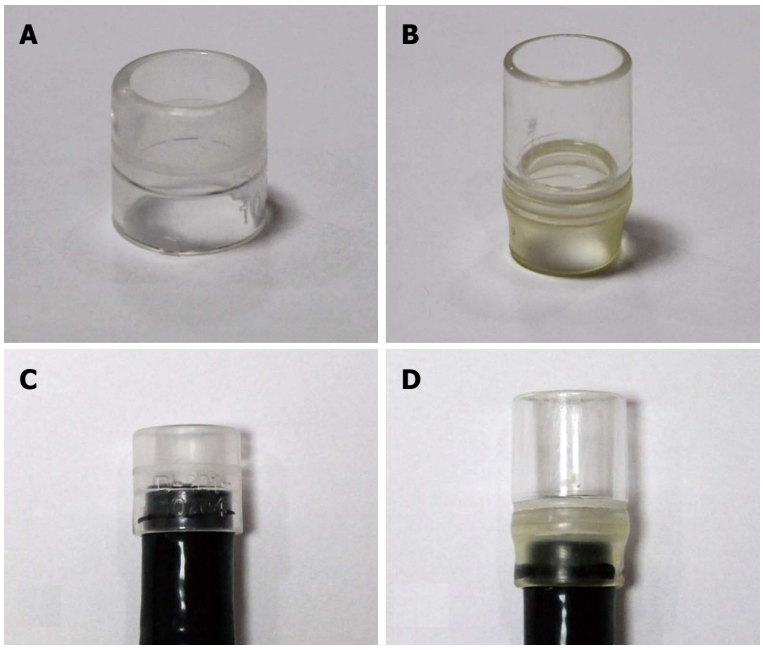

Figure 1 Endoscopic transparent cap. A: Short transparent cap (Olympus distal attachment D-201-10704, outer diameter: 11.35 mm, length from distal end of endoscope: 4 mm; Olympus Tokyo, Japan); B: Long transparent cap (Olympus distal attachment MH-593, outer diameter: 12.9 mm, length from distal end of endoscope: 11 mm; Olympus); C: Short cap attached to the tip of a forward-viewing endoscope; D: Long cap attached to the tip of a forward-viewing endoscope.

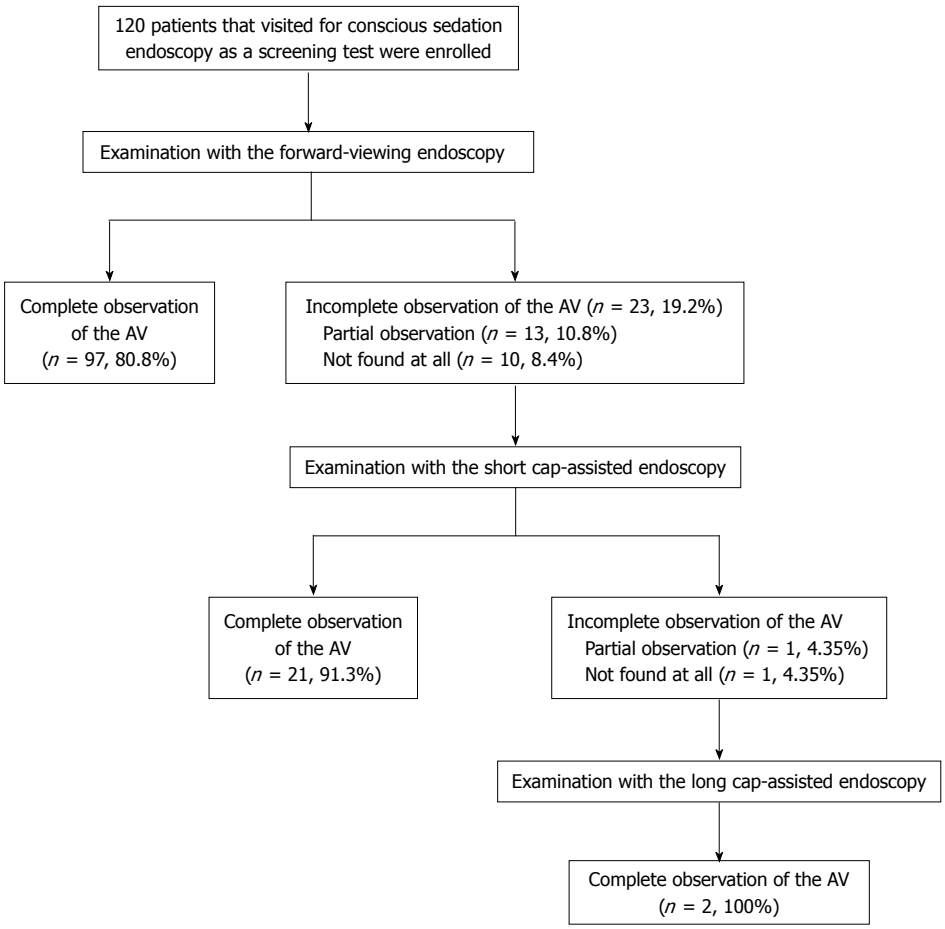

Figure 2 Flow diagram of patient enrollment and examinations. In total, 120 patients were examined by forward-viewing endoscopy. In cases when complete observations of the ampulla of Vater (AV) were unsuccessful, an additional examination for the AV was conducted by short cap-assisted endoscopy. If that examination was unsuccessful, the short cap was replaced with a long cap, and a re-examination was performed.

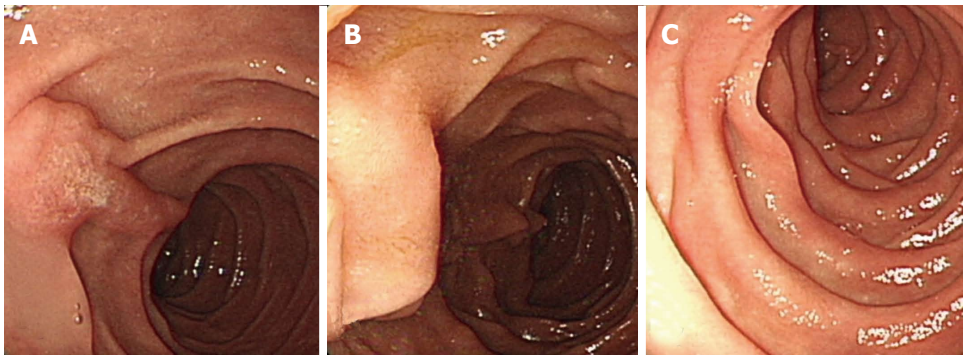

Figure 3 Outcomes of conventional endoscopy. A: Complete observation of the ampulla of Vater (AV), including the orifice, by forward-viewing endoscopy; B: Partial observation of the AV with a folded mucous membrane by forward-viewing endoscopy; C: The AV was not found during forward-viewing endoscopy.

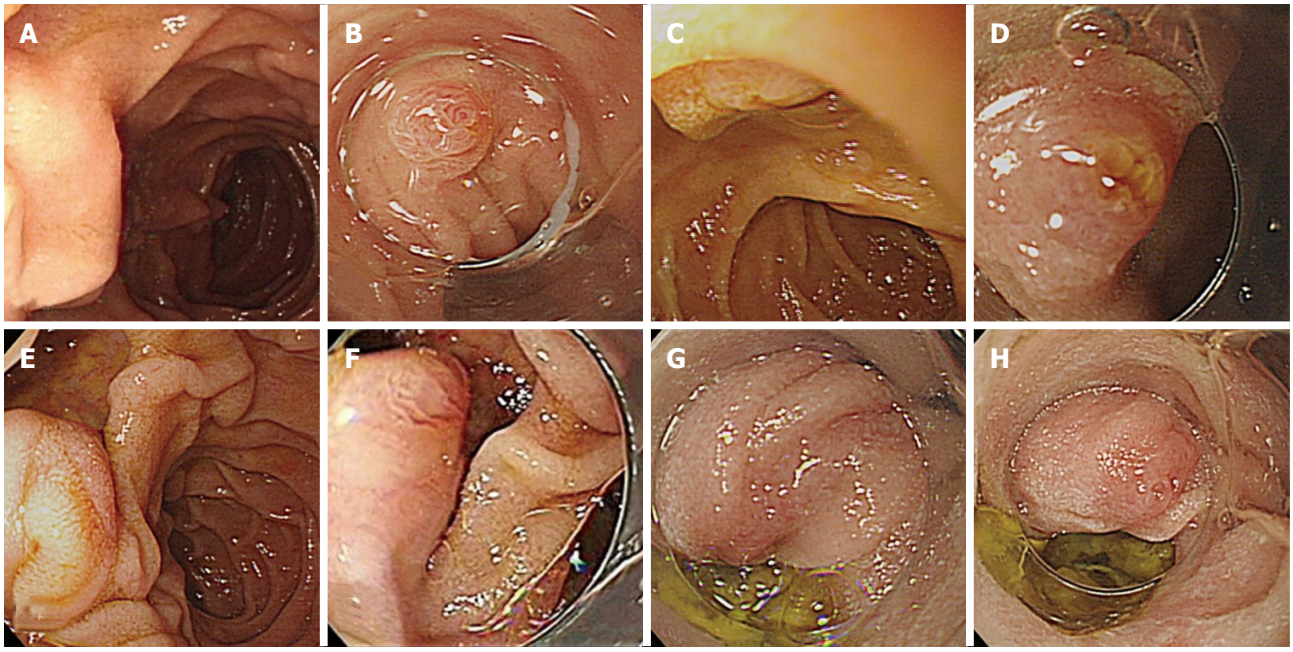

Figure 4 Incomplete observation of the ampulla of Vater by conventional endoscopy and outcomes of cap-assisted endoscopy. A: Incomplete observation of the ampulla of Vater (AV) by forward-viewing endoscopy due to a folded mucous membrane; C: Incomplete observation of the AV due to the close proximity of the endoscope tip to a superior ampulla lesion; E: Incomplete observations of the AV on the edge of diverticulum; B, D, F: Complete observation of the AV, including the orifice, by short cap-assisted endoscopy (A→B, C→D, E→F); G: Incomplete observation of the AV by short cap-assisted endoscopy due to a loop in the scope; H: Complete observation of the AV by long cap-assisted endoscopy (G→H).

for failure to visualize the ampulla were large fold in 16 patients, and diverticulum in 7 patients. Age (mean ± SD) and gender [male (%)] were not significantly different between the complete observation and the incomplete observation groups [55.3 ± 12.6 years, 59 (60.8%); 55.2 ± 16.2 years, 12 (52.2%)], respectively.

Outcomes of CAE

Additional short CAE was performed in patients in whom we could not completely visualize the AV. This group included 13 patients (10.8%) with partial observation of the AV and 10 (8.4%) in which the AV was not found. Short CAE permitted a complete observation of the AV in 21 of the 23 patients (91.3%).

In cases of an incomplete visualization due to folded mucous membranes, complete observation of the orifice areas was achieved by uncovering the fold with a short cap (Figure 4A and B). Due to a too close proximity of the endoscope tip to the superior ampulla lesion, the short cap provided a proper distance and the ability to straighten the mucosal fold by pressing the area surrounding the lesion (Figure 4C and D). The cap made it easier to access the AV at the edge of the diverticulum by directing the force vector along the tip of the endoscope (Figure 4E and F). Patients in whom visualization of the AV failed with short CAE had satisfactory outcomes by replacing the short cap with a long cap; one AV was observed only partially due to the deep location of the AV in the large diverticulum, whereas the other was not found due to a loop in the scope (Figure 4G and H). The time for CAE was 141 ± 88 s. No complications or significant mucosal trauma occurred. In one case of

suspected abnormal lesions, an additional side-viewing endoscopy with biopsy revealed a tubular adenoma, so an endoscopic ampullectomy was performed.

DISCUSSION

The transparent cap was first proposed in 1990 by Inoue *et al*[17] to improve the accessibility of the forward-viewing endoscope. Since then, transparent CAE has been used widely as a method to increase the success rate of the procedure[18,19]. For example, CAE improves cecal intubation times and polyp detection rates during colonoscopy[9,11], it achieves higher success rates of afferent loop intubation and bile-duct cannulation in patients with a Billroth Ⅱ gastrectomy[20-22], and it possible to clip a lesion too tangential to be clipped by routine endoscopy[23-27].

Although there is a growing need to identify CAE as an effective approach to visualize the AV, no studies have investigated the AV observation rate during routine EGD or the efficacy of CAE for AV observation. Although small studies (preliminary data of Leal-Salazar *et al*[28] described the feasibility of visualizing the AV by attaching a long cap from a variceal band ligation set to a conventional endoscope) have been conducted, almost all cases (19 of 20) required emergency endoscopic hemostasis treatment, were not for screening test. That study reported conventional endoscopy permitted an inadequate observation of the AV in all cases (20 of 20), which is questionable, although they reported that observations of the AV using CAE were effective.

Although our cases had a wide variation in procedure duration, complete visualization of the AV was possible in up to 100% by attaching a cap to the tip of a conventional endoscope. The additional time for this procedure was short (average, 141 s). Moreover, the AV could be visualized completely in almost all patients using short CAE. Although a long cap has been used widely, the long cap is hard and difficult to pass over the larynx and pylorus, so it is easy to damage the mucosa, whereas the short cap is soft, safer, and relatively simple and easy to handle.

The transparent cap made the following multiple mechanisms possible: (1) The transparent cap provided a proper distance between the AV and the tip of the endoscope, which may prevent sticking of the endoscope to the duodenal lumen; (2) The cap allowed efficient manipulation of a tangentially placed AV to a more square approach; (3) The cap made it easier to access hidden areas by straightening the mucosal folds by pressing the surrounding lesion areas; and (4) the cap reduced loop formation by "hooking" the tip of the endoscope in the second portion of the duodenum and directing the force vector of the endoscope tip[29-33]. Moreover, the long cap improved all of these functions; thus, it allowed an approach to a deeply AV located deepin the diverticulum[34-36].

This study had some limitations. First, data collection was limited to screening tests in a healthy population that visited the Health Promotion Center; these patients can have lower disease prevalence or anatomical variations in the AV than those in the general population. Thus, the success rate of CAE may be different in the general population. Second, because EGD was performed by one ERCP expert, the results cannot be generalized to a conventional endoscopist with less experience visualizing the AV.

In conclusion, CAE was an effective salvage technique when regular EGD was ineffective for visualizing the AV properly. CAE can increase the diagnostic accuracy of a forward-viewing endoscope and decrease the need for side-viewing endoscopy.

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COMMENTS

Background

Adequate visualization of the ampulla of Vater (AV) is important for early detection of periampullary or pancreaticobiliary diseases. But visualization of the AV with a forward-viewing endoscope is difficult due to the anatomical characteristics of the second portion of the duodenum. Thus, a side-viewing endoscope has been recommended for complete visualization of the AV. However, a side-viewing endoscope is not always available in an ordinary endoscopy suite.

Research frontiers

Cap-assisted endoscopy (CAE) has been used widely to facilitate detection of polyps, improve the success rate of cecal intubation, and to facilitate inspection of lesions situated in blind areas of the colon. The authors have found that the complete visualization rate of the AV in patients who are referred for incomplete visualization of the AV can be improved by attaching a transparent cap.

Innovations and breakthroughs

Although there is a growing need to identify CAE as an effective approach to visualize the AV, no studies have investigated the AV observation rate during routine esophagogastroduodenoscopy or the efficacy of CAE for AV observation. This is the first study to determine the efficacy and safety of an endoscopic examination using a transparent cap to completely visualize the AV in patients failed by conventional endoscopy.

Applications

CAE can increase the diagnostic accuracy of a forward-viewing endoscope and decrease the need for side-view endoscopy. This study may represent a future strategy for effective endoscopic examination as a screening test.

Terminology

Endoscopic caps are commonly used for both diagnosis and therapy during endoscopy. Transparent caps are attached to the distal end of the endoscope. Currently, many different sized caps are available. Cap-assisted endoscopic mucosal resection is the most common application of endoscopic caps. The appropriate selection of an endoscopic cap based on indication and location of the lesion is important for procedural success.

Peer review

The authors examined the efficacy of a CAE to completely visualize the AV in patients failed by conventional endoscopy. It revealed that CAE can increase the diagnostic accuracy of a forward-viewing endoscope and decrease the need for side-view endoscopy. The result are interesting and promising for endoscopic examination as a screening test.

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ORIGINAL ARTICLE

Effects of different resuscitation fluid on severe acute pancreatitis

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Abstract

AIM: To compare effects of different resuscitation fluid on microcirculation, inflammation, intestinal barrier and clinical results in severe acute pancreatitis (SAP).

METHODS: One hundred and twenty patients with SAP were enrolled at the Pancreatic Disease Institute between January 2007 and March 2010. The patients were randomly treated with normal saline (NS group), combination of normal saline and hydroxyethyl starch (HES) (SH group), combination of normal saline, hydroxyethyl starch and glutamine (SHG group) in resuscitation. The ratio of normal saline to HES in the SH and SHG groups was 3:1. The glutamine (20% glutamine dipeptide, 100 mL/d) was supplemented into the resuscitation liquid in the SHG group. Complications and outcomes including respiratory and abdominal infection, sepsis, abdominal hemorrhage, intra-abdominal hypertension, abdominal compartment syndrome (ACS), renal failure, acute respiratory distress syndrome (ARDS), multiple organ dysfunction syndrome (MODS), operation intervention, length of intensive care unit stay, length of hospital stay, and mortality at 60 d were compared. Moreover, blood oxygen saturation (SpO_2), gastric intramucosal pH value (pHi), intra-abdominal pressure (IAP), inflammation cytokines, urine lactulose/mannitol (L/M) ratio, and serum endotoxin were investigated to evaluate the inflammatory reaction and gut barrier.

RESULTS: Compared to the NS group, patients in the SH and SHG groups accessed the endpoint more quickly (3.9 ± 0.23 d and 4.1 ± 0.21 d *vs* 5.8 ± 0.25 d, $P < 0.05$) with less fluid volume (67.26 ± 28.53 mL/kg/d, 61.79 ± 27.61 mL/kg per day *vs* 85.23 ± 21.27 mL/kg per day, $P < 0.05$). Compared to the NS group, incidence of renal dysfunction, ARDS, MODS and ACS in the SH and SHG groups was obviously lower. Furthermore, incidence of respiratory and abdominal infection was significantly decreased in the SH and SHG groups, while no significant difference in sepsis was seen. Moreover, less operation time was needed in the SH and SHG group than the NS group, but the difference was not significant. The mortality did not differ significantly among these groups. Blood SpO_2 and gastric mucosal pHi in the SH and SHG groups increased more quickly than in the NS group, while IAP was significantly decreased in the SH and SHG group. Moreover, the serum tumor necrosis factor-α, interleukin-8 and C-reactive protein levels in the SH and SHG groups were obviously lower than in the NS group at each time point. Furthermore, urine L/M ratio and serum endotoxin were significantly lower in the SH group and further decreased in the SHG group.

CONCLUSION: Results indicated that combination of normal saline, HES and glutamine are more efficient in resuscitation of SAP by relieving inflammation and sustaining the intestinal barrier.

Key words: Microcirculation; Intestinal barrier; Inflammatory reaction; Intra-abdominal hypertension; Capillary leakage syndrome

Zhao G, Zhang JG, Wu HS, Tao J, Qin Q, Deng SC, Liu Y, Liu L, Wang B, Tian K, Li X, Zhu S, Wang CY. Effects of different resuscitation fluid on severe acute pancreatitis. *World J Gastroenterol* 2013; 19(13): 2044-2052 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i13/2044.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i13.2044

INTRODUCTION

Severe acute pancreatitis (SAP) has a mortality rate about 30% and is characterized by pancreatic necrosis, cytokine activation, systemic inflammatory response sydrome (SIRS), and multiple organ dysfunction syndrome (MODS)[1,2]. Accumulative results have demonstrated that microcirculation perfusion and hypoxia have a significant impact on the early stages of disease and play an important role in the pathogenesis of necrosis[3]. Different from normal hypovolemia caused by trauma or bleeding, microcirculatory disorder of SAP is caused by special SIRS. Overexpressed inflammatory media such as tumor necrosis factor (TNF)-α, interleukin (IL)-6 and IL-8 in SIRS will injury the microcirculation endothelium and then increase capillary permeability and fluid sequestration, leading to capillary leakage syndrome and MODS[4].

Moreover, the microcirculatory disorder of the intestinal will lead to intestinal ischemia reperfusion injury and damage the intestinal barrier, which could facilitate translocation of intestinal bacteria and enhance leukocyte activation and inflammatory cytokine release[5]. If the microcirculatory disorder cannot be blocked at the initial stage, it will be exaggerated and form positive feedback. Thus, the excess cytokine will further injury the distal organs and lead to irreversible multiorgan failure[6,7]. Moreover, bacterial translocation from the gut will cause severe infection[8]. Therefore, adequate prompt fluid resuscitation is crucial for prevention of systemic complications[9-11]. Thus, the purpose of effective fluid resuscitation of SAP is not only to supply the deficiency of blood volume, but also especially to stabilize the capillary permeability, modulate the inflammation reaction, and sustain the intestinal barrier function.

Two types of fluids frequently used today for active resuscitation are colloid fluids with large molecules (hetastarch, dextran 40, and albumin) that keep fluid intravascular, and crystalloid fluids with added electrolytes (normal saline, Ringer's, and lactated Ringer's). Both crystalloid and colloid solutions are considered effective for the resuscitation of a hypovolemic patient, because neither fluid provides a survival benefit that is superior to the other[12]. When trying to augment cardiac output and blood pressure, colloids have an advantage over crystalloid solutions, because a larger percentage enters the intravascular space and remains there for a longer period of time. This is because colloids provide the greatest effect on intravascular volume expansion and improve flow secondary to their low viscosity, which is equal to that of water[13].

In the septic shock model with concomitant capillary leakage in the presence of a marked albumin loss, the hydroxyethyl starch (HES) could preserve systemic oxygenation and hemodynamics[14]. Increased capillary permeability leading to fluid loss from the intravascular space and fluid sequestration into the third space are hallmarks of SAP[15]. Clinically, capillary leakage is reflected by intravascular fluid loss leading to hypovolemia [low central venous pressure (CVP)], hemoconcentration (high packed cell volume), and extravascular fluid sequestration in the retroperitoneal, lungs, pleural and abdominal cavities[16]. Although colloids such as HES are supposed more suitable than crystalloids for volume resuscitation of hypovolemia, prospective clinic studies have seldom involved comparing effects of crystalloids and HES in volume resuscitation in SAP[10,11,17].

Some research has shown that HES reduces intestinal permeability by modulating inflammatory response and has a promising effect on survival, together with antibiotics under septic conditions[18]. Although glutamine has been proved to protect the intestinal barrier as a nutrient supplement in nutritional support of SAP, the effects of glutamine delivered as a supplement in resuscitation fluid in the early stage of SAP have not been defined[19-21].

In the present study, we compared the clinical results of different resuscitation fluids including normal saline, combination of normal saline and HES, and combination of normal saline, HES and glutamine in resuscitation of SAP. Moreover, the effects on microcirculation, inflammation reaction and intestinal barrier were investigated.

MATERIALS AND METHODS

Patients

One hundred and twenty patients with SAP were enrolled at the Pancreatic Disease Institute, Union Hospital (Wuhan, China) between January 2007 and March 2010. All of the patients were diagnosed with SAP according to the Atlanta Classification System[22], and were aged 18-60 years. Exclusion criteria were heart disease, severe renal and hepatic dysfunction, coagulation disturbances, and allergy to HES or glutamine. Those patients with manifestation more than 48 h or who received resuscitation from the other hospital were also excluded. Informed consent was obtained from the patients and approval was obtained from the Ethics Committee of Union Hospital, Huazhong University of Science and Technology. The demographic information of the patients is shown in

Table 1 Demographic information of patients with severe acute pancreatitis treated by different resuscitation fluid (mean ± SD)

	NS group (n = 40)	SH group (n = 40)	SHG group (n = 40)
Age (yr)	41.86 ± 13.85	44.50 ± 9.77	45.11 ± 11.57
Sex n (%)			
Male	20 (50)	22 (55)	21 (52.5)
Female	20 (50)	18 (45)	19 (47.5)
Height (cm)	165.86 ± 6.04	165.00 ± 9.03	169.25 ± 6.67
Weight (kg)	66.5 ± 8.63	69.00 ± 9.68	72.38 ± 8.43
APACHE Ⅱ score	11.2 ± 0.7	10.9 ± 0.6	11.3 ± 0.4
MAP (mmHg)	62.3 ± 9.3	64.8 ± 9.2	63.6 ± 8.9
BUN/Cr ratio	23.9 ± 3.6	24.2 ± 3.2	23.7 ± 3.7

No significant differences were observed among the study groups regarding demographic data. APACHE: Acute physiology and chronic health evaluation; MAP: Mean arterial pressure; BUN/Cr: Blood urea nitrogen/creatinine; NS group: Normal saline; SH group: Combination of normal saline and hydroxyethyl starch; SHG group: Combination of normal saline, hydroxyethyl starch and glutamine.

Table 1. If the patient could not successfully achieve a balance of output and input within 7 d, we considered them to have failed resuscitation. However, these cases were not removed from the study and all the results were still included.

Management of resuscitation

The patients were randomly divided into three groups that were resuscitated with normal saline (NS group), combination of normal saline and hydroxyethyl starch (SH group; 130 kD, Sino-Swed Pharmaceutical Corp. Ltd.), or combination of normal saline, hydroxyethyl starch and glutamine (SHG group; glutamine dipeptide, Sino-Swed Pharmaceutical Corp. Ltd.). The ratio of normal saline to HES in the SH and SHG groups was 3:1. Glutamine (20% glutamine dipeptide, 100 mL/d) was supplemented into the resuscitation liquid in the SHG group.

All of the patients with suspected SAP were initially transferred to the pancreatic intensive care unit (PICU). The vital signs, oxygen saturation (SpO_2), gastric intramucosal PH value (pHi), arterial blood-gas analysis, mean arterial pressure (MAP) and intra-abdominal pressure (IAP) were monitored to guide the treatment. All the patients received a central venous catheter capable of measuring central venous oxygen saturation ($ScvO_2$) and CVP.

Meanwhile, the fluid resuscitation was promptly conducted as early as possible. Generally, we infused 1 L normal saline in the NS group or combination of 500 mL normal saline and 500 mL HES in the HS and HSG groups in the first 2 h (500 mL/h) to achieve a CVP of 8-12 mmHg. If the MAP was < 65 mmHg, vasopressors were given to maintain an MAP of at least 65 mmHg. If the MAP was > 90 mmHg, vasodilators were given until it was ⩽ 90 mmHg[23]. If the urine output was < 0.5 mL/kg/h after the CVP and MAP were stabilized, 20 mg dihydrochlorothiazide bolus was infused and followed with 1-2 mL/h continuous infusion *via* syringe pump to maintain the urine output at > 0.5 mL/kg per hour. After that, resuscitation fluid was continually infused at a speed of 150-300 mL/h (approximately 2-3 mL/kg per hour, ratio of normal saline to HES is 3:1) and modulated depending on the reaction of early resuscitation and parameters in the later course, which maintained the urine at 0.5-1 mL/kg per hour and prevented excess resuscitation.

Moreover, if the oliguria continued for > 2 d and the ratio of blood urea nitrogen/creatinine (BUN/Cr) significantly increased, continuous venovenous hemofiltration (CVVH) with ultrafiltration modulation was performed to extra the liquid sequestration in the "3rd space" and overexcited inflammatory mediators. Most of the patients were accompanied with respiratory dysfunction, and for those patients with $ScvO_2$ < 70% after stabilization of CVP and MAP, we did not perform blood transfusion but intensified oxygen supplementation through pressure oxygen mask or tracheal intubation with artificial respirator. Except for the CVP, MAP, urine and $ScvO_2$ adopted as early resuscitation parameters, IAP was also used to assess the microcirculation dysfunction. Even without significant abnormality in the other parameters, the infusion was carefully controlled and the CVVH applied to balance the output and input in those patients with significantly increased IAP. The input of resuscitating liquid depended on the output including ultrafiltration volume of CVVH, urine and non-dominant water loss. The ultimate endpoint of resuscitation was defined as the balance of input and output. Those patients who approached the endpoint in 7 d were considered to have successful resuscitation, otherwise it had failed.

Complications and clinical outcomes

Complications and outcomes were recorded throughout the whole course, including respiratory infection, abdominal infection, sepsis, abdominal hemorrhage, intra-abdominal hypertension (IAH), abdominal compartment syndrome (ACS), renal failure, acute respiratory distress syndrome (ARDS), MODS, operation intervention, length of intensive care unit stay, length of hospital stay and mortality at 60 d.

All the clinical parameters were record from day 1 to day 7 after administration. Resuscitation fluid input, abdominal drainage fluid and urine output were recorded every day. Oxygen supply and microcirculation perfusion was evaluated by pulse SpO_2 and pHi, which were detected by bedside monitor (Life Scope A, Nihon Kohden, Irvine, CA, Untied States) and gastric mucosal pH monitor (Tonocap, Datex-Ohmeda, Finland) respectively[24]. Harvested heparinized blood was centrifuged and the plasma was removed and stored at -80 ℃ for later examination.

Measurements of IAP

IAP was measured according to the method of Oda *et al*[25]. In brief, an 18-gauge catheter was inserted into the culture aspiration port of the urine catheter, and a line filled with saline was connected to the pressure transducer. After urine had been completely drained from the bladder, the urine catheter line was clamped, and then 100 mL of saline was instilled in the bladder and the pres-

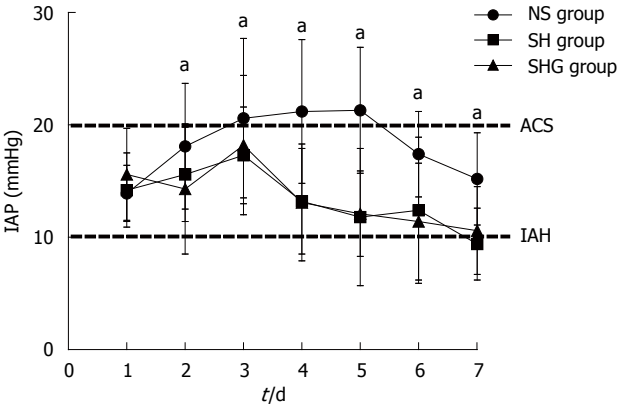

Figure 1 Effect of different resuscitation fluids on changes in intra-abdominal pressure in severe acute pancreatitis. Intra-abdominal pressure (IAP) was indirectly measured *via* a bladder catheter during 7 d and performed twice daily. All of the patients had intra-abdominal hypertension on d 1 (IAP > 10 mmHg). $^{a}P < 0.05$ *vs* normal saline (NS group). SH group: Combination of normal saline and hydroxyethyl starch; SHG group: Combination of normal saline, hydroxyethyl starch and glutamine; ACS: Abdominal compartment syndrome; IAH: Intra-abdominal hypertension.

sure was measured at the end of expiration. IAP was measured first on admission to the PICU, and twice daily thereafter in the morning and evening, in principle. According to the consensus guidelines, IAH was defined as IAP ⩾ 12 mmHg and ACS as IAP > 20 mmHg with evidence of organ dysfunction.

Plasma cytokines assay

Serum TNF-α, IL-8 and C-reactive protein (CRP) from day 1 to day 7 were evaluated by enzyme-linked immuno sorbent assay (R-D Systems, Minneapolis, MN, United States) according to the manufacturer's instructions.

Urine lactulose/mannitol ratio assay

Urine lactulose/mannitol (L/M) ratio during 7 d was analyzed to evaluate intestinal permeability as described before[21]. All the patients fasted for at least 6 h and their bladders were emptied before the test. The test solution consisted of 10 g lactulose and 5 g mannitol in a total volume of 50 mL through nasojejunal tube. The urine volume was collected for the subsequent 6 h. The urine volume was recorded, and 10 mL was frozen and stored at -80 ℃. L/M ratio in urine was analyzed by Hi-Crush Partners LP.

Endotoxin assay

Serum concentration of endotoxin at 7 d was detected by quantitative chromogenic limulus amebocyte lysate assay (QCL-1000; Whittaker MA Bioproducts, Walkersville, MD, United States) according to the manufacturer's instructions. Blood was drawn aseptically into lipopolysaccharide-free tubes. All samples were processed in a laminar flow hood. To minimize nonspecific plasma inhibitors, samples were diluted with pyrogen-free water and heat inactivated at 100 ℃ for 10 min. *Escherichia coli* 055:B5 reference endotoxin (1 endotoxin unit = 0.6 ng/mL) was used for the standard curve (Whittaker MA Bioproducts).

Statistical analysis

Statistical analyses were performed with SPSS version 12.0.2. Data are presented as mean ± SD. χ^2 analysis and one-way repeated-measures analysis of variance were used for the analysis of differences. $P < 0.05$ was considered significant.

RESULTS

Demographic information

As shown in Table 1, no significant differences were observed between the study groups regarding demographic data including age, agenda, height, weight, Acute physiology and chronic health evaluation Ⅱ score, MAP and BUN/Cr ratio at the initial time.

Fluid resuscitation and fluid balance

All patients in the three groups received resuscitation at an early stage after manifestation of SAP (12.8 ± 6.7 h, 13.1 ± 5.4 h, and 12.2 ± 6.3 h). The balance of input and output was considered as the ultimate endpoint of resuscitation. Compared to the NS group, it took a significantly shorter time to approach the resuscitation endpoint in the SH and SHG groups (5.8 ± 0.25 d *vs* 3.9 ± 0.23 d and 4.1 ± 0.21 d, $P < 0.05$). Nevertheless, the volumes of fluid administered in the SH and SHG groups was obviously lower than that in the NS group (67.26 ± 28.53 and 61.79 ± 27.61 *vs* 85.23 ± 21.27 mL/kg per day, $P < 0.05$), while the abdominal drainage fluid in the SH and SHG groups was significantly lower than that in the NS group (Table 2).

Complications and outcomes

When infection was suspected, ultrasound-guided percutaneous aspiration of pancreatic tissue, sputum and blood was performed with Gram's stain and culture. If ultrasound-guided percutaneous aspiration confirmed the infection, antibiotics were administered following the results of culture and antibiotic sensitivity. If the episode is not effectively relieved, the patient should undergo debridement. Compared to the NS group, the incidence of renal dysfunction, ARDS, MODS and ACS in the SH and SHG groups was obviously lower. Furthermore, the incidence of respiratory and abdominal infection was significantly decreased in the SH and SHG groups, while no significant difference in sepsis was seen. Moreover, less operation time was needed in the SH and SHG groups than in the NS group, but the difference was not significant (Table 3). IAP in the NS group was significantly higher than in the SH and SHG groups at each time point (Figure 1). Patients in the SH and SHG groups had a notably shorter length of stay in the intensive care unit (ICU) and hospital than the NS group had. Although mortality in the NS group was higher than in the SH and SHG groups, no significant difference was observed. No significant difference in any of the parameters was noted between the SH and SHG groups.

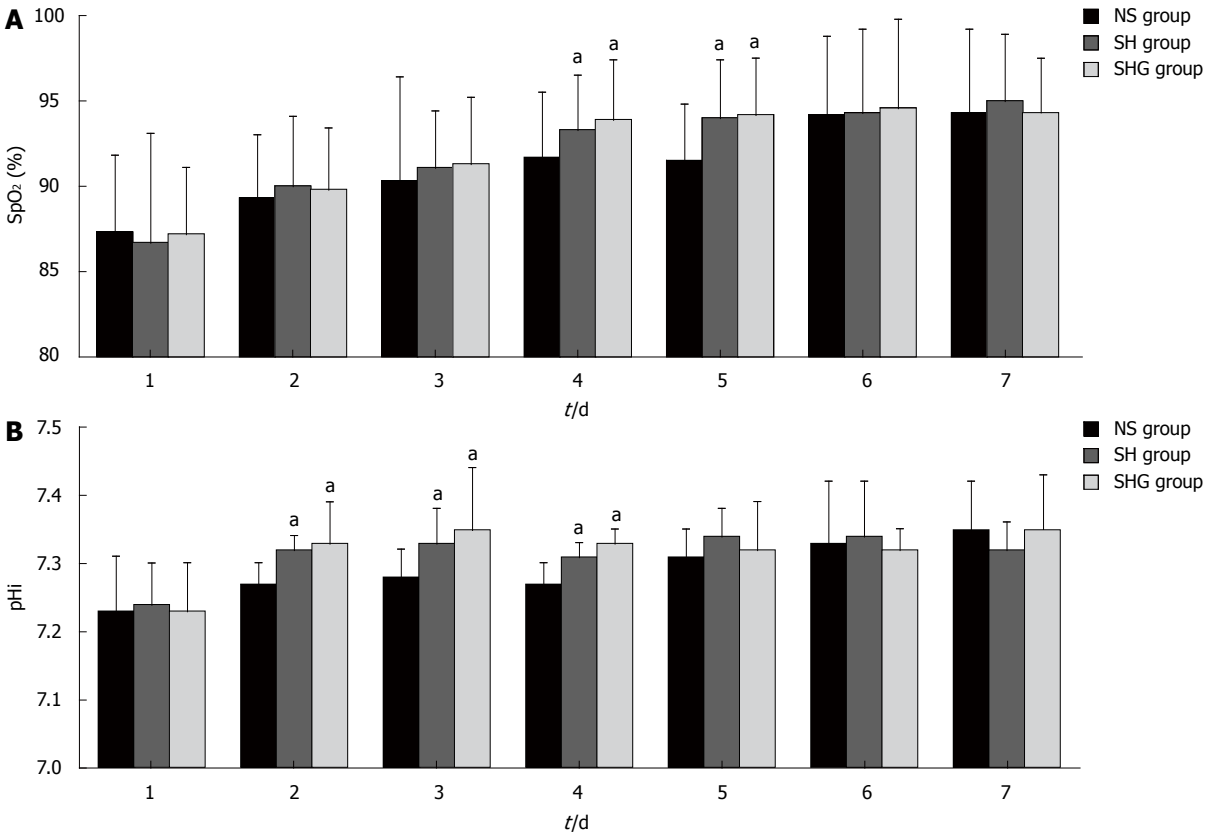

Figure 2 Effect of different resuscitation fluids on circulation oxygen supply and microcirculation perfusion. A: Effect of different fluids on circulation oxygen supply was evaluated with pulse oxygen saturation (SpO_2) by automatic monitoring; B: Microcirculation perfusion was assessed with gastric intramucosal pH value (pHi) by Tonocap monitor. [a]*P* < 0.05 *vs* normal saline (NS group). SH group: Combination of normal saline and hydroxyethyl starch; SHG group: Combination of normal saline, hydroxyethyl starch and glutamine.

Table 2 Window of manifestation to resuscitation, time of resuscitation endpoint, volume of resuscitation fluid, urine output, and abdominal drainage fluid in different groups

	NS group	SH group	SHG group
Window to resuscitation (h)	12.8 ± 6.7	13.1 ± 5.4	12.2 ± 6.3
Time to endpoint (d)	5.8 ± 0.25	3.9 ± 0.23[1]	4.1 ± 0.21[1]
Resuscitation fluid (mL/kg per day)	61.79 ± 7.61	46.93 ± 12.38[1]	44.75 ± 8.53[1]
Urine output/d (mL/kg per day)	31.3 ± 5.47	28.71 ± 11.62	27.94 ± 10.62
Abdominal drainage fluid (mL/kg per day)	11.32 ± 2.13	6.28 ± 3.26[1]	6.35 ± 1.42[1]

[1]*P* < 0.05 *vs* normal saline (NS group). Statistic measure of variation is standard error. SH group: Combination of normal saline and hydroxyethyl starch; SHG group: Combination of normal saline, hydroxyethyl starch and glutamine.

