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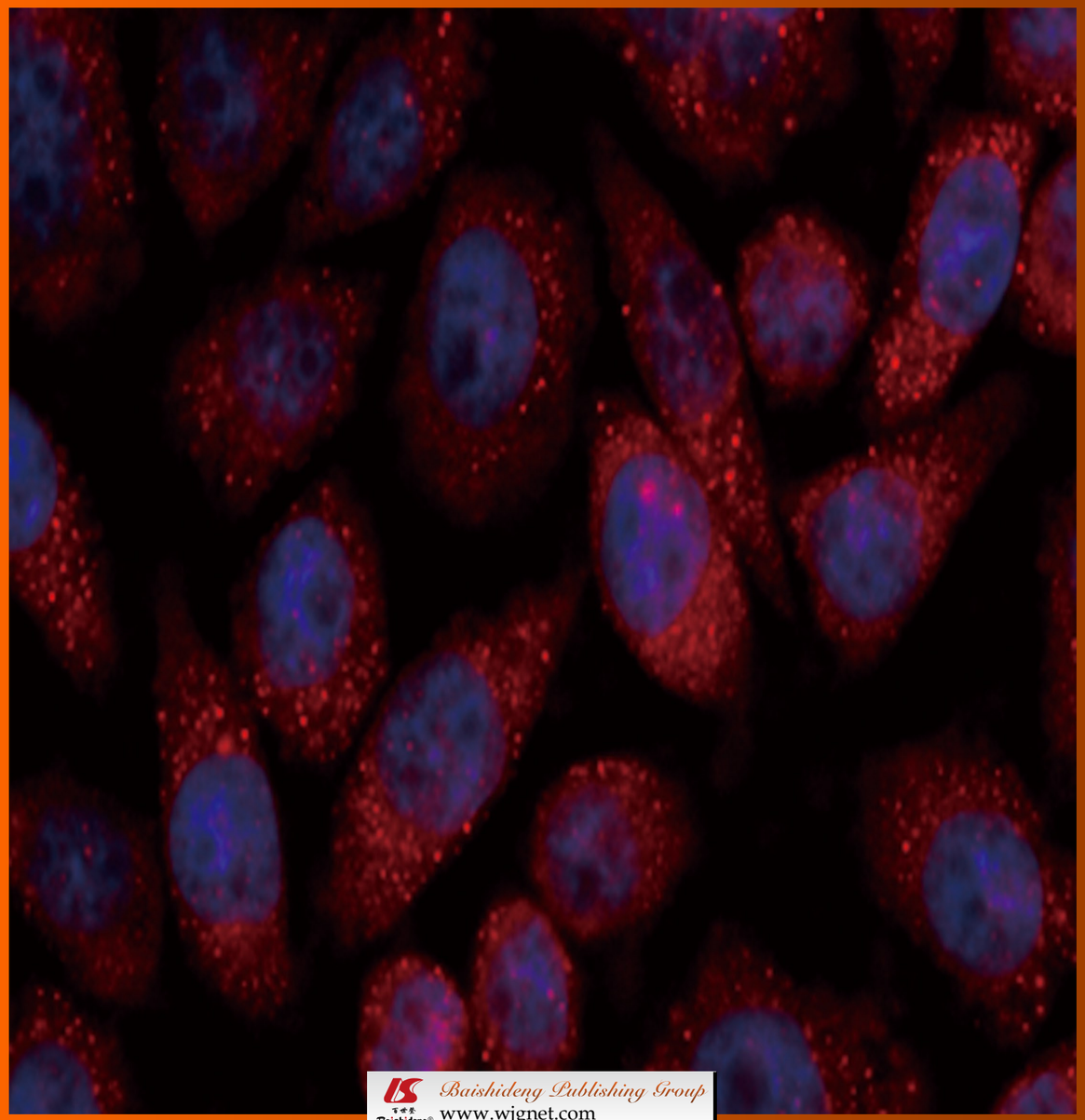

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EDITORIAL

Common misconceptions about 5-aminosalicylates and thiopurines in inflammatory bowel disease

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Abstract

Misconceptions are common in the care of patients with inflammatory bowel disease (IBD). In this paper, we state the most commonly found misconceptions in clinical practice and deal with the use of 5-aminosalicylates and thiopurines, to review the related scientific evidence, and make appropriate recommendations. Prevention of errors needs knowledge to avoid making such errors through ignorance. However, the amount of knowledge is increasing so quickly that one new danger is an overabundance of information. IBD is a model of a very complex disease and our goal with this review is to summarize the key evidence for the most common daily clinical problems. With regard to the use of 5-aminosalicylates, the best practice may to be consider abandoning the use of these drugs in patients with small bowel Crohn' s disease. The combined approach with oral plus topical 5-aminosalicylates should be the first-line therapy in patients with active ulcerative colitis; once-daily treatment should be offered as a first choice regimen due to its better compliance and higher efficacy. With regard to thiopurines, they seem to be as effective in ulcerative colitis as in Crohn' s disease. Underdosing of thiopurines is a form of undertreatment. Thiopurines should probably be continued indefinitely because their withdrawal is associated with a high risk of relapse. Mercaptopurine is a safe alternative in patients with digestive intolerance or hepatotoxicity due to azathioprine. Finally, thiopurine methyltransferase (TPMT) screening cannot substitute for regular monitoring because the majority of cases of myelotoxicity are not TPMT-related.

Key words: Crohn' s disease; Ulcerative colitis; Inflammatory bowel disease; Aminosalicylates; Steroids; Azathioprine; Mercaptopurine; Misconceptions

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INTRODUCTION

Daily clinical practice requires constant decision making, and each is open to possible errors[1-6]. Misconceptions are very common in clinical practice, but can be prevented[1-5]. More than 10 years ago, the Institute of Medicine issued its groundbreaking report, "To err is human: building a safer health system", which revealed that approximately 100 000 Americans die each year from preventable errors in hospitals[7]. The publication fundamentally changed the debate about health care quality in the United States and reconfigured how we think about the quality of care; attracted great interest

among payers and employers for improvement of care and patient safety; and produced substantial increases in research support[2]. In fact, safety issues have been a key factor in many human activities during the past few decades, and it is shocking how late the general culture of safety is reaching the health-care business. As recently summarized in a must-read book[8], "to get things right" can be a complex task but an indispensable one.

It has been pointed out that variation itself is a natural consequence of medicine being as much art as science, and thus some basal level of variation is to be expected[9]. However, in many instances, the current process of care exceeds the expected levels of natural variation, and at times may be extreme to the point of possibly indicating suboptimal overall care[9]. Medical advances have generated an increase in scientific literature and have made decision making more complex. From a scientific point of view, evidence-based medicine provides various highly useful tools for patient treatment, including clinical guidelines or consensus documents. However, frequent digressions from evidence-based recommendations and published guidelines exist, despite the wide dissemination of practice guidelines, which denotes poor quality of care[9-12].

When faced with the same set of facts, healthcare providers often make different diagnoses, employ different tests, and prescribe different therapies[9,13]. Wide practice variations might have several explanations, including the need for more evidence to determine the best course of action; the possibility that multiple approaches might be equally effective for a clinical scenario; or the need for existing evidence to be more effectively consolidated into guidelines and disseminated into practice[12]. Despite the wide dissemination of practice guidelines, clinical pathways and utilization review protocols, extreme variation continues to exist throughout all fields of medicine[9-11]. Within the field of gastroenterology, inflammatory bowel disease (IBD) is likely to generate diversions from clinical guidelines and extreme variations in the process of care[12,14-16]. There are at least three factors that establish IBD as a target for variation[9]: (1) the diagnosis of IBD is often uncertain, and this diagnostic uncertainty may lead to a potentially arbitrary sequence of diagnostic testing with various modalities; (2) the presentation of IBD is heterogeneous, and the multiple presentations of IBD mandate different diagnostic and therapeutic approaches; and (3) the treatments for IBD are themselves varied, and new treatments are always being developed and disseminated. It has been emphasized that demonstration of significant variations in the process of care in IBD indicates a need to disseminate better the available information in this area. Furthermore, identifying specific factors that predict extremes in resource utilization and clinical practice may allow for improved targeting of areas where doctor knowledge or education is inadequate[9].

Although experts and community providers are in general consensus about diagnostic decision making in Crohn' s disease, extreme variation exists both between and within groups for key therapeutic decisions in this disease[9]. When the standard of outpatient care provided has been evaluated, it has been demonstrated that the specialist IBD clinic provides better care than the non-specialist general gastroenterology clinics; even in the specialist clinic, however, the care of a relevant minority of patients does not fulfill certain criteria[17]. Some authors have performed a vignette survey to measure variations in decision making in areas of controversy dealing with ulcerative colitis, and have concluded that community gastroenterologists and ulcerative colitis experts vary dramatically in their approach to many areas of uncertainty, which suggests that current practice patterns are highly disparate and focus attention on specific areas of disconnect that should be further investigated[12]. Finally, a recent study has aimed to determine whether patients referred for a second opinion were receiving therapy in accordance with practice guidelines; it was concluded that patients with IBD often do not receive optimal medical therapy[18].

Our aim was to review several common misconceptions in the management of IBD. We focus on ambulatory patients who have predominantly mild or moderate disease treated with 5-aminosalicylates (5-ASAs) and thiopurines; the two most widely used drugs in IBD. Although decision making in the outpatient setting appears to be less difficult than in hospital situations, the reality of every day care makes human errors even more possible in outpatients. Thus, in the clinical setting, decisions need to be made immediately, with the pressure of limited time, and the understanding that an enormous variety of possible clinical situations exist. The approach taken in this paper is to state the most commonly found misconceptions in clinical practice, to review the related scientific evidence, and finally propose appropriate recommendations.

5-AMINOSALICYLATES

Aminosalicylates are the undisputed first-line option for treating and maintaining remission in ulcerative colitis[19-24]. Furthermore, they may have chemopreventive properties against colorectal cancer[25]. However, the role that these drugs may play in the management of Crohn' s disease has been controversial.

5-ASA drugs are as effective for the treatment of Crohn' s disease as for ulcerative colitis

Initially published trials have shown that oral aminosalicylates are effective treatment for active ileal, ileocolic, or colonic Crohn' s disease[26,27]. Sulfasalazine 3-6 g/d is effective in patients with colonic, but not in those with small bowel disease[28,29]. Asacol is effective in ileocolic or colonic disease[30] and Pentasa has been reported to be effective for ileitis, ileocolitis and colitis[31]. As a consequence, mesalazine has become a popular treatment for mild Crohn' s disease. However, more recently, a meta-analysis of the three placebo-controlled trials of Pentasa 4 g/d for active Crohn' s disease for 16 wk in a total of 615 patients, showed a mean reduction of the Crohn's disease activity index (CDAI) of 63 points, compared to 45 points for placebo (that is, a difference of only 18 points)[32].

Although this confirmed that a time-dependent de-

layed release formulation of mesalazine, Pentasa 4 g/d, is superior to placebo, the clinical significance of the reduction in CDAI is debatable because in individual trials, a 70- to 100-point decrease generally is required to establish clinical efficacy[32]. From these data, an alternative conclusion seems to be more plausible; namely, that Pentasa is ineffective for the treatment of symptomatic Crohn' s disease[33]. Thus, at this stage, mesalazine should be considered clinically no more effective than placebo for active ileal or colonic Crohn' s disease[33]. Accordingly, the European Crohn' s and Colitis Organization (ECCO) have concluded, "the benefit of mesalazine is limited"[26,27]. Therapeutic agents now exist that offer safe and highly effective alternatives to 5-ASA for the treatment of mild-to-moderate Crohn' s disease[34]. Specifically, in ileal or ileocolonic disease, budesonide provides the benefits of prednisone with less systemic side effects[35].

When faced with the same set of facts, healthcare providers often make different diagnoses, employ different tests, and prescribe disparate therapies. Esrailian *et al*[9] have constructed a survey with five vignettes to elicit provider beliefs regarding the appropriateness of therapies in Crohn' s disease. The authors measured agreement between community gastroenterologists and Crohn' s disease experts (the latter following, theoretically, more closely practice guidelines recommendations), and measured variation within each group. In the management of a patient with newly diagnosed Crohn' s disease, 75% of community providers endorsed the use of 5-ASA products, whereas less than half of experts (44%) employed 5-ASA therapies.

In summary, in the setting of modest efficacy and more potent alternatives, the best practice may be to consider abandoning the use of 5-ASA in patients with small bowel Crohn' s disease, until the appropriate patient population where these drugs may theoretically be effective is better delineated[33,34].