Table 3 Complications and outcomes of patients treated by different resuscitation fluid

	NS group	SH group	SHG group
Renal dysfunction	11	31	41
ARDS	16	61	71
MODS	10	31	31
ACS	6	11	11
Lung infection	13	51	51
Abdominal infection	11	31	21
Sepsis	3	2	3
Operation	6	2	2
LOIS	14 ± 8.2	10 ± 9.4[1]	11 ± 6.3[1]
LOHS	31 ± 22.8	22 ± 18.9[1]	21 ± 23.71
Mortality	5	2	3

[1]*P* < 0.05 *vs* normal saline (NS group). SH group: Combination of normal saline and hydroxyethyl starch; SHG group: Combination of normal saline, hydroxyethyl starch and glutamine; ARDS: Acute respiratory distress syndrome; MODS: Multiple organ dysfunction syndrome; ACS: Abdominal compartment syndrome; LOIS: Length of intensive care unit stay; LOHS: Length of hospital stay.

SpO_2 and microcirculation perfusion

The SpO_2 of the SH and SHG groups increased more quickly than in the NS group and was significantly higher at day 4 and day 5. Similarly, pHi in the SH and SHG groups also increased faster than in the NS group from day 2 to day 4 (Figure 2).

Serum cytokine and CRP concentration

Serum TNF-α, IL-8 and CRP were detected daily from day 1 to day 7. Although the TNF-α concentration in all three groups increased to a peak at d 3 and then gradually decreased, it was significantly higher in the NS group at days 3-5 (Figure 3A). Unlike the highest IL-8 concentration in the NS group at day 4, the SH and SHG groups had the highest IL-8 concentration at day 3 and then it

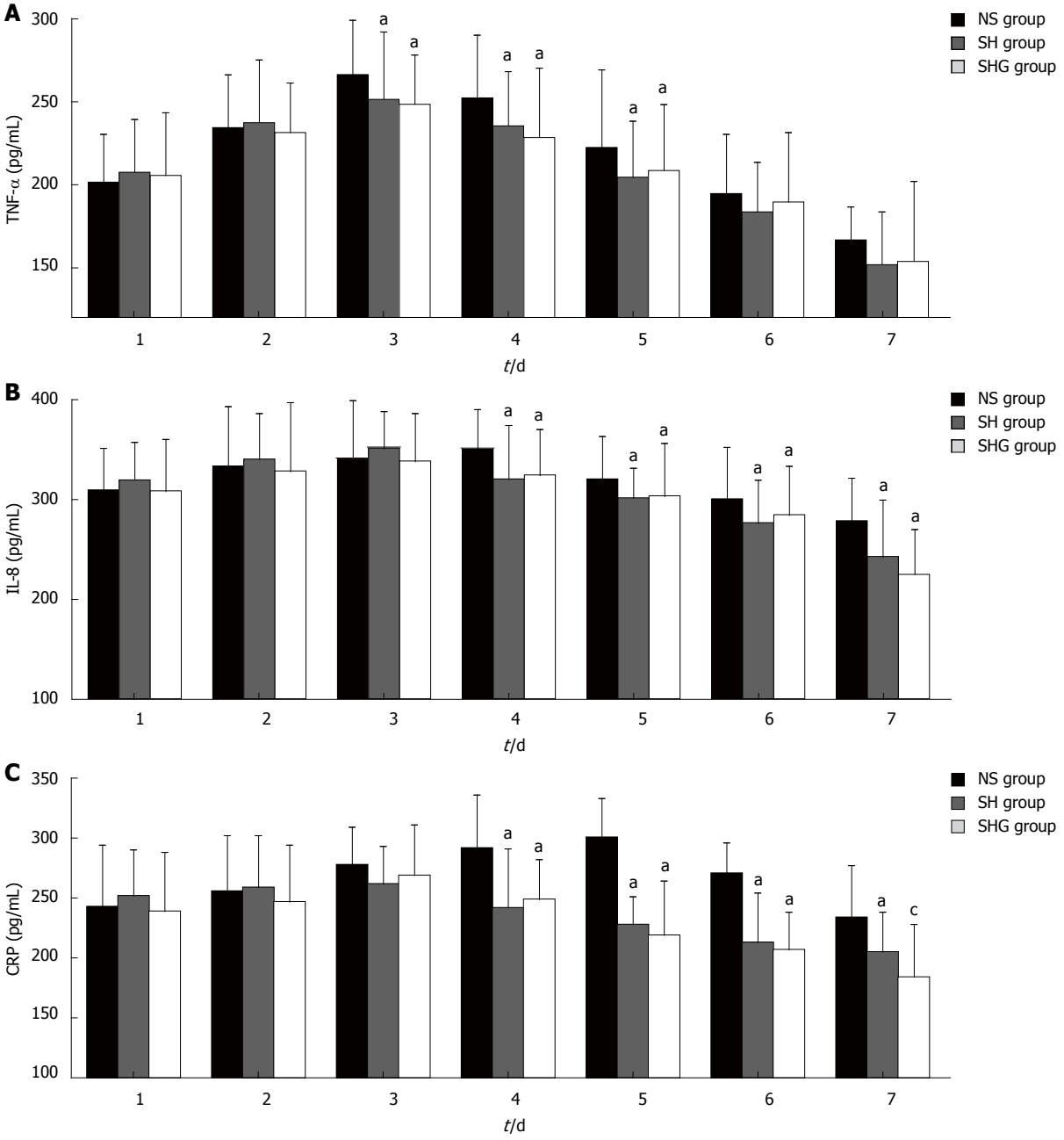

Figure 3 Effects of different resuscitation fluids on serum cytokine and C-reactive protein. Serum tumor necrosis factor (TNF)-α (A), interleukin (IL)-8 (B) and C-reactive protein (CRP) (C) concentration was evaluated by enzyme-linked immuno sorbent assay. $^{a}P < 0.05$ *vs* normal saline (NS) group; $^{c}P < 0.05$ *vs* combination of normal saline and hydroxyethyl starch (SH group). SHG group: Combination of normal saline, hydroxyethyl starch and glutamine.

significantly decreased. Furthermore, the IL-8 concentration in the SHG group was obviously lower than in the SH group at days 4-7 (Figure 3B). The CRP concentration in the NS group continually increased until day 5, while it was significantly decreased in the SH and SHG groups after day 3. Moreover, the CRP concentration in the SHG group was significantly lower than that in the SH group after day 7 (Figure 3C).

Intestinal permeability

Intestinal permeability was assessed by urine L/M ratio and serum endotoxin. Urine L/M ratio in all three groups increased after administration of SAP. Compared to the NS group, urine L/M ratio in the SH and SHG groups was significantly lower after day 4. Moreover, compared to the SH group, it further decreased in the SHG group after day 5. Parallel to urine L/M ratio, serum endotoxin in the NS group was also significantly higher than that in the SH and SHG groups. Nevertheless, serum endotoxin in the SHG group was markedly lower than that in the SH group after day 6 (Figure 4).

DISCUSSION

Many studies have indicated that colloids such as HES are more suitable than crystalline solutions for volume

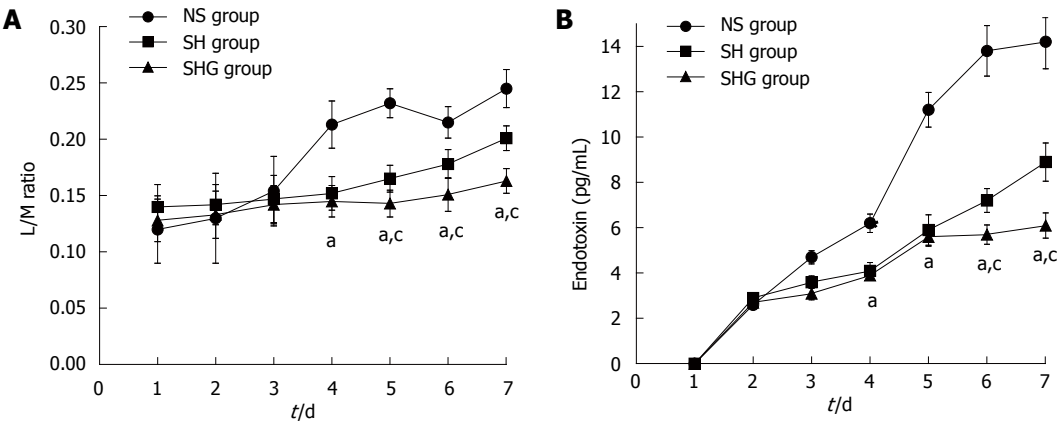

Figure 4 Effect of different fluids on intestinal mucosa barrier function. A: Lactulose/mannitol (L/M) ratio of urine in normal saline (NS group), combination of normal saline and hydroxyethyl starch (SH group) and combination of normal saline, hydroxyethyl starch and glutamine (SHG group) was measured by Hi-Crush Partners LP; B: Serum endotoxin in different groups was detected by quantitative chromogenic limulus amebocyte lysate assay reagent. $^{a}P < 0.05$ *vs* NS group; $^{c}P < 0.05$ *vs* SH group.

resuscitation in hypovolemia of sepsis, of which microcirculation arrangement are same as SAP[26-28]. Our present study demonstrated that less time and volume were needed to achieve the resuscitation endpoint in the SH and SHG groups. Moreover, higher urine output and lower abdominal drainage were seen in the SH and SHG groups. Furthermore, the incidence of MODS and ACS were significantly decreased in the SH and SHG groups.

This indicated that combination of HES and crystalloids might effectively attenuate the capillary leakage by maintaining colloid osmotic pressure and then decreasing extravascular fluid sequestration, such as pleural effusion and abdominal ascites. As an important clinical clue for extensive sequestration, the IAP and IAH in the SH and SHG groups were decreased significantly more than those in the NS group.

Although the mortality was not significantly decreased, the incidence of respiratory infection and abdominal infection was significantly reduced in the SH and SHG groups. This indicated that the deceased liquid sequestration in the abdomen and lungs significantly reduced the infection risk. Furthermore, less operation time was needed and a lower level of organ dysfunction occurred in the SH and SHG groups. Thus, shorter length of ICU and hospital stay was seen in the SH and SHG groups. These results further suggest that combination of HES and normal saline might be effective in resuscitation and effectively decrease infective complications and organ failure in SAP. Although the NS group had a higher incidence of respiratory and abdominal infection, incidence of sepsis and mortality in the NS did not differ significantly from that in the SH and SHG groups. This might because the treatment of SAP is multimodal including resuscitation, organ support, nutrition support, operative intervention, and antibiotics and these different single resuscitation managements were not sufficient to affect mortality.

The target of effective resuscitation is not only maintaining the blood volume, but more importantly, to improve tissue oxygenation and microcirculation perfusion. In our research, arterial SpO_2 and pHi were detected to evaluate the oxygenation and microcirculation perfusion. We showed that SpO_2 and pHi in the SH and SHG groups recovered more quickly than in the NS group. We showed that combination of HES and normal saline was effective in expanding the blood volume and improving the microcirculation and tissue oxygenation.

Our research demonstrated that inflammatory factors including TNF-α, IL-8 and CRP, in the SH and SHG groups were significantly lower and decreased earlier than those in the NS group, which indicates that HES might modulate the inflammatory reaction. Other research has also discovered that HES attenuates capillary leakage through modulation of the inflammation reaction in sepsis and abdominal surgery. It is speculated that HES may inhibit nuclear factor-κB activation and neutrophil adhesion and migration[29-31], but the exact mechanism has not been defined. Other research implies that HES prevents the inflammatory reaction through relieving ischemia-reperfusion injury in the intestine[18,32]. The present study demonstrated that intestinal permeability significantly decreased in the SH and SHG groups, which also implied that the inflammation reaction was modulated by improvement of intestinal ischemia-reperfusion injury.

Flint *et al*[33] have shown that the severity of SAP can be exacerbated by intestinal ischemia-reperfusion injury. When the intestinal barrier is disrupted, the luminal content invades the portal venous and lymphatic systems. This translocation activates immune cells downstream from the intestinal mucosa to release inflammatory mediators that drive the onset of SIRS and MODS in SAP[34-36]. Our present study demonstrated that urine L/M ratio and endotoxin were significantly decreased after treatment with HES and normal saline. It indicated that sustaining the intestinal barrier by HES is one of the important reasons for decreased mortality from infection and MODS.

Many studies have shown that nutrition support supplemented with specific immunonutrients such as glutamine may protect the intestinal barrier and modulate the acute phase responses, thereby potentially improving

outcome in SAP[19-21]. We speculated that supplementation of glutamine in resuscitation could further sustain intestinal function and improve the clinical outcomes of SAP. Our results showed that the IL-8, CRP, urine L/M ratio and serum endotoxin were further decreased after supplementation with glutamine, while the clinical outcomes and complications had no significant change. This implies that ischemia-reperfusion injury is a major reason for intestinal dysfunction in the early stage of SAP.

In summary, the present study showed that combination of HES and normal saline was more efficient in fluid resuscitation of SAP by modulating inflammation and the intestinal barrier, which resulted in a lower level of infection and organ failure. Nevertheless, although supplementation with glutamine could further modulate inflammatory reaction and intestinal, while no significant changes were seen in clinical results.

COMMENTS

Background

Accumulative results demonstrated that microcirculation perfusion and hypoxia leading to capillary leakage syndrome (CLS) and multiple organ dysfunction syndrome (MODS) in severe acute pancreatitis (SAP). Although the colloid such as hydroxyethyl starch (HES) are supposed more suitable than crystalloid in volume resuscitation in hypovolemia, seldom prospective clinic researches have involved in SAP. Given to protect intestinal barrier as nutrient supplement in SAP, the effects of glutamine delivered as supplement of resuscitation fluid in early stage of SAP were not defined. Thus, the present in present set to compare clinical results of different resuscitation fluid including normal saline, combination of normal saline and HES as well as combination of normal saline, HES and glutamine in resuscitation of SAP, respectively.

Research frontiers

Overexpressed inflammatory cytokine of SAP such as tumor necrosis factor-α (TNF-α), interleukin (IL)-6, IL-8 will injury the capillary permeability and lead to CLS, which further damage the intestinal barrier and induce translocation of intestinal bacterial. Thus, the effective fluid resuscitation of SAP should be not only to sustain body volume but also improve capillary permeability and intestinal barrier function. Although the colloid such as HES are supposed more suitable than crystalloid in volume resuscitation in hypovolemia, seldom prospective clinic researches have involved in comparing effects of crystalloid and HES in volume resuscitation in SAP.

Innovations and breakthroughs

Many researches indicated that colloid such as HES are supposed more suitable than crystalline in volume resuscitation in hypovolemia of sepsis, but it is unknown whether the HES is also more efficient in SAP which microcirculation arrangement is similar to septic. The present results firstly demonstrated that less time and volume were needed to get resuscitation endpoint in combination of normal saline and hydroxyethyl starch (HS) group and HES and glutamine (SHG group). Moreover, higher urine output and lower abdominal drainage were showed in SH and SHG groups. Furthermore, the incidence of MODS and abdominal compartment syndrome (ACS), were significantly decreased in SH and SHG groups. The results displayed that oxygen saturation and intramucosal pH value of combination of normal saline and hydroxyethyl starch (SH group) and SHG group recovered more quickly than normal saline (NS) group which implied that combination of HES and normal saline is not only effective expanding blood volume but also improving the microcirculation and tissue oxygenation. This research demonstrated that the inflammatory factor including TNF-α, IL-8 and C-reactive protein of SH and SHG group were significantly lower and decreased earlier than those of NS group. Moreover, our present study demonstrated that urine lactulose/mannitol ration and endotoxin were significantly decreased after treated with HES and normal saline.

Applications

The present study showed that combination of HES and normal saline was more efficient in fluid resuscitation of SAP by modulate inflammation and intestinal barrier, which resulted in lower modality of infection and organ failure. Moreover, the supplementary with glutamine could further modulate inflammatory reaction and intestinal. It implied that combination of normal saline, HES and glutamine might be an efficient resuscitation fluid in SAP which deserves to be investigated in further clinical study.

Terminology

CLS is a rare medical condition characterized by self-reversing episodes during which the endothelial cells which line the capillaries are thought to separate for a few days, allowing for a leakage of fluid from the circulatory system to the interstitial space, resulting in a dangerous hypotension (low blood pressure), hemoconcentration, and hypoalbuminemia. It is a life-threatening illness because each episode has the potential to cause damage to, or the failure of, vital organs due to limited perfusion. ACS occurs when the abdomen becomes subject to increased pressure. Specific cause of ACS is not known, although some causes can be sepsis and severe abdominal trauma. Increasing pressure reduces blood flow to abdominal organs and impairs pulmonary, cardiovascular, renal, and gastro-intestinal function, causing MODS and death.

Peer review

This is an interesting paper with potentially clinical use.

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BRIEF ARTICLE

Noninvasive methods for prediction of esophageal varices in pediatric patients with portal hypertension

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Abstract

AIM: To evaluate clinical and laboratory parameters for prediction of bleeding from esophageal varices (EV) in children with portal hypertension.

METHODS: Retrospective study of 103 children (mean age: 10.1 ± 7.7 years), 95.1% with intrahepatic portal hypertension. All patients had no history of bleeding and underwent esophagogastroduodenoscopy for EV screening. We recorded variceal size (F1, F2 and F3), red-color signs and portal gastropathy, according to the Japanese Research Society for Portal Hypertension classification. Patients were classified into two groups: with and without EV. Seven noninvasive markers were evaluated as potential predictors of EV: (1) platelet count; (2) spleen size z score, expressed as a standard deviation score relative to normal values for age; (3) platelet count to spleen size z score ratio; (4) platelets count to spleen size (cm) ratio; (5) the clinical prediction rule (CPR); (6) the aspartate aminotransferase to platelet ratio index (APRI); and (7) the risk score.

RESULTS: Seventy-one children had EV on first endoscopy. On univariate analysis, spleen size, platelets, CPR, risk score, APRI, and platelet count to spleen size z score ratio showed significant associations. The best noninvasive predictors of EV were platelet count [area under the receiver operating characteristic curve (AUROC) 0.82; 95%CI: 0.73-0.91], platelet: spleen size z score (AUROC 0.78; 95%CI: 0.67-0.88), CPR (AUROC 0.77; 95%CI: 0.64-0.89), and risk score (AUROC 0.77; 95%CI: 0.66-0.88). A logistic regression model was applied with EV as the dependent variable and corrected by albumin, bilirubin and spleen size z score. Children with a CPR < 114 were 20.7-fold more likely to have EV compared to children with CPR > 114. A risk score > -1.2 increased the likelihood of EV (odds ratio 7.47; 95%CI: 2.06-26.99).

CONCLUSION: Children with portal hypertension with a CPR below 114 and a risk score greater than -1.2 are more likely to have present EV. Therefore, these two tests can be helpful in selecting children for endoscopy.

Key words: Portal hypertension; Clinical predictors; Pediatric patients; Esophageal varices

Core tip: Children with portal hypertension (PH) are at risk for variceal bleeding. The standard test for screening varices is endoscopy, an invasive method. We evaluated non-invasive markers for diagnosing esophageal varices (EV) in 103 children (95% intrahepatic PH). All patients had no bleeding history and underwent endoscopy for EV screening. Platelet count (< 115 000), clinical prediction rule (< 114) and risk score (cutoff > -1.2) were the best predictors of EV. Limitations are the retrospective

design and the small number of pre-hepatic PH patients. The strength is the paucity of pediatric studies related to this issue and the assessment of risk score in children.

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INTRODUCTION

Portal hypertension is the underlying pathophysiological process that leads to the formation of portosystemic collaterals and heralds the onset of a severe complication: variceal hemorrhage. It is estimated that approximately 50% of pediatric patients with chronic liver disease and 90% of those with extrahepatic portal vein obstruction (EHPVO) will experience gastrointestinal bleeding[1,2]. Esophagogastroduodenoscopy (EGD) is considered the primary modality for detection and surveillance of esophageal varices (EV) and to determine the risk of bleeding.

Guidelines for adult cirrhotic patients recommend universal EV screening by EGD at the time of the diagnosis of cirrhosis[3-7].

Many studies have sought to determine clinical, laboratory, or other noninvasive methods that could predict the presence of EV. Preliminary data suggests that laboratory tests such as platelet count, albumin and ultrasonographic parameters such as presence of splenomegaly, spleen size z score and platelet count to spleen size ratio and the clinical prediction rule (CPR; calculated from platelet count, spleen size z-score, and albumin concentration) developed by Gana may be useful as first-line tools for identification of adults and pediatric patients at risk of variceal development and thus reduce the number of unnecessary EGDs[8-22].

The aim of this study was to analyze the following non-invasive methods for predicting EV in pediatric patients with portal hypertension submitted to EGD: platelet count, spleen size z score, platelet count to spleen size (cm) ratio, platelet count to spleen size z score ratio, CPR, risk score and the aspartate aminotransferase to platelet ratio index (APRI).

MATERIALS AND METHODS

We conducted a retrospective evaluation of patients aged < 18 years with a diagnosis of chronic liver disease or EHPVO who underwent EGD between the 2000 and 2011 at Hospital de Clinicas de Porto Alegre, a tertiary referral center in Southern Brazil. Portal hypertension was defined after the diagnosis of some conditions which natural progression occurs along with portal hypertension such as chronic liver disease and extra hepatic portal vein thrombosis. The following exclusion criteria were applied: active or previous variceal bleeding, prior variceal treatment (any type) or variceal bleeding prophylaxis (including nonselective β-blocker use, endoscopic variceal ligation or sclerotherapy, surgical portosystemic shunt or transjugular intrahepatic portosystemic shunt insertion), liver transplantation, and malignancy.

The presence of EV on endoscopy was the primary endpoint. Clinical and demographic data, diagnoses, medication use, physical examination findings, and severity of liver disease, as assessed by pediatric end-stage liver disease and model for end-stage liver disease (for children > 12 years old) and the Child-Pugh classification, were reviewed. The results of laboratory tests and ultrasound scans were considered for analysis if performed within 3 mo of EGD.

Endoscopy was carried out as part of routine clinical care. Four different endoscopists, recorded variceal size (F1, F2 and F3), red-color signs, and portal gastropathy, according to Japanese Research Society for Portal Hypertension classification[23], and gastric varices according to the Sarin classification[24].

Three thousand EGDs were reviewed and 127 patients with chronic liver disease or EHPVO were identified. Twenty-four patients were excluded: eleven due to previous variceal bleeding, four due to non-selective β-blocker therapy, four due to liver transplantation, two with laboratory test performed over than 3 mo of EGD, one due to surgical shunting, one due to no etiologic confirmation and one due to band ligation.

Seven non-invasive markers, previously described in adults and pediatric patients with portal hypertension, were evaluated as potential predictors of EV: (1) platelet count; (2) spleen size z score, expressed as a standard deviation score relative to normal values for age[25]; (3) platelet count to spleen size z score ratio; (4) platelet count to spleen size (cm) ratio; (5) the CPR, proposed by Gana *et al*[22] which is calculated according to the following formula: [(0.75 × platelets)/(spleen z score + 5)] + (2.5 × albumin); (6) the APRI test; and (7) a risk score, calculated as follows: [14.2 - 7.1 × $\log_{10}$ platelets (10^9/L)] + [4.2 × $\log_{10}$ bilirubin (mg/dL)][21].

For statistical analyses, patients were classified into two groups: patients with EV and patients without EV.

Statistical analysis

Data are expressed as mean and standard deviation, median and interquartile range, and proportions and 95%CI as appropriate. A P value of < 0.05 was considered statistically significant in all analyses. Continuous variables (such as laboratory data, spleen size z score, CPR, risk score) were compared using the Student t-test or the Mann-Whitney U test. Categorical variables (such as ascites, encephalopathy, and splenomegaly) were compared by the chi-square test or Fisher's exact test.

To determinate test performance for prediction of EV, a receiver operator characteristic (ROC) curve was constructed and the area under the ROC curve (AUROC) was calculated. The cutoff value of the variables was determined at the point of highest sensitivity and specific-

Table 1 Univariate analysis for esophageal varices

Variables	Varices (*n* = 71)	No varices (*n* = 32)	*P* value
Age (yr)	9.1 ± 4.9	8.5 ± 4.4	0.530
AST (U/L)	87 (51-158)	68 (36-178)	0.417
ALT (U/L)	78 (40-141)	54 (22-160)	0.197
INR	1.2 (1.1-1.4)	1.1 (1.1-1.3)	0.066
Bilirubin (mg/dL)	1.4 (0.8-2.4)	0.6 (0.4-2.2)	0.016
Albumin (g/dL)	3.8 ± 0.6	4.1 ± 0.7	0.077
Creatinine (mg/dL)	0.5 ± 0.2	0.5 ± 0.2	0.686
Splenomegaly	64 (95.5%)	22 (73.3%)	0.001
Spleen size (cm)	14.6 ± 3.3 (*n* = 65)	12.2 ± 2.7 (*n* = 24)	0.001
Spleen size z score	6.3 ± 3.2 (*n* = 65)	3.7 ± 2.6 (*n* = 24)	0.000
Platelets (10^3/μL)	102 ± 50.8	195 ± 85.2	0.000
Encephalopathy(1/2/3)[1]	31/0/0	68/3/0	0.245
Ascites (1/2/3)[2]	31/0/1	59/9/3	0.100
Model of end-stage liver disease	6.6 ± 4.6 (*n* = 23)	3.6 ± 7.1 (*n* = 8)	0.290
Pediatric end-stage liver disease	-1.3 ± 8.6 (*n* = 48)	-2.2 ± 10.2 (*n* = 24)	0.708
Child-Pugh A/B/C	35/31/5	22/8/2	0.169
Child score	7.0 ± 1.4	6.6 ± 1.3	0.074
Clinical prediction rule	103.6 ± 17.5 (*n* = 65)	121.1 ± 21.1 (*n* = 24)	0.001
Platelets/spleen size z score	16.7 (7.9-31.1)	47.1 (27.2-123.3)	0.000
APRI	2.3 (1.0-3.7)	1.0 (0.3-2.3)	0.001
Platelets/spleen size	0.7 (0.4-1.1) (*n* = 65)	1.3 (0.8-2.2) (*n* = 24)	0.000
Risk score	1.2 ± 2.6	-1.6 ± 2.9	0.000

[1]Determined clinically or by electroencephalogram: 1 = none; 2 = grade 1 or 2; 3 = grade 3 or 4; [2]Determined clinically or by ultrasound: 1 = no ascites; 2 = controlled or mild; 3 = moderate or tense. AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; INR: International normalized ratio; APRI: Aspartate aminotransferase to platelet ratio index.

ity. Sensitivity, specificity, predictive values and likelihood ratios were calculated for these cutoff values. A logistic regression model was used to evaluate the variables that reached statistical significance on univariate analysis, with EV as the dependent variable. All statistical analyses were performed in the SPSS 18.0. This study was approved by the local Research Ethics Committee.

RESULTS

A hundred and three patients were included, with a mean age of 8.9 (± 4.7) years. Fifty-six (56/103; 54.3%) patients were female. Ninety-eight (98/103; 95%) patients had a diagnosis of chronic liver disease and five (5/103; 4.8%) had EHPVO. Seventy-one of the (71/103; 68.9%) patients had EV. Varices were classified as F2 and F3 in 35 patients, with red spots in 12 patients. Sixteen (16/71; 22.5%) patients presented both esophageal and gastric varices, and one had isolated gastric varices. Twenty (20/103; 19.4%) patients had portal hypertensive gastropathy.

Spleen size, platelet count, CPR, APRI, platelet count to spleen size ratio, platelet count to spleen size z score ratio, and the risk score were able to discriminate patients with and without varices (Table 1).

On ROC curve analysis, the best predictors of EV were: platelet count; platelet count to spleen size z score ratio; CPR; risk score; platelet count to spleen size (cm) ratio; spleen size z score; and the APRI test (Figure 1). The cutoff points were established with the best relationship between sensitivity and specificity for each variable as follows: platelet count, 115 000; platelet count to spleen size z score ratio, 25; CPR, 114; risk score, -1.2; platelet count to spleen size ratio, 1.0; APRI test, 1.4.

Table 2 Odds ratios for esophageal varices

Variables	OR[1]	95%CI	*P* value
CPR < 115	7.99	1.45-43.82	0.017
CPR < 114	20.74	2.66-161.50	0.004
Platelets/spleen size z score < 25	4.27	0.90-20.26	0.067
Platelets/spleen size < 1	2.20	0.65-7.43	0.203
Platelets	0.98	0.97-0.99	0.016
Platelets < 115	3.10	0.97-9.88	0.056
Risk score > -1.2	7.47	2.06-26.99	0.002
APRI > 1.4	1.85	0.56-6.10	0.309

[1]Odds ratio computed by multivariate logistic regression model. CPR: Clinical prediction rule; APRI: Aspartate aminotransferase to platelet ratio index.

A logistic regression model was applied with EV as the dependent variable, corrected by albumin, bilirubin and spleen size z score. Patients with a CPR < 114 were 20.74-fold more likely to have EV compared to children with CPR > 114. Risk score > -1.2 increased the likelihood of varices (odds ratio 7.47; 95%CI: 2.06-26.99) (Table 2). Sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio and negative likelihood ratio values for CPR, platelet count, platelet count to spleen size z score ratio, platelet count to spleen size (cm) ratio, risk score and APRI as EV predictors are presented on Table 3.

DISCUSSION

We evaluated seven non-invasive markers, two of which had never been tested in children, the platelet count to spleen size (cm) ratio and the risk score. We found that platelets, the platelet count to spleen size z score ratio, CPR, and the risk score were able to predict EV. The prevalence of EV observed in our sample was similar to those reported elsewhere[22,26,27].

Thrombocytopenia is a common complication of chronic liver disease, affecting 76% of cirrhotic patients[28]. Unlike in adults[13], isolated platelet count has been described as a predictor of EV in four out of four studies of pediatric patients[22,26,27,29]. Nevertheless, there is still no consensus as to the best cutoff points, ranging from 100 000 to 130 000[22,26]. Gana *et al*[27] demonstrated that platelet count (cutoff point = 115 000) was the best predictor of EV, with an area under the AUROC curve of 0.79 (95%CI: 0.69-0.90). In the present study, the cutoff of point was similar to that observed by Gana *et al*[22] with an area under the ROC curve = 0.82 (95%CI: 0.73-0.91).

Splenomegaly is an important clinical sign of portal hypertension, especially in patients with chronic liver dis-

Table 3 Diagnostic performance of variables as esophageal varices predictors

Variables	Sensitivity (95%CI)	Specificity (95%CI)	Positive predictive value (95%CI)	Negative predictive value (95%CI)	Positive likelihood ratio (95%CI)	Negative likelihood ratio (95%CI)
Clinical prediction rule < 115	76.6 (64.0-85.8)	70.8 (48.7-86.5)	87.5 (75.3-94.4)	53.1 (35.0-70.4)	2.63 (1.38-4.96)	0.33 (0.20-0.53)
Clinical prediction rule < 114	75.0 (62.3-84.6)	79.2 (57.3-92.0)	90.5 (78.5-96.5)	54.2 (36.8-70.8)	3.60 (1.63-7.95)	0.32 (0.20-0.49)
Platelets < 115	67.6 (55.3-77.9)	81.3 (62.9-92.1)	88.8 (76.6-95.4)	53.0 (38.4-67.2)	3.61 (1.72-7.54)	0.40 (0.28-0.56)
Platelets/spleen size z score < 25	68.8 (55.8-79.4)	79.2 (57.3-92.0)	89.8 (76.9-96.2)	48.7 (32.7-64.9)	3.30 (1.48-7.32)	0.39 (0.27-0.58)
Platelets/spleen size < 1	72.3 (59.6-82.3)	66.7 (44.6-83.6)	85.4 (72.8-93.0)	47.0 (30.1-64.6)	2.17 (1.20-3.89)	0.42 (0.27-0.64)
Risk score > -1.2	80.3 (67.2-89.3)	70.9 (51.7-85.1)	86.3 (75.2-93.2)	61.1 (43.5-76.4)	2.77 (1.57-4.85)	0.28 (0.17-0.45)
APRI > 1.4	63.4 (51.0-74.2)	65.6 (46.7-80.8)	80.3 (67.2-89.3)	44.7 (30.4-59.8)	1.84 (1.10-3.07)	0.56 (0.39-0.78)

APRI: Aspartate aminotransferase to platelet ratio index.

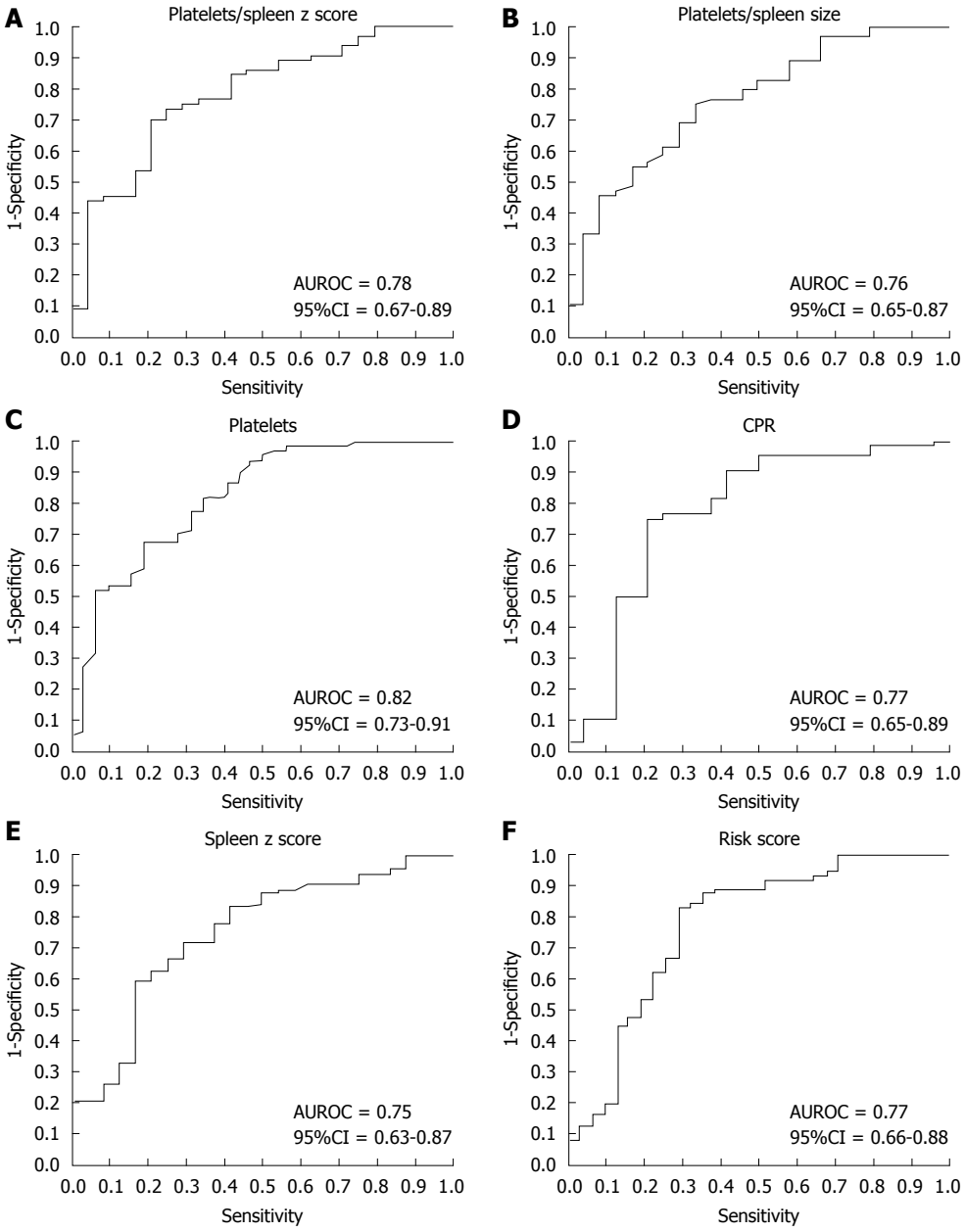

Figure 1 Receiver operator characteristic curves for presence of esophageal varices. AUROC: Area under the receiver operating characteristic curve.

ease[30]. It has been used as such in several studies, both as an isolated parameter and as a component of scores or mathematical algorithms[22,26,27,29]. In cirrhotic children studied by Fagundes *et al*[26] patients with splenomegaly were almost 15-fold more likely to have EV compared with those without splenomegaly. In our study, 83.5%

of patients had splenomegaly on physical examination, and, as observed by others, this variable discriminated patients with and without EV ($P = 0.001$).

Based on the premise that both thrombocytopenia and splenomegaly may depend on several factors related to chronic liver disease *per se*, Giannini *et al*[8] proposed studying the platelet count to spleen diameter ratio as a noninvasive rule predicting EV. According to the authors, a ratio less than 909 was independently associated with the presence of EV, the negative predictive value found was reproducible even in patients with compensated disease, and it was cost effective[8].

In our study, a platelet count to spleen size (cm) ratio < 1.0 was able to discriminate patients with and without EV ($P < 0.000$), but did not reach statistical significance on logistic regression (OR = 2.2; 95%CI: 0.65-7.43; $P = 0.203$). This could be explained by age and gender differences in spleen size. We tried to minimize the impact of this factor by using the spleen size z score, but this parameter was also not able to reach statistical significance on logistic regression (OR = 4.7; 95%CI: 0.90-20.26; $P = 0.067$). A disadvantage of considering platelet count and spleen size is that this evaluation needs to be synchronously due the great variability of both.

More recently, a systematic review and meta-analysis sought to determine the evidence on the diagnostic accuracy of platelet count to spleen diameter ratio < 909 as a noninvasive predictor of EV and concluded that the quality of evidence of these studies was low, raising questions about the reliability of the platelet count to spleen diameter ratio as a good predictor of EV. We agree with Chawla *et al*[20] that the heterogeneity of patients studied may limit the value of this ratio as a noninvasive predictor of EV. The etiological diversity of patients in our sample may have played a role in our findings.

An interesting CPR was developed and validated by Gana *et al*[22] in a retrospective study, using platelet count, spleen size z score and albumin as variables[22]. In a prospective, multicenter clinical trial, a CPR ≤ 116 had a sensitivity of 81%, a specificity of 73% and an AUROC of 0.84. The authors suggested that CPR under 115 could screen patients for endoscopy[27].

Apart from Gana *et al*[22], we are the first group to test the CPR in children. To do so, we used two different cutoff points: 115 and 114. The best specificity was observed with a cutoff of 114 (79%). Others predictors identified by Gana *et al*[22] were platelet count under 115 000 and serum albumin level. On multivariate analysis, CPR (OR 0.62; 95%CI: 0.45-0.84; $P = 0.002$) and albumin (OR 3.1; 95%CI: 1.5-6.7; $P = 0.004$) were independent predictors[27]. In our study, logistic regression, adjusted for albumin, bilirubin and spleen size z score, had an OR of 7.79 (95%CI: 1.45-43.82) with a CPR cutoff of 115 and an OR of 20.74 (95%CI: 2.66-161.5; $P = 0.004$) with a CPR cutoff of 114. This mathematical algorithm is simple, composed by available and noninvasive variables, and our results suggest that it is reproducible.

The degree of fibrosis can determine significant changes in the hepatic venous pressure gradient, and seems related to complications such as the development of EV[7]. Non-invasive markers for fibrosis have been tested in children with biliary atresia[29,31]. There was good correlation between APRI and Metavir scores in patients studied by Kim *et al*[31], suggesting that the APRI test could predict the appearance of fibrosis in those patients (a cutoff of 1.42 was correlated with grade 4 fibrosis).

The APRI was studied by Colecchia *et al*[29] as a noninvasive marker of EV in pediatric patients with chronic liver disease. A cutoff > 0.96 had a total accuracy of 83%. These results were not confirmed on multivariate analysis[29]. APRI, with a cutoff of > 1.4, was also used as a variable in this study, and we did not find this parameter to be statistically significant for prediction of EV on logistic regression. We did not test other cutoffs. In fact, the exact thresholds of APRI for prediction of fibrosis constitute the main issue related to its diagnostic accuracy[32].