The combination of oral and topical 5-ASA treatment is not necessary, as each treatment on its own is similarly effective

Pharmacokinetic studies have demonstrated that, when given *per os*, the active moiety of mesalazine is delivered mainly to the distal ileum and proximal large bowel, thus ensuring a higher mucosal drug concentration in the right than in the left colon, with only negligible amounts of the drug reaching the rectal mucosa[36,37]. The increase in the oral dosage further increases the mucosal concentration in the proximal colonic segments, but does not significantly modify distal drug distribution[38]. Conversely, topical mesalazine administration assures considerable drug availability in the recto-sigmoid sites and, to a lower extent in the descending colon[39-41]. Therefore, it appears that, to increase mucosal mesalazine concentration in ulcerative colitis patients, along the entire length of their large bowel, besides oral dosage, topical treatment should be given[42].

As David Sachar has accurately emphasized, a form of undertreatment is overlooking the benefits of topical formulations[16]. The advantages of the combination of oral and topical aminosalicylates have been demonstrated for both inducing ulcerative colitis remission and for maintaining it. For treatment of an acute flare of the disease, on one hand, an already considered classic trial on patients with distal colitis has shown that combined therapy works more rapidly and effectively compared to oral or topical therapy alone[43]. Accordingly, the ECCO states that "left-sided active ulcerative colitis of mild-moderate severity should initially be treated with topical aminosalicylates combined with oral mesalazine. Mesalazine alone is also effective, but less effective than combination therapy"[44]. The beneficial effect of the combined regimen has also been confirmed in extensive colitis by Marteau *et al*[45]. Furthermore, patient-reported health-related quality of life in data collected from this study was investigated, and it was concluded that combined oral plus topical mesalazine treatment significantly improved this important parameter in patients with active ulcerative colitis[46].

On the other hand, there have been several randomized controlled trials comparing combination treatment, including oral mesalazine plus intermittent mesalazine enema, to oral mesalazine alone for maintaining remission[42,47-49], and success rates have been higher in patients receiving the combination regimen. Furthermore, combined oral and topical 5-ASA therapy also appear to have a favorable cost-effectiveness ratio in pharmacoeconomic analyses[47,48].

Although most authors have claimed that patients find long-term rectal treatment acceptable, a postal survey of British patients has shown that 80% preferred oral treatment alone[50]. Therefore, this form of combination treatment (with the aim of maintaining remission) could be appropriate and may be reserved for patients with a high probability of suffering relapse, because it has been demonstrated that the continuous use of topical mesalazine, associated with a high oral dosage, significantly improves the clinical course of ulcerative colitis in patients at high risk of relapse[42]. Thus, adding rectal therapy is a treatment option for patients who have relapsed on oral 5-ASA alone[44].

In summary, owing to the superiority of the combined approach - oral plus topical 5-ASA - it should be used as first-line treatment in patients with ulcerative colitis; mainly in those with predominant rectal syndrome[51].

Total 5-ASA dose should be divided at least twice daily, because a single daily dose is less effective

Oral 5-ASA is an established treatment for ulcerative colitis and the current standard of care for most patients requiring long-term maintenance treatment throughout their lives[52]. However, adherence rates - particularly in patients in remission - may be as low as 40% outside of the clinical trial setting[53]. It is now becoming relevant to find tools that improve patient adherence to treatment[54], as it has been found that multiple dosing is a predictor of non-compliance in IBD[55] and is related to a significantly increased risk of ulcerative colitis flare-ups[56].

Formulations to deliver 5-ASA to the disease activity

site, both orally and topically, have been often inconvenient and have classically required multiple daily dosing[57]. Such regimens can interfere with normal life and reduce the overall quality of life, with a negative impact on treatment adherence and poorer long-term outcomes[52]. Thus, ulcerative colitis patients cite treatment regimen complexity, tablet quantity and dose frequency as key negative influencers of adherence[52,57].

Pharmacokinetic studies in healthy volunteers have suggested that once-daily dosing may be an effective option in patients with ulcerative colitis. Hussain *et al*[58] have shown that serum, urinary, fecal, and rectal tissue concentrations are similar for once and three times daily mesalamine dosing regimens. Also, in a recent study, 4 g oral ethylcellulose-coated mesalamine given once daily was bioequivalent to a twice-daily regimen after single or repeated administration[59].

A new oral delayed-release formulation of mesalazine utilizing Multi Matrix System (MMX) technology was recently approved[60,61]. It is a high-dose (1.2 g/tablet), delayed-release form. Several studies with MMX have shown that mesalamine can be administered once-daily[62-64]. What is most important is that not only the new once-daily mesalazine formulations, but also older forms of 5-ASA may be administered in a single daily dose; apparently with adequate effects.

Response to 5-ASA is better correlated with tissue concentrations and best predicted by concentrations of the drug within the lumen of the colon. Some authors have used computer simulation to predict colonic 5-ASA levels after Asacol administration[65]. An Asacol dosage of 800 mg, three times daily, was compared to 2400 mg given once daily. The predicted maximum and average 5-ASA concentrations in the total colon and individual colonic segments differed by < 10% between dosing regimens. This model supports once-daily administration of 5-ASA as standard treatment for ulcerative colitis.

In a initial pilot clinical study, patients were randomized to receive either once daily or conventional (twice or three times daily) mesalazine for maintenance of remission in ulcerative colitis[66]. After 6 mo, patients in the once-daily arm appeared more satisfied with their regimen and consumed more medication than those in the conventional arm (90% *vs* 76%). More recently, preliminary results from a randomized trial have confirmed these encouraging results[67].

Data for the administration of a single daily dose of 5-ASA are available for both the induction and maintenance of remission of ulcerative colitis. On one hand, some authors have determined the therapeutic equivalence and safety of once-daily *vs* three times daily dosing of a total daily dose of 3 g Salofalk granules in patients with active ulcerative colitis[68]. On the other hand, other authors have confirmed this equivalence for patients with quiescent ulcerative colitis[69]. The results of the first long-term efficacy trial of maintenance therapy (with Pentasa as the 5-ASA) showed that 71% of patients receiving a single daily dose of 2 g mesalazine remained in remission, as compared to 59% of those taking 1 g twice daily; the differences being statistically significant[69]. Patients with ulcerative colitis given 5-ASA once daily had better remission rates, acceptability, and self-reported adherence to therapy compared with patients given 5-ASA twice daily. Another study was conducted to determine the efficacy and safety of once-daily dosing of delayed release mesalamine (Asacol) compared with twice-daily dosing for maintaining remission in ulcerative colitis patients, and demonstrated equivalent results with both regimens[70].

The totality of these data suggests that the success of once-daily dosing for all of these compounds may be due to the pharmacodynamic properties of 5-ASA, and may not depend on the specific characteristics of the formulation determining drug delivery[70]. In other words, given comparable efficacy between once-daily and divided dosing regimes for the treatment of ulcerative colitis with mesalazine MMX, and also with other 5-ASA formulations, the effect is likely to be generic rather than compound specific[44].

In summary, once-daily treatment should be offered as a first-choice regimen to ulcerative colitis patients. Indeed, the availability of treatments that can be taken once daily allows increased flexibility to tailor therapy according to patient preference and lifestyle, and may also have the potential to enhance compliance[69]. In fact, improved efficacy with once-daily dosing seems to be at least partly related to improved compliance[69]. These results and subsequent recommendations reinforce the principle that continued medication consumption, rather than actual drug regimen, is important in preventing disease relapse[67]. Also, that adherence, rather than medication regimen, appear to be important in disease outcome, mainly in the long term[67].

AZATHIOPRINE AND MERCAPTOPURINE

Thiopurine drugs azathioprine and mercaptopurine have been shown to be effective at inducing and maintaining remission in IBD[71,72]. These drugs are becoming increasingly popular, and their use is, at present, being considered at earlier phases of the disease than before.

Correct dose of azathioprine for Crohn's disease is 1-2 mg/kg, because higher doses are not more effective and are associated with increased adverse effects

The choice of azathioprine and mercaptopurine dose is generally based on the patient's weight, with the intention to achieve the highest therapeutic efficacy and, at the same time, to reduce the incidence of adverse effects[73-75]. Based on reported clinical trials, the most effective doses appear to be azathioprine 2.0-3.0 mg/kg and mercaptopurine 1.0-1.5 mg/kg, although there has not yet been a head-to-head comparison at various dose levels or a comparative trial evaluating the efficacy of mercaptopurine versus azathioprine in patients with IBD[76].

A meta-analysis has been performed to evaluate the

efficacy of these agents for the maintenance of remission of quiescent Crohn' s disease[77]. The pooled analysis for maintaining remission was stratified by the dose of azathioprine. When the maintenance therapy data were analyzed for the effect of azathioprine dose (1.0-2.5 mg/kg per day), the odds ratio (OR) for response increased from 1.20 (95% CI: 0.60-2.41) at 1.0 mg/kg per day to 3.01 (95% CI: 1.66-5.45) at 2.0 mg/kg per day, and to 4.13 (95% CI: 1.59-10.71) at 2.5 mg/kg per day. Thus, a common error is to step up the treatment strategy, giving up on thiopurine drugs (for example changing from these drugs to anti-tumor necrosis factor (TNF)α, before being absolutely sure that they have been administered at correct, maximal doses[16].

In summary, a form of undertreatment with thiopurines is underdosing[16]. The habit of automatically administering mercaptopurine or azathioprine at fixed doses of 50 mg/d should have been long abandoned, as higher doses of azathioprine (2.5 mg/kg per day) are more effective than lower doses (1.0 or 2.0 mg/kg per day) for treating Crohn' s disease.

Azathioprine and mercaptopurine are ineffective in ulcerative colitis (or, at best, much less effective than in Crohn' s disease)

Thiopurine drugs are the gold-standard treatment for steroid-dependent Crohn' s disease, because these drugs have been shown to be effective both at inducing and mainly, maintaining remission of the disease[71,72]. In addition, a clear steroid-sparing effect in active or quiescent Crohn' s disease has been observed with azathioprine/mercaptopurine therapy[71,72]. However, debate exists regarding whether thiopurine therapy is as effective in ulcerative colitis as it is in Crohn' s disease[78]. There have been surprisingly few randomized controlled trials, most of which were performed several decades ago and suffered from small sample sizes, used inadequate dosing of azathioprine, had ambiguous endpoints, and other methodological limitations[79].

Some meta-analyses have evaluated the efficacy of azathioprine/mercaptopurine in patients with ulcerative colitis[80-82]. The first one[80], which included studies up to the year 2003, identified only four clinical trials, and the pooled OR of the response to azathioprine therapy compared with placebo for the maintenance of remission was 2.26 (95% CI: 1.27-4.01). In the second meta-analysis[81], the literature search was performed up to the year 2006, and azathioprine was also shown to be superior for the maintenance of remission compared to placebo. Finally, the results of the most recent meta-analysis comparing azathioprine/mercaptopurine *vs* placebo or 5-ASA for the maintenance of remission in ulcerative colitis[82] has been published in 2009, and included six studies[83-88]. A therapeutic benefit of azathioprine, both overall (OR: 2.56; 95% CI: 1.51-4.34) and, particularly, when azathioprine was compared with placebo (OR: 2.59; 95% CI: 1.26-5.3), was demonstrated[82]. The number needed to treat (NNT) to prevent one recurrence with azathioprine/mercaptopurine, when compared with placebo, was only five (which compares favorably with the NNT of seven reported with azathioprine in Crohn' s disease[71]). These favorable results were confirmed when the experience from the non-controlled studies were reviewed: when these drugs were evaluated for the maintenance of remission of ulcerative colitis, the efficacy rate was as high as 76%[82].

A clinically meaningful steroid-sparing effect is achieved by thiopurine treatment, not only in Crohn' s disease patients but also in ulcerative colitis[89-92]. The number of cumulative hospitalizations significantly decreases during azathioprine treatment, both in Crohn' s disease and in ulcerative colitis patients[92,93]. Furthermore, the cumulative number of surgical interventions in patients treated with azathioprine/mercaptopurine has been reported to also be significantly lower after starting thiopurine treatment than before[92]. Finally, some authors have evaluated mortality by IBD medication, and have found that use of immunomodulators (mainly azathioprine and mercaptopurine) were associated with 50% decreased mortality in ulcerative colitis[94].

Few studies have directly compared thiopurine therapy efficacy between ulcerative colitis and Crohn' s disease. Kull *et al*[95] have compared the 6-mo efficacy of azathioprine in patients with both diseases, and found that clinical remission rates were slightly higher for ulcerative colitis than for Crohn' s disease (77% *vs* 70%); furthermore, complete corticosteroid weaning was obtained significantly more often in ulcerative colitis than in Crohn' s disease patients (59% *vs* 30%). Verhave *et al*[96] have concluded that patients with ulcerative colitis treated with azathioprine respond similarly to their Crohn' s disease counterparts. Moreover, they have determined that the beneficial effect occurs 1 mo sooner in ulcerative colitis patients than in Crohn' s disease patients. Fraser *et al*[97] have shown that azathioprine was more likely to achieve remission in patients with ulcerative colitis than Crohn' s disease (58% *vs* 45%), but was equally effective for the maintenance of remission. This study is also worth mentioning because of the long mean follow-up of patients, which provides valuable information to the clinician. In the study by Bastida *et al*[98], the beneficial effect of azathioprine was independent of the type of IBD. Finally, Gisbert *et al*[92] have found in a recent prospective study that azathioprine was similarly effective for Crohn' s disease and ulcerative colitis patients (49% *vs* 42%). Furthermore, azathioprine treatment resulted in a similar reduction in the number of hospitalizations and surgical procedures in both diseases[92].

In summary, it may be concluded that azathioprine and mercaptopurine seem to be at least as effective in ulcerative colitis as in Crohn' s disease patients.

Withdrawal of azathioprine should be recommended after several years if the patient is in remission

A form of undertreating with antimetabolites is suspending or discontinuing them too soon. Although azathioprine and mercaptopurine are effective for maintain-

ing remission in Crohn' s disease[99], no safe number of years has been determined after which these medications can be withdrawn without risk of relapse[16].

With the acceptance that Crohn' s disease is a chronic illness that needs long-term chronic therapy and the adoption of more aggressive goals of therapy (steroid-free remission, avoidance of surgery, and even mucosal healing), continuing an effective maintenance therapy is increasingly advised[100]. However, given the small but finite risk of significant adverse effects, coupled with the need for long-term therapy in patients who are often young and otherwise healthy, stopping immunomodulators in a patient in remission remains appealing[100,101].

The ECCO states that "for patients in remission on azathioprine as maintenance treatment, cessation may be considered after four years of "remission"[26,27]. It is also stated that "benefit and risks of continuing azathioprine should be discussed with individual patients"[27]. However, there has been no consensus about the duration of the treatment once remission has been obtained.

A retrospective study published in 1996 has suggested that withdrawal of azathioprine might be possible in patients who have been in complete remission without steroids for longer than 3.5 years, because the 2-year relapse rate seems similar whether the treatment is continued or stopped after this time[102]. This uncontrolled observation on a small subset of patients required confirmation by a prospective controlled trial. Therefore, Lemann *et al*[101] subsequently performed a multicenter, randomized, double-blind, noninferiority withdrawal trial. Patients who were in clinical remission on azathioprine for > 42 mo were randomized to continue azathioprine or to receive an equivalent placebo for 18 mo. Kaplan-Meier estimates of the relapse rate at 18 mo were 8% and 21%, respectively. Therefore, this study shows that azathioprine withdrawal is not equivalent to continued therapy with azathioprine for maintenance of remission in patients with Crohn' s disease who have been in remission on azathioprine for > 3.5 years. Consequently, the authors have concluded that azathioprine maintenance therapy should be continued beyond 3.5 years[101].

More recently, a cohort study of 66 patients in prolonged remission while being treated with azathioprine who stopped azathioprine, during or at the end of the aforementioned randomized controlled trial, underwent long-term follow-up evaluation[103]. The cumulative probabilities of relapse at 1, 3 and 5 years were 14%, 53%, and as high as 63%, respectively. In other words, two thirds of subjects still relapsed by 5 years when taken off azathioprine. This suggests that in many patients with Crohn' s disease, azathioprine withdrawal is not a feasible alternative, even after years of control, because it is associated with a high risk of relapse, whatever the duration of remission under this treatment[100].

In addition, two retrospective surveys have reported relapse rates after azathioprine or mercaptopurine withdrawal of 66%[97] and 85%[104], respectively, at 3 years. Another study[105] has reported the outcome of 29 patients in remission under continuous treatment with azathioprine for 2 years or more, randomized for continuation or withdrawal of azathioprine. At 1 year after randomization, the remission rate in each group was 85% and 47%, respectively ($P < 0.05$).

Discussion regarding the duration of an effective azathioprine treatment mainly concerns two points: (1) the magnitude of the relapse risk after stopping the drug; and (2) the toxicity of prolonged treatment[101]. As with all other agents, there will be some cost in relation to potential adverse events, including rare cases of infections and neoplasia that are probably related to the level of immunosuppression[106]. When the overall risks and benefits of prolonged maintenance therapy with azathioprine are balanced, it is likely that most clinicians and patients will accept the small, as yet unquantified, risk of a lymphoid malignancy, and the small risk of opportunistic infections, to prevent the ongoing morbidity and impact on quality of life that are related to the chronic symptomatic activity of Crohn' s disease[106].

In conclusion, even after a long duration of clinical remission under azathioprine, withdrawal of this drug is associated with a high risk of relapse. Therefore, as in transplanted patients, azathioprine maintenance therapy should probably be continued indefinitely in patients with Crohn' s disease once remission has been achieved[103,107].

In IBD patients who develop azathioprine digestive intolerance, thiopurine drugs should be definitively withdrawn

Azathioprine intolerance remains an important clinical problem in patients with IBD, which leads to withdrawal of therapy in up to 30% of patients[73]. In particular, its use is limited due to digestive intolerance in 10%-15% of patients[73]. This often mandates treatment with methotrexate, an alternative second-line immunosuppressive therapy in patients with Crohn's disease, or more recently, anti-TNF therapy. For patients with ulcerative colitis, colectomy may be precipitated in some individuals by azathioprine intolerance.

However, it has been suggested that the thiopurine drugs azathioprine and mercaptopurine could be interchangeable. Thus, an alternative strategy for azathioprine intolerance (mainly due to nausea or vomiting) is treatment with mercaptopurine (or *vice versa*). Several case series have addressed this question and have shown that mercaptopurine is tolerated in > 50% of azathioprine-intolerant patients (range: 47%-73%)[108-113].

In summary, treatment with mercaptopurine is a safe alternative in patients with IBD and previous digestive intolerance of azathioprine. Given the mild character of these symptoms, these patients may be cautiously switched to mercaptopurine (or *vice versa*) before being considered for other therapy or surgery[76].

Systematic blood controls may be avoided if thiopurine methyltransferase phenotype/genotype is normal

Azathioprine and mercaptopurine are inactive compounds that must be metabolized to 6-thioguanine nucleotides

(6-TGNs) to exert their cytotoxic and immunosuppressive properties. Thiopurine methyltransferase (TPMT) metabolizes mercaptopurine into inactive 6-methylmercaptopurine[114]. Therefore, reduction in TPMT activity predisposes to bone marrow suppression because of preferential metabolism of mercaptopurine to 6-TGN[115]. Quantification of TPMT activity has been considered a promising area, because it may identify unique metabolic profiles in patients at high risk of adverse reactions prior to drug exposure[115]. Thus, high concentrations of 6-TGN are detected in patients with low activity of TPMT, while low concentrations of these metabolites are found in patients with high TPMT activity, although not all studies have demonstrated this inverse correlation[116-120].

Several studies have reported a correlation between TPMT phenotype/genotype and the risk of myelotoxicity[115]. Homozygous patients for the low TPMT activity allele have an increased risk of suffering severe myelotoxicity due to excessive accumulation of 6-TGN[115]. It has been reported that the probability of having a complete TPMT deficiency or being homozygous for this enzyme is > 6 times higher among patients who have had a myelosuppression episode, when compared with those patients with good tolerance to thiopurine drugs[121]. Furthermore, other authors have even found an incidence of myelotoxicity of up to 100% in patients who are homozygous for the low activity allele[122]. However, some authors have reported that TPMT genotype/phenotype does not predict myelotoxicity in IBD patients treated with thiopurine drugs[123-132]. In this respect, a recent study has prospectively evaluated whether the choice of azathioprine or mercaptopurine dose based on TPMT activity prevents myelotoxicity in IBD patients. Among the four patients with myelotoxicity, one had intermediate basal TPMT levels, and three even had high levels, but no patient had low levels[133]. Finally, several studies have demonstrated that TPMT deficiency phenotype or genotype explains a variable proportion of myelotoxicity cases, but in no way explains all episodes of bone marrow suppression[116-122,125,128,129,134-142].

In summary, the majority of cases of leukopenia are not TPMT-related and therefore TPMT screening can never be viewed as a substitute for the current practice of regular monitoring of white blood cell counts. For this reason, it may be concluded that several factors (e.g., environmental and pharmacological) not related to TPMT activity may be responsible for azathioprine myelotoxicity, and systematic blood controls (complete blood count; mainly leukocyte count) should be done in these patients despite the function of this enzyme being normal.

Azathioprine should always be stopped and non-thiopurine therapy used instead if liver abnormalities are detected

Acute hepatocellular and cholestatic hepatitis have both been described during thiopurine therapy[143,144]. A small percentage of patients present with slight elevation of liver tests that do not have clinical implications, and abnormalities in liver tests return to normal during follow-up, which indicates that it is not always necessary to adjust immunomodulator dose. For example, abnormal liver tests resolved spontaneously while continuing on mercaptopurine in four out of five patients in the study by George *et al*[145], and in three out of four patients in the study by Markowitz *et al*[146].

When abnormalities in liver tests are more marked, but without associated jaundice, the dose of azathioprine/mercaptopurine may be reduced by 50%. It is probably not necessary to withdraw azathioprine or mercaptopurine completely, but frequent clinical and analytical controls should be strictly performed after reducing its dose. With this strategy, liver tests frequently normalize, and the initial azathioprine/mercaptopurine dose may be cautiously prescribed again[147,148].

A recent long-term follow-up study aimed to assess the incidence of azathioprine/mercaptopurine-induced liver injury in 786 patients with IBD (138 of whom received azathioprine/mercaptopurine)[149]. Among azathioprine/mercaptopurine-treated patients, the incidence of abnormal liver tests [liver tests between N (upper limit of the normal range) and 2 N] and hepatotoxicity (liver tests > 2 N) was, respectively, 7.1% and 2.6% per patient-year. In most patients, liver tests spontaneously normalized despite maintaining thiopurine treatment. These drugs were withdrawn due to hepatotoxicity (liver tests > 5 N, and lack of decrease despite 50% dose reduction) in only 3.6% of the patients, and all of them showed normalized liver tests.

If liver tests do not return to normal values with tapering of thiopurines, it has been recommended that therapy should be withdrawn. However, if azathioprine was initially prescribed, another possibility is to use mercaptopurine instead. Lopez-Sanroman *et al*[150] did so in 4/5 patients, and achieved complete resolution of liver test alterations in all patients. This finding is consistent with another smaller study in which seven out of eight patients with hepatotoxicity during azathioprine treatment tolerated mercaptopurine, and only one patient had hepatotoxicity again with mercaptopurine[151]. In this same way, in the study by Hindorf *et al*[111] 71% of patients with hepatotoxicity during azathioprine treatment subsequently tolerated mercaptopurine and only two of the patients had a recurrence of hepatotoxicity with mercaptopurine. Finally, Bermejo *et al*[152] have assessed tolerance to mercaptopurine in 31 patients with previous azathioprine-related liver injury; in 87% of patients, mercaptopurine was tolerated without further liver injury; and among these, 77% tolerated full mercaptopurine doses.

Nevertheless, it should be noted that in unusual cases, thiopurines may induce severe cholestatic jaundice that, in contrast to acute hepatocellular hepatitis that is generally associated with azathioprine/mercaptopurine, may not regress but even progress despite thiopurine withdrawal[153]. Therefore, these drugs should be completely withdrawn, and not only tapered, in patients who present with clinically significant jaundice during thiopu-

rine treatment[144].

In summary, most of the cases of thiopurine-induced hepatotoxicity in IBD patients are mild, and liver test abnormalities spontaneously returned to normal values despite maintenance of azathioprine/mercaptopurine; therapy withdrawal is necessary in < 5% of patients. However, when liver test abnormalities are more marked, the dose of azathioprine/mercaptopurine may be reduced by 50%. Finally, administration of mercaptopurine is a good alternative in patients with azathioprine-related liver injury before thiopurines are definitely withdrawn.