The risk score was another clinical model tested for predicting EV in adults with advanced fibrosis and portal hypertension. The AUROC of the risk score for prediction of EV was 0.82. The -1.0 cutoff had a sensitivity of 82% and a specificity of 76%. The authors suggested that this score should be validated as a noninvasive test to detect the presence of EV[29]. This was the first pediatric study to use the risk score. The cutoff of -1.2 had a sensitivity of 80.3%, a specificity of 70.9%, an AUROC of 0.77 (95%CI: 0.66-0.88), and an OR of 7.47 (95%CI: 2.06-26.99; $P = 0.002$). This method is also composed by simple and available variables that proved to be good noninvasive parameters for EV detection in our patients. Furthermore, this method avoids the frequent use of ultrasound. It is worth noting that this method has not been tested in patients with pre-hepatic portal hypertension, and may not be an effective method in such patients, whose bilirubin levels are usually normal.

We tried to apply all known non-invasive clinical methods for prediction of EV to the study population. According to other pediatric studies, we also found platelet count to be a good predictor of EV, with a cutoff of 115 000. Children with a CPR under 114, in a logistic regression model, were 20.7-fold more likely to have EV compared to children with CPR > 114. More studies of this rule are required to find the optimal cutoff value.

The risk score, previously studied in adults, was a good and inexpensive predictor of EV in our patients. We believe it should be tested as a tool that can potentially limit the number of endoscopies in pediatric patients.

This study has some limitations. The retrospective design precludes blinding of the endoscopists or controlling for interobserver variability in ultrasound tests. The small number of patients with pre-hepatic portal hypertension could not be compared with those with intrahepatic portal hypertension.

In conclusion, the results of this study suggest that

platelet count, the CPR and risk score could be used to screen children with portal hypertension for endoscopy. Further studies with a prospective design are necessary to confirm these suggestions.

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COMMENTS

Background

Esophageal varices (EV) bleeding is a severe complication of portal hypertension. The standard diagnostic screening tool for EV is endoscopy, which is considered an invasive procedure in pediatric patients. Evaluate clinical and laboratory parameters for prediction of EV is very important to avoid unnecessary endoscopy, especially in children. Some studies have reported that platelet count and spleen size could be used to predict EV, but there is no agreement in the cut-off point.

Research frontiers

The development of mathematical models, such as clinical prediction rule (CPR) and risk score, that involves variables associated with intrahepatic portal hypertension seems to be promising. The research hotspot is to evaluate the parameters that could predict EV in children and reduce the indication of endoscopy.

Innovations and breakthroughs

Few previous studies in pediatric patients evaluated platelet count, CPR splenomegaly isolated in different population. The risk score was studied only in adults and aspartate aminotransferase to platelet ratio index was not used to predict EV in children. The risk score, that use platelet count and bilirubin as variables, should be used as a tool that can limit endoscopies indications in pediatric patients with the advantage of not using spleen size. The predictive value was similar to CPR and better than platelet count isolated.

Applications

The study suggests that both CPR and risk score could be used to screen children with portal hypertension for endoscopy.

Terminology

CPR is a clinical prediction rule, proposed by Gana *et al* that use as independent variables platelet count, spleen size z score (based on age and gender) and albumin. Risk score is a score proposed by Park *et al* to be used in patients with advanced fibrosis to determine clinically significant portal hypertension and was used to predict EV, using platelet count and bilirubin.

Peer review

This is a very interesting manuscript that further delineates clinical variables that are readily available and which can be used to increase the yield of endoscopy for identifying EV in children with portal hypertension. Though the variables studied have all been reported previously, the validation of these variables in children is an important extension of this work.

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BRIEF ARTICLE

Atrophic gastritis: Risk factor for esophageal squamous cell carcinoma in a Latin-American population

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Abstract

AIM: To study the association between atrophic gastritis (AG) and esophageal squamous cell carcinoma (ESCC) in a Latin-America population.

METHODS: A case-control study was performed at two reference Brazilian hospitals including patients diagnosed with advanced ESCC and dyspeptic patients who had been subjected to upper gastrointestinal endoscopy, with biopsies of the gastric antrum and body. All cases with ESCC were reviewed by a single pathologist, who applied standard criteria for the diagnosis of mucosal atrophy, intestinal metaplasia, and dysplasia, all classified as AG. The data on the patients' age, sex, smoking status, and alcohol consumption were collected from clinical records, and any missing information was completed by telephone interview. The association between AG and ESCC was assessed by means of univariate and multiple conditional logistic regressions.

RESULTS: Most patients were male, and the median age was 59 years (range: 37-79 years) in both the ESCC and control groups. Univariate analysis showed that an intake of ethanol greater than 32 g/d was an independent risk factor that increased the odds of ESCC 7.57 times (P = 0.014); upon multiple analysis, alcohol intake of ethanol greater than 32 g/d exhibited a risk of 4.54 (P = 0.081), as adjusted for AG and smoking. Smoking was shown to be an independent risk factor that increased the odds of ESCC 14.55 times (P = 0.011) for individuals who smoked 0 to 51 packs/year and 21.40 times (P = 0.006) for those who smoked more than 51 packs/year. Upon multiple analyses, those who smoked up to 51 packs/year exhibited a risk of 7.85 (P = 0.058), and those who smoked more than 51 packs/year had a risk 11.57 times higher (P = 0.04), as adjusted for AG and alcohol consumption. AG proved to be a risk factor that increased the odds of ESCC 5.33 times (95%CI: 1.55-18.30, P = 0.008) according to the results of univariate conditional logistic regression.

CONCLUSION: There was an association by univariate conditional logistic regression between AG and ECSS in this sample of Latin-American population.

Key words: Atrophic gastritis; Esophagus; Squamous cell carcinoma; Risk factor; Alcohol; Tobacco

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INTRODUCTION

Esophageal cancer (EC) is the eighth most common cancer worldwide with 481 000 new cases (3.8% of all cancers) estimated in 2008, and is the sixth most common cause of death from cancer with 406 000 deaths (5.4%)[1]. The two main histological types of EC are adenocarcinoma and esophageal squamous cell carcinoma (ESCC), which differ regarding their risk factors and demographic distributions. Although adenocarcinoma has been the most frequent subtype among white males in the United States since the beginning of the 90s[2], ESCC remains as the most frequent subtype worldwide, corresponding to more than 80% of all cases[3]. The highest mortality rates of ESCC are found in East Asia, Southern and Eastern Africa[1]. In Brazil, which is the largest Latin-American country, ESCC represents 96% of all cases[4]. The main risk factors for ESCC in the West are alcohol consumption and smoking[5,6]. An increased risk of ESCC was initially reported among patients with pernicious anemia[7], and more recently, patients with atrophic gastritis (AG) were also found to be more susceptible[8]. This hypothesis was strengthened by a Swedish study that assessed the association among *Helicobacter pylori* (*H. pylori*) infection, gastric mucosal atrophy, and ESCC. These researchers discovered that an infection by cytotoxin-associated gene A (CagA)-positive *H. pylori* was associated with a higher risk of ESCC, particularly among patients with gastric atrophy. When the correlation between gastric atrophy and ESCC was assessed independently from the *H. pylori* serotype, gastric atrophy exhibited a strong association with increased risk for ESCC. The authors suggest that gastric mucosal atrophy represents an intermediate step in the pathway from a CagA-positive *H. pylori* infection to ESCC[9]. More recently, a meta-analysis[10] selected and analyzed seven studies that investigated this association and concluded that AG increases the risk of ESCC[3,9,11-15]. However, this meta-analysis did not address any studies that considered populations outside Northern Europe or Asia; therefore, the association has not been studied in Latin-American populations. Considering that the South American and Caribbean populations include 572 million people and represent 8.6% of the world population[16], the present study aimed to investigate the correlation between gastric mucosal atrophy and ESCC in a sample from that unexamined population.

MATERIALS AND METHODS

All of the patients with ESCC who were treated at the Cancer Hospital of Barretos between April 2011 and April 2012 were retrospectively analyzed. Exclusion criteria included the following: a diagnosis of obstructive ESCC, *i.e.*, where endoscopic access to the stomach was hindered; previous gastrointestinal (GI) tract surgery and age greater than 80 years. For each case, a gender- and age-matched control was randomly selected among patients who had been subjected to upper GI endoscopy due to dyspeptic complaints at the General Hospital of the Botucatu Medical School. The controls were chosen from patients with dyspeptic complaints, because in Brazil there is no protocol to screen asymptomatic individuals by means of upper GI endoscopy. Because the two centers that participated in the present study are referral centers for patients nationwide, demographic characteristics were not taken into account for matching.

Following routine protocols at both hospitals, all of the patients were subjected to endoscopic biopsies of the gastric antrum and body. The *H. pylori* infection was diagnosed by urease test (pink color after 30 min) and Warthin-Starry stain (Artisan-Dako, Denmark), was used for the visualization of *H. pylori* (with this method *H. pylori* is stained black while the background is stained golden yellow). The criteria for the diagnosis of ESCC were based on the endoscopy aspects and the final pathology report. The histopathologic features included true invasion of lamina propria, at least, by tumoral isolated cells or tumor clusters from the squamous esophagic epithelium. The cells were generally polygonal with pink cytoplasm and distinct cell borders and the nuclei were enlarged, hyperchromatic and pleomorphic. At high power intercellular bridges and keratinization within the cells were commonly seen, although they were absent in poorly differentiated tumors. The adjacent surface epithelium sometimes exhibited the same neoplastic cells, containing therefore, an intraepithelial ("*in situ*") component. All cases with ESCC were reviewed by a single pathologist, who applied standard criteria for the diagnosis of mucosal atrophy, intestinal metaplasia, and dysplasia. All three conditions were classified as AG in the present study.

The data on the patients' age, sex, smoking status, and alcohol consumption were collected from clinical records, and any missing information was completed by telephone interview. Alcohol consumption was calculated as grams of ethanol per day, and tobacco consumption was calculated as number of packs per year.

Statistical analysis

For statistical analysis, categorical values are given in frequencies and percentages. Means, medians, and standard deviations were calculated for numerical variables. To assess the risk factors of ESCC, univariate and multiple conditional logistic regressions were used. In addition, to assess the correlation between the use of alcohol or smoking and AG, non-conditional logistic regression was performed. The level of significance was established as 5%. Analyses were performed using software SPSS version 19 and Stata/SE. The research ethics committees of both participating centers approved the present study.

Table 1 Clinical, lifestyle, and diagnostic characteristics between cases and controls *n* (%)

Characteristic	Category	Case	Control
Gender	Female	7 (14.3)	7 (14.3)
	Male	42 (85.7)	42 (85.7)
Age group (yr)	36-45	3 (6.1)	3 (6.1)
	46-55	14 (28.6)	14 (28.6)
	56-65	21 (42.9)	21 (42.9)
	66-75	8 (16.3)	8 (16.3)
	76-85	3 (6.1)	3 (6.1)
Alcohol	Never drinker	10 (20.4)	16 (32.7)
	Current drinker	39 (79.6)	16 (32.7)
	Former drinker	0 (0.0)	17 (34.7)
Tobacco	No	8 (16.3)	24 (49.0)
	Yes	41 (83.7)	25 (51.0)
Hot drinks	No	49 (100)	49 (100)
Tylosis	No	49 (100)	49 (100)
Achalasia	No	48 (98.0)	49 (100)
	Yes	1 (2.0)	0 (0.0)
Caustic esophagitis	No	49 (100)	49 (100)
Plummer-Vinson	No	49 (100)	49 (100)
Head and neck SCC	No	49 (100)	49 (100)
Endoscopic gastric	No	10 (20.4)	42 (85.7)
atrophy	Yes	39 (79.6)	7 (14.3)
Gastric atrophy	No	37 (75.5)	45 (91.8)
	Yes	12 (24.5)	4 (8.2)
Intestinal metaplasia	No	34 (69.4)	41 (83.7)
	Yes	15 (30.6)	8 (16.3)
Helicobacter pylori	No	25 (51.0)	22 (44.9)
	Yes	24 (49.0)	27 (55.1)
Low-grade dysplasia	No	47 (95.9)	49 (100)
	Yes	2 (4.1)	0 (0.0)
High-grade dysplasia	No	48 (98.0)	49 (100)
	Yes	1 (2.0)	0 (0.0)
Total		49 (100)	49 (100)

SCC: Squamous cell carcinoma.

RESULTS

The comparison between both investigated groups is described in Table 1. Most patients were male, and the median age was 59 years (range: 37-79 years) in both the ESCC and control groups.

Only one individual in the ESCC group exhibited achalasia, and none of the patients exhibited any of the following classic risk factors: consumption of warm beverages, caustic esophagitis, squamous cell carcinoma of the head and neck, Plummer-Vinson syndrome or tylosis.

Table 2 describes the association between risk factors and ESCC. Univariate analysis showed AG to be an independent risk factor, which increased the odds of ESCC 5.332 times ($P = 0.008$). On multiple analysis, AG exhibited risk of 3.76 ($P = 0.063$), as adjusted for alcohol and smoking.

Univariate analysis showed that an intake of ethanol greater than 32 g/d was an independent risk factor that increased the odds of ESCC 7.57 times ($P = 0.014$); upon multiple analyses, alcohol intake of ethanol greater than 32 g/d exhibited a risk of 4.54 ($P = 0.081$), as adjusted for AG and smoking.

Smoking was shown to be an independent risk factor that increased the odds of ESCC 14.55 times ($P = 0.011$) for individuals who smoked 0 to 51 packs/year and 21.40 times ($P = 0.006$) for those who smoked more than 51 packs/year. Upon multiple analyses, those who smoked up to 51 packs/year exhibited a risk of 7.85 ($P = 0.058$), and those who smoked more than 51 packs/ year had a risk 11.57 times higher ($P = 0.04$), as adjusted for AG and alcohol consumption.

Table 2 Univariate and multiple logistic regression for case-control study according to gastric atrophy, alcohol intake, and tobacco consumption

Variables	Univariate		Multiple	
	OR unadjusted	*P* value	OR adjusted	*P* value
Gastric atrophy	5.332	0.008	3.76	0.063
Alcohol				
Non-drinker	1		1	
0-32 g ethanol/d	1.29	0.736	0.98	0.985
> 32 g ethanol/d	7.57	0.014	4.54	0.081
Tobacco				
No smoker	1		1	
51 pack/yr	14.55	0.011	7.85	0.058
> 51 pack/yr	21.40	0.006	11.57	0.040

OR: Odds ratio.

Non-conditional logistic regression assuming alcohol consumption and smoking as risk factors for AG did not find evidence for an association.

In the present study, *H. pylori* infection did not exhibit association with ESCC [odds ratio (OR) = 0.81, $P = 0.578$] and behaved as a protective factor against AG (OR = 0.3, $P = 0.009$).

DISCUSSION

Recent interest in the correlation between AG and ESCC led to a meta-analysis of seven studies from Asia and northern Europe that demonstrated an association between the conditions[10].

The present case-control study also discovered significant statistical associations from a non-adjusted analysis between the following: AG and ESCC; heavy use of alcohol (more than 32 g of ethanol/d) and ESCC; and smoking and ESCC. Nevertheless, the use of adjusted models to investigate AG, alcohol, and smoking found a statistically significant association between ESCC and heavy smoking (more than 51 packs/d) alone. From the seven studies included in the aforementioned meta-analysis, three did not adjust their analysis for other risk factors besides AG[12,13,15]. The fact that the associations discovered by the present study often lost statistical significance after adjustment could be explained by the small sample sizes ($n = 49$ cases, $n = 49$ controls). The loss of significance attributable to heavy alcohol consumption, in particular, corroborates this interpretation; alcohol consumption is a classic risk factor for ESCC in Western countries. de Vries *et al*[15] suggest that the association between AG and ESCC might be explained by confounding factors, such as smoking, after demonstrating an association between AG and small cell lung car-

cinoma. However, in the present study, univariate non-conditional logistic regression did not indicate an association between alcohol consumption or smoking with AG. It is worth noting that four other studies performed logistic regression adjusted for risk factors, and each study found a statistically significant association between AG and ESCC[3,9,11,14].

The present study did not find an association between ESCC and *H. pylori*, which, in fact, proved to be protective against AG. Although seemingly paradoxical, this finding may be explained by the fact that *H. pylori* does not colonize atrophic mucosa nor areas with intestinal metaplasia.

This study, as well as other similar studies, encountered limitations regarding the selection of healthy controls. Although some Japanese studies, such as the one by Akiyama *et al*[3], have been able to use healthy controls undergoing screening upper GI endoscopy, these studies also report difficulty in selecting appropriate controls. As such screening programs do not exist in Brazil, the controls used in the present study were individuals subjected to endoscopy due to dyspeptic complaints; consequently, higher odds of exhibiting pathological findings in the GI tract may exist.

Although related literature shows an association between AG an ESCC, whether that relationship is causal is still unknown. A possible mechanism of this relationship is that achlorhydria in patients with gastric atrophy may generate an intragastric environment that favors bacterial overgrowth and n-nitrosation, thus resulting in increased exposure of the esophageal mucosa to carcinogenic endogenous nitrosamines[17].

Other explanations for the positive association between gastric atrophy and ESCC are worth exploring. It is possible that both conditions share genetically determined pathogenetic mechanisms that facilitate a similar destructive process (*i.e.*, inflammatory response or defective DNA repair), damaging both gastric and esophageal epithelia[15,18-20].

It is also possible that the observed association between ESCC and AG is due to the fact that patients with advanced ESCC can only ingest small amounts of food, owing to esophageal stenosis and lack of appetite, and this reduced ingestion may lead to disuse atrophy. That hypothesis, however, is contested in the study by Kamangar *et al*[13], which found that a lower serum PGI/Ⅱ ratio was linearly associated with higher risk of esophageal squamous dysplasia, a preneoplastic condition of ESCC. It is worth noting that patients with esophageal dysplasia do not exhibit dysphagia.

Despite the abovementioned limitations, the present study, as far as we know, is the first to identify a statistically significant association by univariate conditional logistic regression between AG and ESCC in the Latin-American population. In conclusion, in the present study the AG was an independent risk factor in this sample from Latin-America population, as has been demonstrated in studies of population samples from Asia and northern Europe. This fact highlights the importance of conducting prospective, multicenter studies enrolling larger populations that represent different ethnic groups, to investigate the causal relationship between AG and ESCC.

COMMENTS

Background

Esophageal cancer (EC) is the eighth most common cancer worldwide with 481 000 new cases (3.8% of all cancers) estimated in 2008, and is the sixth most common cause of death from cancer with 406 000 deaths (5.4%). The highest mortality rates of esophageal squamous cell carcinoma (ESCC) are found in East Asia, Southern and Eastern Africa. In Brazil, which is the largest Latin-American country, ESCC represents 96% of all EC. The main risk factors for ESCC in the Western countries are alcohol consumption and smoking. More recently, a meta-analysis concluded that AG increases the risk of ESCC. However, this meta-analysis did not address any studies that considered populations outside of Northern Europe or Asia; therefore, the association has not been studied in Latin-American populations. Considering that the South American and Caribbean populations include 572 million people and represent 8.6% of the world population, the present study aimed to investigate the correlation between gastric mucosal atrophy and ESCC in a sample from that unexamined population.

Research frontiers

The better knowledge of risk factors and pathophysiological mechanisms of EC can lead to better preventive and therapeutic options in a cancer type that presents with low survival rates.

Innovations and breakthroughs

An increased risk of ESCC was initially reported among patients with pernicious anemia and more recently, patients with atrophic gastritis (AG) were also found to be more susceptible. This hypothesis was strengthened by a Swedish study that assessed the association among *Helicobacter pylori* (*H. pylori*) infection, gastric mucosal atrophy and ESCC. These researchers discovered that an infection by cytotoxin-associated gene A (CagA)-positive *H. pylori* was associated with a higher risk of ESCC, particularly among patients with gastric atrophy. When the correlation between gastric atrophy and ESCC was assessed independently from the *H. pylori* serotype, gastric atrophy exhibited a strong association with increased risk for ESCC. The authors suggest that gastric mucosal atrophy represents an intermediate step in the pathway from a CagA-positive *H. pylori* infection to ESCC.

Applications

The study results suggest an association between AG and ESCC.

Terminology

All three conditions (mucosal atrophy, intestinal metaplasia, and dysplasia) were classified as AG in the present study.

Peer review

This is a small but well conducted study that shows that also in South America AG seems to predispose to ESCC thus showing that the association can be found despite the obvious environmental differences between Asia, Europe and North America. The study thus further generalizes the hypothesis and should encourage research into the mechanism behind this.

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BRIEF ARTICLE

Increased endothelin receptor B and G protein coupled kinase-2 in the mesentery of portal hypertensive rats

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Abstract

AIM: To elucidate the mechanisms of mesenteric vasodilation in portal hypertension (PHT), with a focus on endothelin signaling.

METHODS: PHT was induced in rats by common bile duct ligation (CBDL). Portal pressure (PP) was measured directly via catheters placed in the portal vein tract. The level of endothelin-1 (ET-1) in the mesenteric circulation was determined by radioimmunoassay, and the expression of the endothelin A receptor (ETAR) and endothelin B receptor (ETBR) was assessed by immunofluorescence and Western blot. Additionally, expression of G protein coupled kinase-2 (GRK2) and β-arrestin 2, which influence endothelin receptor sensitivity, were also studied by Western blot.

RESULTS: PP of CBDL rats increased significantly (11.89 ± 1.38 mmHg *vs* 16.34 ± 1.63 mmHg). ET-1 expression decreased in the mesenteric circulation 2 and 4 wk after CBDL. ET-1 levels in the systemic circulation of CBDL rats were increased at 2 wk and decreased at 4 wk. There was no change in ETAR expression in response to CBDL; however, increased expression of ETBR in the endothelial cells of mesenteric arterioles and capillaries was observed. In sham-operated rats, ETBR was mainly expressed in the $CD31^+$ endothelial cells of the arterioles. With development of PHT, in addition to the endothelial cells, ETBR expression was noticeably detectable in the SMA^+ smooth muscle cells of arterioles and in the $CD31^+$ capillaries. Following CBDL, increased expression of GRK2 was also found in mesenteric tissue, though there was no change in the level of β-arrestin 2.

CONCLUSION: Decreased levels of ET-1 and increased ETBR expression in the mesenteric circulation following CBDL in rats may underlie mesenteric vasodilation in individuals with PHT. Mechanistically, increased GRK2 expression may lead to desensitization of ETAR, as well as other vasoconstrictors, promoting this vasodilatory effect.

Key words: Portal hypertension; Mesentery; Endothelin; Endothelin B receptor; G protein coupled kinase-2

Core tip: Portal hypertension (PHT) is a life-threatening condition which frequently develops in patients with liver cirrhosis, and has limited treatment options. For many years, endothelin-1 (ET-1) has received considerable interest in the area of liver cirrhosis for its potential contribution to PHT. The aim of the present study was to directly examine the expression of ET-1 and its receptors in the mesentery of rats with PHT, and to clarify how the ET-1 signaling system changed with the development of PHT.

Du QH, Han L, Jiang JJ, Li PT, Wang XY, Jia X. Increased en-

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INTRODUCTION

Portal hypertension (PHT) is one of the most significant complications associated with liver cirrhosis, which can give rise to many other severe and often lethal conditions, such as bleeding esophageal varices. Increased resistance to portal blood flow is the primary factor leading to PHT and is aggravated by a hyperdynamic, vasodilated, splanchnic circulation[1]. Though the pathophysiology of PHT is becoming better understood, excepting β-receptor blockers, there is no effective treatment approach for PHT. One reason to explain this shortfall is that the mechanism of splanchnic vasodilation is unclear. The organs involved in splanchnic hyperdynamic circulation are those whose blood flows into the portal vein, including the intestine, mesentery, colon, spleen and stomach. Previous studies have indicated that vasodilation of the mesenteric vascular bed plays the greatest role in PHT, by increasing portal inflow[2]. In previous work from our laboratory, we also observed vasodilation of the mesenteric vascular bed; thus in the present study we focused on this tissue to try to explain splanchnic vascular dilation and possibly to identify new therapeutic targets for treating PHT.

Splanchnic vasodilation is associated with the imbalance of vasoactive mediators[3,4] and hyporeactivity to vasoconstrictors[5]. Endothelin-1 (ET-1) is a potent endothelium derived vasoactive peptide. For many years, ET-1 has received considerable interest in the area of liver cirrhosis for its potential contribution to PHT[6-9]. This has led to many studies being focused upon the effect of ET-1 and its receptors in the liver; however, there are only a few studies examining the possible mechanism of the endothelin signaling system in hyperdynamic circulation. It has been established that the divergent effect of ET-1 on blood vessels depends on the different expression of endothelin receptors on smooth muscle and endothelial cells[10]. There are two known types of ET-1 receptor: the endothelin A receptor (ETAR) and the endothelin B receptor (ETBR)[11]. ETBR has two recognized subtypes: ETB1 and ETB2[10-13]. ETAR and ETB2 are predominantly expressed in vascular smooth muscle cells, whereas ETB1 is characteristic of endothelial cells. ET-1 binds to ETAR and ETB2 to induce vasoconstriction, while ET-1 binds to ETB1 to cause vascular relaxation[14]. Both mixed ETAR-ETBR antagonists and selective ETBR antagonists have been proven to decrease portal pressure (PP) and increase mean arterial pressure (MAP), while ETAR antagonists have been shown to have no effect on MAP[14,15]. Moreover, selective ETBR inhibition *in vivo* significantly ameliorated hepatopulmonary syndrome (HPS)[16-18], which is also known to be caused by dilation of the microcirculation, similar to PHT[18]. Based on this literature, we speculate that ETBR may play a primary role in the hyperdynamic circulation associated with PHT.

In addition to the localization and expression level of ET-1, its signaling is known to be affected by other regulators. For example, it has been observed that in some instances of high splanchnic ET-1 expression, the vascular bed of this tissue was still dilated, leading the authors to speculate that the sensitivity of the ET-1 receptor(s) was decreased[19]. Endothelin receptors may be desensitized by phosphorylation through G-protein-coupled receptor kinases (GRKs) and binding of β-arrestin 2[20]. So far, seven kinds of GRKs have been cloned[21]. GRK2 is the most likely of the GRKs to initiate human human endothelin A and B receptor desensitization[22]. Endothelin signalling in arterial smooth muscle is tightly regulated by GRK2[23]. As such, in this study we also focused on two known regulators, GRK2 and β-arrestin 2. The current literature has indicated a possible role for ET-1 signaling in splanchnic vasodilation, though there is a lack of experimental data to support this hypothesis. The aim of the present study was to directly examine the expression of ET-1 and its receptors in the mesentery of rats with PHT, and to clarify how the ET-1 signaling system changed with the development of PHT.

MATERIALS AND METHODS

Animal models

Male Sprague-Dawley rats (approximately 250 g; Vital River Laboratory Animal Technology Co. Ltd., Beijing, China) underwent sham surgery or common bile duct ligation (CBDL). In brief, the common bile ducts of rats were exposed after median laparotomy and ligated twice. In each animal, the segment between the 2 ligations was resected, and the animal's abdomen was sutured closed. Sham-operated rats served as controls. In these rats, the common bile duct was exposed, but no ligation or resection was performed. Seven animals were used in each group. The study was approved by the local committee for animal studies.

Measurement of portal blood pressure and ET-1

In brief, after 2 wk or 4 wk following CBDL, a PE-50 catheter was inserted into the portal vein to measure portal blood pressure. After stable recordings of portal venous pressure were obtained, blood was drawn from the superior mesenteric artery (SMA) for further analysis. The level of ET-1 was measured in the mesenteric circulation using a commercial RIA kit (PLA Institute of RIA, Beijing, China) according to the manufacturer's protocol.

Immunofluorescence

For immunofluorescence double staining of ETAR and ETBR, mesentery tissues were harvested at 2 and 4 wk

and fixed in 4% neutral paraffin. After antigen retrieval, all sections were incubated with PBS containing 1% bovine serum albumin (block buffer) for 60 min in a wet chamber at room temperature. Then, for ETAR, sections were incubated with primary anti-ETAR (polyclonal antibody; Santa Cruz Biotechnology, Santa Cruz, CA; 1:100 dilution) and anti-smooth muscle actin SMA (Santa Cruz Biotechnology, Santa Cruz, CA, United States; 1:200 dilution); for ETBR, slides were incubated with primary anti-ETBR (polyclonal antibody; Santa Cruz Biotechnology, Santa Cruz, CA, United States; 1:300 dilution) and anti-CD31 (polyclonal antibody; Santa Cruz Biotechnology, Santa Cruz, CA, United States; 1:100 dilution) antibodies at 4 ℃ overnight. Then all sections were washed with PBS. For the ETAR, the slides were incubated with goat anti-rabbit/mouse secondary antibodies at the same time. For the ETBR, the sections were incubated with rabbit anti-sheep and goat anti-rabbit antibodies at the same time (Invitrogen, San Diego, CA, United States) for 1 h at room temperature. Subsequently, sections were washed with phosphate-buffered saline (PBS). Control sections were incubated with secondary antibody in the absence of primary antibody. The results were analyzed using confocal laser scanning microscope.

Western blot analysis

For Western blot analysis, samples of rat mesentery were homogenized in radio immunoprecipitation assay (RIPA) lysis buffer containing 50 mmol/L Tris (pH 7.4), 150 mmol/L NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 5 mmol/L ethylenediamine tetraacetic acid, 1 mmol/L sodium orthovanadate, 20 mmol/L pepstatin A, 20 mmol/L leupeptin and 1 mmol/L phenylmethanesulfonyl fluoride. The protein content of the cleared homogenates was assessed with bicinchoninic acid assay kit (Applygen, Beijing, China). After boiling with SDS sample buffer (Applygen, Beijing, China), 50 μg of protein per lane of each sample was subjected to SDS-polyacrylamide gel electrophoresis (10% gels for GRK2, 12.5% gels for ETAR, ETBR and β-arrestin 2). After blotting on polyvinylidene difluoride membrane (Millipore, Bedford, MA, United States), the membranes were probed with primary antibodies diluted in TBS containing blocking protein and 0.1% Tween, and left to incubate overnight at 4 ℃. The following primary antibodies in the indicated dilutions were used: mouse anti-GRK2, 1:500 (Abcam); rabbit anti-ETAR/ETBR and mouse β-arrestin 2, 1:200 (Santa Cruz Biotechnology, Santa Cruz, CA, United States). Thereafter, the membranes were washed and incubated with appropriate peroxidase-coupled secondary antibodies diluted 1:5000 in TBS containing blocking protein and 0.1% Tween for 45 min (goat anti-rabbit or goat anti-mouse; Jackson, West Grove, PA, United States). Detection was performed with enhanced chemiluminescence (Applygen, Beijing, China), and films were developed using Kodak film. Densitometric quantification was performed using Phoretix 1D gel image analysis software for free.

Statistical analysis

All data are presented as the mean ± SD; statistical comparisons were performed using one way analysis of variance. Physiological and biochemical findings represent averages of seven rats. Results of molecular assays represent averages of samples from at least five rats in each group. P values < 0.05 were considered statistically significant.

RESULTS

Increased PP following CBDL

PP was measured in sham and experimental rats 2 and 4 wk after CBDL; PP of CBDL rats increased significantly (16.34 ± 1.63 mmHg *vs* 11.89 ± 1.38 mmHg for sham-operated animals). The increase in PP in CBDL rats was statistically significant compared with sham-operated rats at the 2 and 4 wk timepoint ($P < 0.005$).

Concentration of ET-1 in the mesenteric and systemic circulation

The concentration of ET-1 was measured in the mesenteric and systemic circulation 2 and 4 wk after CBDL. At 2 and 4 wk, ET-1 levels in the mesenteric circulation of CBDL rats were 92.09 ± 13.26 pg/mL and 100.35 ± 16.36 pg/mL, which were significantly lower than that in sham-operated rats. Furthermore, in the systemic circulation, compared with sham-operated rats, ET-1 levels of CBDL rats were also significantly increased at 2 wk (142.77 ± 27.67 pg/mL); however, systemic ET-1 levels were decreased at 4 wk (88.62 ± 15.40 pg/mL).

ETAR and ETBR immunofluorescence

The expression pattern of ETAR and ETBR in mesenteric tissues was determined by immunofluorescence (Figures 1 and 2). From these pictures, the expression of ETAR was observed on SMA^+ smooth muscle cells (Figure 1). In sham-operated rats, ETBR was mainly expressed in $CD31^+$ endothelial cells of the vasculature, though the microcirculation also had weak immunostaining (Figure 2A). Our data indicates that with PHT development, in addition to endothelial cells, ETBR expression was noticeably detectable in the $CD31^+$ capillaries (Figure 2B and C). We also noted increased vasodilation in the mesentery and formation of hyperdynamic circulation in CBDL rats, which was associated with increased angiogenesis (Figure 2B and C).

Quantification of ETAR, ETBR, GRK2 and β-arrestin 2 expression by Western blot

To confirm our immunofluorescence results, we investigated ETAR and ETBR expression by Western blot; we also used this method to assess GRK2 and β-arrestin 2 expression, as it relates to the sensitivity of the ET-1 receptors during the development of a hyperdynamic circulation (Figure 3). In agreement with our immunostaining, we found no statistically significant difference in ETAR expression between sham-operated and CBDL

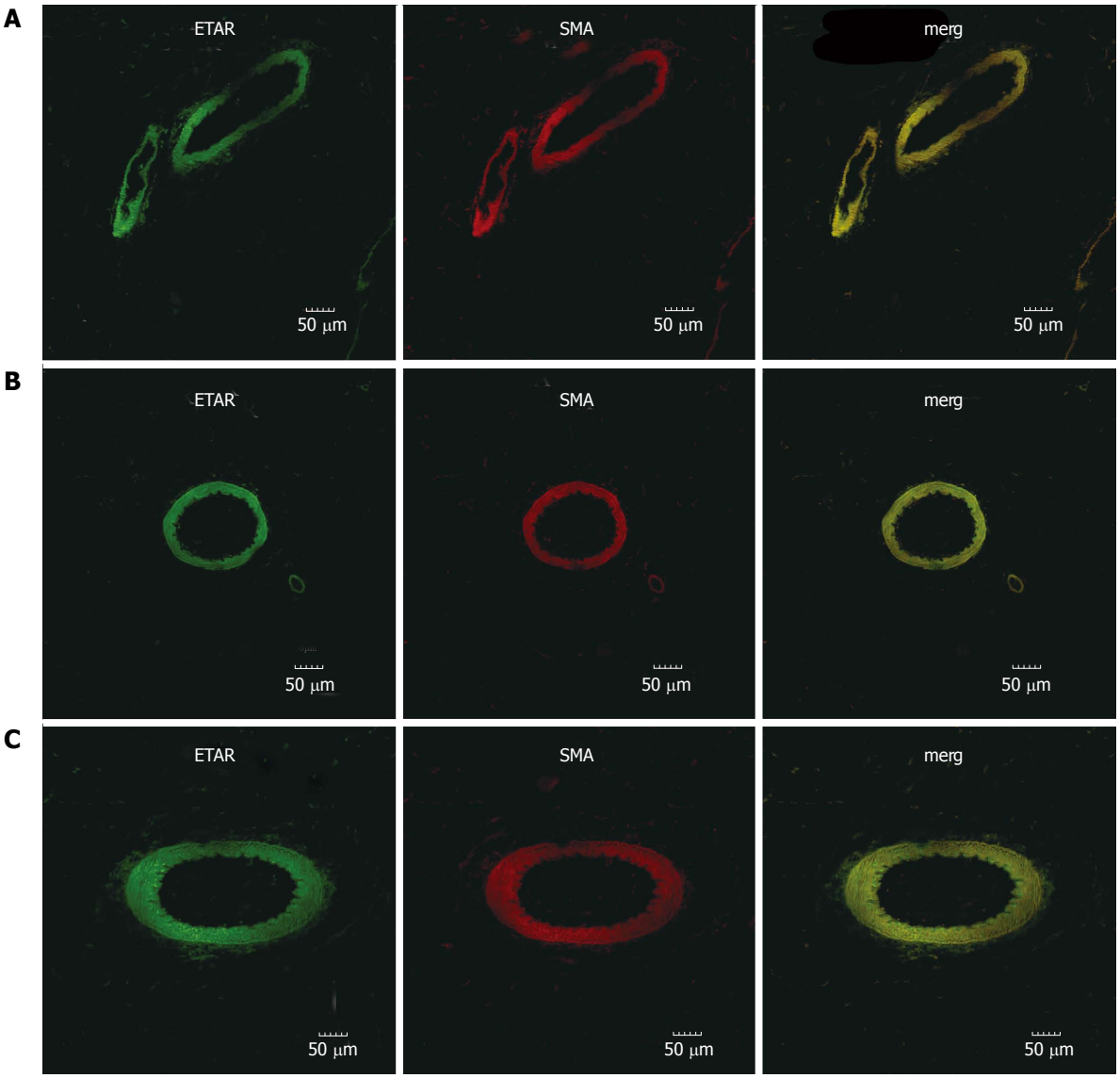

Figure 1 Expression of endothelin A receptor in rat mesentery. Expression of endothelin A receptor (ETAR, green) on smooth muscle cells (red) in mesentery of sham-operated rats (A), and common bile duct ligation rats at 2 wk (B) and 4 wk (C). ETAR was expressed extensively in smooth muscle cells of the vascular.

rats at the 2 and 4 wk timepoints (Figure 3A). ETBR was significantly upregulated in CBDL rats after 2 wk ($P < 0.01$) and 4 wk ($P < 0.01$), as compared to sham-operated rats (Figure 3B). Similar to previous research, which has shown that GRK2 levels are increased in the aortas of CBDL rats[24], we also observed an upregulation of GRK2 protein levels in the mesentery of CBDL rats at both timepoints, which was statistically significant ($P < 0.005$; Figure 3C). We also observed no significant change in the protein expression level of β-arrestin 2 between sham-operated and CBDL rats after 2 or 4 wk ($P > 0.05$; Figure 3D).

DISCUSSION

In accordance with Ohm's law, PP depends on intrahepatic resistance and portal inflow. In cases of cirrhosis, both intrahepatic resistance and splanchnic blood flow are increased. The initiating factor is an increase in intrahepatic vascular resistance, whereas the increase in splanchnic blood flow is a secondary phenomenon that maintains or worsens the increased PP and gives rise to the hyperdynamic systemic state[25]. In terms of the relevant literature[16,17], it has been speculated that the ET-1 signaling system may play an important role in the hyperdynamic circulation, even though there is no direct experimental evidence. Moreover, there are also articles about endothelin receptor antagonism treatment in humans with PHT[26,27]. In this regard, this is the first *in vivo* study examining the expression of the ET-1 signaling system in the mesentery of PHT rats.