CONCLUSION

Misconceptions are common in medical practice in general and, in particular, in the health care of IBD patients. Many of these misconceptions are related to the use of 5-ASAs and thiopurines, the two most widely used drugs in IBD. A proportion of medical errors directly affects patient safety and causes accidental deaths, but the vast majority of them are effectiveness errors. However, we must not focus all our attention on prevention of safety errors while forgetting effectiveness ones. Prevention of errors needs knowledge to avoid errors being caused by ignorance. In fact, throughout history the main reason for medical errors has simply been ignorance[8]. However, at present, the amount of knowledge has increased so quickly that one new danger is overabundance of information. IBD is a model of a very complex problem, and our goal with this review is to summarize the key evidence for the most common daily clinical problems faced by physicians and patients.

With regard to the use of 5-ASAs, the best practice may to be consider abandoning the use of these drugs in patients with small bowel Crohn' s disease. The combined approach with oral plus topical 5-ASAs should be the first-line therapy in patients with active ulcerative colitis, because this is more effective than monotherapy; once-daily treatment should be offered as a first-choice regimen due to its better compliance and higher efficacy. With regard to thiopurine therapy, it seems to be as effective in ulcerative colitis as in Crohn' s disease. Underdosing with thiopurines is a form of undertreatment with these drugs. Thiopurine treatment should probably be continued indefinitely because its withdrawal is associated with a high risk of relapse. Mercaptopurine is a safe alternative in patients with digestive intolerance or hepatotoxicity due to azathioprine. Finally, TPMT screening cannot substitute for regular monitoring because the majority of cases of myelotoxicity are not TPMT-related.

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ORIGINAL ARTICLE

How albumin administration for cirrhosis impacts on hospital albumin consumption and expenditure

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Abstract

AIM: To assess the impact of guidelines for albumin prescription in an academic hospital, which is a referral center for liver diseases.

METHODS: Although randomized trials and guidelines support albumin administration for some complications of cirrhosis, the high cost of albumin greatly limits its use in clinical practice. In 2003, a multidisciplinary panel at Sant'Orsola-Malpighi University Hospital (Bologna, Italy) used a literature-based consensus method to list all the acute and chronic conditions for which albumin is indicated as first- or second-line treatment. Indications in hepatology included prevention of post-paracentesis circulatory dysfunction and renal failure induced by spontaneous bacterial peritonitis, and treatment of hepatorenal syndrome and refractory ascites. Although still debated, albumin administration in refractory ascites is accepted by the Italian health care system. We analyzed albumin prescription and related costs before and after implementation of the new guidelines.

RESULTS: While albumin consumption and costs doubled from 1998 to 2002, they dropped 20% after 2003, and remained stable for the following 6 years. Complications of cirrhosis, namely refractory ascites and paracentesis, represented the predominant indications, followed by major surgery, shock, enteric diseases, and plasmapheresis. Albumin consumption increased significantly after guideline implementation in the liver units, whereas it declined elsewhere in the hospital. Lastly, extra-protocol albumin prescription was estimated as < 10%.

CONCLUSION: Albumin administration in cirrhosis according to international guidelines does not increase total hospital albumin consumption if its use in settings without evidence of efficacy is avoided.

Key words: Human serum albumin; Cost analysis; Liver cirrhosis; Critical illness; Ascites

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INTRODUCTION

Serum albumin represents approximately 50% of the total

plasma protein content in healthy individuals, and generates about 70% of plasma oncotic pressure. The fairly elevated albumin concentration in extracellular fluids and its strong negative charge, which attract sodium, make albumin the main modulator of fluid distribution throughout body compartments. In addition, albumin carries many other biological properties, with implications for drug metabolism, free radical detoxification, inflammatory response, vascular integrity, and coagulation[1,2].

Human albumin is widely employed in clinical practice, but its administration is often inappropriate. This is largely due to a common belief in its efficacy, whereas many indications are still under debate or have been disproved by evidence-based medicine. Indeed, the high cost, the theoretical risk of viral disease transmission, and the availability of cheaper alternatives should be carefully weighed in a cost/effectiveness analysis of albumin prescription. Thus, it is not surprising that several clinical and economic studies have been performed to establish recommendations and guidelines for albumin prescription, but controversy persists[3-9].

At present, it is generally accepted that the administration of non-protein colloids and crystalloids represents the first-line treatment of resuscitation, while the use of albumin in critically ill patients should be reserved for specific conditions[10-15]. Albumin administration is not recommended to correct hypoalbuminemia *per se* (i.e. not associated with hypovolemia) or for nutritional intervention[6,7,9,16], but this is often disregarded in clinical practice. Albumin is also prescribed in certain conditions and diseases, such as kernicterus, plasmapheresis, and graft-*vs*-host disease[9], even though these indications are not supported by definite evidence.

Hepatology is a setting where the use of albumin is particularly common, since this treatment is currently employed to treat or prevent severe complications of cirrhosis. Indeed, randomized studies have shown that it is effective in preventing circulatory dysfunction after large-volume paracentesis[17] and renal failure induced by spontaneous bacterial peritonitis[18], and to treat hepatorenal syndrome in association with vasoconstrictors[19-21]. Although the recommendations on the use of albumin in cirrhosis have been endorsed by the International Ascites Club and other international scientific societies[22-26], albumin is not widely administered in clinical practice, even in specialized centers, mainly because of its high cost.

With the aim of rationalizing albumin prescription and reducing health care costs, clinical practice guidelines were devised in 2003, and subsequently implemented at the S Orsola-Malpighi University Hospital in Bologna, Italy, a third-level referral centre for many diseases, including liver cirrhosis and transplantation.

We here report the annual albumin consumption and costs comparing the 5 years before (January 1998-June 2003) with the 6 years after (July 2003-December 2008) implementation of the guidelines. We also analyze the indications for albumin, adherence to the protocol, and the distribution of albumin prescription among specialties in 2008.

MATERIALS AND METHODS

Protocol drafting

In the first half of 2003, clinical practice guidelines for the appropriate prescription of albumin were devised at the Sant'Orsola-Malpighi University Hospital of Bologna, Italy, using a systematic, literature-based consensus method. Briefly, a panel of experts from various disciplines (internal medicine, anesthesia, surgery, gastroenterology, nephrology, hematology, public health, and pharmacy) reviewed the available clinical literature and drew up draft guidelines which were submitted to a second panel of physicians from the same scientific areas, but not involved in the writing of the first draft. Then a consensus was reached by the two working groups and a final version was approved and distributed among the physicians employed at the hospital. Since July 2003, the in-hospital prescription of albumin has been regulated according to the recommendations reported in Table 1. Schematically, they list a series of acute and chronic clinical conditions for which albumin administration is indicated as a first- or second-line treatment or is not indicated at all. The level of scientific evidence and the strength of the recommendation are also reported according to the criteria summarized in Table 2. The guidelines were updated in 2007, but only minor changes were made regarding specific and limited indications.

Table 1 Levels of evidence and strength of recommendation

Levels of evidence	
1	Randomized clinical trials and/or meta-analyses
2	Single randomized clinical trail
3	Prospective observational studies
4	Retrospective studies
5	Cross-sectional surveys and descriptive studies
6	Opinion of experts in guidelines or consensus
Strength of recommendations	
A	Strong (levels of evidence 1 and 2)
B	Relatively strong (levels of evidence 3, 4 and 5)
C	Weak (level of evidence 6)

Data collection

Since the protocol was implemented, each order of albumin has been filled in by the prescribing physician using a specific form listing the amount requested, the diagnosis and the indication for albumin use, and the unit of the prescribing physician. All the data regarding in-hospital use of albumin are collected in a database at the hospital pharmacy service and a quarterly report on albumin prescription is sent by mail to all physicians.

Statistical analysis

Trends of albumin consumption before and after implementation of the clinical practice guidelines were analyzed using the Pearson correlation test. $P < 0.05$ were considered statistically significant. Data were analyzed using Graph PAD 4.0 software.

RESULTS

Consumption data

Albumin consumption and costs were monitored at the S Orsola-Malpighi University Hospital from 1998. The number of albumin vials (50 mL containing 10 g albumin,

Table 2 Practical recommendations for the prescription of albumin at the S Orsola-Malpighi University Hospital, Bologna, Italy

Acute diseases	First-line treatment	Second-line treatment
Hypovolemic shock [1, A]	Colloid/crystalloid solutions	Human albumin if: Sodium intake restriction Hypersensitivity to colloids or crystalloids Lack of response to combined use of colloids and crystalloids
Major surgery [6, C]		
(1) Cardiovascular surgery	Colloid/crystalloid solutions	Human albumin if: Lack of response to combined use of colloids and crystalloids
(2) Other surgery	As for hypovolemic shock	As for hypovolemic shock
Burns [6, C]	Colloid/crystalloid solutions	Human albumin plus crystalloid solutions if: Lack of response to colloid or crystalloid solutions alone Severe burns (> 50% body surface)
Chronic diseases	First-line treatment	Second-line treatment
Cirrhosis	Human albumin	
(1) Paracentesis [1, A]	8 g/L of removed ascites if paracentesis > 4 L	
(2) Spontaneous bacterial peritonitis [1, A]	1.5 g/kg at diagnosis and 1 g/kg on third day + antibiotic therapy	
(3) Hepatorenal syndrome [1, A]	1 g/kg at diagnosis followed by 20-40 g/d + vasoconstrictors	
(4) Ascites [1, A]	Diuretic treatment	Human albumin if: Ascites resistant to diuretics
Plasmapheresis [6, C]	Human albumin if plasma changes > 20 mL/kg per week	
Protein wasting enteropathy/ malnutrition	Enteral or parenteral nutrition	Human albumin only if: severe diarrhea (> 2 L/d) albuminemia < 2 g/dL clinical hypovolemia

Albumin is not recommended for cerebral ischemia (3, B), wound-healing (6, C), chronic pancreatitis (6, C), nephritic syndrome (6, C). Albumin is also provided for the following specific procedures: pulmonary endarterectomy, molecular adsorbent recirculating system (MARS), immunomagnetic selection system (CLINIMACS).

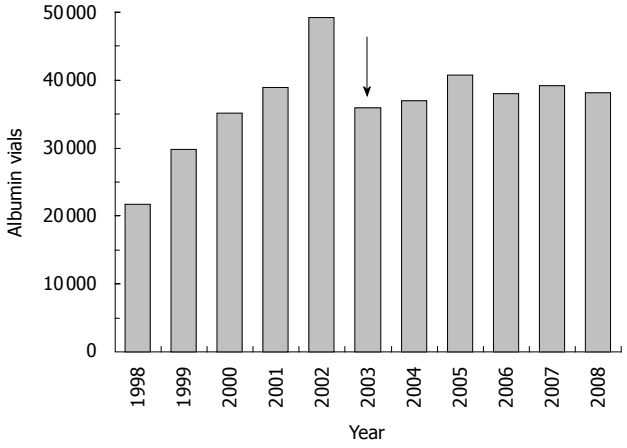

Figure 1 Annual consumption of albumin vials (50 mL, 20%) at the S Orsola-Malpighi University Hospital, Bologna, Italy. The black arrow indicates when the practical guidelines for in-hospital albumin prescription were implemented in July 2003.

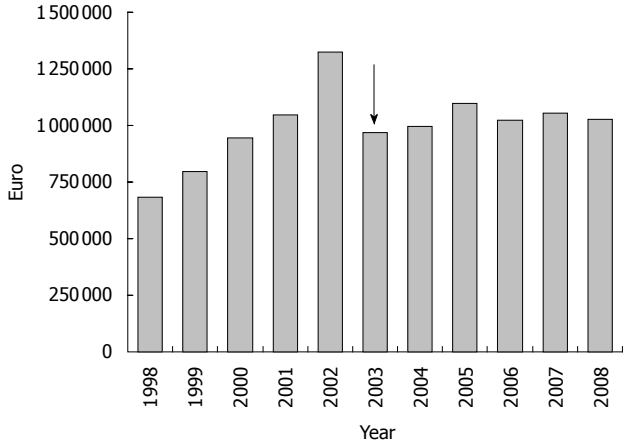

Figure 2 Global annual cost of albumin use at the S Orsola-Malpighi University Hospital, Bologna, Italy. The black arrow indicates when the practical guidelines for in-hospital albumin prescription were implemented in July 2003.

concentration 20%) and the related expenditure more than doubled from 1998 to 2002 (Figures 1, 2). The implementation of recommendations in July 2003 yielded a rapid 15%-20% reduction in albumin use (Figure 1), which was associated with a similar fall in albumin cost both expressed in absolute terms (Figure 2) or as percentage of the global pharmaceutical expenditure (Figure 3). Thereafter, albumin consumption and related costs remained substantially stable during the following 6 years (Figures 1-3). The data for the 2003 represent the sum of two periods with different prescription modalities (before and after guidelines implementation). The cost of each albumin vial paid for by our hospital remained stable throughout the study period.