We have found that the concentration of ET-1 in the mesenteric circulation decreases as the liver becomes fibrotic. We also found that ETBR expression, but not ETAR expression, increased in vascular smooth muscle cells and in the microcirculation of mesentery tissue, which may mean decreased vasoconstriction and increased vasodilatation induced by local ET-1 in the mesen-

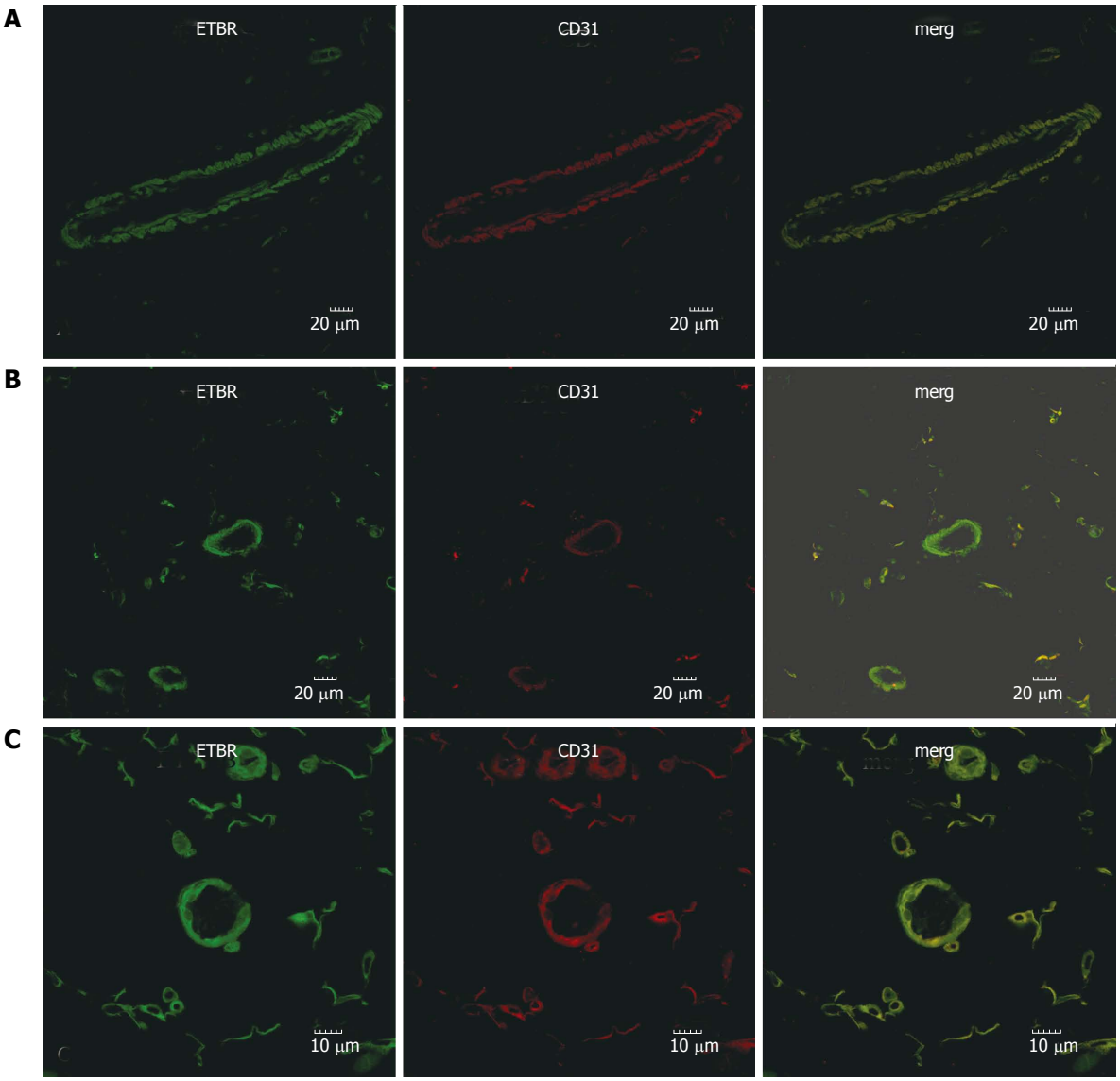

Figure 2 Expression of endothelin B receptor in rat mesentery. Expression of endothelin B receptor (ETBR) in the mesentery of sham-operated rats (A), and common/L on bile duct ligation rats at 2 wk (B) and 4 wk (C). In the mesentery of sham-operated rats, ETBR (green) was mainly expressed in $CD31^+$ endothelial cells (red) of the vascular, with weak staining in the microcirculation. With portal pressure rising, ETBR expression was detected in the smooth muscle cells of arterioles and the microcirculation.

tery of PHT rats. Furthermore, the expression of GRK2 increased significantly in CBDL rats, which may imply that desensitization of ETAR and other vasoconstrictor receptors also promotes splanchnic vasodilatation.

The ET-1 signaling system is associated with vascular dysfunction at three levels, changes in: ET-1 concentration, ET-1 receptor expression and sensitivity, and the ET-1 signaling transduction pathway. Thus, the ability to modulate ET-1 signaling at either of these levels may provide ways to improve splanchnic vascular dysfunction. Previous studies have shown that ET-1 was upregulated in the liver tissue and systemic circulation of patients[9], but the expression level of ET-1 in the splanchnic circulation is not consistent. The observed decrease in ET-1 in the splanchnic circulation 2 and 4 wk after CBDL leads us to speculate that one reason for mesenteric vascular bed dilation in this model is a local decrease in ET-1.

ET-1 must bind to its receptor to modulate vascular tone, so variations in receptor subtype expression and quantity affect the action of ET-1 on blood vessels. Using an animal model of HPS, which is also applicable to PHT, it was found that the extensive dilation of the pulmonary microcirculation was related to a selective increase in ETBR expression[16,17]. PHT and HPS can both be induced by CBDL in rats, and dilation of the microcirculation exists in both syndromes, raising the question whether ET-1 signaling through ETBR plays the same role in the hyperdynamic circulation as it does in HPS? Further research will be required to address this important question and establish if this hypothesis is correct. In the present study, we observed that ETAR expression in the mesentery was not different between sham-operated and CBDL rats. Interestingly however, ETBR expression

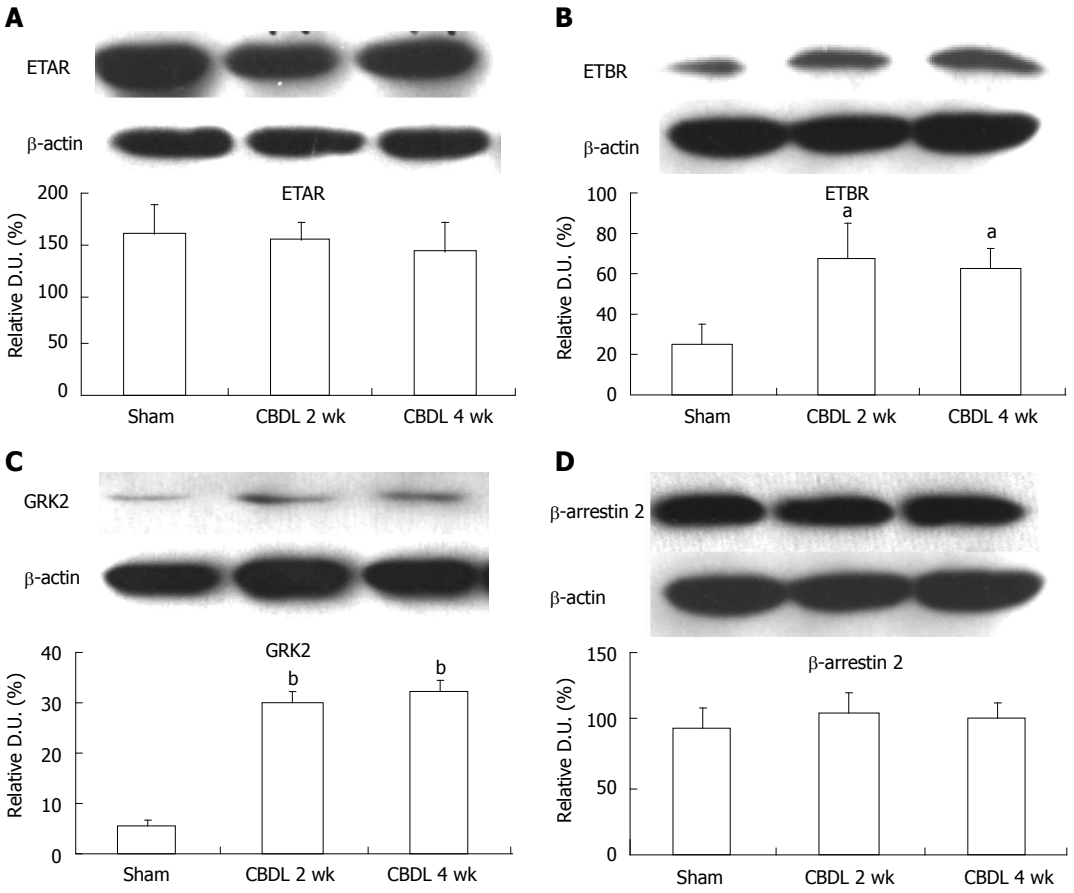

Figure 3 Western blots of endothelin A receptor (A), endothelin B receptor (B), G protein coupled kinase-2 (C) and β-arrestin 2 (D) in rat mesentery. Expression of the investigated proteins in mesentery from sham-operated rats and mesentery from common bile duct ligation (CBDL) rats was compared by Western blot. Representative Western blots are shown for each protein and densitometric quantification of all experiments are provided (data are mean ± SE; n = 3-6/group). $^{a}P < 0.05$, $^{b}P < 0.01$ for CBDL rats *vs* sham-operated rats. ETAR: Endothelin A receptor; ETBR: Endothelin B receptor; GRK2: G protein coupled kinase-2; D.U.: Densitometric units.

was obviously increased by CBDL. Our immunofluorescence data indicates that, under normal physiological conditions, ETBR is primarily expressed in the endothelial cells of the vasculature, whereas the microcirculation also has weak positive staining. With the development of liver fibrosis and liver cirrhosis, ETBR was not only expressed in endothelial cells but was also strongly observed in the microcirculation. Given that the primary function of the microcirculation is to accumulate blood, we interpreted from our data that in the mesentery of CBDL rats, signaling through ETBR mediates vasodilation *via* release of nitrogen oxide from endothelial cells, leading to an increased blood volume. It is possible that ETBR expression in the smooth muscle cells is a compensatory mechanism to facilitate contraction of blood vessels and oppose the overdilation. Analysis of ETBR by Western blot confirmed the increase in expression following CBDL. Though we observed immune-positive staining for both ETAR and ETBR in fat tissue, this would not affect our Western blot data because the fat tissue in mesentery cannot be dissolved by the RIPA lysis buffer and was discarded during homogenization.

ET-1 binds to its Gq protein-coupled receptor to send an extracellular signal into the cell, an event which is limited by the sensitivity of the receptor. Under normal conditions, the receptor is desensitized to prevent excessive stimulation by vasoactive substances. Available experimental evidence demonstrates that augmentation of receptor sensitivity, by factors like norepinephrine, vasopressin, ET-1 or angiotensin, impairs the transduction of vasoconstrictor signals and promotes dilation of the splanchnic vascular bed[24]. It is known that GRKs and arrestins are key participants in the canonical pathways leading to phosphorylation-dependent or independent G protein-coupled receptor desensitization and endocytosis[28]. GRK2 is one member of the GRK family and has been shown to be able to specifically phosphorylate human ETAR and ETBR[20]. Our findings demonstrate that the increased PP following CBDL resulted in an upregulation of mesenteric GRK2 expression, but not β-arrestin 2. GRK2 and β-arrestin 2 may modulate ETAR and ETBR desensitization through the following mechanisms: (1) phosphorylation of ETAR by GRK2 promotes the receptor binding to β-arrestin 2, which blocks the activation of the G proteins and leads to rapid homologous desensitization; and (2) independent of phosphorylation, GRK2 interacts with G alpha(q) directly, which results in uncoupling of ET-1 receptor and its associated G proteins, thus impairing the ET-1 signal transduction pathway[29]. Increased GRK2 expression in the mesentery of

PHT rats not only results in desensitization of ETAR, but also the receptors of norepinephrine and angiotensin, which indicates that such vasoactive substances are unable to mediate vasoconstriction, regardless of ligand concentration or the level of receptor expression.

In summary, PHT induced by CBDL in rats was associated with decreased levels of ET-1 in the mesenteric circulation, and increased mesenteric expression of ETBR and GRK2. We conclude that these changes underlie mesenteric vasodilation in an animal model of PHT, and are also applicable to patients with this condition. We interpret our data to indicate that the ET-1 signaling pathway is an important factor in the development of splanchnic vasodilation associated with PHT. These findings have major therapeutic implications not only for individuals with liver disease, but also for other diseases with vascular dysfunction.

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COMMENTS

Background

Portal hypertension (PHT) is a life-threatening condition which frequently develops in patients with liver cirrhosis, and has limited treated options. During PHT, vasodilation results in increased blood flow into the mesenteric circulation, thereby increasing flow into the portal circulation, which can worsen PHT. For many years, endothelin-1 (ET-1) has received considerable interest in the area of liver cirrhosis for its potential contribution to PHT. The aim of the present study was to directly examine the expression of ET-1 and its receptors in the mesentery of rats with PHT, and to clarify how the ET-1 signaling system changed with the development of PHT.

Research frontiers

PHT can give rise to many other severe and often lethal conditions, such as bleeding esophageal varices. Increased resistance to portal blood flow is the primary factor leading to PHT and is aggravated by a hyperdynamic, vasodilated, splanchnic circulation. Though the pathophysiology of PHT is becoming better understood, excepting β-receptor blockers, there is no effective treatment approach for PHT. One reason to explain this phenomenon is that the mechanism of splanchnic vasodilation is unclear. So in this study, the authors choose mesentery tissue of hypertensive rats to research the mechanisms of vasodilation based on ET-1 and its receptors.

Innovations and breakthroughs

The current literature has indicated a possible role for ET-1 signaling in splanchnic vasodilation, though there is a lack of experimental data to support this hypothesis. This is the first *in vivo* study examining the expression of the ET-1 signaling system in the mesentery of PHT rats.

Applications

These findings have major therapeutic implications not only for individuals with liver disease, but also for other diseases with vascular dysfunction.

Terminology

PHT is the main complication of cirrhosis and is defined as a hepatic venous pressure gradient (HVPG) of more than 5 mmHg. Clinically significant PHT is defined as HVPG of 10 mmHg or more; Hyperdynamic circulation: The hyperdynamic circulatory state of PHT is characterized by splanchnic and peripheral vasodilation, increased plasma volume and increased cardiac output.

Peer review

In this paper, the authors show that expression of G protein coupled kinase-2 is downregulated while expression of the endothelin B receptor is increased in the mesentery after common bile duct ligation, a model for PHT. These findings are interesting and might represent some mechanisms underlying vasodilatation in the mesentery.

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BRIEF ARTICLE

Clinicopathological and prognostic significance of galectin-1 and vascular endothelial growth factor expression in gastric cancer

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Abstract

AIM: To evaluate the expression of galectin-1 and vascular endothelial growth factor (VEGF) in gastric cancer and investigate their relationships with clinicopathologic factors and prognostic significance.

METHODS: Galectin-1 and VEGF were immunohistochemically investigated in tumor samples obtained from 214 gastric cancer patients with all tumor stages. Immunohistochemical analyses for galectin-1 and VEGF expression were performed on formalin-fixed, paraffin-embedded sections of surgical specimens. The relationship between the expression and staining intensity of galectin-1 and VEGF, clinicopathologic variables, and patient survival were analyzed. All patients underwent follow-up until cancer-related death or more than five years after tumor resection. P values < 0.05 were considered statistically significant.

RESULTS: Immunohistochemical staining demonstrated that 138 of 214 gastric cancer samples (64.5%) were positive for galectin-1, and 116 out of 214 gastric cancer samples (54.2%) were positive for VEGF. There was a significant association between galectin-1 and VEGF expression; VEGF was detected in 60.1% of galectin-1-positive samples and 43.4% of galectin-1-negative samples (P < 0.05). Galectin-1 expression was associated with tumor size, tumor location, stage, lymph node metastases, and VEGF expression (all P < 0.05). VEGF expression was related to tumor size, stage, and lymph node metastases (all P < 0.05). The 5-year survival rate was 56.6% for galectin-1-positive patients and 69.2% for galectin-1-negative patients, and the prognosis for galectin-1-positive patients was significantly poorer compared with galectin-1-negative patients (χ^2 = 13.880, P = 0.000). The 5-year survival rates for VEGF-positive and VEGF-negative patients were 53.4% and 70.5%, respectively (χ^2 = 4.619, P = 0.032). The overall survival rate of patients with both galectin-1 and VEGF overexpression in gastric cancer tissue samples was significantly poorer than other groups (both P < 0.05).

CONCLUSION: Galectin-1 expression was positively associated with VEGF expression. Both galectin-1 and VEGF can serve as independent prognostic indicators of poor survival for gastric cancer after gastrectomy.

Key words: Galectin-1; Vascular endothelial growth factor; Gastric cancer; Prognosis

Core tip: Galectin-1 and vascular endothelial growth factor (VEGF) played important roles in angiogenesis and progression of malignant tumor, while their expression in Chinese gastric cancer and relationship between the two parameters and clinicopathological features, as well as prognostic value remained largely unknown. In this present study, we examined 214 gastric cancer samples

for the presence of galectin-1 oncoprotein and VEGF by immunohistochemistry and found that overexpressions of galectin-1 and VEGF were related with tumor progression and poor survival, and our findings supported an association between galectin-1 and VEGF expression. These two molecules may serve as independent predicative markers for patient prognosis in gastric cancer.

Chen J, Zhou SJ, Zhang Y, Zhang GQ, Zha TZ, Feng YZ, Zhang K. Clinicopathological and prognostic significance of galectin-1 and vascular endothelial growth factor expression in gastric cancer. *World J Gastroenterol* 2013; 19(13): 2073-2079 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i13/2073.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i13.2073

INTRODUCTION

Gastric cancer is one of the most common cancers in the world. It is the second leading cause of cancer death after lung cancer[1,2]. The incidence of gastric cancer is highest in Eastern Asia, including China, Japan and South Korea[3]. Despite advances in diagnostic techniques, neoadjuvant chemoradiotherapy, and surgery, the 5-year survival rate for gastric cancer remains poor, particularly in more advanced stages[4]. Recently, therapeutic strategies have been improved by the availability of monoclonal antibodies. Additionally, studies have evaluated new biologic and molecular targets for their potential roles as prognostic markers and targets for therapy in patients with gastric cancer.

Galectin-1, a β-galactoside-binding protein, is a 14 KDa homodimer and the first protein discovered in the galectin family[5-7]. Accumulating evidence clearly shows that galectin-1 is involved in numerous essential cancer-related processes, including immunosuppression, angiogenesis, and metastasis[8-15]. Galectin-1 overexpression in tumor stromal cells has been detected in several malignant tumors, such as colon cancer[16], breast cancer[17], hepatocellular cancer[18], prostate cancer[19], and pancreatic ductal adenocarcinoma[20]. Furthermore, high galectin-1 expression was shown to correlate with poor survival in several types of cancer[21-25].

Vascular endothelial growth factor (VEGF) is an angiogenic factor produced by tumor cells that stimulates intratumoral microvessel proliferation. Angiogenesis is a fundamental process in tumor growth and metastasis, and it contributes to the metastatic process by providing large numbers of leaking blood vessels for vascular invasion[26,27]. VEGF is the most potent and specific promoter of tumor angiogenesis[28]. It is able to stimulate the growth of epithelial cells of various origins, promote vasculature construction, and enhance blood vessel permeability, especially microvessels[29]. A few published studies have shown that VEGF overexpression in gastric cancer is associated with poor prognosis[30-32]. However, no previous studies have clarified the correlation between galectin-1 and VEGF overexpression in gastric cancer. In this study, we performed immunohistochemical staining and extensively examined galectin-1 and VEGF expression in gastric cancer tissues. The aims of this study were to determine whether the expression levels of galectin-1 and VEGF were correlated with each other and with clinicopathological features and prognosis, including patient survival.

MATERIALS AND METHODS

Patient information

From January 2004 to October 2006, a total of 214 patients with gastric cancer who underwent gastrectomy at the Department of General Surgery of the Affiliated Hospital of Jiangsu University were enrolled in this retrospective study. There were 129 men and 85 women between the ages of 31 and 84 years (mean, 64.5 years). None had received chemotherapy or radiotherapy before surgery. Follow-up was completed on 30 October 2012. Patient clinicopathologic parameters were collected, including age, gender, differentiation, and pathological tumor-node-metastasis classification (according to the International Union Against Cancer).

Immunohistochemistry

Immunohistochemical analyses of galectin-1 and VEGF expression were performed on formalin-fixed, paraffin-embedded sections of surgical specimens. Tissue microarray blocks were serially cut into 4 μm-thick sections and stained. Paraffin sections were deparaffinized in xylene and rehydrated in a gradient of ethanol solutions. Endogenous peroxidases were blocked with 3% hydrogen peroxide in methanol for 10 min. The slides were immersed in 10 mmol/L citrate buffer (pH 6.0) and heated for 30 min for antigen retrieval. The slides were then cooled at room temperature for 20 min and washed with phosphate-buffered saline (PBS). Non-specific binding was blocked by pre-incubation with 10% fetal calf serum in PBS with 0.01% sodium azide. The slides were then incubated in a humidified chamber for 1 h with antibodies against galectin-1 (titer 1:100, New Castle, United Kingdom) and VEGF (titer 1:50, DakoCytomation, Denmark). After washing three times in PBS, the slides were incubated with the envision-HrP complex (undiluted; Dako) for 60 min and visualized with diaminobenzidine and counterstained with hematoxylin. For substitute negative controls, the primary antibodies were replaced with PBS. Positive controls were provided by the kit supplier. The results were assessed by two independent pathologists who had no knowledge of the patient clinical status.

Evaluation of immunohistochemical staining

A scoring system was used to evaluate the immunoreactivity of gastric cancer. Galectin-1 staining was scored semiquantitatively using the following criteria: 0, no staining and less than 10% of tumor cells or stromal cells with membrane staining; 1+, more than 10% of tumor cells or stromal cells with faint partial membrane staining; 2+,

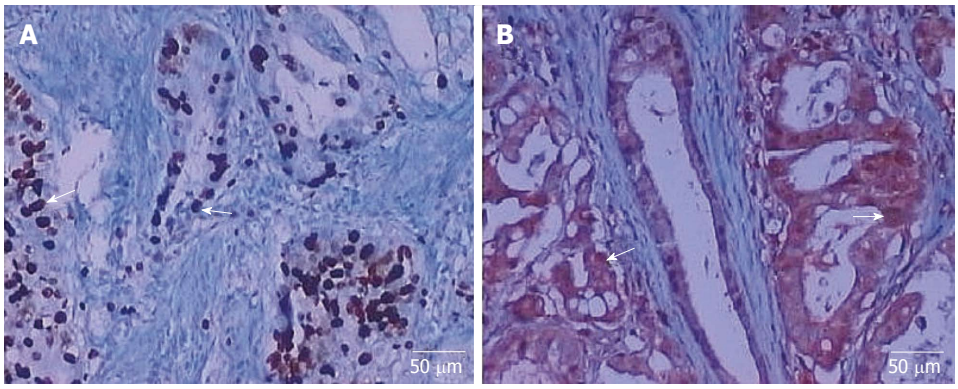

Figure 1 Immunohistochemical staining for galectin-1 and vascular endothelial growth factor (original magnification, × 200). A: Positive galectin-1 expression in gastric cancer tissue; B: Positive vascular endothelial growth factor expression in gastric cancer tissue. The over-expressed markers are shown with arrows.

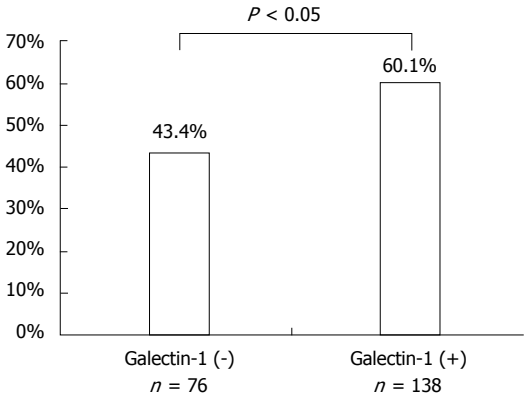

Figure 2 Vascular endothelial growth factor was expressed more frequently in galectin-1-positive gastric cancers than galectin-1-negative.

more than 10% of tumor cells or stromal cells with weak to moderate partial membrane staining; 3+, more than 10% of tumor cells or stromal cells with strong partial membrane staining. Specimens with scores of 0 or 1+ were considered negative, and scores of 2+ or 3+ were considered positive for galectin-1 expression. VEGF staining was considered positive when at least 10% of the tumor cells were stained, as previously described[33,34].

Follow-up

Patients underwent continuous follow-up up to September 2012. No patients were lost to follow-up. The median follow-up duration was 48.5 mo (range, 0-60 mo) after surgery.

Ethics

This work was performed in accordance with the Declaration of Helsinki of the World Medical Association. This study was ethically approved by the Affiliated Hospital of Jiangsu University (JSUH-EC-189923). All patients provided written informed consent.

Statistical analysis

Statistical analyses were performed with SPSS 16.0 for Windows (SPSS, Chicago, IL, United States). The correlations between galectin-1 and VEGF expression and clinicopathological features were analyzed using the χ^2 test. The Kaplan-Meier test was employed to evaluate the survival rate, and the survival rate curves were compared using the log-rank test. P values < 0.05 were considered statistically significant.

RESULTS

Galectin-1 and VEGF expression in gastric cancer tissues

Galectin-1 expression in tumor stromal cells was detected in 197 (92.1%) of 214 tumor tissues. Galectin-1 expression was positive in 138 out of 214 gastric cancer samples (64.5%) and negative in the remaining 76 samples (35.5%); 86 samples were 2+ (40.2%), and 52 were 3+ (24.3%). VEGF expression was positive in 116 of 214 gastric cancer samples (54.2%) and negative in the remaining 98 samples (45.8%); 30 samples were 1+ (14.0%), 53 were 2+ (24.8%), and 33 were 3+ (15.4%). Figure 1 shows galectin-1 and VEGF staining in gastric cancer tissues.

Correlation between galectin-1 and VEGF expression and clinicopathological features

There was a significant association between galectin-1 and VEGF expression; VEGF was detected in 60.1% of galectin-1-positive tumors and 43.4% of galectin-1-negative tumors (P < 0.05, Table 1, Figure 2). The correlations between galectin-1 and VEGF expression and clinicopathological features are shown in Table 2. Galectin-1 expression was positively associated with tumor size, tumor location, stage, and lymph node metastases (all P < 0.05), but it was not correlated with gender, age, or differentiation grade (all P > 0.05). VEGF expression was positively correlated with tumor size, stage, and lymph node metastases (all P < 0.05), but it was not correlated with the other clinicopathological features assessed (all P > 0.05).

Correlation between galectin-1 and VEGF expression and patient survival

All patients underwent follow-up until cancer-related death or more than five years after tumor resection. The median follow-up interval was 48.5 mo. The 5-year sur-

Table 1 Relationship of galectin-1 and vascular endothelial growth factor expression in gastric cancer tissues

VEGF	Galectin-1		*P* value
	Positive (n = 138)	Negative (n = 76)	
Positive (n = 116)	83	33	
Negative (n = 98)	55	43	0.022

VEGF: Vascular endothelial growth factor.

vival rate was 56.6% for galectin-1-positive patients and 69.2% for galectin-1-negative patients, and the prognosis for galectin-1-positive patients was significantly poorer than that of galectin-1-negative patients (χ^2 = 13.880, P = 0.000). The 5-year survival rates for VEGF-positive and VEGF-negative patients were 53.4% and 70.5%, respectively (χ^2 = 4.619, P = 0.032). Additionally, VEGF-positive patients had a shorter survival time than VEGF-negative patients. The cumulative overall survival rates for these two populations were determined (Figure 3A and B).

To evaluate the combined effect of galectin-1 and VEGF expression on the prognosis of gastric cancer, we classified patients into four subgroups according to galectin-1 and VEGF expression: group Ⅰ, low expression of both markers; group Ⅱ, high galectin-1 expression and low VEGF expression; group Ⅲ, low galectin-1 expression and high VEGF expression; and group Ⅳ, high expression of both markers. We found that the 5-year survival rate in group Ⅳ was 40.9%, which was significantly lower compared with groups Ⅰ (53.5%), Ⅱ (49.1%), and Ⅲ (48.5%) (Figure 3C, P < 0.05).

DISCUSSION

Despite the development of surgical techniques and new cytotoxic agents, which have improved the prognosis of gastric cancer, once patients develop resistance to chemotherapeutic regimens, no other treatment options are available. Given the frequent failure of conventional treatment strategies, many cancer-related molecules have been characterized with the goal of developing novel anticancer therapies[35]. To guide clinical decision making in therapy and prognosis prediction, efforts have been made to identify prognostic biomarkers for patients with gastric cancer.

Galectin-1 is a β-galactoside-binding protein that is abundantly secreted by almost all types of malignant tumor cells. Galectin-1 expression is regulated by hypoxia-inducible factor-1, and it plays vital protumorigenic roles within the tumor microenvironment. Furthermore, galectin-1 suppresses T cell-mediated cytotoxic immune responses and promotes tumor angiogenesis. Recent evidence has demonstrated that galectin-1 plays an important role in tumor progression and metastasis[36]. The amplification and overexpression of galectin-1 have been demonstrated in several tumors, including colon cancer, breast cancer, and hepatocellular cancer. The frequency

Table 2 Relationship of galectin-1 and vascular endothelial growth factor expression to clinicopathological variables in gastric cancer tissues

Variable	Galectin-1		*P* value	VEGF		*P* value
	(+)	(-)		(+)	(-)	
Age (yr)			0.200			0.784
≤ 60	58	39		54	48	
> 60	80	37		62	50	
Gender			0.308			0.583
Male	87	42		62	48	
Female	51	34		54	50	
Tumor size			0.026			< 0.001
< 3 cm	43	36		48	73	
≥ 3 cm	95	40		68	25	
Tumor location			0.004			0.287
Upper third	18	3		35	21	
Middle third	60	50		44	38	
Lower third	60	23		37	39	
Differentiation			0.112			0.998
Well	12	14		30	25	
Moderate	53	27		26	22	
Poor	73	35		60	51	
TNM stage			< 0.001			< 0.001
T1	3	37		25	65	
T2-T4	135	39		91	33	
Lymph node status			< 0.001			0.002
Positive	30	46		66	35	
Negative	108	30		50	63	

TNM: Tumor-node-metastasis; VEGF: Vascular endothelial growth factor.

of positivity appears to increase with the clinical stage of the disease and is associated with a worse prognosis[16-18]. However, the prevalence of galectin-1 overexpression in gastric cancer and its relationship with prognosis is not clear. There are only two studies in the literature evaluating the correlation between galectin-1 expression and survival. In these studies, galectin-1 expression in tumor cells was significantly correlated with short survival in astrocytic neoplasms and colon cancer[16,37]. In the present study, we examined 214 gastric cancer samples for the presence of the galectin-1 oncoprotein by immunohistochemistry. In all, 138 samples (64.5%) showed positive galectin-1 expression, and galectin-1 expression was related to tumor size, differentiation grade, stage, and lymph node metastases, suggesting that this protein may participate in tumor growth and distant metastasis. We also confirmed a significant prognostic value of galectin-1 in gastric cancer using a Kaplan-Meier survival analysis. The outcome of galectin-1-positive patients was significantly poorer than galectin-1-negative patients. Thus, detecting galectin-1 expression in gastric cancer tissues might be helpful for predicting patient prognosis.

Angiogenesis is essential for tumor growth and metastasis[38]. VEGF is the most potent angiogenic factor identified to date. Tumor angiogenesis and neovascularization require VEGF expression[39]. VEGF is primarily secreted by tumor cells, and its functions are largely restricted to endothelial cells[40]. VEGF strongly stimulates the growth of endothelial cells, leading to the formation of new blood vessels and providing essential nutrients

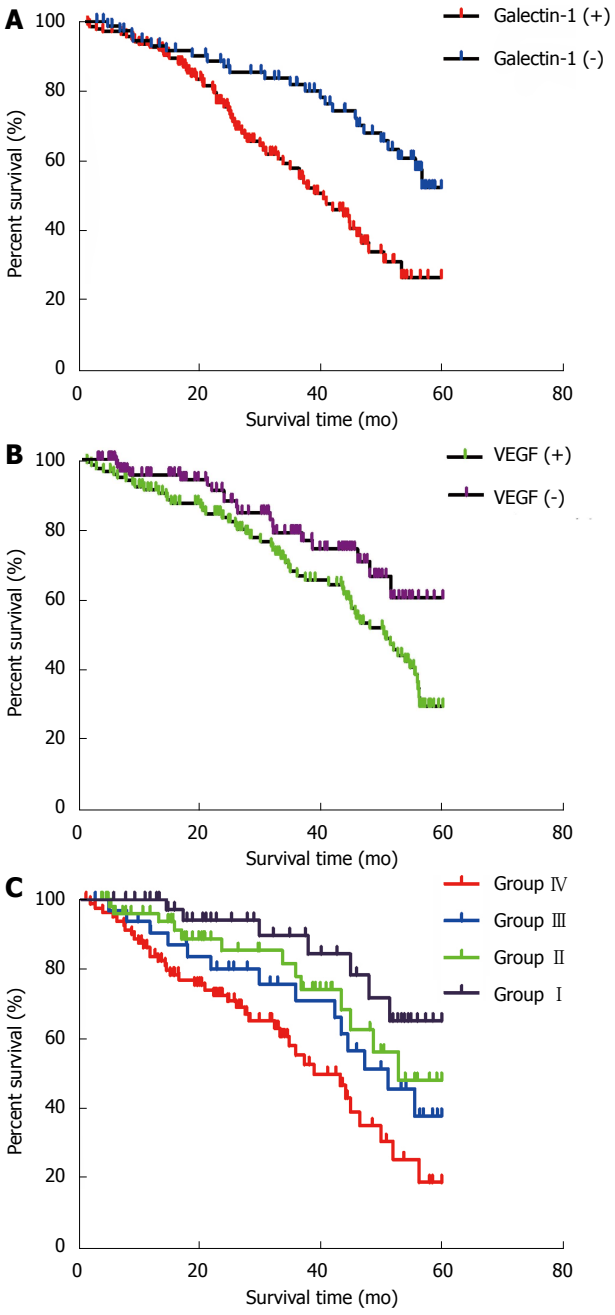

Figure 3 Overall survival rate of patients. A: The overall survival rate of patients relative to galectin-1-positive expression and galectin-1-negative expression in gastric cancer tissue samples. Galectin-1 overexpression was significantly associated with poor patient survival ($P < 0.05$); B: The overall survival rate of patients relative to vascular endothelial growth factor-positive expression and vascular endothelial growth factor -negative expression in gastric cancer tissue samples. Vascular endothelial growth factor overexpression was significantly associated with poor patient survival ($P < 0.05$); C: The overall survival rates of patients relative to groups Ⅰ, Ⅱ, Ⅲ and Ⅳ. Both galectin-1 and vascular endothelial growth factor overexpression was significantly associated with poor patient survival ($P < 0.05$).

for tumor growth. Therefore, VEGF-based antiangiogenesis therapy may be of therapeutic benefit against solid tumors and has been tested in several tumor types, including gastric cancers. In our study, VEGF expression was detected in more than half of gastric cancers (54.2%). Both the incidence and proportion of VEGF expression increased with the progression of gastric cancer, and it was correlated with tumor size, stage, and lymph node metastases. VEGF expression has been identified as a significant marker for tumor recurrence and reduced survival independent of conventional clinicopathological variables in gastric cancer[41,42]. In this study, using Kaplan-Meier analysis, we also demonstrated a significant association between VEGF expression and poor survival.

VEGF is one of the most potent inducers of angiogenesis, whereas galectin-1 has been implicated in the regulation of VEGF. Koopmans *et al*[43] demonstrated that galectin-1 activation led to the translational up-regulation of VEGF and increased angiogenesis through the JAK/STAT pathway in myeloproliferative neoplasia. Fischer *et al*[44] demonstrated that galectin-1 inhibited rearranged during transfection and Janus kinase 2 signals and up-regulated vascular endothelial growth factor receptor 3 signaling in trophoblast tumor cells. Hsieh *et al*[45] found that galectin-1 was overexpressed in the connective tissue surrounding cancer cells in tumor-associated vascular endothelial cells. Galectin-1 can increase angiogenesis by interacting with neuropilin-1 on the endothelial cell surface. Galectin-1 binding to neuropilin-1, which acts as a co-receptor of VEGF in endothelial cells, enhances VEGF receptor phosphorylation and the subsequent activation of mitogen-activated protein kinases[16]. However, few studies have evaluated the correlation between VEGF and galectin-1 in gastric cancer.

The present study showed that VEGF expression was increased in galectin-1-positive tumors compared to galectin-1-negative tumors. Meanwhile, galectin-1 expression was also increased in VEGF-positive tumors compared to VEGF-negative tumors. Galectin-1 expression was positively associated with VEGF expression. Galectin-1 and VEGF played concordant roles in tumor angiogenesis, progression, metastasis, and prognosis, which suggests a connection between them. Our results also indicated that galectin-1 and VEGF overexpression was significantly correlated with poor survival in Chinese gastric cancer patients, especially patients with both galectin-1 and VEGF expression. Therefore, detecting galectin-1 and VEGF expression might help to identify gastric cancer patients with a poor prognosis and could therefore be a novel prognostic marker. To date, the galectin-1 regulatory mechanism of VEGF in gastric cancer has not been well explored and requires further study.

COMMENTS

Background

Galectin-1 and vascular endothelial growth factor (VEGF) played important roles in angiogenesis and progression of malignant tumor. The high expression of galectin-1 and VEGF are correlated with disease behavior in some cancers, while their expression in Chinese gastric cancer and relationship between the two parameters and clinicopathological features, as well as prognostic value remained largely unknown.

Research frontiers

Even with the advancement of diagnosis, neoadjuvant chemoradiotherapy and surgery, the 5-year survival for gastric cancer remains poor, especially in more

advanced stages. Recently therapeutic strategies have been improved by the availability of monoclonal antibodies. Researches have been evaluating new biologic and molecular targets for their potential role as prognostic markers and as targets for therapy in patients with gastric cancer.

Innovations and breakthroughs

Given the frequent failure of conventional treatment strategies, many cancer-related molecules have been characterized with the goal of developing novel anticancer therapies. In order to guide clinical decision-making in therapy and prognosis prediction, efforts have been invested in identifying prognostic biomarkers for patients with gastric cancer. Galectin-1 is a β-galactoside binding protein that is abundantly secreted by almost all types of malignant tumor cells. The expression of galectin-1 is regulated by hypoxia-inducible factor-1 (HIF-1) and it plays vital protumorigenic roles within the tumor microenvironment. However, the prevalence of galectin-1 overexpression in gastric cancer as well as its relationship with prognosis is not clear. There are only two studies in the literature evaluating the correlation between galectin-1 expression and survival. In these studies, galectin-1 expression in tumor cells significantly correlated with short survival in astrocytic neoplasms and in colon cancer. In this present study, the authors examined 214 gastric cancer samples for the presence of galectin-1 oncoprotein by immunohistochemistry.

Applications

The study aimed at evaluating the expression of galectin-1 and VEGF in gastric cancer by immunohistochemical methods. The authors found that overexpressions of galectin-1 in tumor stroma cells and VEGF in tumor cells were related with tumor progression and poor survival in gastric cancer, and our findings supported an association between galectin-1 and VEGF expression. These two molecules may serve as independent predicative markers for patient prognosis in gastric cancer.

Terminology

Galectin-1 is a β-galactoside binding protein that is abundantly secreted by almost all types of malignant tumor cells. The expression of galectin-1 is regulated by HIF-1 and it plays vital protumorigenic roles within the tumor microenvironment. Galectin-1 suppresses T cell-mediated cytotoxic immune responses and promotes tumor angiogenesis. VEGF is the most potent angiogenic factor identified so far. Tumor angiogenesis and neovascularization require VEGF expression. VEGF is mainly secreted by tumor cells with its functions largely restricted to endothelial cells, and it strongly stimulate the growth of endothelial cells leading to the formation of new blood vessels, thus providing essential nutrients for tumor growth.

Peer review

This manuscript describes convincingly the expression of galectin-1 and VEGF in gastric cancer patients. The paper is well prepared and its publication in the journal is recommended with minor corrections.

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BRIEF ARTICLE

Synchronous *vs* sequential laparoscopic cholecystectomy for cholecystocholedocholithiasis

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Abstract

AIM: To compare synchronous laparoscopic cholecystectomy (LC) combined with endoscopic sphincterotomy (EST) and sequential LC combined with EST for treating cholecystocholedocholithiasis.

METHODS: A total of 150 patients were included and retrospectively studied. Among these, 70 were selected for the synchronous operation, in which the scheme was endoscopic retrograde cholangiopancreatography combined with EST during LC. The other 80 patients were selected for the sequential operation, in which the scheme involved first cutting the papillary muscle under endoscopy and then performing LC. The indexes in the two groups, including the operation time, the success rate, the incidence of complications, and the length of the hospital stay, were observed.

RESULTS: There were no significant differences between the groups in terms of the numbers of patients, sex distribution, age, American Society of Anesthesiologists score, serum bilirubin, γ-glutamyl transpeptidase, mean diameter of common bile duct stones, and previous medical and surgical history (P = 0.54, P = 0.18, P = 0.52, P = 0.22, P = 0.32, P = 0.42, P = 0.68, P = 0.70, P = 0.47 and P = 0.57). There was no significant difference in the surgical operation time between the two groups (112.1 ± 30.8 min *vs* 104.9 ± 18.2 min). Compared with the sequential operation group, the incidence of pancreatitis was lower (1.4% *vs* 6.3%), the incidence of hyperamylasemia (1.4% *vs* 10.0%, P < 0.05) was significantly reduced, and the length of the hospital stay was significantly shortened in the synchronous operation group (3 d *vs* 4.5 d, P < 0.001).

CONCLUSION: For treatment of cholecystocholedocholithiasis, synchronous LC combined with EST reduces incidence of complications, decreases length of hospital stay, simplifies the surgical procedure, and reduces operation time.

Key words: Laparoscopic cholecystectomy; Endoscopic sphincterotomy; Endoscopic retrograde cholangiopancreatography; Cholecystolithiasis; Choledocholithiasis

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INTRODUCTION

Cholelithiasis, including cholecystolithiasis and common

bile duct stones (CBDSs), is common in clinical practice. The incidence of concurrent cholecystolithiasis and CBDSs is 10%-33%, and varies according to age[1]. Cholelithiasis can be associated with serious complications, including biliary pancreatitis and suppurative cholangitis. Therefore, it is important to regularize and improve the process of clinical diagnosis and treatment of this disease.

Laparotomy for gallbladder excision, with common bile duct (CBD) exploration or endoscopic sphincterotomy (EST) through duodenal papilla, was once the standard treatment plan for concurrent cholecystolithiasis and CBDSs. In the past 10 years, with the rapid development of laparoscopic techniques, laparoscopic cholecystectomy (LC) has become the main treatment for cholecystolithiasis. However, many studies have shown that LC combined with laparoscopic common bile duct exploration (LCBDE) has a high success rate (up to 83%-89%) for concurrent cholecystolithiasis and CBDSs. It also has many merits, such as a significantly shortened hospital time and synchronous minimally invasive surgery[2-6]. Moreover, there is no significant difference in the incidence of complications with this technique when compared with EST[7]. Unfortunately, it is not widely applied because of the complex surgical technique[3,8].

With the rapid development of endoscopic retrograde cholangiopancreatography (ERCP), a variety of operations can be chosen on the basis of the LC scheme for concurrent cholecystolithiasis and CBDS. Besides LC with LCBDE, the so-called double endoscopy joint operation is also an option, which comprises LC combined with ERCP and EST before, during, or after the operation to remove CBDSs[9-11]. The most widely used operation scheme is LC combined with preoperative ERCP and EST. This scheme often requires two hospitalizations, longer hospital stays, and correspondingly higher medical costs. Even after strict preoperative screening, a proportion of CBDS cases with preoperative diagnoses is still found to be biliary stone negative during the ERCP process. Therefore, some patients must pay unnecessary ERCP-related medical expenses and undergo potential risks of surgery[12]. In recent years, there have been reports on the laparoendoscopic rendezvous (LRV) operation to treat concurrent cholecystolithiasis and CBDSs. The LRV operation has the advantages of high stone clearance, a low incidence of complications, and reduced hospital time, but it also has disadvantages that include a complex surgical procedure and a longer single operation time[13,14].

In our study, we used synchronous LC combined with EST to treat concurrent cholecystolithiasis and CBDSs. This approach combined LRV with conventional surgical procedures to perform endoscopic retrograde bile duct intubation. We compared the efficacy and safety of synchronous LC with LRV *vs* sequential LC with the conventional operation.

MATERIALS AND METHODS

Patients

A total of 167 patients with cholecystolithiasis and CBDSs were enrolled in this study from June 2009 to October 2012 at the Second Clinical Medical School, Yangzhou University. The preliminary diagnosis was established by the clinical symptoms (abdominal pain and vomiting), signs (right upper-quadrant abdominal pain and jaundice), serum biochemical index (high bilirubin or transaminase level), and abdominal ultrasound (gallstones and suspicious CBDSs, or CBD diameter > 8 mm). All of these cases were further examined by magnetic resonance cholangiopancreatography (MRCP) to diagnose cholecystolithiasis and choledocholithiasis.

The exclusion criteria were: (1) age > 80 years or < 18 years; (2) American Society of Anesthesiologists (ASA) score[15] ≥ 4; (3) suppurative cholangitis (body temperature > 38.5 ℃, with right upper-quadrant abdominal pain and pressure pain, or hyperbilirubinemia); (4) acute pancreatitis (serum amylase 3 times higher than normal); (5) pregnancy; (6) abdominal surgical history; and (7) decompensated cirrhosis that is not suitable for endoscopic and laparoscopic surgery.

A total of 150 patients were retrospectively studied and the treatment procedure is shown in Figure 1. Among these, 70 were selected for the synchronous operation, in which ERCP was combined with EST during LC. The other 80 patients were selected for the sequential operation, in which the papillary muscle was cut under endoscopy, and then LC was performed after 24-72 h. All ERCPs were performed by one of two endoscopic technologists, while LC was performed by one of three expert surgeons. Our study was approved by the Ethics Committee of the Second Clinical Medical School, Yangzhou University, and signed informed consent was obtained from each patient for the operative procedures.

Surgical procedures

The entire procedure was performed with the patient under general anesthesia. Patients in the synchronous group were placed on a C-arm-compatible table. Pneumoperitoneum was routinely established and laparoscopic instruments were put into the peritoneal cavity. The triangle of Calot was first dissected, then the gallbladder artery was ligated close to the gallbladder side, the gallbladder duct was exposed and cut open near the CBD side to make an oblique incision, and the angiographic catheter was inserted (Figure 2A). The contrast agent was injected to confirm the presence of bile duct stones (Figure 2B). The duodenoscope was inserted into the descending part of the duodenum, and a selective CBD intubation was made. Stones were removed by balloon or basket after successful intubation, and lithotripsy or balloon expansion was carried out if it was difficult to remove the stones (Figure 2C). If selective bile duct intubation failed, a yellow zebra guide wire was intubated using an angiographic catheter under laparoscopy (Figure 2D). The yellow zebra was across the duodenal papilla to the descending part of duodenum (Figure 2E), drawn out, and plugged into the duodenum again with the end of guide wire. The duodenoscope was inserted in the descending part of duodenum through the mouth, and the guide wire was pulled

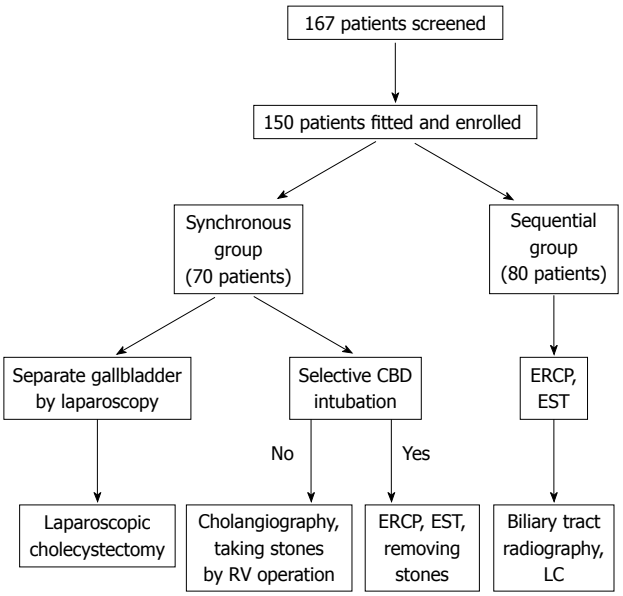

Figure 1 Treatment procedure for this study. CBD: Common bile duct; ERCP: Endoscopic retrograde cholangiopancreatography; EST: Endoscopic sphincterotomy; RV: Rendezvous; LC: Laparoscopic cholecystectomy.

out with the duodenal trap of the duodenoscope. The duodenal papillary muscle was cut with an incision knife, which followed the guide wire retrograde to the duodenal papilla (Figure 2F). Gas inside the gastrointestinal tract was exhausted at the end of the endoscopic operation, the gallbladder duct was ligated by routine laparoscopic procedure, and the gallbladder was removed.

Patients in the sequential operation group were placed in the left supine or prone position. The duodenoscope was inserted, and radiography was performed to confirm the situation of the biliary tract. The duodenal papillary muscle was cut, and stones were removed by balloon or basket. Endoscopic nasobiliary drainage was performed and biliary tract radiography was completed during 24-48 h. Residual stones were removed, and LC was carried out if no residual stone was observed.

Operation time was defined as the time from anesthesia to when the patient awoke after the operation in the synchronous group. In the sequential group, the operation time was the sum of the time for the ERCP operation before LC and the LC operation time. Major complications were defined as any intraoperative or postoperative (42 d) events that altered the clinical course, such as ERCP complications (including pancreatitis, hyperamylasemia, perforation, and bleeding) and LC complications (bile duct leakage, bleeding, pneumonia, and organ failure).

The success rate included the ERCP and LC success rates. ERCP success was defined as smoothly cannulating the CBD and achieving complete CBD stone clearance at the time of final cholangiography. LC success was defined as performing LC smoothly without converting to open surgery. Postoperative hospitalization time was the hospital time for LC combined with ERCP in the synchronous group, while it was the length of the hospital stay after ERCP in the sequential group.

Table 1 Basic characteristics and intraoperative and postoperative parameters of patients who underwent synchronous and sequential operations

	Synchronous group	Sequential group	*P* value
Total patients	70	80	
M/F ratio	46/24	53/27	0.54
Mean age, yr	59.0 (38-75)	56.6 (36-74)	0.18
ASA score (Ⅰ-Ⅱ/Ⅲ)	62/8	70/10	0.52
Symptoms			
Abdominal pain	59 (84.3)	72 (90.0)	0.22
Jaundice	51 (71.4)	62 (77.5)	0.32
Nausea or vomiting	39 (55.7)	47 (58.8)	0.42
Mean serum bilirubin, mg/dL	5.4 (0.5-24)	5.9 (0.6-27)	0.68
Mean γ-GGT, μ/dL	116.2 (27-342)	122.8 (35-396)	0.70
MRCP diagnosis			
Mean diameter of CBDS, mm	9.7 (7-21)	9.2 (6-20)	0.47
Stone number (single/multi)	49/21	56/24	0.57
Mean operative time, min	112.1 ± 30.8	104.9 ± 18.2	0.08
Success rate			
Endoscopic sphincterotomy	70 (100)	77 (96.3)	0.15
Laparoscopic cholecystectomy	69 (98.6)	80 (100)	0.74
Major complications rate			
Acute pancreatitis	1 (1.4)	5 (6.3)	0.14
Hyperamylasemia	1 (1.4)	8 (10)	0.03
Bleeding/perforation/infection	0 (0)	0 (0)	
Hospital stay, d	3 (2-6)	4.5 (3-12)	< 0.001

Data are expressed as absolute *n* (%) or median (range). M: Male; F: Female; γ-GGT: γ-glutamyl transpeptidase; ASA: American Society of Anaesthesiologists; MRCP: Magnetic resonance cholangiopancreatography; CBDS: Common bile duct stone.

Follow-up procedure

Patients were scheduled for follow-up 2 and 6 wk after surgery. During that time, no patients were lost to follow-up. The patients were reviewed by color ultrasound and for liver function. MRCP was performed if there was a question of residual bile duct stones, and stones were removed by remedial ERCP if they were confirmed.

Statistical analysis

The SPSS software package (versions 17.0, SPSS, Chicago, IL, United States) was used for all statistical analyses. Categorical variables were compared with the χ^2 test. Continuous variables were compared with the Student's *t* test or the Mann-Whitney *U* test, depending on the distribution. $P < 0.05$ was considered statistically significant.

RESULTS

The baseline characteristics of the patients are shown in Table 1. There were no significant differences between the groups in terms of the numbers of patients, sex distribution, age, ASA score, serum bilirubin, γ-glutamyl transpeptidase, mean diameter of CBDSs, and previous medical and surgical history ($P > 0.05$ each).

The intraoperative and postoperative parameters are shown in Table 1. The mean operation time in the synchronous group was 112.1 ± 30.8 min. The LRV operation was performed in 15 cases, because it was difficult to complete selective bile duct intubation during the endo-

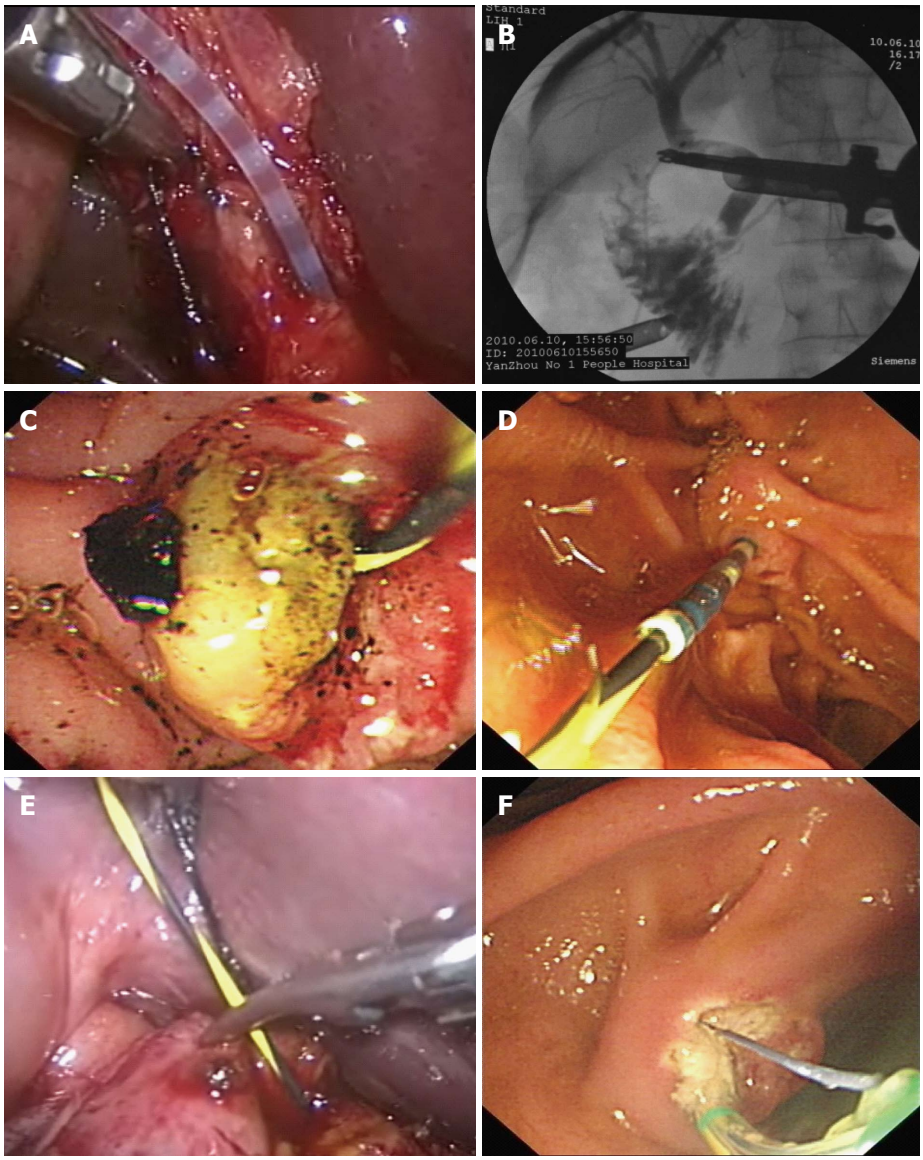

Figure 2 Surgical procedures. A: Angiographic catheter was inserted; B: Choledochography was performed; C: Stones were removed by balloon or basket; D: Yellow zebra guide wire was inserted into cystic duct; E: Angiographic catheter was inserted following the guide wire; F: Duodenal papillary muscle was cut.

scopic process, and the average operation time of the 15 cases was 132.3 ± 29.0 min. The mean ERCP operation time in the sequential operation group was 38.4 ±12.1 min, the LC operation time was 66.6 ± 14.4 min, and the overall operation time was 104.9 ± 18.2 min. There was no significant difference in the average operation time between the two groups ($P > 0.05$).

The hyperamylasemia incidence in the synchronous group was 1.4% (1/70), and 10.0% (8/80), in the sequential group, and there was a significant difference in incidence between the two groups ($P < 0.05$). The incidence of acute pancreatitis in the synchronous group was 1.4% (1/70) and 6.3% (5/80) in the sequential group, and there was a trend toward significance between the two groups ($P > 0.05$). Bleeding, perforation, death, and serious complications were not observed in either of the groups. The acute pancreatitis that occurred after the operation was mild, and it did not develop into severe pancreatitis after timely treatment.

The length of the hospital stay in the sequential group was 4.5 d (range, 3-12 d), and five patients with acute pancreatitis had lengthened hospital stays. The length of hospital stay in the synchronous group was 3 d (range, 2-6 d), which was significantly lower than that in the sequential group ($P < 0.001$).

All 150 patients were followed up for a mean 65 wk (range, 8-135 wk). At the 6-wk follow up, color Doppler ultrasound, liver function tests, and MRCP did not identify recurrence of stones and complications related to the operation, except for one patient in the synchronous group. This patient was readmitted 8 wk after the LRV procedure with residual choledocholithiasis and treated successfully with repeat ERCP and CBD clearance.

DISCUSSION

LC combined with EST is the most commonly used minimally invasive treatment for concurrent cholecystolithi-

asis and CBDS[16,17]. LC combined with postoperative EST is an important remedial treatment measure for stones, which appear in LC but are not removed by instant LCBDE. Its weakness is that EST has a greater need for operative success because, if EST fails to remove stones, patients could require additional surgical procedures. The success rate of ERCP is 85%-90%[18]. Even if the postoperative ERCP is successful, the hospitalization time is longer than for synchronization[19,20]. The scheme in most medical units is conventional LC combined with preoperative ERCP, which also has some disadvantages. Even if the preoperative ERCP is successful in removing the stones, the few cases for which LC fails still require laparotomy. If preoperative ERCP is complicated by acute pancreatitis, it is not possible to perform LC. In this study, there were five patients with acute pancreatitis in the sequential group for whom LC had to be delayed, and these patients had extended hospital stays. In addition, intraoperative exploration confirms only 27%-54% of stones, in spite of the clinical history characteristics, medical examination, serum biochemical index, abdominal ultrasound diagnosis, and CBDS preoperative examination, which means that a considerable proportion of patients incur unnecessary ERCP-related medical expenses and potential risks of surgery[12]. The ERCP serious complication rate was 2.5%-11%, and the mortality rate was 0.5%-3.7%[18].

In recent years, there have been reports that synchronous ERCP and EST are carried out in LC to treat concurrent cholecystolithiasis with CBDSs[21]. One meta-analysis of 27 published intraoperative ERCP studies including a total of 795 patients by La Greca *et al*[22] showed that the operation success rate was 69.2%-100%, with an average of 92.3%; the average intraoperative endoscopic operation time was 35 min; and the average surgical operation time was 104 min. In these 27 studies, 4.7% of cases required laparotomy, the complication incidence was 5.1%, and the mortality rate was 0.37%. Intraoperative synchronous EST in LC has no obvious differences in terms of complications, such as acute pancreatitis and hyperamylasemia, compared with sequential LC and EST operations, but it significantly improves the operation success rate, shortens the average hospitalization time, and decreases the medical treatment charges[23]. A randomized study with 120 cases of concurrent cholecystolithiasis with CBDSs observed the risk factors of postoperative ERCP-related pancreatitis, and found that no case was complicated by acute pancreatitis in synchronous surgery, and six patients suffered from iatrogenic acute pancreatitis in sequential surgery[24]. These data suggest that the synchronous operation has the advantages of high stone clearance, high success rate, and a low complication rate for treating CBDSs when compared to sequential double endoscopy.

Although synchronous surgery has obvious advantages, its implementation faces a few difficulties. First, the synchronous double endoscopy combined operation mostly uses the LRV operation during laparoscopic transcystic intubation into the filar guide and can extend the operation time. A clinical study with 45 patients showed that the average time for double endoscopy synchronous surgery was 119.09 ± 14.4 min[13]. Another study showed that the operation time for LC combined with intraoperative ERCP was 192.0 ± 8.9 min, which was 85 min longer than for separate laparoscopic gallbladder resection and CBD exploration[14]. In the beginning, we used the LRV operation, which is similar to the approach used by ElGeidie's team[25]. We found that there were certain difficulties in the operation that extended the time required. Now, we prefer LC combined with conventional endoscopic retrograde bile duct intubation, and turn to the LRV operation when there is difficulty in selective intubation. This method can avoid associated risks, including acute pancreatitis and bleeding caused by repeated intubation, contrast agent injection, and precut sphincterotomy. It can also simplify the operation process and reduce the time. In our study, there were difficulties during the selective intubation of 15 patients in the synchronous operation group, so we turned to the LRV operation. There was no difference in the operation time between the synchronous and sequential treatment groups. The incidence of hyperamylasemia and iatrogenic pancreatitis was lower in the synchronous than in the sequential operation group. Besides the operation time, time was required for the positional adjustment of the X-ray machine and endoscopic equipment by the operators. This timing can be addressed after improving the surgical process. Second, the synchronous operation required cooperation between the surgeons and endoscopic physicians. The latter must perform intraoperative ERCP immediately and synchronously with surgery once biliary angiography has confirmed CBDSs. Thus, we can try to reduce the operation time. However, clinical practice often faces certain difficulties. All of the cases in our study were diagnosed with CBDSs by MRCP preoperatively, because the surgeons, endoscopic physicians, and equipment were in the right place from the beginning. This design guaranteed the effective organization of the synchronous double endoscopy operation. The sensitivity and specificity of MRCP diagnosis in CBDS are 95% and 97%, respectively[26], and all cases diagnosed with CBDSs by MRCP were confirmed in the perioperative period in this study. Third, some researchers think that general anesthesia by endotracheal intubation is an unfavorable factor in duodenoscopy operations[15], so we used general anesthesia by nasal intubation to reduce this negative influence.

Our study also had several limitations. First, it was a retrospective study that was not performed in a double-blind and randomized fashion. Second, our work was in the preliminary stage, and it did not assess the learning curves for the two types of surgery. Third, the length of follow up was short, and the number of patients was small. Therefore, further studies with larger patient populations are needed to draw more valid conclusions.

In conclusion, we found that both synchronous and sequential laparoscopic operations combined with endoscopic operations were minimally invasive surgical proce-

dures for effective treatment of concurrent cholecystolithiasis and CBDSs. Moreover, the synchronous double endoscopy combined operation may selectively apply the LRV scheme. Synchronous surgery has advantages, such as reducing complications and shortening hospital stay, and it can also simplify the operation process and reduce the time required.

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COMMENTS

Background

Cholecystolithiasis, combined with common bile duct stones (CBDSs), is common in clinical practice. In the management of cholelithiasis, laparoscopic cholecystectomy (LC) is the treatment of choice, but the ideal management of choledocholithiasis with LC is controversial. Today a number of options exist, including endoscopic sphincterotomy (EST) before LC, laparoscopic common bile duct exploration, and postoperative endoscopic retrograde cholangiopancreatography.

Research frontiers

Several studies have shown the efficacy of the combined laparoendoscopic rendezvous (LRV) technique for treatment of cholecystolithiasis and CBDSs. Studies have demonstrated that this method has advantages of easier cannulation, prevention of pancreatic trauma, and reduced hospital time, but it also has disadvantages, including a complex surgical procedure and a longer single operation time.

Innovations and breakthroughs

In this study, the authors used synchronous LC combined with EST to treat concurrent cholecystolithiasis and CBDS, with selective application of the LRV procedure. Study data showed that synchronous surgery had advantages, such as reducing complications and shortening hospital stay, and it also simplified the surgical procedure and reduced the operation time in most cases.

Applications

Elective application of the LRV procedure in a synchronous double endoscopy combined operation is a minimally invasive surgical procedure for the effective treatment of concurrent cholecystolithiasis and CBDSs.

Terminology

LRV is a technique in which the sphincterotome is driven across the papilla into the choledochus by a Dormia basket passed into the duodenum through the cystic duct during LC.

Peer review

This was a well-designed retrospective study in which the authors compared the efficacy and safety of synchronous LC with LRV *vs* sequential LC with the conventional operation. The results are interesting and suggest that synchronous surgery has advantages, such as reducing complications, and shortening operation time and hospital stay.

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BRIEF ARTICLE

Contrast-enhanced ultrasonographic findings of hepatic paragonimiasis

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Abstract

AIM: To investigate the features of hepatic paragonimiasis on contrast-enhanced ultrasound (CEUS) imaging.

METHODS: Fifteen patients with hepatic paragonimiasis who were admitted to our hospital between March 2008 and August 2012 were enrolled to this study. The conventional ultrasound and CEUS examinations were performed with a Philips IU22 scanner with a 1-5-MHz convex transducer. After conventional ultrasound scanning was completed, the CEUS study was performed. Pulse inversion harmonic imaging was used for CEUS. A bolus injection of 2.4 mL of a sulfur hexafluoride-filled microbubble contrast agent (SonoVue) was administered. CEUS features were retrospectively reviewed and correlated with pathological findings.

RESULTS: In total, 16 lesions were detected on CEUS. The mean size of the lesions was 4.4 ± 1.6 cm (range, 1.7-6.6 cm). Subcapsular location was found in 12 lesions (75%). All the lesions were hypoechoic. Six lesions (37.5%) were of mixed content, seven (43.8%) were solid with small cystic areas, and the other three (18.8%) were completely solid. Ten lesions (62.5%) were rim enhanced with irregular tract-like nonenhanced internal areas. Transient wedge-shaped hyperenhancement of the surrounding liver parenchyma was seen in seven lesions (43.8%). Areas with hyper- or iso-enhancement in the arterial phase showed contrast wash-out and appeared hypoenhanced in the late phase. The main pathological findings included: (1) coagulative or liquefactive necrosis within the lesion, infiltration of a large number of eosinophils with the formation of chronic eosinophilic abscesses and sporadic distribution of Charcot-Leyden crystals; and (2) hyperplasia of granulomatous and fibrous tissue around the lesion.

CONCLUSION: Subcapsular location, hypoechogenicity, rim enhancement and tract-like nonenhanced areas could be seen as the main CEUS features of hepatic paragonimiasis.

Key words: Paragonimiasis; Liver; Infection; Contrast-enhanced ultrasonography

Core tip: We retrospectively investigated the contrast-enhanced sonographic features of hepatic paragonimiasis. Hepatic paragonimiasis has its own features on contrast-enhanced ultrasound. Knowledge of these findings is helpful in differentiating hypoechoic lesions in the liver. When a subcapsular hypoechoic lesion with irregular tract-like non-enhancing necrosis is presented in non-

cirrhotic liver, the diagnosis of hepatic paragonimiasis should be suspected.

Lu Q, Ling WW, Ma L, Huang ZX, Lu CL, Luo Y. Contrast-enhanced ultrasonographic findings of hepatic paragonimiasis. *World J Gastroenterol* 2013; 19(13): 2087-2091 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i13/2087.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i13.2087

INTRODUCTION

Paragonimiasis is a parasitic infestation caused by the lung fluke. Although the primary site of paragonimiasis is the lungs, ectopic infestation can occur in locations such as the brain, muscles, retroperitoneum, and liver[1-6]. The liver is known to be an organ in which ectopic paragonimiasis may occur. Hepatic paragonimiasis often appears as a mass that should be differentiated from other cancerous lesions. Contrast-enhanced ultrasound (CEUS) has been widely used in characterization of focal liver lesions (FLLs)[7-14]. The enhancement patterns of several FLLs have been described and are well known[7,15-17]. However, to the best of our knowledge, the CEUS features of hepatic paragonimiasis have not been investigated or reported in the English-language literature. In this study, we retrospectively investigated the CEUS features of hepatic paragonimiasis.

MATERIALS AND METHODS

Patients

We retrospectively reviewed the results of conventional and CEUS examination of 15 patients with hepatic paragonimiasis who were admitted to our hospital between March 2008 and August 2012. There were eight men and seven women with a mean age of 42.5 ± 12.3 years (range, 29-65 years). All patients in this study were residents of China's Sichuan Province, which is an endemic area of paragonimiasis, especially the paragonimiasis skrjabini variety, and a majority of them (10/15) had a history of eating crayfish. The study was approved by the Ethical Committee of the hospital. All the patients underwent surgery and the diagnoses were confirmed histologically.

Ultrasound examination

The conventional ultrasound and CEUS examinations were performed with a Philips IU22 scanner (Philips Medical Solutions; Mountain View, CA, United States) with a 1-5-MHz convex transducer. The CEUS imaging technique used in this study was pulse inversion harmonic imaging. The mechanical index for CEUS was 0.06. After conventional ultrasound scanning was completed, the CEUS study was performed. A bolus injection of 2.4 mL sulfur hexafluoride-filled microbubble contrast agent (SonoVue; Bracco SpA, Milan, Italy) was administered through a 20-gauge needle placed in the antecubital vein. A flush of 5 mL 0.9% sodium chloride solution was followed after the injection of SonoVue. On completion of the SonoVue injection, the timer was started simultaneously. The target lesion and surrounding liver parenchyma were observed continuously for 6 min. As previously described by Albrecht *et al*[18], the arterial phase was defined as 7-30 s after contrast agent injection; the portal phase was 31-120 s after injection; and the late phase was 121-360 s after injection. The entire CEUS examination was stored as a dynamic digital video file on the hard disk of the ultrasound system and recorded on a digital video recorder. All of the procedures were performed by Lu Q or Luo Y who had > 5 years of experience of CEUS study of the liver.

Image analysis

The diameters and echogenicity of the tumors on conventional ultrasound were recorded. The enhancing pattern and enhancement level in different phases of CEUS imaging of the lesion were reviewed. The degree of enhancement was divided into nonenhancement, hypoenhancement, isoenhancement, and hyperenhancement according to the enhancement level of the lesion compared with that of the surrounding normal liver parenchyma. Contrast enhancement patterns were classified as homogeneous, heterogeneous, and rim enhancement.

RESULTS

CEUS findings

In total, 16 lesions were detected on CEUS. The mean size of the lesions was 4.4 ± 1.6 cm (range: 1.7-6.6 cm). Subcapsular location was found in 12 lesions (75%). All the lesions were hypoechoic. Six lesions (37.5%) were of mixed content, seven (43.8%) were solid with small cystic areas, and the other three (18.8%) were completely solid. Ten lesions (62.5%) were rim enhanced with irregular tract-like nonenhanced internal areas (Figure 1). Transient wedge-shaped hyperenhancement of the surrounding liver parenchyma was seen in seven lesions (43.8%). Areas with hyperenhancement or isoenhancement in the arterial phase showed contrast wash-out and appeared hypoenhanced in the late phase.

Pathological findings

Microscopy revealed that there was an egg present in one case, but no larvae were present in any of the lesions. There were areas of track-like or sinus structures. The main pathological findings included: (1) coagulative or liquefactive necrosis within the lesion, infiltration of a large number of eosinophils with the formation of chronic eosinophilic abscesses and sporadic distribution of Charcot-Leyden crystals; and (2) hyperplasia of granulomatous and fibrous tissue around the lesion.

DISCUSSION

Hepatic paragonimiasis is an infestation caused by inges-

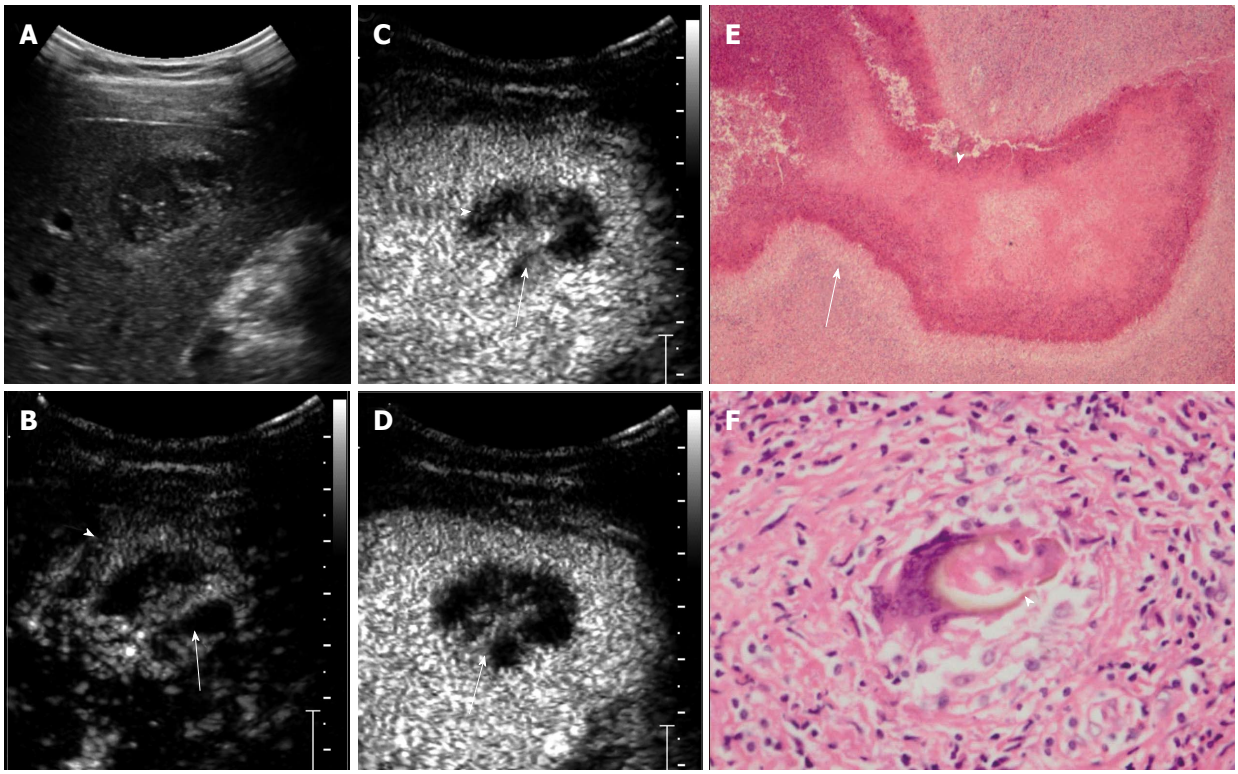

Figure 1 A 29-year-old woman with hepatic paragonimiasis. A: Hypoechoic lesion measuring 3.2 cm × 2.2 cm was seen in the right posterior inferior segment of the liver; B: Contrast-enhanced ultrasound showed rim enhancement (arrow head) and hyperenhanced internal septa (arrow) with irregular unenhanced areas in arterial phase; C and D: In portal phase (C) and late phase (D), contrast agent wash-out was seen at the enhanced septa (arrow), and the unenhanced area remained unenhanced (arrow head); E: Pathological findings showed coagulative necrosis (arrow head) within the lesion, surrounded by infiltration of a large number of barrier-like arrayed epithelioid cells (arrow); F: An egg (arrow head) was engulfed by a macrophage.

tion of raw or incompletely cooked freshwater crabs or crayfish infected with metacercariae. Only two species pathogenic to humans exist in Sichuan Province, namely, *Paragonimus skrjabini* and *Paragonimus westermani*[19]. During the journey from the intestine to the lung where juvenile worms mature, the juvenile worms often cause damage to the liver capsule and parenchyma[20,21]. Definitive diagnosis of paragonimiasis is based on the presence of eggs in patients' sputum or feces, or flukes in histological specimens. Polypide and eggs usually cannot be found in most of the lesions. However, with the epidemiological information, diagnosis can be made histopathologically[22].

The lesion is often incidentally detected by ultrasonography in routine examination. Accurate diagnosis of suspected FLLs is important to determine the most effective therapy. If hepatic infection is correctly diagnosed, the need for surgery can be reduced or even avoided, compared with other abnormalities such as malignant tumors[12,23].

Like other inflammatory lesions, hepatic paragonimiasis typically shows heterogeneous hyperenhancement in the arterial phase and hypoenhancement in the late phase on CEUS. Pathologically, the imaging feature of these lesions was eosinophilic abscesses, in which the enhanced septa in mixed-content lesions and enhanced area in solid lesions represented hyperplasia of granulomatous and fibrous tissue, whereas the unenhanced area represented necrotic debris, and Charcot-Leyden crystals.

The preponderance of subcapsular involvement and tract-like necrosis is characteristic and it may be attributed to the penetrating behavior of juvenile worms and eosinophilic abscess. The wedge-shaped enhancement in adjacent parenchyma in the arterial phase was similar to that reported by Kim *et al*[5], and can be explained as inflammatory congestion adjacent to eosinophilic abscess[19].

When a hypoechoic lesion in the liver is encountered by sonographic imaging, the differential diagnoses should include hepatocellular carcinoma, pyogenic abscesses, and hemangioma. In hepatocellular carcinoma, the hepatic parenchyma is more likely to be cirrhotic[24]. Necrosis is readily visible by CEUS and is less common in small hepatocellular carcinoma. In pyogenic abscess, fever and pain in the right upper abdomen are more frequent[12]. On CEUS, nonenhancing abscess and enhancing septa are often seen in pyogenic abscess, and lobulated abscess coalesces into a larger abscess cavity, whereas the eosinophilic abscess of hepatic paragonimiasis is irregular and arranged in tract-like fashion. Hepatic hemangioma may present as a hypoechoic lesion, whereas the CEUS manifestations typically show peripheral nodular enhancement in the arterial phase and gradual filling in the portal phase and hyperenhancement in late phase.

In our review of the literature, besides the imaging findings, blood eosinophilia was often seen in hepatic paragonimiasis patients, which was suggestive of parasitic

infection[21]. For patients with symptoms of acute infection, praziquantel is the drug of choice to treat paragonimiasis, whereas partial liver resection is more suitable for those who have localized lesions without acute infection symptoms[25,26].

The main limitation of this study was the small number of patients presented. Although hepatic paragonimiasis is rare, further investigation is mandatory.

In conclusion, hepatic paragonimiasis has its own features at CEUS. Thus, knowledge of these findings is helpful in differentiating hypoechoic lesions found in the liver. When a subcapsular hypoechoic lesion with irregular tract-like nonenhancing necrosis is present in noncirrhotic liver, diagnosis of hepatic paragonimiasis should be suspected.

COMMENTS

Background

Hepatic paragonimiasis is rare, but it often appears as a mass that should be differentiated from other cancerous lesions. Accurate diagnosis of suspected focal liver lesions (FLLs) is important to determine the most effective therapy. For hepatic infection, the need for surgery can be reduced or even avoided if it is correctly diagnosed, as compared with other abnormalities such as malignant tumors. Therefore, it is necessary to investigate the contrast-enhanced ultrasonography (CEUS) features of hepatic paragonimiasis.

Research frontiers

CEUS has been widely used in characterization of FLLs. The enhancement patterns of several FLLs have been described and are well known. However, the CEUS features of hepatic paragonimiasis have not been investigated or reported in the English-language literature.

Innovations and breakthroughs

The CEUS feature of hepatic paragonimiasis has been reported in this study. When a subcapsular hypoechoic lesion with irregular tract-like nonenhancing necrosis is present in noncirrhotic liver, a diagnosis of hepatic paragonimiasis should be suspected.

Applications

CEUS is a convenient and useful method for the detection and discrimination of hepatic paragonimiasis. Hepatic paragonimiasis could be better managed if ultrasound technicians and physicians are familiar with its features on CEUS.

Terminology

CEUS is the application of ultrasound contrast medium to traditional medical sonography. Microbubble contrast agents produce a unique sonogram with increased contrast due to the high echogenicity difference. CEUS can be used to image blood perfusion in organs.

Peer review

The authors described the CEUS findings of hepatic paragonimiasis. They analyzed 16 lesions of hepatic paragonimiasis, and demonstrated several specific findings. The article is well organized and well written.