Finally, as shown in Figure 4, the trend analysis of albumin consumption clearly indicates that its time-dependent increase was interrupted by the implementation of the recommendations, supporting their efficacy in regulating in-hospital albumin prescription.

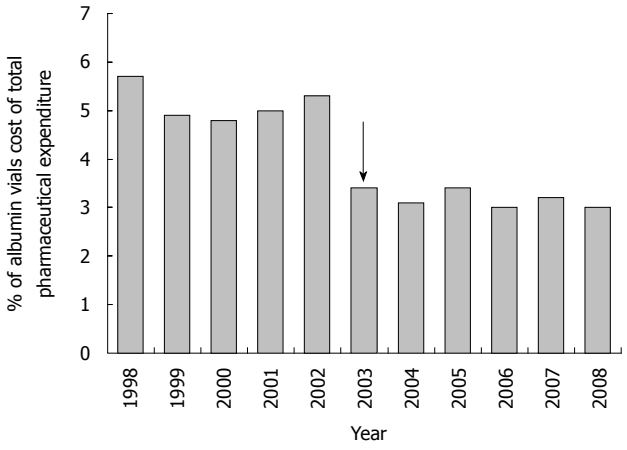

Figure 3 Annual cost of albumin use at the S Orsola-Malpighi University Hospital, Bologna, Italy, expressed as a percentage of the total pharmaceutical expenditure. The black arrow indicates when the practical guidelines for in-hospital albumin prescription were implemented in July 2003.

Prescription analysis

As the annual breakdown of albumin prescriptions among the major indications did not change significantly in the period 2003-2008, we present only the prescription analysis performed on the data for 2008 (Figure 4). Complications of cirrhosis represent the predominant indication for albumin use, accounting for 52% of vials utilized and 36% of patients treated. Major surgery was the second commonest indication for albumin use. Taking into account that liver transplantation and hepatic resection constitute approximately 30% of these surgical cases, it is evident that liver diseases represent the setting where the majority of albumin was prescribed. Other relatively common indications were shock not responsive to crystalloid/colloids in intensive care units (ICUs), bowel diseases associated with malnutrition, and plasmapheresis (Table 3).

The mean number of albumin vials per patient and the related cost were higher in patients with cirrhosis than in those presenting other indications, with the two expected exceptions being plasmapheresis and albumin dialysis with the molecular adsorbent recirculating system (MARS) (Table 3). Finally, the use of albumin outside protocol indications occurred only in about 10% of cases and mainly in patients with edematous and/or anasarcatic states (Table 3).

Ascites not responding to diuretic treatment represented the major indication for albumin prescription in patients with liver cirrhosis, and the high number of albumin vials per patient likely reflected the prolonged length of treatment. Repeated procedures also resulted in increased albumin consumption in patients after large-volume paracentesis to prevent post-paracentesis circulatory dysfunction (Table 4).

Although it appears that hypoalbuminemia was the major indication before guideline implementation (data not shown), we could not perform an accurate comparison between the two 5-year periods of prescription since the data were not systematically collected before 2003.

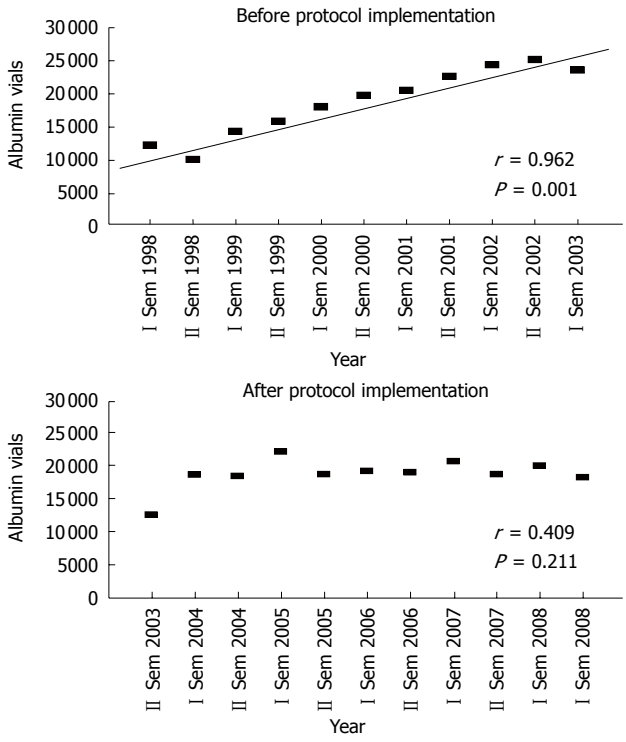

Figure 4 Top panel. Trend in albumin consumption at the S Orsola-Malpighi University Hospital, Bologna, Italy before implementation of the practical guidelines for albumin prescription. Albumin consumption is expressed per semester from January 1, 1998 to June 30, 2003. Data are analyzed using the Pearson correlation. Bottom panel. Trend in albumin consumption at the S. Orsola-Malpighi University Hospital, Bologna, Italy after implementation of the practical guidelines for albumin prescription. Albumin consumption is expressed per semester from July 1, 2003 to December 31, 2008. Data are analyzed using the Pearson correlation.

In an attempt to overcome this limitation of the study, we analyzed albumin consumption after grouping all the units into three main categories: hepatological medical and surgical units, i.e. units representing referral centers for liver diseases, non-hepatological medical and surgical units, and ICUs. While the proportion of albumin consumption remained stable over the years in the ICU, the increase observed in the "hepatological" units after guideline implementation was mirrored by a parallel decrease in the "non-hepatological" units (Figure 5).

DISCUSSION

Albumin utilization remains highly controversial in a variety of clinical settings in terms of indications, efficacy, and cost-benefit ratio. Over the last 10-15 years, specific conditions for which albumin administration is indicated have been defined, and liver disease possibly represents a field where this has been most clearly and convincingly demonstrated. However, besides inappropriate prescribing, the high cost of albumin infusion is the main issue leading health authorities and hospital administrators to restrict its use, and this has often prevented the widespread application of indications emerging from international guidelines in hepatology.

Table 3 Distribution of albumin consumption and cost among clinical indications in 2008

	Vials (number)(%)	Cost (euros)	Patients (number)(%)	Vials/patients (number)	Cost/patient (euros)
Cirrhosis	19 871 (52)	534.532	807 (36.3)	24.6	662
Major surgery	6196 (16.2)	166.982	495 (22.2)	12.5	337
Shock	5069 (13.3)	136.558	447 (20)	11.3	305
Enteric disease	2982 (7.8)	80.215	146 (6.5)	20.4	549
Plasmapheresis	2333 (6.1)	62.757	54 (2.4)	43.2	1162
Mars	196 (0.5)	5.272	6 (0.2)	32.6	878
Others[1]	201 (0.5)	5353.000	149 (6.7)	7.1	260
Extra-protocol	1240 (3.7)	33.418	119 (5.3)	10.5	281

[1]Others: Clinimacs system, neonatal jaundice, pediatric cardio-pulmonary bypass.

Table 4 Distribution of albumin consumption and cost among the clinical indications for cirrhosis in 2008

	Vials (number)(%)	Cost (euros)	Patients (number)(%)	Vials/patients (number)	Cost/patient (euros)
Ascites	12.540 (63.3)	337.976	453 (56.4)	27.7	746
Paracentesis	4.564 (23.1)	122.988	222 (27.6)	20.6	554
Hepatorenal syndrome	2.340 (11.8)	63.070	106 (13.2)	22.0	595
Spontaneous bacterial peritonitis	367.000 (1.8)	9.880	22 (2.7)	14.0	380

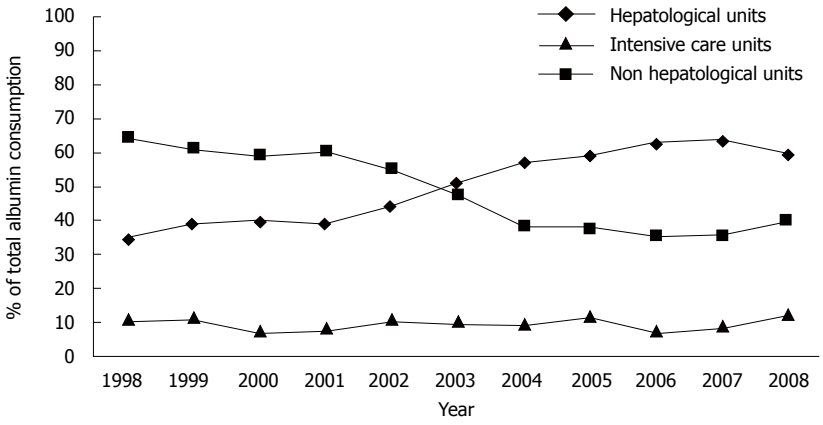

Figure 5 Distribution of the annual consumption of albumin vials (50 mL, 20%) among various Units of the S Orsola-Malpighi University Hospital, Bologna, Italy, grouped into three main categories: hepatological medical and surgical units, i.e. units representing a referral centre for liver diseases, non-hepatological medical and surgical units, and intensive care units. The black arrow indicates when the practical guidelines for in-hospital albumin prescription were implemented in July 2003.

The present study clearly shows that in a general hospital the recommendations for the use of albumin in patients with cirrhosis can be followed without increasing the global pharmaceutical costs, despite the large amounts of albumin required. This can be achieved by implementing rational albumin prescription guidelines, thereby avoiding its futile administration in settings where there is a lack of clinical evidence of efficacy. The clinical practice guidelines devised by our hospital to regulate albumin prescription used a literature-based consensus method and adopted all the recognized indications for albumin administration in hepatology, such as prevention of post-paracentesis circulatory dysfunction, prevention of renal impairment in patients with spontaneous bacterial peritonitis, and treatment of hepatorenal syndrome in association with vasoconstrictors. These guidelines managed to curb the incremental trend of albumin consumption and related costs recorded in previous years. In our view, these results are particularly important given the characteristics of our hospital, that acts both as an academic third-level and primary referral centre, hosts a program for liver transplantation, and where several medical and surgical units are devoted to the management of chronic liver diseases. Not surprisingly, the prevention or treatment of complications of cirrhosis was the commonest reason for prescribing albumin in our institution, and albumin use in this setting increased after our in-hospital clinical practice guidelines were implemented.

Refractory ascites was the most common specific indication for albumin infusion, and the high number of vials given to each patient likely reflects the need for sustained albumin administration in these cases. This finding merits some comment as the use of albumin to treat cirrhotic ascites is controversial with a lack of clear evidence in its

favor. Despite this debate, the Italian Drug Agency (AIFA) permits albumin reimbursement by the National Health Service in patients with refractory ascites based on the results of two Italian studies[27,28]. First, the Albumin Delphi Study, designed to gain a consensus among Italian physicians, showed that 80% of hepatologists agree that albumin treatment shortens the length of hospitalization, enhances the response to diuretics, lowers the relapse rate of ascites when given at home, and also improves patient general condition and well-being[26]. Second, a controlled clinical trial in 126 patients with decompensated cirrhosis reported that treatment with diuretics plus albumin favored the disappearance of ascites, shortened the hospital stay, and reduced the rate of ascites recurrence and readmission to hospital due to ascites compared with diuretics alone[27]. More recently, the same research group showed that long-term albumin administration increased patient survival and reduced the risk of ascites recurrence in patients with first-onset ascites followed for a median of 7 years[28]. No other controlled clinical trials have so far been performed to evaluate the effectiveness of prolonged albumin administration in the treatment of cirrhotic ascites. Thus, the lack of confirmatory multicenter randomized studies together with the high cost of albumin infusion explains why albumin is not usually included among the therapeutic options for difficult-to-treat ascites in countries other than Italy. For instance, the most recent international guidelines for the management of adult patients with ascites due to cirrhosis[25,29] do not even mention albumin as a possible therapeutic tool for either responsive or refractory ascites.

In the last 15 years, large randomized trials and meta-analyses have focused on the use of human albumin in the setting of critically ill patients, the clinical field where international guidelines for albumin prescription were first established. These studies showed that beside being cheaper, non-protein colloids and crystalloids are equally or even more effective than albumin for fluid resuscitation and blood volume expansion in patients with hypovolemic shock and critical illnesses, representing the first-line treatment in these cases. Thus, albumin should only be administered in the presence of contraindications to the use of colloids and/or crystalloids or specific conditions, such as the need for salt intake restriction[10-15]. Interestingly, the implementation of our in-hospital guidelines has not produced significant changes in albumin prescription by physicians working in ICUs, probably because they were already accustomed to established international guidelines, despite the ongoing dispute on the matter.