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BRIEF ARTICLE

Missed diagnosis of early gastric cancer or high-grade intraepithelial neoplasia

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Abstract

AIM: To investigate the causes of missed diagnosis of early gastric cancer (EGC) or high-grade intraepithelial neoplasia (HGIN) in Chongqing, China.

METHODS: The present study summarizes 103 cases of EGC/HGIN detected by esophagogastroduodenoscopy (EGD) and pathological analysis from January 2010 to December 2011. Dimethyl silicone oil was administrated orally 15 min before the EGD procedures. The stomach was cleaned by repeated washing with saline when the gastroscope entered the stomach cavity. Suspected EGC lesions were subject to conventional biopsy sampling and pathological examinations. The correlation between lesion locations, endoscopic morphology of cancerous sites, training level of the examiners, pathological biopsies, and missed diagnosis was analyzed.

RESULTS: Twenty-three cases were missed among the 103 cases (22.23%) of EGC/HGIN. The rate of missed EGC in the gastroesophageal junction (8/19, 42.1%) was significantly higher than at other sites (15/84, 17.86%) (χ^2 = 5.253, P = 0.022). In contrast, the rate of missed EGC in the lower stomach body (2/14, 14.29%) was lower than at other sites (21/89, 23.6%), but there were no significant differences (χ^2 = 0.289, P = 0.591). The rate of missed EGC in the gastric antrum (5/33, 15.15%) was lower than at other sites (18/70, 25.71%), but there were no significant differences (χ^2 = 1.443, P = 0.230). Endoscopists from less prestigious hospitals were more prone to not diagnosing EGC than those from more prestigious hospitals (χ^2 = 4.261, P = 0.039). When the number of biopsies was < 4, the rate of missed diagnosis was higher (20/23, 89.96%) than for when there were > 4 biopsies (3/23, 13.04%) (P < 0.001). In addition, there was no significant difference in the rate of missed diagnosis in patients with 1-3 biopsy specimens (χ^2 = 0.141, P = 0.932).

CONCLUSION: Endoscopists should have a clear understanding of the anatomical characteristics of the esophagus/stomach, and endoscopic identification of early lesions increases with the number of biopsies.

Key words: Missed diagnosis; Early gastric cancer; High-grade intraepithelial neoplasia; Endoscopic diagnosis; Biopsies

Core tip: Early gastric cancer (EGC) detection rate in China is much lower than that in Japan, where > 80% of EGC is detected. How to avoid missed diagnosis of EGC is most important for digestive endoscopy practice. We found that there were many influencing factors for missed diagnosis of EGC. The most critical issue for endoscopists to avoid missed diagnosis is being cautious about each individual patient.

Ren W, Yu J, Zhang ZM, Song YK, Li YH, Wang L. Missed diagnosis of early gastric cancer or high-grade intraepithelial neoplasia. *World J Gastroenterol* 2013; 19(13): 2092-2096 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i13/2092.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i13.2092

INTRODUCTION

Gastric cancer (GC) is one of the most common malignant carcinomas, which is highly prevalent worldwide. There were 989 600 estimated new cases and 738 000 deaths in 2008, and > 70% of new cases and deaths occurred in developing countries[1-5]. Although the incidence of GC has been declining in recent years, China still has the most GC patients in the world. Recent statistics show that > 400 000 new cases of *GC* are confirmed in China annually[6-8]. The prognosis of advanced GC is poor and its 5-year survival rate is only 20%-40%, but there could be a 5-year survival rate of 90% for early gastric cancer (EGC) after surgical treatment[9]. Therefore, timely and accurate diagnosis is important for the treatment and prognosis of patients with GC. However, detection rate of EGC in China is generally about 2%-5%. Although at some hospitals in Shanghai, the detection rate of EGC has increased to 20%-28% in recent years, the detection rate is still significantly lower than that in Japan or South Korea[10,11]. Therefore, it is imperative for endoscopists to make efforts to improve the detection rate of EGC and reduce missed diagnosis in China[12]. Our study summarizes the missed cases among 103 patients with EGC or high-grade intraepithelial neoplasia (HGIN) admitted to our hospital from 2010 to 2011, and explored the causes of missed diagnosis.

MATERIALS AND METHODS

General information

From January 2010 to December 2010, gastroscopic examinations were performed on 21 500 patients in Xin Qiao Hospital, Chongqing, China and 245 of these were diagnosed with GC. EGC/ HGIN accounted for 17.56% (43/245) of all the cases of GC. From January to December 2011, gastroscopic examinations were performed on 23 000 patients and 230 were found to have GC, and 26.08% (60/230) of them had EGC/HGIN. There were 69 men and 34 women, aged 44-79 years, with an average age of 60.2 years.

Examination methods

In this study, all cases were examined by gastroscopy (Olympus H260 and PENTAX EPK-i-san, Japan). The date of examination and the results, along with the attending doctor and hospital, were recorded. Dimethyl silicone oil was administered orally, 15 min before the esophagogastroduodenoscopy procedures. The stomach was cleaned by repeated washing with saline, when the gastroscope entered the stomach cavity. Suspected EGC lesions were subject to conventional biopsy sampling and pathological examination. The shape and location of the lesions, as well as the extent and site of the biopsies were recorded. Endoscopic diagnosis was performed in accordance with the Paris endoscopic classification of superficial neoplastic lesions[13]. Pathological diagnosis of EGC/HGIN followed the 2010 version of the World Health Organization classification of tumors of the digestive system[14]. Missed diagnosis was defined as follows. Patients who were previously diagnosed with other diseases (*e.g.*, gastric polyps or chronic gastritis) at two examinations at < 3 mo apart, and were later confirmed to have EGC/ HGIN.

Statistical analysis

Data analysis was conducted using SPSS 20.0 software (Chicago, IL, United Sates). Comparison between the groups was performed by using χ^2 test or Fisher's exact probability test and $P < 0.05$ was considered statistically significant.

RESULTS

Different lesion locations correlated with different rates of missed diagnosis of EGC/HGIN

There were 23 cases of EGC/HGIN that were not found by endoscopy but were diagnosed later by pathological examination, so the overall rate of missed EGC/HGIN was 22.23% (23/103) (Table 1). In detail, 42.1% (8/19) of cases of EGC/HGIN that occurred in the gastroesophageal junction were missed, and the rate was higher than for other parts of the stomach (15/84, 17.86%; $\chi^2 = 5.253$, $P = 0.022$). The rate of missed EGC in the lower stomach body (2/14, 14.29%) was lower than at other sites (21/89, 23.60%), but there were no significant differences ($\chi^2 = 0.289$, $P = 0.591$). The rate of missed EGC in the gastric antrum (5/33, 15.15%) was also lower than at other sites (18/70, 25.71%), but there were no significant differences ($\chi^2 = 1.443$, $P = 0.230$).

Endoscopists from hospitals of different standing had different rates of missed diagnosis

Among the 23 missed cases of EGC/HGIN, 15 were found to have no abnormalities or were diagnosed with other diseases (*e.g.*, gastric polyps or chronic gastritis) by endoscopy in the less prestigious hospitals (15/23, 65.21%), but were later diagnosed with EGC/HGIN in our hospital (a more prestigious hospital). The other eight cases (8/23, 34.78%) were initially diagnosed with other diseases by endoscopy physicians in our hospital and then with EGC/HGIN after further examinations. The rate of missed diagnosis of EGC/HGIN by endoscopists from less prestigious hospitals was higher than that from more prestigious hospitals ($\chi^2 = 4.261$, $P = 0.039$) (Table 2).

Endoscopic appearance of cancerous lesions affected missed diagnosis of EGC/HGIN

The rate of missed diagnosis of 0-Ⅱc type lesions was 91.3% (21/23), which was higher than that for 0-Ⅰ (1/23, 4.35%) or 0-Ⅱb (1/23, 4.35%) lesions. However, one 0-Ⅱb lesion in the lesser curvature was missed. At the first gastroscopy examination, no cancerous lesions were found and the patient was treated for gastritis for 1 mo but the symptoms did not improve. The second gastroscopic examination was performed at the request of the

Table 1 Missed diagnosis of early gastric cancer or high-grade intraepithelial neoplasia in different parts of the stomach *n* (%)

Locations	Total EGC/HGIN	Missed cases
Gastroesophageal junction	19	8 (42.10)[a]
Upper stomach body	12	2 (16.67)
Middle stomach body	11	3 (27.27)
Lower stomach body	14	2 (14.29)[b]
Antrum of stomach	33	5 (15.15)[b]
Gastric angle	14	3 (21.42)
Total	103	23 (22.33)

[a]$P < 0.05$ *vs* non-missed diagnosis cases; [b]No statistical differences. EGC: Early gastric cancer; HGIN: High-grade intraepithelial neoplasia.

Table 2 Early gastric cancer or high-grade intraepithelial neoplasia missed by endoscopists at our and other hospitals

Lesion locations	Other hospitals	Our hospital
Gastroesophageal junction	7	1
Upper stomach body	1	1
Middle stomach body	2	1
Lower stomach body	1	1
Antrum of stomach	2	3
Gastric angle	2	1
Total	15	8

Table 3 Number of biopsies and missed diagnosis *n* (%)

Biopsies	Missed cases
1	7 (30.43)
2	7 (30.43)
3	6 (26.09)
≥ 4	3 (13.64)
Total	23 (100.00)

patient's family, and flaky red regions were observed in the middle of the gastric body near the lesser curvature. It was further confirmed as intramucosal differentiated-type GC by pathological diagnosis after endoscopic submucosal dissection.

More biopsies resulted in less missed diagnosis

There were 20 patients in whom diagnosis was missed from 1-3 biopsy specimens. The rate of missed diagnosis was 86.96% (20/23). There were three patients in whom diagnosis was missed with four biopsy specimens. The rate of missed diagnosis was 13.04% (3/23). When the number of biopsies was < 4, the rate of missed diagnosis (20/23, 89.96%) was higher than for > 4 biopsies (3/23, 13.04%) ($P < 0.001$). In addition, there was no significant difference in the rate of missed diagnosis in patients with 1, 2 or 3 biopsy specimens ($\chi^2 = 0.141$, $P = 0.932$) (Table 3).

DISCUSSION

The incidence of GC is about 30/100 000 in East Asian countries including China and Japan[1]. In some regions of China, the incidence even exceeds 100/100 000[15,16]. Every year, mass screening in Japan shows the presence of GC in a low proportion of patients receiving gastroscopic examination. It has been reported that in some Japanese hospitals that the diagnosed cases of GC account for only approximately 0.4% of the gastroscopy examinations each year[17], but the incidence of GC was 1%-1.2% in our endoscopy center, which was significantly higher than that in Japan. In addition, although there are no accurate statistics for EGC detection rate, it is believed that the rate in China is much lower than that in Japan, where > 80% of EGC is detected[17]. Many factors contribute to the low detection rate of EGC in China, but how to avoid missed diagnosis of EGC is important for digestive endoscopy practice.

We found that lesion location, training level of doctors (doctors from less prestigious hospitals and fewer years of endoscopy experience are considered to have a low level of training), lesion morphology, and the number of biopsies can affect the diagnosis when EGC is not identified. Previous studies have shown that lesion location has a significant effect on EGC missed diagnosis. Hosokawa *et al*[18-20] have conducted a survey in Fukui Hospital, where 562 cases of GC were diagnosed from 51 411 (1.05%) gastroscopic examinations, and 188 cases were confirmed as GC within the next 3 years, with an overall missed diagnosis rate of 25.8%. They have also found that doctors with < 10 years experience as an upper gastrointestinal endoscopist missed 32.4% of GC cases, but the missed diagnosis rate was only 19.5% when doctors with > 10 years of experience conducted the examination ($P < 0.01$).

Importantly, EGC in the gastric cardia or body, especially at the lesser curvature or posterior wall, is usually overlooked[18,19]. Consistent with this, we found that the rate of missed EGC in the gastroesophageal junction near the stomach side was significantly higher than at other sites. Due to the anatomical structure of the cardia, cancerous lesions in these parts are often difficult to observe when the endoscope is withdrawn or reversed. This requires that the endoscopists should carefully investigate the morphological changes in the gastric fundus near the cardia. On one hand, we should not withdraw the gastroscope too quickly. On the other hand, to avoid the shield of scope itself, it is necessary to observe from both sides of the scope when it is reversed.

Additionally, we found that the proportion of EGC occurring in the gastroesophageal junction was higher than that previously reported. This may have been due to the increased incidence of GC at this site, which needs further large epidemiological investigations. Compared to other sites, the gastric antrum is easy to expose and the rate of missed diagnosis was lower at this site. The rate of missed diagnosis in the gastric antrum was still approximately 15%, therefore, every part of the stomach should be fully and carefully investigated. We also noticed that there was a significant difference in missed diagnosis of EGC between doctors with different training levels in endoscopy, suggesting that the standardization

of endoscopy records and long-term cognitive training for EGC are crucial. The main cause of missed diagnosis of EGC in many primary hospitals is the inadequate knowledge and cognitive ability[20]. For example, in our study there were several cases of EGC that were misdiagnosed as gastric erosion. Thus, it is urgent for physicians to strengthen their endoscopic training for diagnosis of EGC. Endoscopic appearance also has a significant effect on EGC diagnosis. Compared to the protruding lesions, the depressed lesions were more prone to be missed. This may have been due to the high proportion of depressed lesions in EGC, because we found that superficially depressed lesions accounted for the vast majority of all cases of EGC. However, the rate of missed diagnosis of EGC 0-Ⅱc lesions (Ⅱc, Ⅱc + Ⅱa, Ⅱa + Ⅱc) was still significantly higher than that of 0-Ⅱ or 0-Ⅲ lesions. Although the rate of missed diagnosis of 0-Ⅱb EGC was 100% (1/1) in this study, it still calls for more observation on a larger scale. However, we can conclude that 0-Ⅱ lesions are more easily missed than 0-Ⅰ and 0-Ⅲ lesions. Number of biopsies also affected the rate of missed diagnosis. For cases with ≥ 4 biopsies, the rate of missed diagnosis was significantly lower than for those with < 4 biopsies[21]. In our study, no patients were subjected to other techniques, such as chromoendoscopy, narrow-band imaging (NBI), magnified endoscopy, and NBI + magnified endoscopy, because these new techniques have not been widely adopted in most hospitals in China. If we use these techniques, the results will be better[22-25]. In addition, whether targeted biopsy sampling can increase the positive rate of EGC/HGIN requires further research.

In summary, there are many influencing factors for the missed diagnosis of EGC. We agreed with Axon that the most critical issue for endoscopists to avoid missed diagnosis is being cautious about each individual patient[26].

COMMENTS

Background

Gastric cancer (GC) is one of the most common malignant carcinomas. China has the most GC patients in the world. The prognosis of advanced GC is poor but there could be a 90% 5-year survival rate for early gastric cancer (EGC) after surgical treatment. However, detection rate of EGC in China is very low.

Research frontiers

Research of EGC is a hotspot in digestive diseases at present and how to improve the diagnosis rate of EGC is a key problem. This study aimed to analyze the reasons for missed diagnosis of EGC and provide methods to avoid missed diagnosis. With rapid EGC research and development, more new technology will apply.

Innovations and breakthroughs

Few studies have been carried out focusing on the rate of diagnosis of EGC, due to lack of recognition. The present study was a detailed and systematic study in this field. Furthermore, the study also provides a brighter future in the diagnosis and treatment of EGC, with the development of understanding and new technology.

Applications

This study provides reference data for the diagnosis of EGC. It can be applied to gastroenterologists in hospitals of different rank. There are many influencing factors for missed diagnosis of EGC, but the most critical issue for endoscopies to avoid missed diagnosis is to be cautious about each individual patient.

Peer review

This is an important analysis of the factors involved in the missed diagnosis of EGC. The authors should use other important techniques, for example: chromoendoscopy, narrow-band imaging (NBI), magnified endoscopy, and NBI + magnified endoscopy. These techniques could change the results.

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BRIEF ARTICLE

Chemotherapy and resection for gastric cancer with synchronous liver metastases

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Abstract

AIM: To investigate the effect of surgery and chemotherapy for gastric cancer with multiple synchronous liver metastases (GCLM).

METHODS: A total of 114 patients were entered in this study, and 20 patients with multiple synchronous liver metastases were eligible. After screening with preoperative chemotherapy, 20 patients underwent curative gastrectomy and hepatectomy for GCLM; 14 underwent major hepatectomy, and the remaining six underwent minor hepatectomy. There were 94 patients without aggressive treatment, and they were in the non-operative group. Two regimens of perioperative chemotherapy were used: S-1 and cisplatin (SP) in 12 patients, and docetaxel, cisplatin and 5-fluorouracil (DCF) in eight patients. These GCLM patients were given preoperative chemotherapy consisting of two courses chemotherapy of SP or DCF regimens. After chemotherapy, gastrectomy and hepatectomy were preformed. Evaluation of patient survival was by follow-up contact using telephone and outpatient records. All patients were assessed every 3 mo during the first year and every 6 mo thereafter.

RESULTS: Twenty patients underwent gastrectomy and hepatectomy and completed their perioperative chemotherapy and hepatic arterial infusion before and after surgery. Ninety-four patients had no aggressive treatment of liver metastases because of technical difficulties with resection and severe cardiopulmonary dysfunction. In the surgery group, there was no toxicity greater than grade 3 during the course of chemotherapy. The response rate was 100% according to the Response Evaluation Criteria in Solid Tumors Criteria. For all 114 patients, the overall survival rate was 8.0%, 4.0%, 4.0% and 4.0% at 1, 2, 3 and 4 years, respectively, with a median survival time (MST) of 8.5 mo (range: 0.5-48 mo). For the 20 patients in the surgery group, MST was 22.3 mo (range: 4-48 mo). In the 94 patients without aggressive treatment, MST was 5.5 mo (range: 0.5-21 mo). There was a significant difference between the surgery and unresectable patients (P = 0.000). Three patients in surgery group were still alive at the end of the cut-off date.

CONCLUSION: Perioperative weekly DCF and SP achieved a good response, and combined with surgery, they could improve prognosis of GCLM.

Key words: Gastric cancer; Liver metastases; Surgery; Chemotherapy; Pilot study

Core tip: We investigated the effect of surgery and chemotherapy for gastric cancer with multiple synchronous liver metastases (GCLM). Perioperative weekly docetaxel, cisplatin and 5-fluorouracil and S-1 and cisplatin achieved a good response, and combined with surgery, they could improve prognosis of GCLM.

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INTRODUCTION

Surgery for gastric cancer with multiple synchronous liver metastases (GCLM) is a major challenge to every surgeon; not only because of coexisting factors, but each GCLM patient has his/her own clinicopathological features. It is difficult to determine the suitable candidates for treatment. At present, the justification for surgical resection is still controversial[1], and the prognosis is dismal. In contrast, for patients with colorectal carcinoma with liver metastases, a second liver resection is safe and feasible. Hepatic resection has been widely accepted as a potentially curative approach in patients with liver metastases of colorectal carcinoma[2].

One study demonstrated that patients with GCLM limited to one lobe, who underwent radical gastrectomy with D2 lymphadenectomy, had the most favorable outcomes following hepatic surgical treatment[3]. A further study found that the number of metastases was no longer considered to be an important predictor of long-term survival[4]. Some positive effect of liver resection in these patients seemed to imply that hepatic surgical treatment should be recommended for appropriate GCLM candidates[5-7]. The United Kingdom myoblast autologous grafting in ischemic cardiomyopathy (MAGIC) trial of perioperative chemotherapy in gastric cancer found that perioperative systemic chemotherapy improved 5-year survival from 23% to 36%[8], compared with surgery alone. What is the optimal dosing appropriate for Chinese patients, and how do we schedule perioperative chemotherapy that could improve tolerability while maintaining efficacy? In our previous pilot study, we found that liver resection combined with a weekly docetaxel-based regimen (docetaxel, cisplatin and 5-fluorouracil, DCF) were well tolerated, with a good response. In the present study, we assessed more GCLM patients who underwent aggressive treatment, in comparison with non-surgical treatment.

MATERIALS AND METHODS

From July 2007 to October 2012, 1821 patients with gastric cancer were treated in Beijing Cancer Hospital of Beijing University and Qingdao Municipal Hospital. Only patients with adenocarcinoma were enrolled in this study. Among these patients, 114 developed multiple liver metastases. The inclusion and exclusion criteria are described in our previous study[9]. Patients had adequate physical condition and received two course of preoperative chemotherapy. After effective screening with preoperative chemotherapy, 20 patients underwent curative gastrectomy and hepatectomy for GCLM. Two regimens of perioperative chemotherapy were used. Twelve patients received the S-1 and cisplatin (SP) regimen: 40 mg S-1 orally, twice daily for 3 consecutive weeks, and 60 mg/m^2 cisplatin intravenously on day 8, followed by a 2-wk rest period, within a 5-wk cycle[10]. Eight patients received the DCF regimen: 20 mg/m^2 cisplatin over 1 h; 20 mg/m^2 docetaxel, over 30 min; and 350 mg/m^2 5-fluorouracil over 15 min on day 1. This was administered weekly for 6 wk, followed by a 2-wk break[9].

According to the Japanese Research Society for Gastric Cancer guidelines, our surgical procedure was total or subtotal gastrectomy, at a minimum of 5 cm clearance. Hepatic resection with D2 lymphadenectomy was performed[11].

After surgery, two courses of chemotherapy (SP or DCF regimen) were administered. After completion of chemotherapy, patients without other distant disease, except for hepatic metastasis, underwent hepatic arterial infusion (HAI). If liver lesions progressed in the course of postoperative chemotherapy, HAI was commenced immediately. Safety evaluation was standardized by the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 (May 28, 2009). Evaluations were classified by the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines[12]. The study was approved by the medical ethics committees of Qingdao Municipal Hospital and Beijing Cancer Hospital. Written informed consent was obtained according to the principles of the institution.

Evaluation of patient survival was by follow-up contact using telephone and outpatient records. All patients were assessed every 3 mo during the first year and every 6 mo thereafter. Patient follow-up lasted until death or the cut-off date of October 1, 2012. Three patients (2.6%) were lost to follow-up, and survival information was censored at their last visit. Four (3.5%) patients were still alive and were censored at the cut-off date. The median follow-up period for the 114 patients was 10 mo (range: 2-53 mo).

Statistical analysis

Statistical analysis was performed with SPSS version 13.0 (SPSS, Chicago, Illinois, United States). For univariate analysis, binomial and categorical data were evaluated by cross-linked tables and the Fisher's exact test. Results were regarded as being statistically significant when $P < 0.05$. For survival analysis, the Kaplan-Meier method was used.

Table 1 Clinicopathological characteristics of patients with and without hepatectomy

Clinicopathological characteristics	With hepatectomy	Without hepatectomy
Sex		
Male	12	59
Female	8	35
Primary gastric tumors		
Median diameter of primary gastric tumors (cm)	4.3 (2.4-8.8)	4.5 (2.1-9.3)
Tumor location		
Upper	7	27
Lower	13	67
Pathological T-stage of the primary[1]		
pT1	2	9
pT2	4	21
pT3	12	51
pT4	2	13
N stage of the primary tumor		
N0	3	18
N1	9	48
N2	5	21
N3	3	7
Differentiation of the primary tumor		
Well	2	9
Moderate	14	64
Poor	4	21
Liver metastases		
Median diameter of liver metastases (cm)	4.1 (1.7-16)	4.5 (1.5-18)
No. of metastases		
Solitary	8	43
⩾ 2	12	51
Vascular invasion of metastases		
Present	3	26
Absent	17	68
Site of metastases		
Left lobe	4	18
Right lobe	7	31
Bilobar	9	45
Interruption of hepatic hilum		
Present	5	28
Absent	15	66

[1]According to tumor-nodes-metastasis-classification.

Table 2 Response evaluation after first two courses of preoperative chemotherapy

No. of cases	Diameter of metastases (mm^3)		Evaluation of response (according to RECIST)	Adverse events grade
	Pre-chem	Post-chem		
1	128	0-10	CR	2
2	135	56	PR	3
3	188	65	PR	1
4	64	22	PR	2
5	48	24	PR	2
6	148	38	PR	1
7	205	83	PR	1
8	162	65	PR	2
9	78	30	PR	2
10	228	94	PR	3
11	206	108	PR	2
12	144	56	PR	1
13	67	41	PR	1
14	104	67	PR	3
15	163	103	PR	2
16	134	92	PR	3
17	88	61	PR	3
18	225	134	PR	2
19	143	96	PR	2
20	78	43	PR	3

When the number of liver lesions was > 5, the diameters of the five largest lesions were summed. RECIST: Response Evaluation Criteria in Solid Tumors; CR: Complete response; PR: Partial response; Pre-chem: Pre-chemotherapy; Post-chem: Post-chemotherapy.

RESULTS

Patient characteristics

The mean age of the 114 patients was 56.7 years (range: 33-75 years), and the male to female ratio was 3.1:1. Twenty patients underwent gastrectomy and hepatectomy. These 20 patients completed their perioperative chemotherapy and HAI before and after surgery. The other 94 patients were not considered for aggressive treatment of liver metastases. In most cases (n = 91), the reason for deciding against aggressive treatment was patient refusal; the remaining three were not eligible for surgery due to severe cardiopulmonary dysfunction. There was no perioperative mortality. There were no obviously different clinicopathological characteristics between patients with and without hepatectomy.

Surgery

There were 12 male and 8 female patients in the surgery group. The median age of this group was 54 years (range: 31-74 years). Seventeen patients had a lymph-node-positive stage of the primary tumor, and only three had no lymph node involvement. There were 13 patients with distal gastric cancer, seven had proximal gastric cancer, and nine had bilobar metastases. The clinicopathological characteristics of the patients who underwent hepatectomy are listed in Table 1.

The patients in the surgery group finished two courses of SP or DCF chemotherapy before the operation. In the two courses of chemotherapy with different regimens, no patients had toxicity greater than grade 3. The most common adverse effects in the two regimens were diarrhea, nausea, leukopenia, neutropenia and thrombocytopenia, at grade 1 or 2 intensity. Most adverse effects could be modified by premedication, such as dexamethasone and antiemetics. Granulocyte colony-stimulating factor support was given to 12 patients. Response to treatment was assessed by monthly magnetic resonance imaging or computed tomography. All patients achieved a partial response according to the RECIST[12] criteria (Table 2). The response rate was 100% according to the RECIST (Figures 1 and 2). There was no treatment-related mortality.

We performed gastric and liver resection only in cases that were potentially curative. The common complications in the perioperative course were impaired wound healing (surgical therapy in two patients), and pleural effusion in four. Fourteen patients underwent major hepatectomy (hepatic resection of more than three segments:

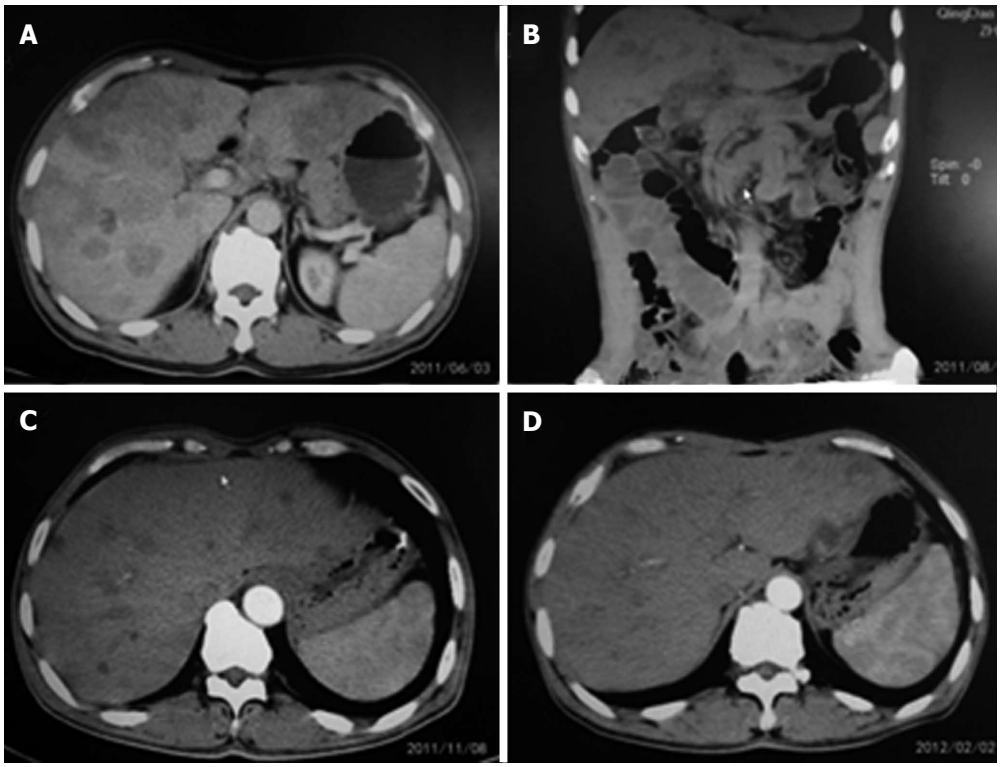

Figure 1 Patients with complete response. A: Abdominal computed tomography (CT) in gastric cancer with multiple synchronous liver metastases (GCLM) patient treated with preoperative chemotherapy (June 3, 2011); B: Abdominal CT in patient with GCLM after neoadjuvant chemotherapy (August 29, 2011); C: Abdominal CT in patient with GCLM after neoadjuvant chemotherapy (November 8, 2011); D: Abdominal CT in patient with GCLM after neoadjuvant chemotherapy (February 2, 2012).

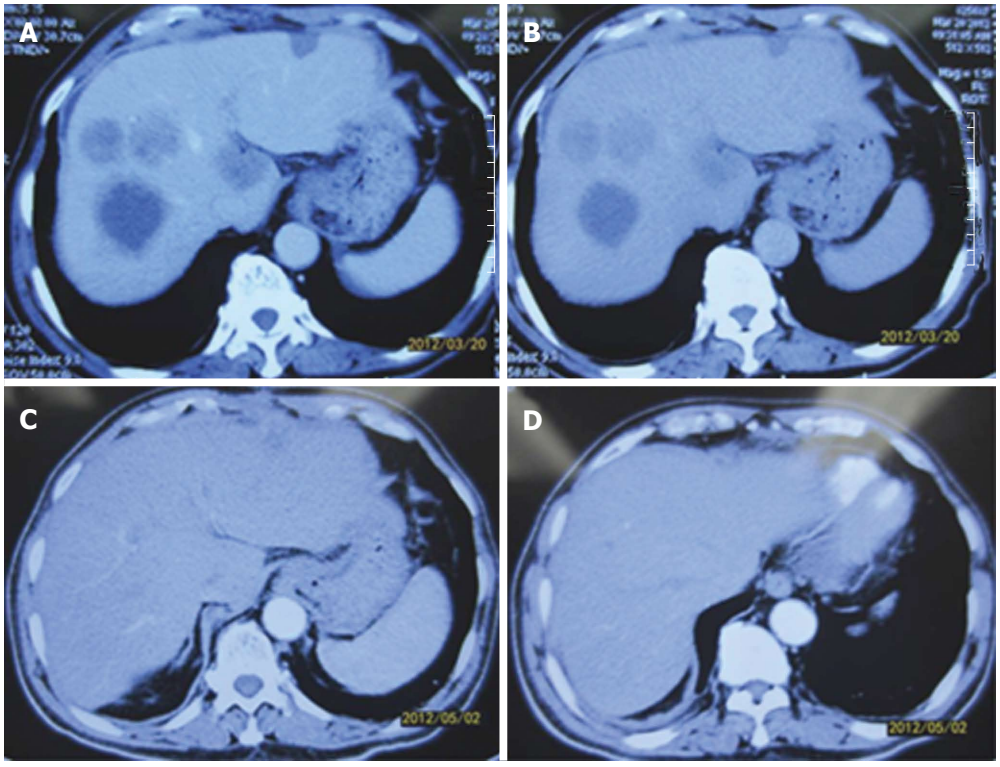

Figure 2 Patients with partial response. A, B: Abdominal computed tomography (CT) in patients with gastric cancer with multiple synchronous liver metastases (GCLM) after preoperative chemotherapy; C, D: Abdominal CT in patients with GCLM after neoadjuvant chemotherapy.

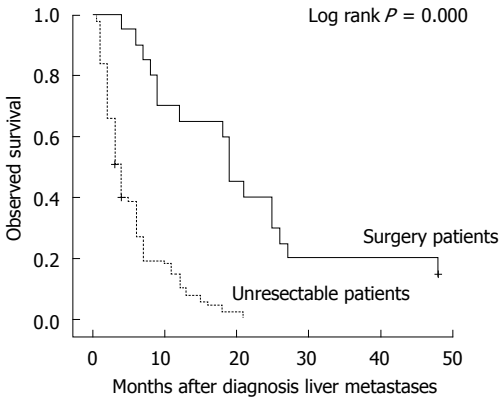

Figure 3 Overall survival of patients with hepatic metastases from gastric cancer.

hemihepatectomy in 12 and trisectionectomy in 2); and the remaining six patients underwent minor hepatectomy (sectionectomy in 2 and limited resection in 4). The types of hepatectomy were classified according to the Brisbane 2000 terminology[13].

Survival rate in surgery and nonoperative groups

For all 114 patients, the overall survival rate was 8.0%, 4.0%, 4.0% and 4.0% at 1, 2, 3 and 4 years, respectively, with an median survival time (MST) of 8.5 mo (range: 0.5-48 mo). For the 20 patients in the surgery group, MST was 22.3 mo (range: 4-48 mo). In the 94 patients without aggressive treatment, MST was 5.5 mo (range: 0.5-21 mo). A significant difference was observed between the surgery and nonoperative patients ($P = 0.000$, Figure 3). Three patients in the surgery group were still alive at the end of the cut-off date.

DISCUSSION

We reviewed retrospectively 20 macroscopically complete liver resections for patients with GCLM at two institutions. After hepatectomy, their MST was 22.3 mo. These results compare favorably with patients without surgery, whose MST was only 5.5 mo. The survival time in patients with hepatectomy was longer than in those without hepatectomy. However, our MSTs were shorter than the 34 mo reported by Takemura *et al*[14]. The discrepancy may have been caused by the different operating procedures. In the Takemura *et al*[14] study, 14/64 (21.9%) patients underwent major hepatectomy and the remaining 50 (78.1%) minor hepatectomy. In our study, 70% patients had major hepatectomy and 30% had minor hepatectomy. Both studies indicate that hepatectomy is beneficial for some patients with GCLM despite the remaining controversy surrounding surgical resection.

Liver metastases is reported to develop in 5%-9% of patients with gastric cancer[15]. One study has shown that only a limited number of GCLM patients are eligible for surgical treatment[4]. After the promising results of the MAGIC trial, in Europe, current practice for treatment of GCLM patients has become surgery with perioperative chemotherapy[10,16]. However, the optimal surgical strategy for GCLM remains a matter of debate. Only some patients with GCLM are ideal candidates for hepatectomy, therefore, many patients are unsuitable for surgical resection, either due to other distant metastases, extensive lymph node metastases, multiple bilateral metastases, or comorbidity.

In recent decades, multimodality approaches using chemotherapy, radiotherapy, or both have been evaluated in an attempt to improve outcomes following gastric cancer surgery. Some benefit has been seen in adjuvant chemotherapy after gastric cancer resection. One recent trial conducted in East Asia, ACTS-GC30, evaluated S-1 chemotherapy and found significant 10% improvement in 3-year overall survival with adjuvant chemotherapy after surgery[17]. A more compelling study of perioperative chemotherapy was the phase 3 United Kingdom MAGIC trial. This trial demonstrated that perioperative chemotherapy could significantly improve overall survival and progression-free survival in 503 patients with resectable adenocarcinoma. However, this trial also highlighted the challenges involved in delivering postoperative treatment; only 50% of patients were able to receive postoperative chemotherapy, compared with nearly 91% who received preoperative chemotherapy.

In the late stage of gastric cancer, with high rates of toxicity in perioperative chemotherapy, adoption of the perioperative approach could be useful for a large proportion of GCLM patients. Our results also showed that weekly SP and low-dose DCF in perioperative chemotherapy had a positive effect in GCLM. In our study, two patients with initially unresectable multiple liver metastases were converted to resectable after preoperative chemotherapy. Our results also showed that D2 resection provides better locoregional control and significantly better survival compared with unresectable patients. We recommend more personally tailored multimodality treatment approaches (surgery + chemotherapy ± radiation) in patients with GCLM.

Some researchers have reported that even a generous surgical margin may not be essential for curative hepatic resection of liver metastases, because recurrence is strongly associated with systemic spread rather than local invasion[6]. This conclusion highlights the essentiality of perioperative chemotherapy. GCLM recurrence after surgery is most likely due to occult metastatic disease in the tumor bed and at distant sites, so locoregional resection alone is not a complete 100% successful procedure. Therefore, multimodality approaches using systemic chemotherapy or radiation, or a combination of both have been used in an attempt to improve outcomes following surgery, especially in patients with multiple metastases.

However, adequate chemotherapy can lead to intolerability and morbidity and mortality. In the present study, we wanted to explore some safe and effective regimens available to Chinese patients with GCLM. We investigated the safety and efficacy of liver resection combined

with perioperative S1 regimen in patients with GCLM. We performed a retrospective analysis based on recent prospectively collected data. S-1 is an orally active combination of tegafur (5-fluorouracil prodrug), gimeracil (an inhibitor of dihydropyrimidine dehydrogenase, which degrades fluorouracil), and oteracil (which inhibits phosphorylation of 5-fluorouracil in the gastrointestinal tract) in a molar ratio of 1:0.4:1. S-1 has been the standard regimen for adjuvant chemotherapy for advanced primary gastric cancer[18], and its mild side effect profile and ease of administration make it a preferred choice. The DCF regimen has major myelotoxicity[19-25]. However, weekly DCF in our study was well tolerated, and both the regimens were well tolerated and achieved a good response. All GCLM patients with adequate physical condition obtained a benefit from preoperative chemotherapy, which assisted with their subsequent surgical procedure. Appropriately modified chemotherapy is necessary for the improvement of the GCLM resection rate and complete elimination of micrometastases[26-31]. In our initial results, weekly DCF yielded an unexpected high response as preoperative chemotherapy for GCLM[9]. We found that S-1 combined with cisplatin also yielded a high response and had better applicability. These modifications of altering the dose and frequency of the cytotoxic agents are an individualized approach for treatment of GCLM. Our aim is to improve the generally poor prognosis of this aggressive disease and further phase Ⅱ and Ⅲ trials are warranted to confirm the feasibility and efficacy of preoperative chemotherapy for GCLM.

COMMENTS

Background

Liver metastasis is a fatal event in gastric cancer patients, and remains a major cause of cancer-related death. Surgery for multiple liver metastases from gastric cancer (GCLM) has favorable outcomes. However, the efficacy and safety of perioperative chemotherapy is still a matter of debate.

Research frontiers

The glycosylated and myristoylated smaller surface antigen trial was conducted to compare gastrectomy with metastasectomy plus systemic therapy versus systemic therapy alone. The results of this trial showed that aggressive surgical resection in combination with systemic chemotherapy may improve the outcomes of the patients with metastatic gastric cancer.

Innovations and breakthroughs

In a previous pilot study, the authors found that liver resection combined with weekly docetaxel-based chemotherapy were well tolerated and had a good response. In the present study, the authors found that perioperative weekly docetaxel, cisplatin and 5-fluorouracil (DCF), and S-1 and cisplatin (SP) in patients with GCLM who underwent aggressive surgical treatment could improve prognosis and overall survival.

Applications

The study results suggest that perioperative weekly DCF and SP could be used to treat GCLM patients who underwent aggressive surgical treatment.

Terminology

Synchronous liver metastases are detected before or during surgery, or occur within 1 year after gastrectomy. Metachronous liver metastases are usually detected within a 2-year period following initial gastrectomy.

Peer review

This is a good study in which the authors evaluated the effect of perioperative weekly DCF and SP in GCLM patients who underwent aggressive surgical treatment. The results are interesting and suggest that perioperative weekly DCF and SP combined with resection could be applied in patients with GCLM.