As global albumin consumption at our hospital declined by 15%-20%, the greatest saving occurred in non-hepatological medical and surgical units where hypoalbuminemia *per se* was probably the main reason for prescribing albumin before implementation of our in-hospital guidelines, despite the lack of scientific evidence supporting albumin administration to correct plasma albumin concentration and/or improve nutritional status[6,7,9,16].

Our hospital guidelines, like other local recommendations[3-9], also authorize albumin prescribing for specific procedures and clinical situations. These niche indications differ among guidelines and reflect the particular settings of highly specialized centers, such as university hospitals. Although these indications only affect a small number of patients, the albumin consumption per patient can be very high, as in the MARS procedure[30], or the treatment of orphan diseases, such as intestinal lymphangiectasia.

Several aspects of our study must be addressed for a correct evaluation of the results. The recommendations for albumin prescription in our hospital were devised using a systematic, literature-based consensus method, but not all of them were supported by solid scientific evidence derived from large randomized clinical trials, meta-analyses of trials or universally accepted guidelines. Despite this limitation, implementation of the protocol clearly interrupted the incremental trend in albumin consumption and related expenditure seen in previous years. Of course, most physicians must strictly adhere to the guidelines in order to control and, possibly, curb albumin consumption. Several *ad hoc* strategies were adopted: (1) involvement of experts from many disciplines in drafting the consensus document; (2) use of an albumin order form restating the recommendations at the time of prescription, a measure known to enhance compliance[31]; and (3) regular information sent to every physician in a quarterly report on in-hospital albumin prescription. Such a policy achieved a formal adherence to the protocol in approximately 85% of prescriptions. This figure, however, relied on the assumption that data reported on the order form correctly describe the patient's clinical status, because this concordance was not monitored on a regular basis. Finally, our study cannot provide any information on the impact of the recommendations on the patients' clinical outcomes and global hospital costs. This was beyond the scope of the present analysis and can only be determined by prospective studies designed to assess the cost/benefit ratio of albumin treatment for each specific disease.

In conclusion, this study indicates that prescribing albumin according to the current guidelines in hepatology does not increase total albumin consumption or costs in a hospital acting as both an academic third-level and primary referral center. This result can be achieved provided that albumin prescription is strictly regulated by practical recommendations designed to avoid ineffective albumin infusion in settings without scientific evidence of efficacy. The present data may promote the appropriateness of albumin prescription, particularly in clinical settings where the use of albumin is dogmatically limited rather than regulated on the basis of current scientific evidence. In this sense, our results may foster the acceptance of internationally endorsed indications for the use of albumin in hepatology by health authorities and hospital administrations.

COMMENTS

Background

Human albumin is widely employed in clinical practice, but its administration is often inappropriate. This is largely due to a common belief in its efficacy, whereas

many indications are still under debate or have been disproved by evidence-based medicine. In the field of hepatology, albumin is currently used to treat or prevent severe complications of cirrhosis. Although the recommendations on the use of albumin in cirrhosis have been endorsed by the International Ascites Club and other international scientific societies, albumin is not widely administered in clinical practice, even in specialized centers, mainly because of its high cost.

Research frontiers

As albumin utilization remains highly controversial in a variety of clinical settings in terms of indications, efficacy, and cost-benefit ratio, the implementation of practical recommendations and guidelines, based on solid scientific evidence, appears to be a necessary step to rationalize the use of this expensive hemoderivate.

Innovations and breakthroughs

The present study clearly showed that in an academic general hospital, the adherence to the international scientific guidelines for the use of albumin in patients with cirrhosis does not increase the global pharmaceutical costs, despite the large amounts of albumin required. This can be achieved by implementing rational albumin prescription guidelines, thereby avoiding its futile administration in settings that lack scientific evidence of efficacy.

Applications

The present data may promote the appropriateness of albumin prescription, particularly in clinical settings where the use of albumin is limited rather than regulated on the basis of current scientific evidence. The results may foster the acceptance of internationally endorsed indications for the use of albumin in hepatology by health authorities and hospital administrations. However, this study suffers an important limitation as the effects of the recommendations on the patients' clinical outcomes and global hospital costs could not be determined in the present investigation and only prospective studies specifically designed for this purpose will be able to provide the real cost-effectiveness of treatment for each specific disease.

Peer review

This study shed more light on the continued dilemma of consumption and costs of albumin administration particularly in patients with liver disease.

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ORIGINAL ARTICLE

Integrin-linked kinase in gastric cancer cell attachment, invasion and tumor growth

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Abstract

AIM: To investigate the effects of integrin-linked kinase (ILK) on gastric cancer cells both *in vitro* and *in vivo*.

METHODS: ILK small interfering RNA (siRNA) was transfected into human gastric cancer BGC-823 cells and ILK expression was monitored by real-time quantitative polymerase chain reaction, Western blotting analysis and immunocytochemistry. Cell attachment, proliferation, invasion, microfilament dynamics and the secretion of vascular endothelial growth factor (VEGF) were also measured. Gastric cancer cells treated with ILK siRNA were subcutaneously transplanted into nude mice and tumor growth was assessed.

RESULTS: Both ILK mRNA and protein levels were significantly down-regulated by ILK siRNA in human gastric cancer cells. This significantly inhibited cell attachment, proliferation and invasion. The knockdown of ILK also disturbed F-actin assembly and reduced VEGF secretion in conditioned medium by 40% ($P < 0.05$). Four weeks after injection of ILK siRNA-transfected gastric cancer cells into nude mice, tumor volume and weight were significantly reduced compared with that of tumors induced by cells treated with non-silencing siRNA or by untreated cells ($P < 0.05$).

CONCLUSION: Targeting ILK with siRNA suppresses the growth of gastric cancer cells both *in vitro* and *in vivo*. ILK plays an important role in gastric cancer progression.

Key words: Gastric cancer; Integrin-linked kinase; Small interfering RNA; Cell attachment; Cell proliferation; Cell invasion; Cell microfilament dynamics; Vascular endothelial growth factor; Nude mice

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INTRODUCTION

Gastric cancer is one of the most commonly diagnosed malignant tumors, and is also one of the most frequent causes of cancer mortality worldwide[1]. Its incidence is highest in Japan, China, Eastern Europe and Latin America. In 2002, 934 000 new cases were diagnosed, making it the fourth most common cancer, causing ap-

proximately 700 000 deaths[2]. The 5-year survival rate of gastric cancer is poor, being approximately 20%, even in patients treated with surgical resection, chemotherapy, radiotherapy and other approaches. However, in Japan, according to a systematic screening program, this figure reached 60%[3-5]. Therefore, an understanding of the molecular mechanisms involved in gastric cancer formation and progression, and the identification of specific targets for gene therapy should be helpful in developing more effective strategies. Integrin-linked kinase (ILK) is an ankyrin repeat-containing serine/threonine protein kinase[6] that interacts with the cytoplasmic domain of β1 and β3 integrins[7] and regulates integrin dependent functions. It mediates a diversity of cell functions by coupling integrins and growth factors to cascades of downstream signaling events. ILK is a downstream substrate of phosphoinositide 3-kinase, and is an important upstream kinase for the regulation of protein kinase B (PKB/Akt) and glycogen synthase kinase 3 (GSK-3)[8,9]. ILK is now recognized to play an important role in linking extracellular signaling to the regulation of survival, cell cycle progression, migration, and invasion. The expression and activity of ILK are increased in a range of tumors, and small-molecule inhibitors of ILK activity have been identified and shown to inhibit tumor growth, invasion and angiogenesis[10-12], although certain tumors have decreased or no ILK expression. In gastric cancer, there is no ILK expression in non-neoplastic gastric epithelia, while the number of cells expressing ILK increased to 69% in neoplastic gastric epithelia, which was associated with tumor cell invasion and nodal metastasis[13]. To further investigate the role of ILK in gastric cancer progression and to determine if ILK can be used as a therapeutic target, we specifically knocked down ILK expression using small interfering RNA (siRNA) in gastric cancer cell line, BGC-823. We also analyzed these cancer cells' spontaneous attachment, proliferation, invasion and cell morphology *in vitro* and their tumor growth *in vivo*.

MATERIALS AND METHODS

Cell culture and reagents

Human gastric cancer cell line, BGC-823, was obtained from the Institute of Geriatrics, Ministry of Health (Beijing, China), and was cultured in RPMI-1640 medium (Gibco BRL, Grand Island, United States) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco) in a 5% CO_2 humidified atmosphere at 37 ℃. HiPerFect Transfection Reagent was purchased from Qiagen (Hilden, Germany). A First-Strand cDNA Synthesis kit and SYBR-green real-time polymerase chain reaction (PCR) Mastermix were purchased from Toyobo (Osaka, Japan). For Western blotting analysis, an anti-ILK antibody, purchased from Abcam (Cambridge, United Kingdom) was used at a dilution of 1:2000, and an HRP-goat anti-rabbit secondary antibody obtained from Rockland Immunochemicals (Gilbertsville, PA, United States) was used at a dilution of 1:3000. For immunocytochemistry, the ILK antibody was used at a dilution of 1:1000, and a goat anti-rabbit TRITC conjugated antibody was used at a dilution of 1:400. Anti-β-actin-HRP antibody was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, United States), and SuperSignal West Pico Chemiluminescent Substrate was purchased from Pierce (Rockford, IL, United States). Matrigel was obtained from BD Biosciences (Bedford, MA, United States), while Alexa Fluor 488 phalloidin was purchased from Molecular Probes (Invitrogen, Carlsbad, CA, United States). All other reagents were purchased from Sigma (St. Louis, MO, United States).

siRNA and transfection assays

The ILK-specific (GenBank accession No. NM004517) siRNAs (ILK siRNA, forward: 5'-GAAUCUCAACCGUAUUCCATT-3'; reverse: 5'-UGGAAUACGGUUGAGAUUCTG-3') were chemically synthesized by Qiagen. BGC-823 cells were transfected with siRNA using HiPer-Fect Transfection Reagent according to the manufacturer's instructions. Briefly, the original stock of siRNA was suspended in siRNA suspension buffer provided by the manufacturer. The resulting suspension was aliquoted in a required amount for each experiment and stored at -20 ℃ until use. On the day of transfection, cells were seeded in plates at the recommended density according to the manufacturer's instructions. The siRNA was then gently introduced onto the cells by mixing with the required amount of HiPer-Fect Transfection Reagent as recommended by the manufacturer. In our study, the final concentration of siRNA was 10 nmol/L. Non-silencing siRNA (NS siRNA, forward: 5'-UUCUCCGAACGUGUCACGUTT-3'; reverse: 5'-ACGUGACACGUUCGGAGAATT-3')-treated cells were used to control any effects of the transfection reagent and the non-specific siRNA effects. The *in vitro* assays described here were performed 48 h after transfection. Chemically modified siRNAs used in animal models were synthesized by Qiagen according to the sequences described above.

Real-time quantitative RT-PCR

Isolation of total RNA was performed using Trizol solution according to the manufacturer's protocol. Reverse transcription was then performed using 100 ng RNA and the First-Strand cDNA Synthesis kit. Real-time quantitative PCR analysis was performed with the DNA Engine Opticon 2 System (Bio-Rad, Richmond, CA, United States) using the SYBR® green Real-time PCR Mastermix. We used the following primers: for ILK, forward 5'-TTTGCAGTGCTTCTGTGGGAA-3' and reverse 5'-CTACTTGTCCTGCATCTTCTC-3'; for GAPDH, forward 5'-GAAGGTGAAGGTCGGAGTC-3' and reverse 5'-GAAGATGGTGATGGGATTTC-3'. After initial denaturation at 95 ℃ for 3 min, reactions were cycled 40 times. Each cycle consisted of denaturation at 95 ℃ for 15 s, primer annealing at 60 ℃ for 15 s and primer extension at 72 ℃ for 45 s[14,15]. Results were collected and analyzed using MJ Opticon Monitor Analysis software (Bio-Rad). The quantity data of mRNA input was

controlled by measuring the reference gene, GAPDH. Experiments were performed in triplicate and repeated three times.

Western blotting analysis

Cells were washed with ice-cold phosphate buffered saline (PBS), and whole-cell extracts were prepared using cell lysis buffer [20 mmol/L Tris (pH 7.5), 0.1% Triton X, 0.5% deoxycholate, 1 mmol/L phenylmethylsulfonyl fluoride, 10 μg/mL aprotinin and 10 μg/mL leupeptin] and cleared by centrifugation at 12000 × *g* at 4 ℃. Total protein concentration was measured using the bicinchoninic acid assay with bovine serum albumin (BSA) as a standard. Equal amounts of protein were loaded and analyzed by immunoblotting. Enhanced chemiluminescence detection was performed in accordance with the manufacturer's instructions[16,17]. The ILK signal was quantified using BandScan software version 5.1 (Glyko, Novato, Calif., United States) and normalized to that of β-actin. Experiments were performed in triplicate and repeated 3 times.