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META-ANALYSIS

Extended antimicrobial prophylaxis after gastric cancer surgery: A systematic review and meta-analysis

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Abstract

AIM: To investigate the efficacy of extended antimicrobial prophylaxis (EAP) after gastrectomy by systematic review of literature and meta-analysis.

METHODS: Electronic databases of PubMed, Embase, CINAHL, the Cochrane Database of Systematic Reviews, the Cochrane Controlled Trials Register and the China National Knowledge Infrastructure were searched systematically from January 1980 to October 2012. Strict literature retrieval and data extraction were carried out independently by two reviewers and meta-analyses were conducted using RevMan 5.0.2 with statistics tools risk ratios (RRs) and intention-to-treat analyses to evaluate the items of total complications, surgical site infection, incision infection, organ (or space) infection, remote site infection, anastomotic leakage (or dehiscence) and mortality. Fixed model or random model was selected accordingly and forest plot was conducted to display RR. Likewise, Cochrane Risk of Bias Tool was applied to evaluate the quality of randomized controlled trials (RCTs) included in this meta-analysis.

RESULTS: A total of 1095 patients with gastric cancer were enrolled in four RCTs. No statistically significant differences were detected between EAP and intraoperative antimicrobial prophylaxis (IAP) in total complications (RR of 0.86, 95%CI: 0.63-1.16, P = 0.32), surgical site infection (RR of 1.97, 95%CI: 0.86-4.48, P = 0.11), incision infection (RR of 4.92, 95%CI: 0.58-41.66, P = 0.14), organ or space infection (RR of 1.55, 95%CI: 0.61-3.89, P = 0.36), anastomotic leakage or dehiscence (RR of 3.85, 95%CI: 0.64-23.17, P = 0.14) and mortality (RR of 1.14, 95%CI: 0.10-13.12; P = 0.92). Likewise, multiple-dose antimicrobial prophylaxis showed no difference compared with single-dose antimicrobial prophylaxis in surgical site infection (RR of 1.10, 95%CI: 0.62-1.93, P = 0.75). Nevertheless, EAP showed a decreased remote site infection rate compared with IAP alone (RR of 0.54, 95%CI: 0.34-0.86, P = 0.01), which is the only significant finding. Unfortunately, EAP did not decrease the incidence of surgical site infections after gastrectomy; likewise, multiple-dose antimicrobial prophylaxis failed to decrease the incidence of surgical site infection compared with single-dose antimicrobial prophylaxis.

CONCLUSION: We recommend that EAP should not be used routinely after gastrectomy until more high-quality RCTs are available.

Key words: Gastric cancer; Gastrectomy; Extended antimicrobial prophylaxis; Intraoperative antimicrobial prophylaxis; Meta-analysis

Core tip: We investigated the efficacy of extended antimicrobial prophylaxis (EAP) after gastrectomy through systematic review of literature and meta-analysis. We recommend that EAP should not be used routinely after

gastrectomy until more high-quality randomized controlled trials are available.

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INTRODUCTION

Although the incidence of gastric cancer is sharply declining, it still remains the second cause of cancer-related death worldwide[1,2]. Administration of a first-generation cephalosporin as intraoperative antimicrobial prophylaxis (IAP) to prevent surgery-associated infection has been recommended[3]. Nevertheless, most patients after gastrectomy still receive further extended antimicrobial prophylaxis (EAP) routinely to reduce surgical site infection even until 3-4 postoperative days[4-6]. Few randomized controlled trials (RCTs) have investigated the efficacy of EAP[7-10]. Moreover, EAP administration is controversial and there is no worldwide accepted validation as a result of its scarce efficacy.

However, the administration of antimicrobial prophylaxis may result in antibiotics-associated diarrhea (AAD), which can occur as early as few hours after the first dose of antibiotics[11]. The incidence of AAD varies from 10% to 30%, and AAD has been identified as the leading cause of diarrhea in hospitalized patients, especially in patients with surgery of gastrointestinal tract[12]. Abuse of antibiotics also aggravates the burden of patient hospital costs.

A total of 21 320 new gastric cancer cases and 10 540 deaths from gastric cancer were projected to occur in the United States in 2012[2]. Generally, complete surgical resection of gastric cancer with negative margin (R0 resection) and D2 lymphadenectomy is considered as the most effective treatment strategy for gastric cancer in East Asia[13-15]. Surgical site infections have suggested the essential administration of IAP. However, only few RCTs have investigated the efficacy of EAP[7-10], and almost no meta-analysis has been conducted to assess the efficacy of EAP. Meta-analysis is considered a more powerful evidence for clinical decision marking compared with RCTs. In light of these considerations, we performed this meta-analysis to assess the efficacy of EAP in patients after gastrectomy.

MATERIALS AND METHODS

Literature search

To identify additional studies and published abstracts, electronic databases of PubMed, Embase, CINAHL, the Cochrane Database of Systematic Reviews, the Cochrane Controlled Trials Register and the China National Knowledge Infrastructure were searched systematically from January 1980 to October 2012. MeSH terms of "stomach neoplasm", "gastrectomy", "antibiotic prophylaxis" and "randomized controlled trial" were used. The reference lists of all retrieved articles were reviewed for further identification of potentially relevant trials.

Data collection process

Two reviewers (Zhang CD and Zeng YJ) in our group independently extracted relevant data, including: study and population features, outcomes, titles, abstracts, and even full articles when it was necessary. They compared the results and synthesized the same opinions, and disagreements were solved by discussion with a third reviewer in our group.

Inclusion and exclusion criteria

The inclusion criteria and exclusion criteria were established based on the Cochrane Handbook for Systematic Review of Interventions (Version 5.0.2). The inclusion criteria were: (1) all originally published and unpublished high-quality RCTs; (2) trials concerning antimicrobial prophylaxis after gastrectomy; (3) if studies were from the same author or institution, the most informative and latest ones were selected; and (4) no restriction on publishing language. Exclusion criteria were as follows: (1) studies with little information about the items to be investigated; (2) loss to follow-up exceeding 10%; and (3) non-RCTs.

The following data were acquired: study and year, country, sample size, sex ratio, median age, body-mass index, operation time, blood loss, median follow-up time, participants, interventions, total complications, surgical site infection, incision infection, organ/space infection, anastomotic leakage/dehiscence, and mortality (Tables 1-3).

Quality evaluation

Methodological quality of RCTs was evaluated according to the Cochrane Risk of Bias Tool with regard to randomization, allocation concealment, blind, withdrawal and dropout, and selective reporting bias (Table 4).

Statistical analysis

Data analysis was conducted using Review Manager 5.0.2 (RevMan 5.0.2) with statistics tools risk ratios (RRs). Intention-to-treat analyses were performed. Dichotomous variables were analyzed with RRs. $P < 0.05$ was defined as statistically significant and 95%CI was applied. Fixed model was used if $I^2 < 50\%$ and $P > 0.1$, while random model was selected if $I^2 \geqslant 50\%$ or $P \leqslant 0.1$. Likewise, forest plot was conducted to display RR.

RESULTS

Among a total of 52 studies retrieved, 48 studies were found unrelated to our selection criteria after further assessment. Thus, only four RCTs[7-10] were eligible for the meta-analysis: three RCTs[7-9] comparing EAP with IAP and one RCT[10] comparing multiple-dose with single-

Table 1 Primary characteristics of the randomized controlled trails included in the meta-analysis

Ref.	Country	Sample size	Male	Median age (yr)	Body mass index (kg/m^2)	Operation time (min)	Blood loss (mL)	Median follow-up time (d)
Schardey *et al*[7]	Germany	102	60	63.7 ± 11.4	NM	301.6 ± 87.7	NM	42
		103	59	62.6 ± 11.9	NM	314.8 ± 107	NM	
			$P > 0.05$	$P > 0.05$	NM	$P > 0.05$	NM	
Farran *et al*[8]	Spain	22	33	57(31-87)	NM	NM	NM	> 22
		27			NM	NM	NM	
Imamura *et al*[9]	Japan	179	125	65	22.5 (12.4-32.9)	200 (64-415)	210 (1-1700)	30
		176	115	66	22.3 (16.3-33.0)	209 (58-428)	200 (1-880)	
			$P = 0.536$	$P = 0.429$	$P = 0.190$	$P = 0.499$	$P = 0.903$	
Mohri *et al*[10]	Japan	243	174	68 (22-91)	21.6 (13.4-31.6)	232 (43-70)	338.0 (10-2811)	30
		243	164	68 (23-90)	21.4 (13.6-34.0)	234 (70-492)	405.7 (10-2917)	
			$P = 0.375$	$P = 0.642$	$P = 0.446$	$P = 0.798$	$P = 0.028$	

NM: Not mentioned.

Table 2 Secondary characteristics of the randomized controlled trails included in the meta-analysis

Ref.	Participants	*n*	Interventions	Complications
Schardey *et al*[7]	205 patients August 1991-March 1994 Germany, multi-centre, ⩾ 18 yr, total gastrectomy	102 *vs* 103	Polymyxin B 0.1 g, tobramycin 0.08 g, vancomycin 0.125 g and amphotericin B 0.5 g four times per day orally from the day before operation until 7th postoperative day plus perioperative intravenous prophylaxis: cefotaxime 2 × 2 g *vs* placebo plus perioperative intravenous prophylaxis: cefotaxime 2 × 2 g	Infections: Pulmonary, urinary tract; abscess; Insufficiency: Pancreatic, esophagointestinal; miscellaneous; pancreatic fistula
Farran *et al*[8]	49 patients January 2000-March 2005, single centre, ⩾ 18 yr, total gastrectomy	22 *vs* 27	20 mL oral suspension of erythromycin 0.5 g + gentamicine 0.08 g + nystatin sulfate 0.1 g *vs* 20 mL placebo solution. Both groups started treatment 12 h before surgery and continued until the 5th postoperative day	Dehiscence; sepsis; abscess; pulmonary infection; pulmonary distress syndrome
Imamura *et al*[9]	355 patients June 2005-December 2007, Japan, multi-centre, ⩾ 35 yr, distal gastrectomy	179 *vs* 176	Intraoperative administration plus cefazolin 1 g once after closure and twice daily for 2 postoperative days *vs* intraoperative administration: cefazolin 1 g before surgical incision and every 3 h as intraoperative supplements	Anastomotic leakage; remote infections; surgical site infections
Mohri *et al*[10]	486 patients May 2001-December 2004 Japan, single-centre, ⩾ 20 yr, elective gastrectomy	243 *vs* 243	Intraoperative schedule: cefazolin 1 g or ampicillin-sulbactam 1.5 g by intravenous infusion > 15 min and an additional dose was administrated if operation > 3 h *vs* intraoperative schedule plus further treatment at 12-h intervals, a total of 7 doses	Surgical site infection: incision or organ or space; abscess

Table 3 Basic data of the comparisons included in the randomized controlled trails

Ref.	Total complication	Surgical site infection	Incision infection	Organ/space infection	Remote site infection	Anastomotic leakage/dehiscence	Mortality
Schardey *et al*[7]	31/102	NM	NM	NM	16/102	NM	5/102
	46/103	NM	NM	NM	31/103	NM	11/103
Farran *et al*[8]	2/22	NM	NM	NM	1/22	1/22	2/22
	3/27	NM	NM	NM	3/27	0/27	0/27
Imamura *et al*[9]	22/179	16/179	5/179	11/179	6/179	4/179	NM
	17/176	8/176	1/176	7/176	9/176	1/176	NM
Mohri *et al*[10]	NM	23/243	14/243	12/243	NM	NM	NM
	NM	21/243	11/243	10/243	NM	NM	NM

NM: Not mentioned.

dose antimicrobial prophylaxis after gastrectomy, including 1095 patients (Tables 1-5).

Primary outcomes: Intraoperative vs EAP

Total complications: Three RCTs[7-9] were included (303 EAP and 306 IAP) and fixed model was applied (I^2 = 42%, P = 0.18). No statistically significant difference was detected (RR of 0.86, 95%CI: 0.63-1.16, P = 0.32).

Surgical site infection, incision infection and organ/space infection: Only one RCT[9] comparing EAP and IAP reported surgical site infection, which showed no statistical difference (RR of 1.97, 95%CI: 0.86-4.48, P = 0.11). There were also no significant differences in the analysis of incision infection (RR of 4.92, 95%CI: 0.58-41.66, P = 0.14) and organ or space infection (RR of 1.55, 95%CI: 0.61-3.89, P = 0.36).

Table 4 Quality assessment of the randomized controlled trails included based on the Cochrane Risk of Bias Tool

Ref.	Randomization	Allocation concealment	Blind	Withdrawal and dropout	Presence of selective reporting bias
Schardey *et al*[7]	Without details	Envelope	Double-blind	Well reported	Unclear
Farran *et al*[8]	Well reported	Envelope	Double-blind	Well reported	No
Imamura *et al*[9]	Well reported	Envelope	No	Well reported	No
Mohri *et al*[10]	Well reported	Without details	No	Well reported	Unclear

Table 5 Summary of comparisons between extended antimicrobial prophylaxis and intraoperative antimicrobial prophylaxis

Items	Heterogeneity		Analysis model	Overall effect		RR (95%CI)	Ref.
	I^2	*P*		*Z*	*P*		
Total complications	42%	0.18	Fixed	0.99	0.32	0.86 (0.63-1.16)	[7-9]
Surgical site infections	NP	NP	Fixed	1.61	0.11	1.97 (0.86-4.48)	[9]
Incision infections	NP	NP	Fixed	1.46	0.14	4.92 (0.58-41.66)	[9]
Organ/space infections	NP	NP	Fixed	0.92	0.36	1.55 (0.61-3.89)	[9]
Remote site infections	0%	0.90	Fixed	2.58	0.01	0.54 (0.34-0.86)	[7-9]
Anastomotic leakage/dehiscence	0%	0.97	Fixed	1.47	0.14	3.85 (0.64-23.17)	[8,9]
Mortality	62%	0.10	Random	0.10	0.92	1.14 (0.1-13.12)	[8,9]

NP: Not applicable; RR: Risk ratio.

Remote site infection: Three RCTs (303 EAP and 306 IAP)[7-9] evaluated remote site infection and fixed model was conducted (I^2 = 0%, *P* = 0.90), however, there was a significantly decreased remote site infection rate in EAP compared with IAP (RR of 0.54, 95%CI: 0.34-0.86, *P* = 0.01).

Anastomotic leakage or dehiscence: Two RCTs (201 EAP and 203 IAP)[8,9] were evaluated, showing no statistical difference in anastomotic leakage or dehiscence (RR of 3.85, 95%CI: 0.64-23.17, *P* = 0.14).

Mortality: Two RCTs (124 EAP and 130 IAP)[7,8] were included, which suggested no survival benefit of EAP compared with IAP (RR of 1.14, 95%CI: 0.10-13.12, *P* = 0.92).

Secondary outcomes: Multiple-dose antimicrobial prophylaxis vs single-dose antimicrobial prophylaxis

Only one RCT[10] compared the efficacy of multiple-dose antimicrobial prophylaxis with single-dose antimicrobial prophylaxis, however, no significant differences were detected in surgical site infection (RR of 1.10, 95%CI: 0.62-1.93, *P* = 0.75), incision infection (RR of 1.27, 95%CI: 0.59-2.75, *P* = 0.54) and organ/space infection (RR of 1.20, 95%CI: 0.53-2.73, *P* = 0.66). The incidence of surgical site infection after gastrectomy was similar by the two antimicrobial prophylaxis regimens.

DISCUSSION

Meta-analysis is considered an ideal statistical tool increasing the statistical power, in other words, meta-analysis is a more powerful evidence for clinical decision marking compared with RCTs. In light of these considerations, this meta-analysis was conducted to assess the efficacy of EAP after gastrectomy.

Although it has been widely accepted that patients with gastrectomy will benefit from preoperative antimicrobial prophylaxis and IAP[16,17], there is still no worldwide accepted validation for EAP. In this meta-analysis, we found that postoperative EAP did not decrease the incidence of total complications in patients with gastrectomy. Additionally, EAP failed to improve surgical site infection rate, including incision infection and organ/space infection; likewise, no significant difference was detected in anastomotic leakage/dehiscence and mortality between EAP and IAP. The same striking finding was that patients did not benefit from multiple-dose antimicrobial prophylaxis compared with single-dose antimicrobial prophylaxis; yet, only one RCT[10] was included in this meta-analysis. Based on the present evidence, we do not recommend the administration of EAP after gastrectomy; however, our results need to be validated and re-evaluated by more high-level RCTs.

Surgical site infections remain a substantial cause of postoperative mortality[18]. We therefore conjecture that if EAP can decrease the surgical site infection rate, it may subsequently decrease the postoperative mortality. Unfortunately, EAP failed to decrease the surgical site infection rate. We assessed the mortality of EAP and IAP groups, and found no significant differences between the two groups. In light of these considerations, our findings suggested that EAP fails to decrease mortality in patients after gastrectomy; in other words, no survival benefit can be observed from EAP after gastrectomy based on the present evidence.

Many factors, such as male ratio, median age, obesity, operation time and intraoperative blood loss, may affect the postoperative infection risk[18-23]. For example, the effect of antibiotics will be diminished as a result of intraoperative blood loss; likewise, longer operation time will increase blood loss; meanwhile, obesity may increase

the difficulty of operation and the operation time. Taking all these factors into consideration, we evaluated the statistical difference systematically; fortunately, we did not detect any significant difference among these items. Therefore, these factors have not affected the outcomes in these RCTs (Table 1).

The only significant difference between EAP and IAP is the remote site infection rate. However, we recommend that EAP should not be applied routinely unless the individuals experience a remote site infection. Therefore, we suggest delivering an "individualized treatment" rather than a routine treatment. The drugs used for EAP in these trials varied from cefazolin 1-1.5 g[9,10], erythromycin 0.5 g + gentamicine 0.08 g + nystatin sulfate 0.1 g[8], polymyxin B 0.1 g, tobramycin 0.08 g, vancomycin 0.125 g to amphotericin B 0.5g[7]. The incidence of surgical site infection in these RCTs ranged from 4.5% to 9.5%, which is in keeping with published rates of 5%-14%[24,25]. Despite these differences, the infection rates were similar, and no difference was detected (Table 2). However, our results still need to be validated for patients who require surgery on other sites of the body because the micro-flora in these operation sites differs from that in gastrointestinal tract[26].

There was no country or language restriction in the data search process for this meta-analysis. It is the first meta-analysis concerning the efficacy of EAP after gastrectomy. There are some limitations of these studies, such as various antimicrobial prophylaxis regimens used and disappointing statistical power, thus, more high-level RCTs are needed to validate our results.

Based on the present evidence, EAP fails to decrease the incidence of surgical site infections after gastrectomy; multiple-dose antimicrobial prophylaxis fails to decrease the incidence of surgical site infection compared with single-dose antimicrobial prophylaxis. Therefore, we believe that our findings are significant to all patients with gastrectomy, and suggest that EAP should not be used routinely after gastrectomy until more high-level RCTs are available.

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COMMENTS

Background

Administration of intraoperative antimicrobial prophylaxis (IAP) to prevent surgery-associated infection has been recommended. Although extended antimicrobial prophylaxis (EAP) has been recommended to be discontinued within 24 h of surgery, most patients after gastrectomy still receive further EAP routinely, as a result of insufficient evidence. This meta-analysis was conducted to investigate the effectiveness of EAP aimed at guiding clinical practice.

Research frontiers

Meta-analysis was conducted to evaluate the effectiveness of EAP *vs* IAP for patients undergoing gastric cancer surgery.

Innovations and breakthroughs

The evidence obtained from this meta-analysis proved that EAP failed to demonstrate the advantages over IAP for patients with gastric cancer surgery with regard to total complications, surgical site infection, incision infection, organ or space infection, anastomotic leakage or dehiscence and mortality. These findings suggested that EAP should not be used routinely after gastrectomy.

Applications

The results of this meta-analysis suggest that EAP should not be administrated routinely after gastric cancer surgery and IAP is a standard treatment strategy for gastric cancer surgery.

Terminology

Surgical site infection: Infection occurs within one month after operation and involves superficial incision, deep incision, organ or space, which may generate some symptoms of infection, such as pain or tenderness, local swelling, redness, heat and so on.

Peer review

The period of the investigation extended over 32 years, during which different types and combinations of antibiotics have been used, but does not permit any evaluation of any particular antibiotics which might have been valuable. It is a valuable finding that intra-operative antibiotic prophylaxis is as efficient as an extended post-operative course in preventing post-operative infections.

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CASE REPORT

Hepatocellular carcinoma in a non-cirrhotic patient with Wilson's disease

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Abstract

We report the exceptional case of hepatocellular carcinoma in a non-cirrhotic patient, whose Wilson's disease was diagnosed at the unusual age of 58 years. The liver histology revealed macrovesicular steatosis with fibrosis, but no cirrhosis. The disease was treated with D-penicillamine for 3 years until acute discomfort in the right upper quadrant led to detection of multifocal hepatocellular carcinoma, which was successfully resected. The histological examination confirmed the malignant nature of the 4 lesions, which were classified according to Edmondson and Steiner as poorly differentiated hepatocellular carcinoma grade 3. The non-tumoral parenchyma showed 80% steatosis with ballooned cells, lobular inflammation, septal fibrosis but no cirrhosis. Hepatocellular carcinoma is rare in Wilson's disease, especially in the absence of cirrhosis. The literature's 28 published cases are reviewed and the contributory role of copper in the hepatocarcinogenic process is discussed.

Key words: Wilson's disease; Hepatocellular carcinoma; Hepatocarcinogenesis; Copper; Liver; Fibrosis; Cirrhosis

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INTRODUCTION

Wilson's disease is an autosomal recessive disorder of copper metabolism. Wilson's disease has a worldwide prevalence between 1 in 30 000 and 1 in 100 000[1]. The responsible gene *ATP7B* is located on chromosome 13 and encodes a copper transporter. In Wilson's disease, the copper transporter is mutated and its function is impaired[1]. Wilson's disease has hepatic, neurological, psychiatric and ophthalmic manifestations. Hepatic manifestations are characterized histologically by steatohepatitis, which evolves into cirrhosis if left untreated. Because most cases of Wilson's disease are diagnosed and treated early, hepatocellular carcinoma is a rare sequela. We report the unusual case of a Wilson's disease patient diagnosed at an advanced age and who developed hepatocellular carcinoma in a non-cirrhotic liver.

CASE REPORT

The patient underwent cholecystectomy due to symptomatic gallstones at 51 years of age. Liver biopsies showed macrovesicular steatosis. The circulating levels of gamma-glutamyltransferase remained chronically elevated and the ALT levels were at the upper limit of the normal. A computed tomography (CT) scan performed 5 years later revealed the presence of a 3 cm subcapsular lesion in liver segment VI as well as several ≤ 1 cm lesions. All lesions displayed a discrete enhancement dur-

Table 1 Synopsis of patients with hepatocellular carcinoma and Wilson's disease

Ref.	Sex	WD-age (yr)	HCC-age (yr)	Cirrhosis	Medical therapy	Invasive therapy	Status
Guan *et al*[2]	Female	23	27	Yes	Penicillamin	Hepatic resection	Alive
Iwadate *et al*[3]	Male	17	23	Yes	Penicillamin, low copper diet	-	Dead
Lowette *et al*[4]	-	-	-	Yes	Penicillamin	Transplant	Alive
Ikegawa *et al*[5]	Male	28	37	Yes	Penicillamin, zinc acetate dehydrate	Radiofrequency ablation	Alive
Kumagi *et al*[6]	Male	26	66	Yes	Penicillamin, transplant	Transcatheter arterial chemoembolisation	Dead
Kumagi *et al*[6]	Male	27	36	Yes	Penicillamin, transplant	-	Dead
Kumagi *et al*[6]	Male	27	46	Yes	Penicillamin	-	Alive
Lygren *et al*[15]	Male	15	16	Yes	-	-	Dead
Girard *et al*[16]	Male	22	41	Yes	Penicillamin	-	Dead
Kamakura *et al*[17]	Male	26	32	Yes	Penicillamin	-	Dead
Terao *et al*[18]	Male	29	40	Yes	Penicillamin, dimercaprol potassium sulfate	-	Dead
Wilkinson *et al*[19]	Male	31	41	Yes	Penicillamin	-	Dead
Buffet *et al*[20]	Male	45	57	Yes	Penicillamin	-	Dead
Imhof *et al*[21]	Male	18	40	-	Penicillamin	Hepatic resection	Alive
Madden *et al*[22]	Male	61	61	Yes	Penicillamin	-	Dead
Polio *et al*[23]	Male	32	33	Yes	Penicillamin, low copper diet	Chemotherapy	Dead
Cheng *et al*[24]	Female	39	72	Yes	Penicillamin, dimercaprol potassium sulfate	-	Dead
Agret *et al*[25]	Male	73	73	Yes	-	-	Dead
Walshe *et al*[26]	Male	8	46	Yes	Penicillamin	Transplant	Alive
Walshe *et al*[26]	Male	11	42	Yes	Penicillamin	-	Dead
Kumagi *et al*[27]	Male	66	66	Yes	-	Transcatheter arterial chemoembolisation	Dead
Ozçay et al[28]	Male	-	13	Yes	Penicillamin	Transplant	Alive
Aydinli *et al*[29]	Male	22	22	Yes	-	Radiofrequency ablation, transplant	Alive
Xu *et al*[30]	Male	29	29	Yes	-	Transcatheter arterial chemoembolisation, transplant	Alive
Reyes *et al*[31]	Male	59	59	Yes	-	-	Dead
Emlakçioglu *et al*[32]	Female	30	50	-	Penicillamin	Transcatheter arterial chemoembolisation	Alive
Ikubo *et al*[33]	Female	28	54	Yes	Penicillamin, pyridoxal-phosphate	Hepatic resection	Alive
Savas et al[34]	Male	6	12	Yes	Penicillamin, low copper diet	Transplant	Alive

WD: Wilson's disease; HCC: Hepatocellular carcinoma diagnosis.

ing the arterial phase without washout during the portal phase. Radiological controls over the next 2 years revealed no evolution of these lesions. The biopsy of the largest lesion showed fibrotic remodelling corresponding to Metavir F3 or a modified Ishak score of 4 without evidence of cirrhosis, a 25% macrovascular steatosis, a moderate chronic hepatic inflammation and an area with small cell dysplasia. A broad clinical examination was negative for neurological and ophthalmic (Kayser-Fleischer-rings) signs of Wilson's disease. However ceruloplasmin levels below the limit of detection (0.1 g/L) and urinary copper excretion was elevated. A genetic test confirmed a frameshift-mutation in exon 14 and 2 missense-mutations in exons 18 and 21 of the *ATP7B* gene. The patient was treated with D-penicillamine and pyridoxal-phosphate. This treatment was well tolerated. An magnetic resonance imaging with hepatocellular specific contrast confirmed the known lesions, which were stable in size and interpreted as regeneration nodules.

The patient worked as a mechanic, never smoked and consumed less than 10 g alcohol per day. His mother had metastatic breast carcinoma and died of a cerebral haemorrhage. His father suffered from an undefined psychiatric condition. His 2 brothers and 1 sister are in good health. He has no children.

At the age of 61 years, the patient presented with acute pain in the right upper quadrant, which was preceded for several weeks by discomfort. Contrast-enhanced CT revealed 4 hepatic lesions showing enhancement during the arterial phase and washout during the portal phase. Two lesions of 3.7 cm and 2.1 cm were in segment Ⅳ, 1 lesion of 3.2 cm was in the segment Ⅴ and the largest lesion of 9.7 cm was in segments Ⅵ and Ⅶ. A curative resection was performed. The histological examination confirmed the malignant nature of the 4 lesions, which were classified according to Edmondson and Steiner as poorly differentiated hepatocellular carcinoma grade 3. The non-tumoral parenchyma showed 80% steatosis with ballooned cells, lobular inflammation, septal fibrosis but no cirrhosis. Moreover, there was a mild iron hepatocellular accumulation (Rowe 1), which was absent on the previous biopsy.

DISCUSSION

All the published cases of hepatocellular carcinoma occurring in patients with Wilson's disease are listed in Table 1. As expected for hepatocellular carcinoma, males predominate whereas female constitute a lower than expected percentage (14%) of this group. Females constitute 30% of overall hepatocellular carcinoma cases. The reason may be that cirrhosis initiated by Wilson's disease

is less carcinogenic than that linked to other cirrhotic conditions and that male gender provides additional susceptibility to initiate hepatocarcinogenesis. Because it is uncertain from the information in previous reports whether other risk factors had been considered and excluded[2-6], it is not possible to fully assess whether common features could link them to the hepatocarcinogenic process in our male patient with longstanding, untreated Wilson's disease. However, our patient was exceptional in that he was non-cirrhotic, whereas all previous cases of hepatocellular carcinoma in Wilson's disease occurred in cirrhotic livers (Table 1).

Long-Evans cinnamon rats, which have a mutated *ATP7B* gene and are therefore an experimental model for Wilson's disease, develop hepatocellular carcinoma spontaneously. However, these animals accumulate iron in addition to copper, and an iron-deficient iron diet can abrogate the development of liver tumors[7]. This was attributed to the role of iron in promoting reactive oxygen species and DNA strand breaks[8]. Copper can assume a similar role. Mice receiving copper develop hepatocellular carcinoma, preventable by the concurrent administration of thiamine, which reduces the production of reactive oxygen species in the mitochondria[9]. In addition, copper stabilizes hypoxia-inducible factor-1α (HIF-1α)[10-12] by restraining the activity of the HIF-1α-inhibition factor[10], thereby ensuring the formation of the HIF-1α transcriptional complex[11,12] and the expression of target genes important for angiogenesis, such as vascular endothelial growth factor (VEGF)[10]. Indeed, Martin showed that copper increases VEGF in human hepatoma cells[12]. Another potential carcinogenic property of copper is its ability to stimulate fibroblast growth factor-2[13]. Treatment with D-penicillamine promotes hepatocellular iron accumulation[14]. It is possible that the D-penicillamine treatment of our patient contributed to the oxidative stress through and increase in iron.

When our case is combined with the 28 published cases of hepatocellular carcinoma (Table 1), the mean age at diagnosis of Wilson's disease was 31 ± 18 years: 30 ± 7 years for women and 32 ± 19 years for men. The diagnosis of this genetic disease at such an advanced age suggests that longstanding, untreated Wilson's disease may represent a risk factor for hepatocellular carcinoma. This notion is supported by the observation that the mean age at diagnosis of hepatocellular carcinoma was younger than that observed in patients with other underlying liver diseases (43 ± 18 years).

In conclusion, this case report illustrates that hepatocellular carcinoma does occur in patients with Wilson's disease and that those with longstanding, untreated disease may be particularly vulnerable. Therefore, the importance of determining the fibrosis stage of Wilson's disease patients and of enrolling them in a surveillance program when cirrhotic can only be emphasized.

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CASE REPORT

Case of rectal angioleiomyoma in a female patient

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Abstract

Angioleiomyoma represents a benign stromal tumor, which usually occurs in the subcutaneous tissue of the extremities, although its occurrence in the gastrointestinal tract is very rare. A case of rectal angioleiomyoma in a 40 year-old female patient is described here. Six months earlier, the patient suffered from periodical prolapse of an oval tumor from the anus, along with difficulties in bowel movement. A transanal extirpation of the tumor was performed. This is the first reported case in the English literature of a patient presenting with prolapsed angioleiomyoma of the rectum. During the immediate postoperative period, as well as 6 mo later, the patient had an unremarkable postoperative recovery.

Key words: Prolapsed tumor; Angioleiomyoma; Rectum; Leiomyoma

Stanojević GZ, Mihailović DS, Nestorović MD, Radojković MD, Jovanović MM, Stojanović MP, Branković BB. Case of rectal angioleiomyoma in a female patient. *World J Gastroenterol* 2013; 19(13): 2114-2117 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i13/2114.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i13.2114

INTRODUCTION

Angioleiomyomata are a vascular variant of leiomyomata, which are benign tumors of smooth muscle. Angioleiomyoma of the large bowel is an extremely rare benign tumor. This is the first reported case in the English literature where a patient presenting with perineal discomfort/tenesmus and symptoms with a rectal mass passed via the anus had underlying angioleiomyoma.

CASE REPORT

A 40 year-old woman presented to an examination complaining of pain-related difficulties, feelings of pressure, and a prolapsing rectal tumor during defecation. The patient's difficulties began three years earlier, in the form of a sporadic feeling of discomfort in the anus and an occasional tumor prolapse 3 cm × 2 cm in size, which was spontaneously moving back inside. During the last six months, the tumor has grown up to 7 cm × 5 cm × 6 cm, and had been permanently falling out and hardly withdrawing. Due to bowel movement and personal hygiene problems, the patient came to the proctology unit.

On admission, the woman was hemodynamically stable and the laboratory analyses were within the normal limits, except for a slightly lower level of calcium ions (2.17 mmol/L) and slightly increased chloride (106 mmol/L). She was examined at the Proctology Section of the General Surgery Clinic. The tumor was 7 cm × 5 cm × 6 cm in size, "stuck out" from the anus, had an intact smooth surface, and was painless on palpation, on a 3 cm wide pedicle, and reducible (Figure 1).

After pre-operative preparation, transanal tumor ex-

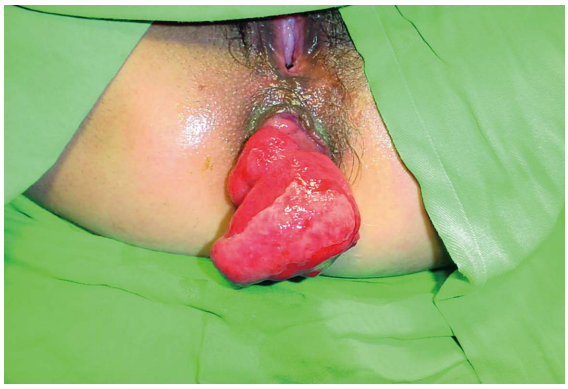

Figure 1 *In situ* prolapsed angioleiomyoma of the rectum.

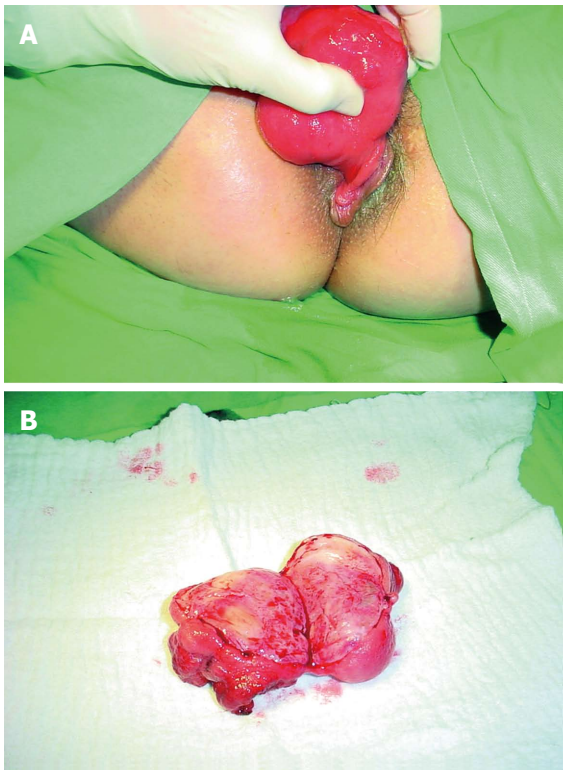

Figure 2 Cross-section of the lobular structure of the tumor showed a visible capsule and a partially bleeding parenchyma. A: Pedunculated tumor; B: Cross-section of the lobular structure tumor with visible capsule and partially bleeding parenchyma.

tirpation surgical intervention was performed. A cross-section of the tumor's lobular structure showed a visible capsule and a partially bleeding parenchyma (Figure 2).

Histopathological examination of the tumor revealed combined capillary and venous angioleiomyoma of the rectum, with thick vascular channels and intervascular smooth muscle bundles (Figure 3).

Results of immunohistochemical analyses revealed that the angioleiomyoma was negative for C-kit and CD34, and positive for smooth muscle actin and desmin (Figure 4).

The postoperative course passed quite regularly, without complications, and the patient was released from

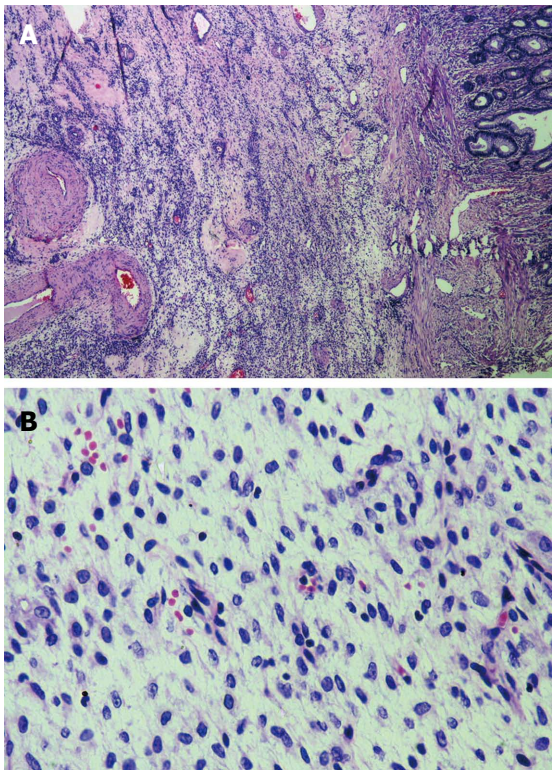

Figure 3 Histopathological examination of a combined capillary and venous angioleiomyoma of the rectum. A: Hematoxylin and eosin staining (× 10); B: Hematoxylin and eosin staining (× 40).

hospital on the second postoperative day. Six months after the operation, during the control examination, the patient was feeling well; digital rectal examination and laboratory findings were in order.

DISCUSSION

Primary leiomyomata are very rare in the gastrointestinal tract; mostly appearing in the stomach and small intestine. They are much less frequent in the esophagus and colon. Leiomyomata in the anorectal region make up 3% of all gastrointestinal benign tumors of smooth muscles (*i.e.*, they appear in 1 out of every 2000-3000 rectal neoplasms)[1,2]. The first rectum leiomyoma was described by Vander Espt in 1881[3]. With regard to macroscopic appearance, colon leiomyomata can be sessile (*i.e.*, pedunculer, intraluminal, intramural and extraluminal). The clinical picture goes mainly without symptoms, except in the case of large tumors, which present occasional bleeding, a palpable and often prolapsing mass, and occasional pain in the last part of the colon[1,4].

In 1995, Ezinger divided all leiomyomata into three groups: superficial, vascular (*i.e.*, angioleiomyomata) and deep leiomyomata[5]. Angioleiomyomata are benign tumors of smooth muscle with expressed abnormal small blood vessels with thickened walls. They usually occur on the skin or in the subcutaneous tissue of the lower extremities, representing one out of five characteristic painful skin lesions, together with angiolipoma, glomus

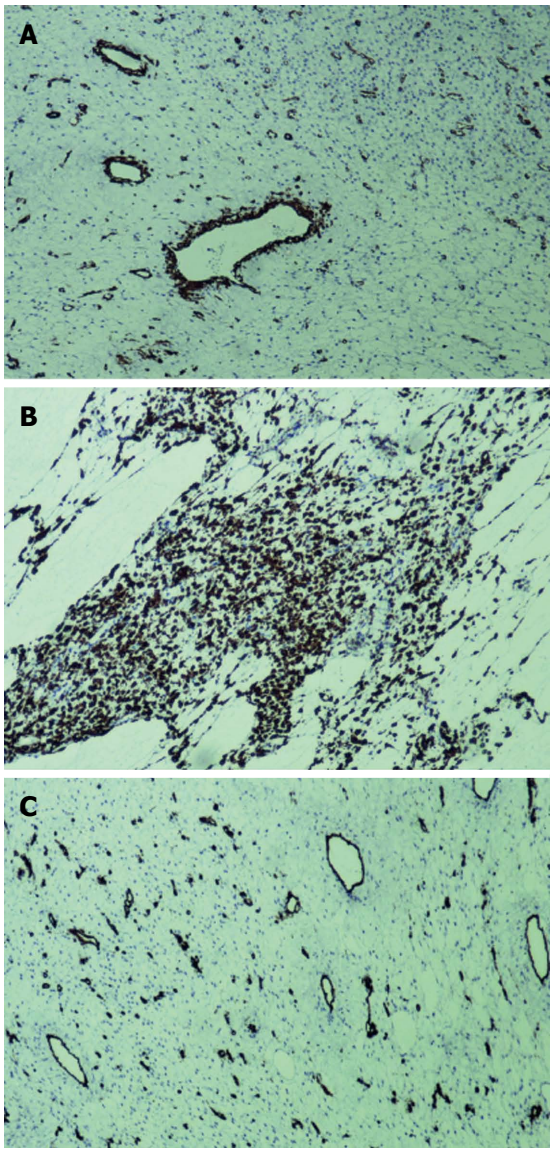

Figure 4 Immunohistochemical analyses. A: Tumor cells positive to actin (× 10); B: Tumor cells positive to desmin (× 10); C: Tumor cells negative for CD34 (× 10).

tumors, spiradenoma, and neurinoma[6]. Cases of localization have also been described, including the mouth cavity, female reproductive organs, scrotum, and kidneys[7-11]. They originate from the muscularis mucosa or muscularis propria of the rectum wall. Pathophysiological division includes 4 types of angioleiomyomata: (1) capillary/solid, which are characterized by the existence of stratified smooth muscle fibers that surround a few thin vascular small channels; (2) venous, with numerous vascular small channels with thickened walls; (3) cavernous, with wide vascular small channels, surrounded by a thin layer of smooth muscle cells; and (4) combined capillary and venous angioleiomyomata[5,12].

The presence of angioleiomyomata in the gastrointestinal tract is exceptionally rare, although in the literature there are several publications which describe cases with complications, such as bleedings, volvulus, peritonitis, and perforation[13-16]. In the English literature, there are no data about the existence of rectum angioleiomyomata, making this the first described case.

Considering the nature of the tumor, the treatment implies that its transanal extirpation was a minimally invasive procedure.

Prolapsed peduncular intraluminal rectal angioleiomyoma represents an exceptionally rare type of tumor, which has now been described in literature for the first time. Its treatment implies transanal extirpation with further patient follow-up.

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CASE REPORT

Endoscopic drainage for duodenal hematoma following endoscopic retrograde cholangiopancreatography: A case report

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Abstract

Intramural duodenal hematoma (IDH) is a rare complication following endoscopic retrograde cholangiopancreatography (ERCP). Blunt damage caused by the endoscope or an accessory has been suggested as the main reason for IDH. Surgical treatment of isolated duodenal hematoma after blunt trauma is traditionally reserved for rare cases of perforation or persistent symptoms despite conservative management. Typical clinical symptoms of IDH include abdominal pain and vomiting. Diagnosis of IDH can be confirmed by imaging techniques, such as magnetic resonance imaging or computed tomography and upper gastrointestinal endoscopy. Duodenal hematoma is mainly treated by drainage, which includes open surgery drainage and percutaneous transhepatic cholangial drainage, both causing great trauma. Here we present a case of massive IDH following ERCP, which was successfully managed by minimally invasive management: intranasal hematoma aspiration combined with needle knife opening under a duodenoscope.

Key words: Duodenal hematoma; Duodenal obstruction; Endoscopic retrograde cholangiopancreatography; Non-operative method

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INTRODUCTION

Intramural duodenal hematoma (IDH) following endoscopic retrograde cholangiopancreatography (ERCP) is very rare. It is usually caused by blunt damage induced by the endoscope or accessory equipment. The typical clinical symptoms of duodenal hematoma include abdominal pain and vomiting, which are directly induced by duodenal obstruction. The hematoma may also lead to obstruction of the papilla opening, and pancreatitis and cholestasis may follow. The clinical presentations and imaging techniques, *e.g.*, ultrasound, upper gastrointestinal endoscopy, magnetic resonance imaging (MRI) or computed tomography (CT), can confirm the diagnosis. A search of PubMed revealed that there has been no previous report on transnasal endoscopic drainage for treatment of duodenal hematoma. Duodenal hematoma is mainly treated by open surgery drainage and percutaneous transhepatic cholangial drainage (PTCD)[1,2]. However, both cause great trauma. In this paper we report our successful experience on minimally invasive management of a massive IDH following ERCP using intranasal hematoma aspiration combined with needle knife opening under duodenoscopy, which can be done under direct vision, with less trauma and risk.

CASE REPORT

A 48-year-old male patient presented with epigastric distention and abdominal pain after removing a stone in the common bile duct by ERCP. Before ERCP, the patient had abdominal discomfort, and examination found a common bile duct stone, with no history of hypertension or coagulation disorders. Laboratory findings before ERCP were as follows: total/direct bilirubin (Tbil/Dbil): 10.2/4.0 mmol/L; white blood cells (WBC) 4.0 × 10^9/L, 59.4%; γ-glutamyltransferase (γ-GT): 196 U/L; coagulation function: fibrinogen (Fbg): 1.6 g/L; prothrombin time (PT): 12.6 s. The patient began to vomit after 1 wk. A CT scan showed a 59 mm × 53 mm intact cyst near the head of pancreas in the duodenum (Figure 1A), suspicious of duodenal hematoma. Hepatic function was as follows: γ-GT: 337 U/L; Tbil/Dbil: 19.2/8.0 mmol/L; WBC: 5.6 × 10^9/L, 73.4%; Fbg: 2.7 g/L; PT: 13.9 s; and blood amylase was normal.

Liver function examination showed a γ-GT of 196 U/L. Emergency duodenoscope inspection revealed a huge cystic bulging object in the intestinal wall of the duodenum, obstructing the duodenum (Figure 1B). A duodenoscope could not pass the obstruction, and the opening of the duodenal papilla was covered by the hematoma. The diagnosis was confirmed as a duodenum hematoma after ERCP. We punctured the hematoma with a needle knife, then inserted a guidewire and catheter (Figure 2) and extracted 100 mL dark red bloody fluid. The fluid became light red after washing with 1:10 000 epinephrine:saline and metronidazole. When the tension of hematoma became smaller, it was found that the hematoma was located at the lateral descending duodenum, 3 cm from the opening of the main papilla. There was no blood at the opening of papilla, and normal bile flowed out from the opening. X-ray showed the bile duct was full of gas. Then we placed a 8.5F per nasal drainage pipe into the hematoma for continuous drainage (Figure 2).

The epigastric distention and abdominal pain were greatly relieved after hematoma drainage, and the patient could ingest semi-fluid without vomiting. Dark red hydatid fluid (about 20 mL) was continuously extracted from the hematoma each day for 2-3 d, and the drainage volume was 5 mL at 5 d after the operation; the patient developed a low fever (38.2 ℃) on the 2nd day after the operation. Duodenoscopy examination was performed again after drainage for 5 d, and it was found that the hematoma was smaller, but the local mucosa still had hyperemia and edema (Figure 3A); the duodenoscope could be easily passed without bleeding and edema at opening of the papilla. The drainage tube was removed and the puncture site was closed with hemostatic clips (Figure 3B). Then we explored the extra-hepatic bile ducts again with a stone basket and found no stones. We placed an endoscopic nasobiliary drainage (ENBD) tube in the common bile duct to avoid puncture site infection caused by bile, and it was pulled out 4 d after ERCP. Then the patient was discharged from hospital without any symptoms. The hematoma had completely disappeared at 1 mo follow-up.

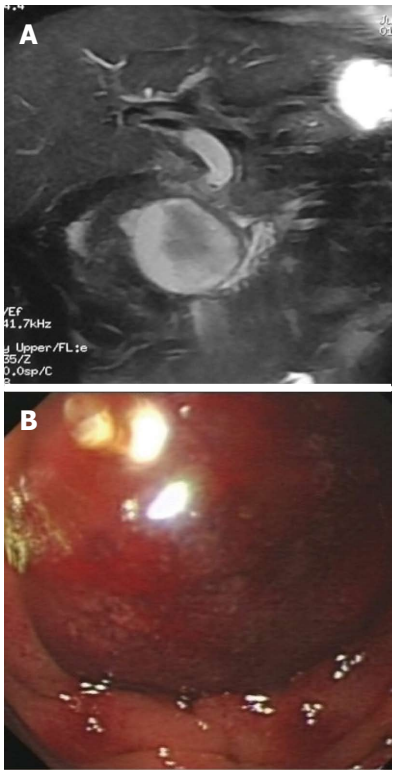

Figure 1 Computed tomography scan and liver function examination of the patient. A: Computed tomography scan showed a 59 mm × 53 mm intact cyst near the head of the pancreas in the duodenum; B: Duodenoscopy inspection showing a large cystic bulging object in the intestinal wall of the duodenum, obstructing the duodenum.

DISCUSSION

A duodenal hematoma is usually caused by trauma, anticoagulant therapy, rupture of a duodenal aneurysm or biopsy. A duodenal hematoma following ERCP is very rare[1-4]. Some studies reported that the guidewire was inserted into the liver capsule and caused bleeding beneath the liver capsule due to rupture of small vessels in the liver parenchyma. When it is difficult to insert a duodenoscope into the duodenum, the long route of the endoscope and abdominal pressure may cause hematoma in the peritoneal cavity[5,6].

The position of the duodenum is generally stationary. When it is compressed, the pylorus will close, and a close circuit is formed between the pylorus and Treiz ligament. The air pressure in the duodenum will be acutely increased, causing the rupture of vessels in the intestinal wall. In some cases, direct damage to the intestinal wall during the operation will cause the hematoma, and if not treated in time, it will result in intestine necrosis and perforation.

The diagnosis of IDH is likely if there are symptoms such as abdominal pain, vomiting and duodenal obstruction after ERCP[6]. Laboratory evaluations are non-specific and usually show only a mild decrease in hemoglobin

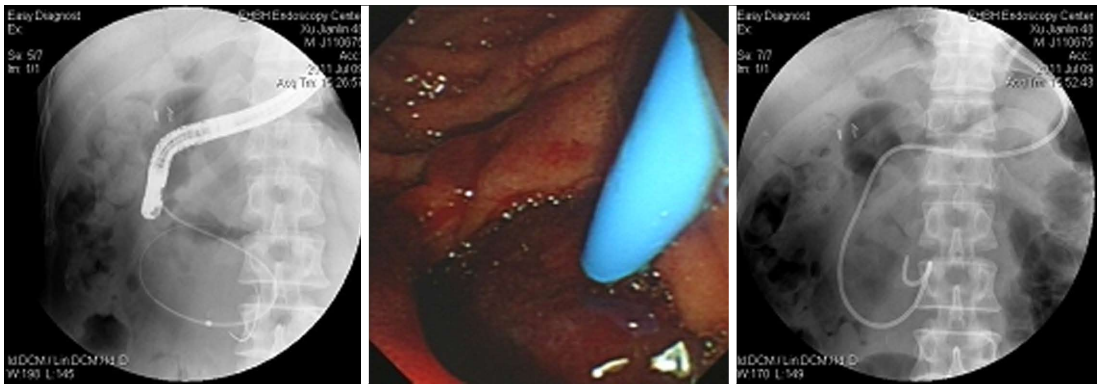

Figure 2 Drainage of the hematoma by the nasal method.

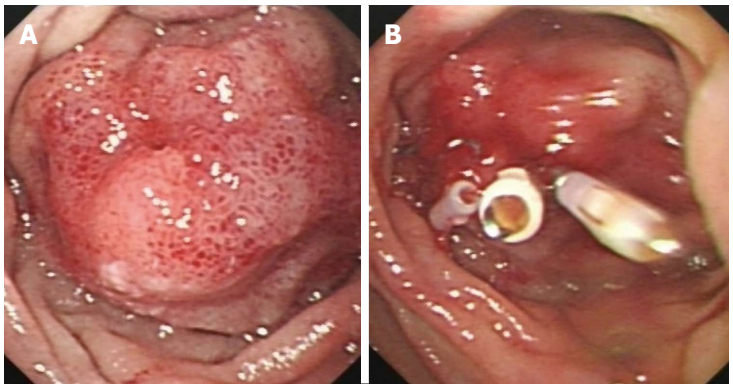

Figure 3 Duodenoscopy examination. A: Hematoma was greatly decreased in size; B: The puncture area was closed with hemostatic clips.

concentration. Imaging techniques, including gastrointestinal endoscopy, CT and MRI, can be used to confirm the diagnosis. Gastrointestinal endoscopy can demonstrate not only duodenal obstruction, but also perforation. CT and MRI can determine the exact extent of the hematoma and may also indicate perforation[7,8]. Since perforation needs a surgical operation, imaging techniques have to be performed immediately.

Once IDH is confirmed, conservative management with fasting, total parenteral nutrition and nasogastric suction should be given promptly. Traditionally, a duodenal hematoma is mainly treated by open surgery drainage and PTCD[9], both causing great trauma. The method used in the present case is minimally invasive and has less risk. We would like to emphasize the following key points in our method. First, the opening should be made at the center of the hematoma with the needle knife under a duodenoscope, and the head of the drainage tube should be soft and flexible to avoid damage to the hematoma wall. Transnasal drainage can be performed by vacuum aspiration, which allows easy observation of the drainage volume. Secondly, after opening the hematoma, the catheter is introduced and the contents are extracted, and then a transnasal guidewire is placed for drainage. Thirdly, the opening on the hematoma should be clamped and an ENBD tube should be placed to drain the bile duct to avoid infection by bile and intestinal juice.

A duodenal hematoma is a rare complication of ERCP, and blunt damage by the endoscope and an accessory should be avoided during ERCP. A CT scan shows specific signs of ERCP-associated duodenal hematoma, such as a high density mass in the enteric cavity and a narrowed enteric cavity. A duodenal hematoma can be easily diagnosed by endoscopy. When the hematoma is very big or causes obstruction of the intestine, a drainage tube can be used to aspirate the contents of the hematoma. Transnasal drainage combined with needle knife puncture under endoscopy has less injury risk and is easy to perform, making it superior to percutaneous drainage. Since ERCP has become a common approach for the treatment of biliary and pancreatic diseases, the incidence of IDH is likely to increase. Our method is worth popularizing in clinical practice.

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CASE REPORT

Small intestinal tubular adenoma in a pediatric patient with Turner syndrome

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Abstract

Turner syndrome (TS) is a female chromosomal disorder caused by the lack of an X chromosome. The loss of this chromosome may result in the deficiency of tumor-suppressive or DNA repair genes, leading to tumorigenesis. Recombinant human growth hormone (GH) has been popularly used for treatment in TS patients for growth promotion. Although treatment with GH has been correlated with precancerous and cancerous lesions in TS children, its associations with gastric or colonic tumors, especially ileal tubular adenomas, have not been reported frequently. We here report a case of a 16-year-old patient with TS and tubular adenoma of the small intestine. Whether the ileal adenoma was caused by TS itself or GH therapy was discussed.

Key words: Tubular adenoma; Turner syndrome; Growth hormone; Pediatric patient; Ileocolonoscopy

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INTRODUCTION

Turner syndrome (TS) is a female chromosomal disorder in which all or part of the X chromosome is missing. Clinically, it is not uncommon for patients with TS to develop tumors from various tissues. In adults with TS, gastric or colonic tumors have been reported. However, these tumors are rarely reported in children, and the occurrence of ileal tubular adenoma in pediatric TS patients has not been described. In this case report, ileal tubular adenoma was detected in a 16-year-old girl with TS after receiving growth hormone (GH) therapy for three years. Whether the ileal adenoma was caused by TS itself or GH therapy was discussed.

CASE REPORT

A 16-year-old girl first admitted to the hospital in July 2009 because of short stature. On admission, she was 127.5 cm in height and 25 kg in weight. Her bone age was 9 years. Ultrasonography revealed a small uterus and right ovary and possible absence of left ovary. A chromosomal analysis showed genetic mosaicism of the X chromosome, namely 46Xi (45X, 46XX). Diagnosis of TS was made and daily nocturnal GH injections were initiated. In 2 years, the patient gained 10 cm in height.

In the patient's health history, she experienced per rectal bleeding at the age of 9 mo that had subsided spontaneously and no definitive diagnosis was made. The patient also had recurrent abdominal cramping associated with loose bowel movements, although the pain usually subsided spontaneously. In August 2009, an ultrasono-

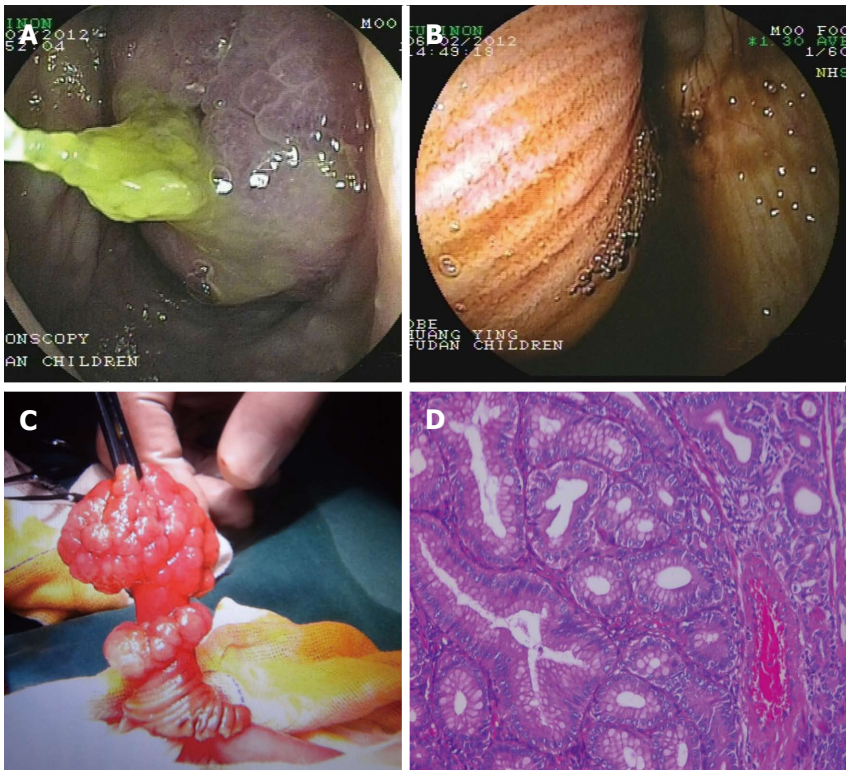

Figure 1 The image of the polyp. A: A large polyp was seen in the terminal ileum when ileocolonoscopy was performed. The tumor appeared hyperemic; B: Double balloon enteroscopy was performed through the anus. The enteroscope was advanced to the ileocecal region and a mass covered with small intestinal mucosa was seen protruding through the ileocecal valve. The appearance was indicative of intussusception; C: A polyp of about 2 cm × 4 cm in diameter and 55 cm from the ileocecal region was seen during operation, with a wide base and multi-nodular appearing surface. The pedicle also appeared nodular; D: Histopathological section of the polyp showing pleomorphic glandular hyperplasia with formation of nodules. The polarity of cell was well presented (hematoxylin and eosin stain, original magnification, × 200).

gram was performed when the abdominal pain became more severe, and an abdominal mass on the right side was noted and suggested intussusception. The pain and mass disappeared spontaneously and no further treatment was undertaken.

The patient again developed abdominal pain on January 27, 2012. The pain was para-umbilical, colicky and associated with bloody stool. There was no tenesmus, and the abdominal colic dissipated after bowel movements. The blood in the stool appeared bright red in color. The patient vomited twice and the vomit contained no blood. She also had a fever of 38 ℃ and was admitted to the hospital for investigation of gastrointestinal bleeding in February 2012.

On admission, her general condition was normal. There was developmental delay in height and no secondary sexual characteristics were noted. Intellectually, her development was normal. Other features of TS were not evident.

On admission, her blood test revealed a hemoglobin level of 79 g/L, positive stool occult blood, white blood cell 10-15/high power, red blood cell 3-5/high power, and no macrophages. Stool culture was negative. Coagulation function was normal. The patient had several dark-colored, bloody bowel movements after admission and abdominal pain that was relieved after bowel movement. She also vomited several times after admission, and the vomit consisted of ingested food. No coffee-ground material was noted. A radionuclide scan for Meckel's diverticulum was negative.

Since she has a history of administration of GH, both insulin-like growth factor-1 (IGF-1) and IGF binding protein levels were checked and both were within normal limits (119 ng/mL and 3.05 μg/mL, respectively). Gastroscopy revealed gastritis and a positive rapid urease test for *Helicobacter pylori*. In ileocolonoscopy, the ileocecal valve was petulant and a large polyp was seen in the terminal ileum (Figure 1A). The polyp shifted in position and was soon noted to have retracted to a more proximal position. It was not seen again until the colonoscope had advanced to more than 10 cm proximally.

Enhanced computed tomography and ultrasound of the abdomen were both suggestive of intussusception of the small intestine with a focal segmental increase in the thickness of the bowel wall. A double-balloon enteroscope was advanced through the anus to the ileocecal region. There a mass covered with small intestinal mucosa was seen protruding through the ileocecal valve (Figure 1B). The appearance was indicative of intussusception of the ileum into the colon and an operation was performed to reduce the invagination. Further examination of the small intestine found a mass of about 2 cm × 4 cm in diameter 55 cm from the ileocecal region. The intestinal segment was decompressed and opened. A small

Table 1 Reported cases of gastroenteric tumors in association with Turner syndrome in children

Ref.	Study location	Age (yr)	Tumor location
Siqueira *et al*[6]	Japanese	14	Adenocarcinoma of colon
Krishnamurthy *et al*[7]	India	13	Adenocarcinoma of rectum
Eriguchi *et al*[8]	United States	13	Carcinoid of appendix
Present case	China	16	Tubular adenoma of small intestine

bowel polyp with a wide base and multi-nodular appearing surface was observed (Figure 1C). The pedicle also appeared nodular. The whole segment of small intestine was resected with the polyp *in situ*.

The pathological findings of the polyp are as follows: macroscopically, brownish polyp 3.3 cm × 2.8 cm in size with nodular protuberances on the surface. The cut surface was grayish in color and of medium firmness; microscopically, pleomorphic glandular hyperplasia with formation of nodules, the polarity of the cell was well presented (Figure 1D); immunohistochemically, β-catenin positive, adenomatous polyposis coli protein negative, 10% Ki-67 positive cells, cyclin D1 positive, carcinoembryonic antigen weak positive, neuron specific enolase positive, synaptophysin positive, and CD56 positive. The final diagnosis was small intestinal tubular adenoma.

DISCUSSION

TS is a female chromosomal disorder and is caused by the lack of an X chromosome. It causes delays in both general growth and sexual development. TS is characterized by physical abnormalities such as short stature, swelling of the hands and feet, a broad chest, low hairline, low-set ears, and a webbed neck. Girls with TS typically experience gonadal dysfunction, which results in primary amenorrhea and sterility.

Many tumor-suppressive and DNA repair genes are located on the X chromosome; the lack of one or many genes may result in chromosomal instability and the deficiency of critical DNA repair mechanisms. Therefore, TS may be associated with oncogenesis in some patients[1]. Clinically, TS has been associated with some tumor types[2-4] with the most common tumors arising in the sex glands. However, other reports have found that patients with TS have a higher incidence of tumors of the central nervous system (including ophthalmic tumors) and of the bladder and urethra. Some reports indicated that adults with TS have a higher incidence of gastric or colonic tumors, although the mechanism is not clear[5] and the association was not common in children[6-8]. Importantly, ileal tubular adenoma in patients with pediatric TS has not been described. The reported and present pediatric cases in the literature are summarized in Table 1.

Recombinant human GH has been popularly used in TS patients for growth promotion. GH exerts its action through binding and activation of its receptor (hGHR), which is ubiquitously expressed in the human body. In TS patients, GH is therapeutically used for growth enhancement, especially for height. GH also influences many metabolic processes, and its association with tumorigenesis has been the subject of intensive research. In patients with acromegaly, the incidence of precancerous (adenomatous polyps) and cancerous lesions in the colon is increased[9]. Carroll *et al*[10] reported that GH deficiency in mice reduced the incidence of colonic tumors. Likewise, hGHR expression in patients with colonic cancer is upregulated, and its expression level is positively correlated with the stage and differentiation of the tumor[11]. A cohort study by Swerdlow *et al*[12] that followed 1848 patients receiving GH treatment determined that the incidence and mortality of both rectal and colonic tumors were increased compared with the non-treated patients. These studies suggest that GH may be related to the pathogenesis of gastrointestinal tumors. Moreover, IGF-1 is an important mediator of the action of GH and has been implicated in the pathogenesis, development, invasion and metastasis of many human tumors. In China, studies have demonstrated that IGF-1 mRNA expression level was higher in gastric cancer tissues in contrast to non-tumorigenic tissues[9]. Other studies have also demonstrated that IGF-1 receptor expression in colonic cancer is elevated and that this over-expression results in tumor cell growth, independent of other growth factors such as platelet-derived growth factor, basic fibroblast growth factor, and epidermal growth factor[13].

Conversely, insulin-like growth factor binding proteins (IGFBPs), particularly IGFBP-3, can inhibit IGF-stimulated growth of tumor cells, facilitating apoptosis[14]. Although the role of the GH-IGF-1 axis in gastrointestinal tumors has not been well defined, evidences from both basic and clinical studies[15] indicate that it may play an important role in the pathogenesis of these tumors because GH enhances tumorigenesis through the action of IGF-1; IGF-1 induces tumorigenesis in the absence of GH; and IGFBP-3 inhibits tumorigenesis.

In conclusion, a small intestinal tumor was detected in a 16-year-old TS patient who had received GH therapy for three years. The patient had a history of per rectal bleeding, recurrent abdominal pain, and intussusception, which indicated the presence of an intestinal polyp. Although neither IGF-1 nor IGFBP-3 levels were elevated, their effects on tumorigenesis could not be ruled out. As the concentration of free IGF is proportional to the ratio of IGF-1/IGFBP-3, and IGF-1 impacts both growth and tumorigenesis, these factors shuld be closely monitored in patients receiving GH treatment. Future studies should address the relationship between GH therapy, the IGF-axis, and incidence of intestinal carcinomas.

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CASE REPORT

Obstructive jaundice and melena caused by hemocholecyst: A case report

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Abstract

A hemocholecyst (HC) is a clot-filled gallbladder caused by bleeding into its lumen. Obstructive jaundice caused by the compression of HC to the hilar biliary tract is likely to be misdiagnosed as cholangiocarcinoma and is extremely rare. We herein report a case of obstructive jaundice and melena caused by HC. A 57-year-old male patient presented with right upper quadrant pain associated with icteric sclera and melena was suspiciously diagnosed as having malignant cholangiocarcinmoa by abdominal ultrasonography, computed tomography and magnetic resonance imaging. Laparotomy found a hematoma in the gallbladder. The hematoma spread to the left hepatic lobe forming an exogenous mass which compressed the hilar biliary tract. Radical cholecystectomy and bile duct exploration with T-tube drainage were performed. Histopathological examination revealed massive necrosis of the gallbladder mucosa with inflammatory cells infiltration as well as intraluminal hematoma formation. One month after operation, a T-tube cholangiography revealed a normal biliary tree. We suggest that HC should be considered in patients with obstructive jaundice and melena after common causes are ruled out.

Key words: Hemocholecyst; Biliary tract; Obstruction; Jaundice; Melena

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INTRODUCTION

A hemocholecyst (HC) is a clot-filled gallbladder caused by bleeding into its lumen. As a cause of hemobilia, HC is rarely reported with a variety of etiology including trauma[1], iatrogenic manipulation[2,3], gallbladder tumor[4,5], cholecystitis[6] and ruptured cystic artery aneurysm[7]. Hematological disorders such as hemophilia are also implicated in some cases[8,9]. Here, we report a rare case of obstructive jaundice and melena caused by HC. Though abdominal ultrasonography (US), computed tomography (CT) and magnetic resonance imaging (MRI) have proved to be highly accurate methods for evaluating gallbladder disorders, a definitive diagnosis cannot be established preoperatively. In addition, our patient had not experienced abdominal trauma, and had no history of obvious bleeding disorders.

CASE REPORT

A 57-year-old man presented at our emergency department with the right upper quadrant pain with icteric sclera for seven days, and melena for two days. The pain was dull in character, sudden in onset, and did not radiate to the back. It was not aggravated by food intake or related to movement. The patient had no history of abdominal trauma or peptic ulcer with bleeding. On admission, the patient was febrile with right upper

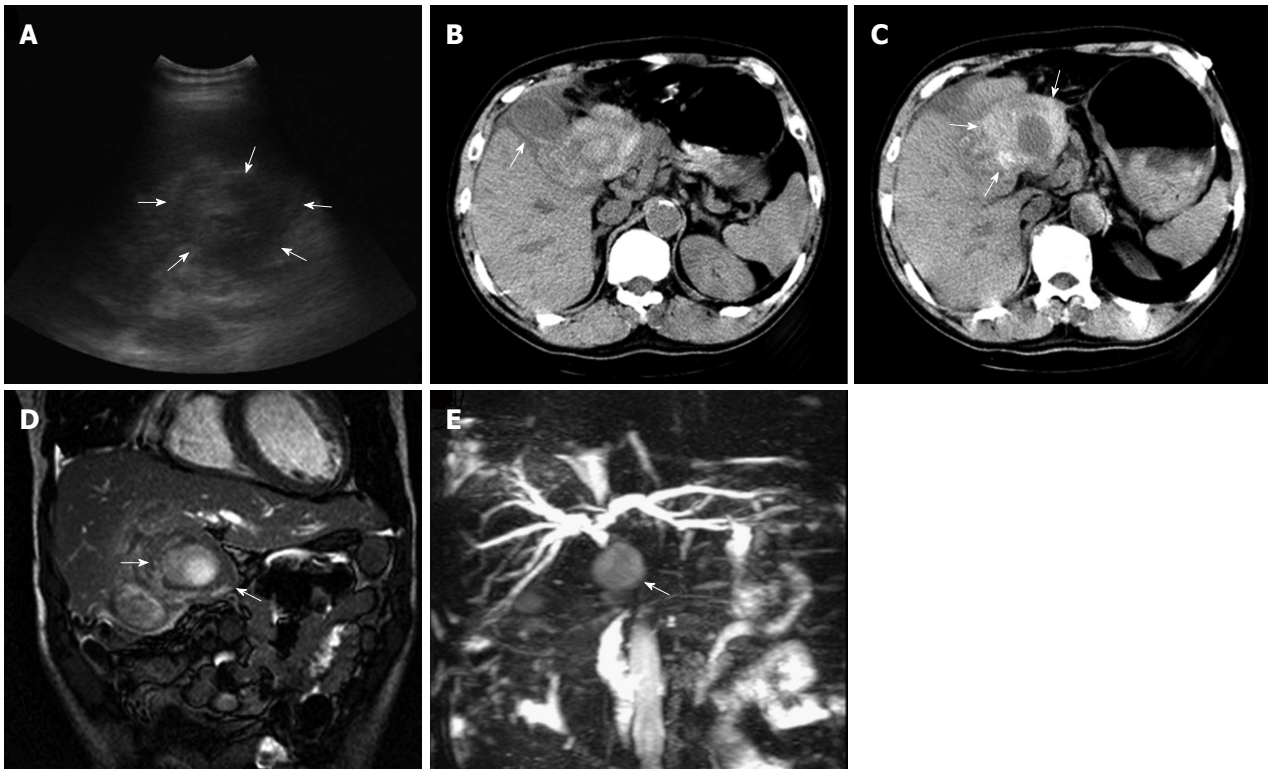

Figure 1 Preoperative imaging findings. A: Ultrasonography revealed a mass with heterogenous echo at the gallbladder region (arrows); B: Computed tomography (CT) showed thickening of the fundus and body of gallbladder wall (arrow); C: CT showed a mass with high-density shadow located at the gallbladder neck without clear demarcation from the left hepatic lobe (arrows); D: Magnetic resonance imaging (MRI) showed a mass located at the gallbladder neck and left hepatic lobe (arrows); E: MRI showed slight dilation of intrahepatic ducts and an obscure image of hilar biliary tract (arrow).

quadrant tenderness, rebound tenderness, slight muscular rigidity and a positive Murphy's sign. Laboratory examinations revealed abnormal hepatic enzyme levels: aspartate aminotransferase 145 U/L (normal, 5-34 U/L), alanine aminotransferase 95 U/L (normal, 0-40 U/L), increased total bilirubin 135.5 μmol/L (normal, 3.4-20.5 μmol/L) and increased alkaline phosphatase 240 U/L (normal, 40-150 U/L). The patient had leukocytosis (14 200/μL), with decreased hemogram (hemoglobin, 8.9 g/dL; hematocrit, 32.7%), but normal platelet count (242 × 10^3/μL). However, prothrombin time and partial thromboplastin time were both normal. Serum amylase and lipase levels were also within normal limits. Tumor markers including alpha foetoprotein, carcinoembryonic antigen and carbohydrate antigen were also negative.

Ultrasound of the abdomen revealed a mass with heterogenous echo at the gallbladder region (Figure 1A). CT showed thickening of the gallbladder fundus and body wall (Figure 1B) and a mass with high-density shadow located at the gallbladder neck without clear demarcation from the left hepatic lobe (Figure 1C). MRI showed a mass located at the gallbladder neck and left hepatic lobe (Figure 1D) with slight dilation of intrahepatic ducts and an obscure image of hilar biliary tract (Figure 1E). Gastrointestinal tract endoscopic study found no common causes of melena such as malignancy, ischemic colitis, *etc*. Initial diagnosis of malignant cholangiocarcinoma was made. He was treated with antibiotics, hemostatics and fluid supplement before transferred to the operating room for exploratory laparotomy. On exploration, a hematoma was found in the neck-side lumen of the gallbladder. The hematoma spread to the left hepatic lobe forming an exogenous mass which compressed the hilar biliary tract (Figure 2). Cholecystectomy and bile duct exploration with T tube drainage were performed. Histopathology revealed massive necrosis of the gallbladder mucosa with inflammatory cell infiltration as well as intraluminal hematoma formation. The patient was discharged on the 7th postoperative day in a stable condition. One month after operation, a T-tube cholangiography revealed a normal biliary tree (Figure 3).

DISCUSSION

Hemorrhagic cholecystitis is an extremely rare cause of hemobilia. Up to now, only three cases of hemobilia which was clearly attributable to acalculous cholecystitis, have been reported by Shah *et al*[10] and Ellington *et al*[11]. Our patient is the 4th reported case. We think that acalculous cholecystitis may cause hemorrhage through mucosal necrosis and ulceration with erosion into one or more vessels in this patient.

Hemobilia classically presents as biliary colic pain, jaundice and gastrointestinal tract bleeding. However, the clinical presentation varies. Depending on the amount and rate of bleeding, blood may clot in various locations. If the blood does not clot in the biliary tract, hematemesis or melena may occur. If blood clots within the bile

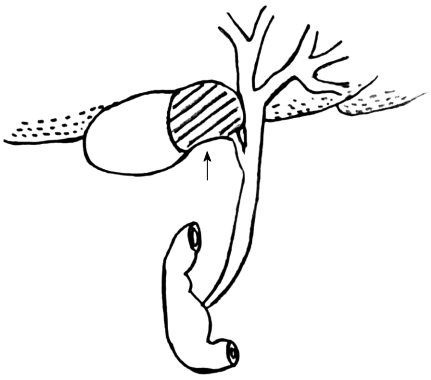

Figure 2 Schema of the hematoma found at the neck of the gallbladder during operation. The arrow indicates hematoma.

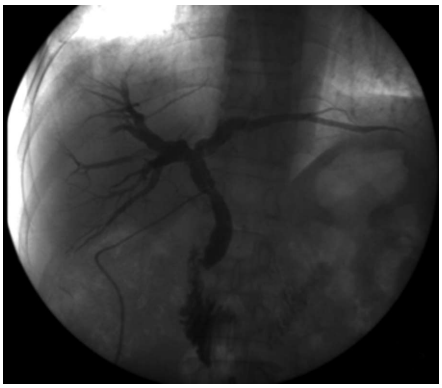

Figure 3 T-tube cholangiography revealed a normal biliary tree after operation.

duct, it may cause obstructive jaundice or pancreatitis. It was unusual in this patient that jaundice was not caused by blood clots within the bile duct, but resulted from the compression of the HC to the hilar biliary tract.

Our patient did not present with melena as a primary symptom. He had symptoms, signs, and laboratory studies that suggested choledocholithiasis and cholangitis. He developed melena only on the 5th day. The preoperative diagnosis of gallbladder hematoma is very difficult, or even impossible, to make in the absence of a history of trauma or bleeding diatheses. Preoperative US, CT and MRI are main techniques for diagnosing gallbladder diseases. Although the US and CT findings of gallbladder hematoma might not be exactly the same as those of gallbladder carcinoma or invasive hilar cholangiocarcinoma, a definitive diagnosis often cannot be made confidently. MRI is useful for differential diagnosis. It was reported that hematomas are similar in signal intensity to skeletal muscle on T1-weighted imaging, with conversion to marked hypointensity on T2-weighted imaging[8]. The hematoma in our patient was not confined to the gallbladder but spread to the left hepatic lobe forming an exogenous mass which compressed the hilar biliary tract, thus making the diagnosis even more difficult. All preoperative imaging studies gave an impression of a highly suspected cholangiocarcinoma with invasion to the gallbladder neck and left hepatic lobe. In our case, cholangiocarcinoma could not be ruled out. Therefore, the correct diagnosis could only be made through dynamic image observation, aggregate analysis and resolute operation exploration.

In conclusion, we suggest that HC should be considered in patients with obstructive jaundice and melena after common causes are ruled out.

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Alcohol consumption and fatty liver disease

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Abstract

Hamaguchi *et al* recently reported some interesting observations on alcohol consumption and risk of fatty liver disease from a large population. However, we feel that it might be necessary to discuss some concerns in this study. As the alcohol consumption categorization was defined by the same criteria in both men and women, which might affect their results. As another factor is soft drinks consumption. It has been proved that soft drinks, especially fructose, contributes to the development of obesity, diabetes, metabolic syndrome, and nonalcoholic fatty liver disease. However, this confounding factor was not adjusted or discussed in this article. The third is the genetic background, for some genetic factors are related with the development of fatty liver disease, which was also not considered yet.

Key words: Alcohol; Fatty liver disease; Obesity; Diabetes; Metabolic syndrome

Core tip: Modest alcohol consumption was significantly inversely associated with fatty liver disease in recent studies. However, some studies did not consider some important potential confounding factors when they conclude their findings. Herein, we raised and discussed these important factors in this letter.

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TO THE EDITOR

We read with great interest the article by Hamaguchi *et al*[1] published in January 2012 at *World J Gastroenterol*. This cross-sectional study reported some interesting observations on alcohol consumption and risk of fatty liver disease from a large population. However, we feel that it might be necessary to discuss some concerns in this study.

The authors clearly indicated that alcohol consumption was significantly inversely associated with fatty liver disease, especially in men. However, they did not find this association in their previous cohort study[2]. The reason for this contradiction might be that some important confounding factors were not considered. As the alcohol consumption categorization was defined by the same criteria in both men and women, only 84 female subjects (1.1%) were defined as excess alcohol consumers and 207 (2.7%) were defined as moderate alcohol consumers, the numbers being much lower than those in men (13.5% and 14.7%, respectively). Although the authors analyzed the data in both men and women, the initial categorization was not separated, which might affect their results.

Another factor is soft drinks consumption. It has been proved that soft drinks, especially fructose, contributes to the development of obesity, diabetes, metabolic syndrome, and nonalcoholic fatty liver disease[3]. However, this confounding factor was not adjusted or discussed in this article. The last point is about the genetic background. Although the mechanisms of this inverse association between alcohol consumption and fatty liver disease are still unclear, some genetic factors are related with the development of fatty liver disease[4-6], such as peroxisome proliferator-activated receptor gamma and hemochromatosis gene polymorphisms[7,8]. Therefore, as some genetic factors might interact with alcohol consumption in fatty liver disease, it could be an interesting topic for further investigations.

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Patent (list all authors)

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