Immunocytochemistry

Immunocytochemical assays were performed as previously described[18]. Briefly, cells were grown on fibronectin coated coverslips, washed in PBS, and fixed for 15 min in 4% paraformaldehyde. Cell monolayers were permeabilized in 0.1% Triton X-100, washed, and blocked in 10% normal goat serum. Cells were incubated with the anti-ILK antibody overnight at 4 ℃. Cells were then washed and incubated with fluorescently labeled secondary antibodies for 1 h at room temperature in the dark. Cells were washed and coverslips were mounted using Kaiser's glycerin gelatin (Merck, Darmstadt, Germany). Fluorescence signals were visualized and acquired using an epifluorescence microscope (Leica, Heidelberg, Germany) with appropriate excitation and emission filters under 40 × magnification. Pictures of observed fields were recorded digitally. Experiments were performed in triplicate and repeated three times.

Cell attachment assay

Plates of 96 wells were coated with 1.25 mg/mL fibronectin in 100 mL PBS overnight at 4 ℃. The plates were blocked with 2.5 mg/mL BSA for 2 h in DMEM at 37 ℃. Transfected cells were trypsinized and 1.5×10^4 cells were seeded in each well for 1 h at 37 ℃. Cells were then washed twice with PBS and the unattached cells were discarded. After the washing step, the number of attached cells was determined by the MTT assay in accordance with the manufacturer's instructions. Absorbance was measured using an enzyme-linked immunosorbent assay (ELISA) plate reader at 570 nm[19]. Experiments were performed in triplicate and repeated three times.

Cell proliferation assay

Cell proliferation was assessed using the MTT assay[20]. Gastric cancer BGC-823 cells were plated at 5×10^3 cells/well in 96-well plates in RPMI-1640 medium containing 10% FBS. After 24 h, the culture medium was replaced by fresh medium containing ILK siRNA or non-silencing siRNA. Six duplicate wells were set up for each group. Untreated cells served as control. After 4, 24, 48 or 72 h of incubation, 20 μL MTT (5 g/L, Sigma) was added to each well and incubation continued for 4 h. Cells were collected by centrifugation at 1000 × *g* for 5 min at room temperature. The reaction was stopped by the addition of 150 μL dimethyl sulfoxide. The absorbance of samples was measured at 570 nm. Each assay was performed in triplicate and repeated three times. Cell proliferation inhibition rate [proliferation inhibition rate = (1-A_{570} experiment group/A_{570} control group) × 100%] was plotted *vs* time.

Cell invasion assay

Polycarbonate membranes (8.0 μm pore size) of the upper compartment of 24-well Transwell culture chambers were coated with 18 μL of 5 mg/mL Matrigel (BD Biosciences) in serum-free medium. Cells (5×10^4) suspended in 250 μL of serum-free medium were applied on the upper compartment, and the lower compartment was filled with 750 μL of DMEM containing 10% fetal bovine serum. After incubation for 24 h, cells were fixed with 10% trichloroacetic acid at 4 ℃ for 1 h. Non-invaded cells on the upper surface of the filter were removed carefully with a cotton swab. Invading cells on the lower side of the filter were stained with 0.5% crystal violet for 2 h and the stained filters were photographed. The crystal violet dye retained on the filters was extracted with 30% acetic acid and cell invasion was measured by reading the absorbance at 590 nm[19]. Each assay was performed in triplicate and repeated three times.

Immunofluorescence analysis of microfilaments

Microfilament organization of RF/6A cells was assessed by a modification of an immunofluorescence protocol using rhodamine-phalloidin[21]. After transfection, cells were trypsinized and seeded onto coverslips for 6 h at 37 ℃ in 5% CO_2. Following this, the medium was aspirated, and adherent cells were fixed with 4% paraformaldehyde in PBS for 20 min. After they had been washed with PBS (pH 7.4) 3 times, cells were permeabilized with 0.1% Triton X-100 for 20 min and blocked with 1% BSA in PBS for 5 min. Cells were then incubated with rhodamine-phalloidin (200 U/mL) for 30 min and diamidinophenylindole (0.1 μg/mL) for 1 min in the dark. PBS was used as the base of all solutions and intervening rinses, and incubations were performed at room temperature. After mounting (Kaiser's glycerin gelatin; Merck, Darmstadt, Germany), slides were examined under an epifluorescence microscope (Leica, Heidelberg, Germany) with appropriate excitation and emission filters under a magnification × 40. Pictures of observed fields were recorded digitally. Experiments were performed in triplicate and repeated 3 times.

Enzyme-linked immunosorbent assay

siRNA-transfected cells were seeded in 6-well plates (3×10^5 cells/well) and incubated at 37 ℃. After 24 h, the cell culture supernatant was harvested, and cell counts were made after trypsinization. After collection, the medium was spun at 800 × *g* for 3 min at 4 ℃ to remove cellular debris[17]. The supernatants were frozen and stored at -80 ℃ until use. The levels of vascular endothelial growth factor (VEGF) were measured in culture medium samples with a VEGF ELISA kit according to the manufacturer's instructions. Experiments were performed in triplicate and repeated 3 times.

Tumor growth in nude mice

An equal number (1×10^7) of BGC-823 cells transfected with ILK siRNA, non-silencing siRNA, or untreated cells was harvested 48 h after transfection, washed twice with 1 × PBS, and resuspended in 0.2 mL of saline. Three groups (each group with 5 mice) of 4-6 wk old male BALB/c nude mice (Institute of Zoology, Chinese Academy of Sciences) were housed in a specific pathogen-free environment at the Animal Laboratory, then given subcutaneous injections with either untransfected cells, cells transfected with non-silencing siRNA, or cells transfected with ILK siRNA. The mice were monitored every 3 d for tumor formation. The date at which a palpable tumor first arose and the volume of the tumor ($V = L \times W^2 \times \pi/6$) were recorded[22]. At week 4 after injection of the cells, the mice were killed and the weights of tumors were recorded. The animal experiments performed on nude mice were approved by the Animal Ethics Committee of Peking University.

Statistical evaluation

Statistical analysis was performed using SPSS software (SPSS V 14.0; SPSS). All results were expressed as mean ± SD. To determine the significance of differences, ANOVA was performed. Differences with $P < 0.05$ were considered statistically significant.

RESULTS

SiRNA down-regulation of ILK expression in gastric cancer cells

Expression of ILK was significantly suppressed in gastric cancer BGC-823 cells transfected with ILK siRNA. The suppression of ILK occurred within 24 h after transfection and lasted a week. ILK siRNA caused a reduction of ILK mRNA of more than 85% after 48 h (88.2% ± 9.3% inhibition, $P < 0.01$) (Figure 1A), and a reduction of ILK protein of more than 85% after 48 h (86.8% ± 8.2% inhibition, $P < 0.01$) (Figure 1B) compared with non-silencing siRNA. Down-regulation of ILK expression in BGC-823 cells was further confirmed by immunocytochemistry (Figure 2): the fluorescence intensity representing the expression of ILK in untreated or non-silencing siRNA-treated cells was very strong, but that in ILK siRNA-transfected cells was barely detectable. Control cells also showed a higher degree of cell spreading when compared with the ILK-knockdown cells.

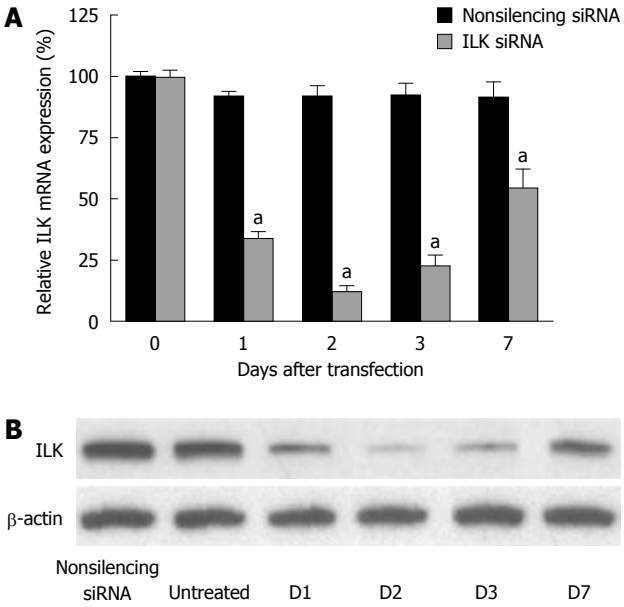

Figure 1 Effects of siRNA on integrin-linked kinase expression in gastric cancer cells. Integrin-linked kinase expression levels were analyzed by (A) real-time polymerase chain reaction and (B) Western blot analysis at different time points after incubation. Data are the mean ± SD. $^aP < 0.05$ *vs* control. Non-silencing siRNA is set to 100%.

ILK regulates cell attachment, proliferation and invasion in gastric cancer cells

We investigated the role of ILK in attachment of gastric cancer cells. In the cell attachment assay, we found that down-regulation of ILK lowered the ability of cells to attach to fibronectin compared with the non-silencing siRNA group ($P < 0.01$, Figure 3). The non-silencing siRNA and untreated groups had no significant difference in cell attachment ability ($P > 0.05$).

MTT assay was performed to observe whether down-regulation of ILK had an inhibitory effect on BGC-823 cell proliferation. We found that treatment of BGC-823 cells with ILK siRNA was associated with a time-dependent inhibition of cell proliferation, whereas no significant inhibitory effect was observed in cells treated with non-silencing siRNA (Figure 4).

Next, we investigated the role of ILK in the invasion of gastric cancer cells. We found that down-regulation of ILK reduced the ability of cells to invade through Matrigel-coated Boyden chambers to 10.7% ± 1.5% of that achieved by the non-silencing siRNA group ($P < 0.01$, Figure 5). The non-silencing siRNA and untreated groups had no significant difference in cell invasion ability ($P > 0.05$).

ILK regulates microfilament dynamics in gastric cancer cells

In the microfilament dynamics assay, ILK siRNA-transfected gastric cancer cells displayed different patterns of

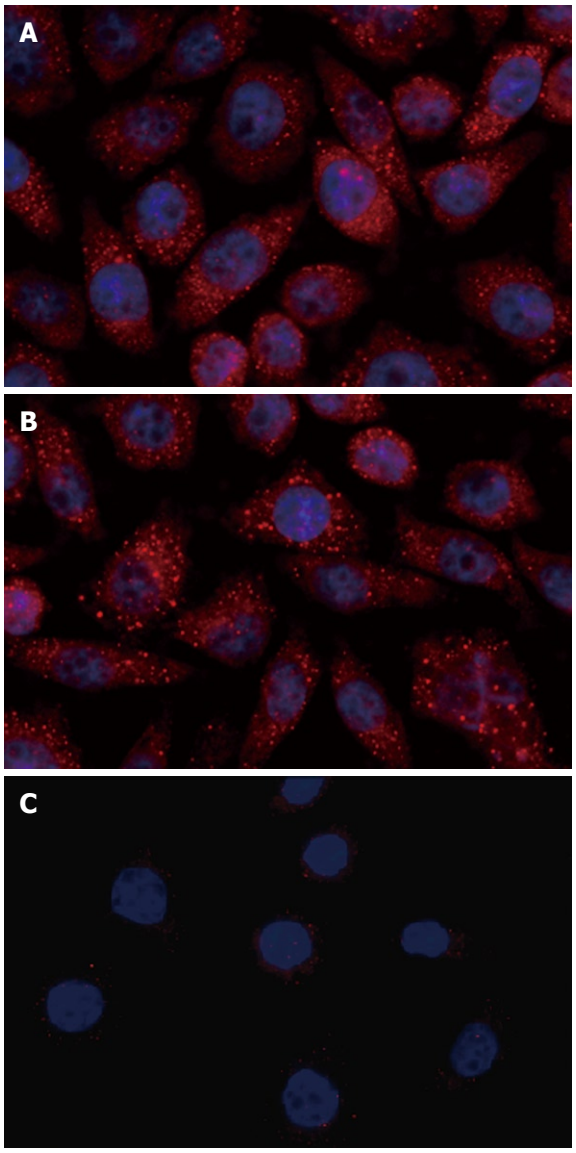

Figure 2 Immunocytochemical assays for integrin-linked kinase in gastric cancer cells. The fluorescence intensity represents the expression of integrin-linked kinase (ILK) in gastric cancer cells. A: Untransfected cells (Untreated); B: Non-silencing siRNA-transfected cells (Non-silencing siRNA); C: ILK siRNA-transfected cells (ILK siRNA). Experiments were repeated three times.

F-actin assembly and cell morphologies compared with the non-silencing siRNA group (Figure 6). F-actin assembly in the ILK siRNA group was significantly disturbed. There was less lamellipodia and filopodia formation in ILK-knockdown cells. However, cells in the non-silencing siRNA group displayed a well-organized actin skeleton with fibers extending throughout the cytoplasm into the cell membrane. Non-silencing siRNA-treated cells also showed a higher degree of cell spreading compared with the ILK siRNA-treated cells.

ILK regulates VEGF secretion from gastric cancer cells

VEGF plays an important role in tumor angiogenesis. To explore whether down-regulation of ILK can reduce VEGF activity in BGC-823 cells, we examined the levels of VEGF secreted into the culture medium by ELISA. We found that ILK knockdown led to a decrease in the level of VEGF secreted into the culture medium. As

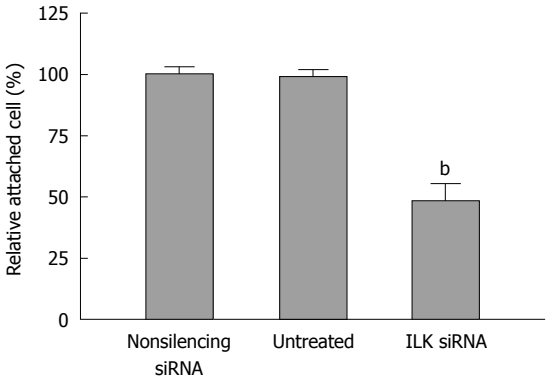

Figure 3 Effects of integrin-linked kinase on attachment of gastric cancer cells. Cell attachment was assessed after 1 h incubation and subsequent MTT test. Data are mean ± SD of three independent experiments ($^bP < 0.01$ *vs* control). Non-silencing siRNA is set to 100%. ILK: Integrin-linked kinase.

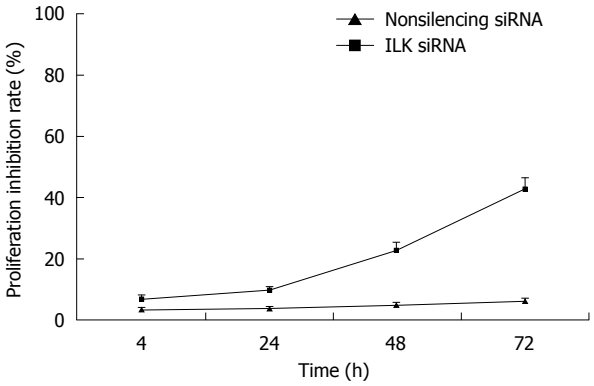

Figure 4 Effects of integrin-linked kinase on proliferation of gastric cancer cells. Gastric cancer BGC-823 cells were treated with integrin-linked kinase siRNA or non-silencing siRNA. Six duplicate wells were set up for each sample. After 4, 24, 48 or 72 h incubation, 20 μL MTT (5 g/L) was added to each well. Data are the mean ± SD of three independent experiments. Cell proliferation is plotted against time.

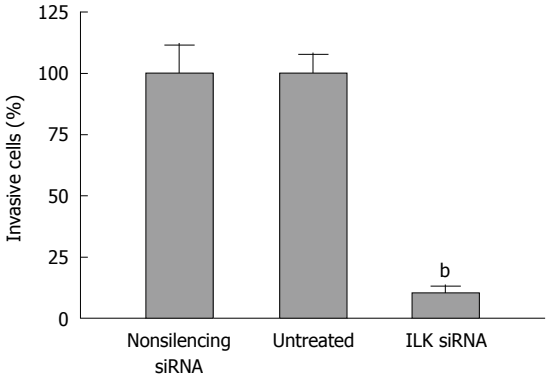

Figure 5 Effects of integrin-linked kinase on invasion of gastric cancer cells. Cell invasion was assessed 24 h after incubation in Matrigel coated transwell culture cambers. Data are the mean ± SD of three independent experiments ($^bP < 0.01$ *vs* control). Non-silencing siRNA is set to 100%. ILK: Integrin-linked kinase.

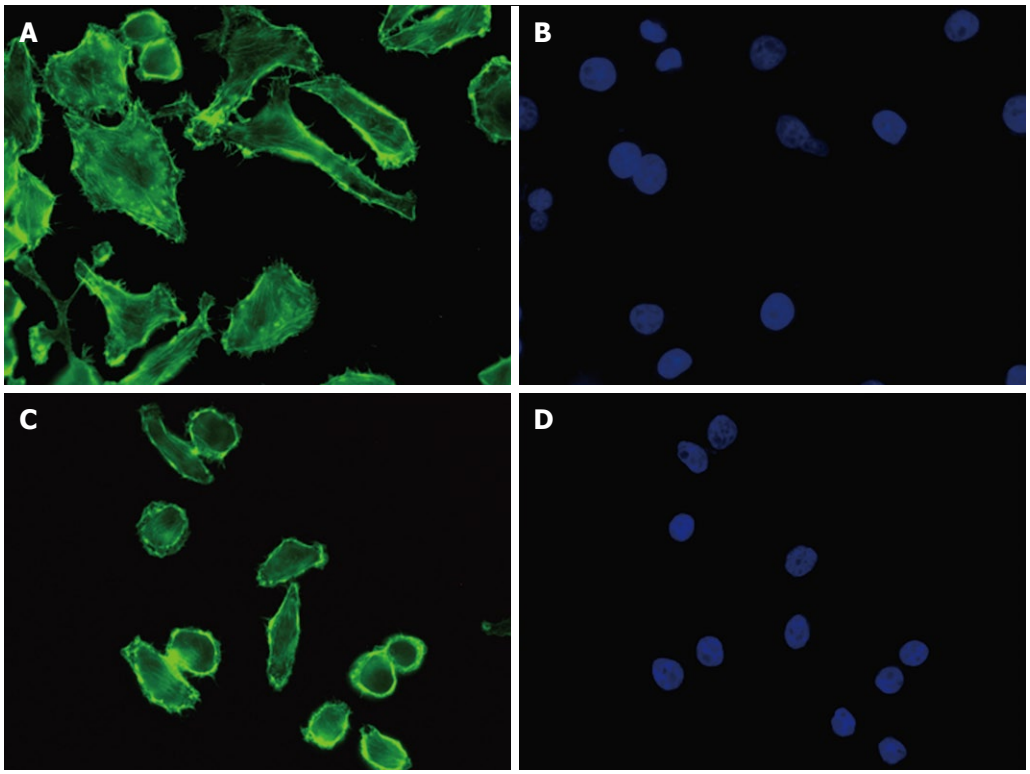

Figure 6 Effects of integrin-linked kinase on microfilament dynamics of gastric cancer cells. Microfilament organization was assessed by immunofluorescence analysis with rhodamine-phalloidin. A, B: Non-silencing siRNA-transfected cells displayed an elaborate network of precisely organized F-actin filaments and a high degree of cell spreading; C, D: The F-actin filament architecture became significantly disturbed with less lamellipodia and filopodia formation in integrin-linked kinase knockdown cells compared with control cells. Experiments were repeated three times.

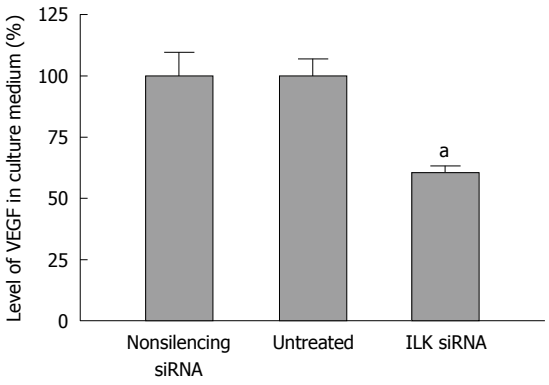

Figure 7 Effects of integrin-linked kinase on vascular endothelial growth factor secretion by gastric cancer cells. After transfection, the culture medium was harvested and cell counting was performed. Vascular endothelial growth factor (VEGF) released into the culture supernatant was measured by enzyme-linked immunosorbent assay. Data are the mean ± SD of three independent experiments ($^aP < 0.05$ *vs* control). Non-silencing siRNA cells are set to 100%. ILK: Integrin-linked kinase.

shown in Figure 7, the level of VEGF in the culture medium was decreased by 40% compared with non-silencing siRNA treated cells ($P < 0.05$). In contrast, there was no significant difference in the level of VEGF secretion between the non-silencing siRNA treated and untreated cells ($P > 0.05$).

ILK is important for gastric cancer growth in vivo

To further investigate the role of ILK in gastric cancer tumorigenesis, equal numbers (1×10^7) of BGC-823 cells transfected with ILK siRNA, non-silencing siRNA, or untreated cells were subcutaneously injected into nude mice. The growth of tumors was measured every 3 d. Four weeks after injection of the cells, mice were sacrificed and the weights of tumors were recorded. As shown in Figure 8, cells with down-regulation of ILK produced significantly smaller tumors in nude mice compared with untreated cells and cells treated with non-silencing siRNA (volume: 2.19 g ± 0.58 g *vs* 6.52 g ± 1.42 g, 6.68 g ± 1.40 g, $P < 0.05$; weight: 1.0 cm^3 ± 0.2 cm^3 *vs* 2.5 cm^3 ± 0.4 cm^3, 2.6 cm^3 ± 0.4 cm^3, $P < 0.05$, respectively) indicating that targeting ILK by siRNA may exert a strong anti-tumor effect on BGC-823 cells *in vivo*. All the tumors were analyzed by H&E staining and were verified to have similar cell morphologies that were consistent with gastric cancer.

DISCUSSION

Gastric cancer is a life-threatening disease with a high mortality worldwide, especially in Asia. Therefore, it is important to understand the molecular mechanisms of gastric cancer progression to discover useful targets to treat advanced gastric cancer. Previous findings showed that ILK has an increased expression and an association with tumor cell invasion and nodal metastasis in gastric cancer[13]. We, therefore, investigated whether ILK was important in different processes involved in gastric cancer progression, including cell attachment, growth, inva-

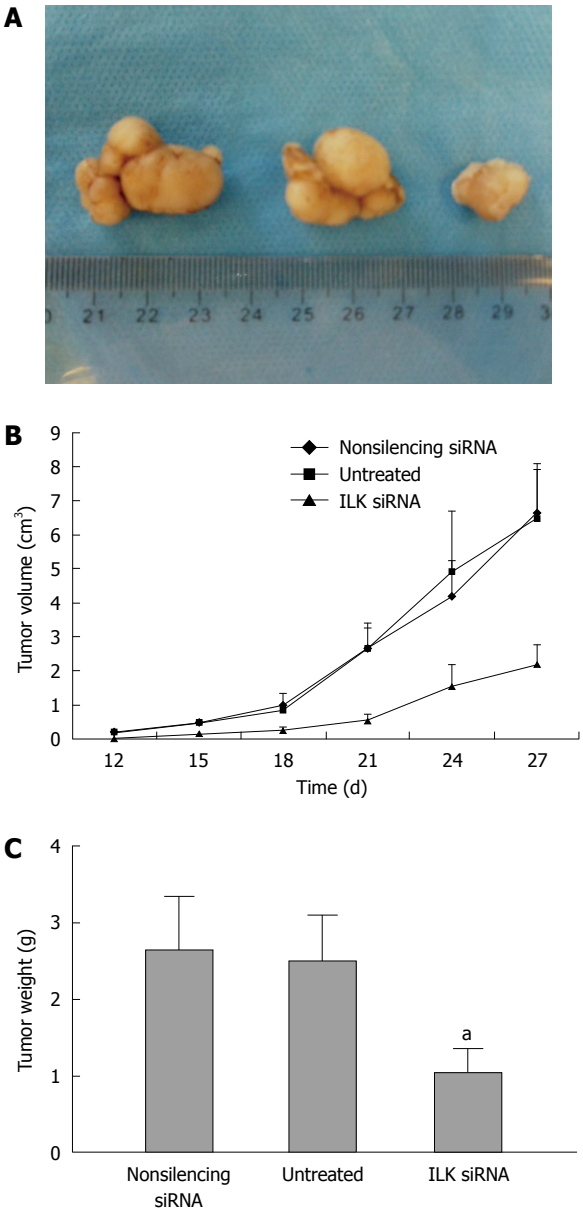

Figure 8 Integrin-linked kinase siRNA inhibits the growth of gastric cancer xenografts in nude mice. BGC-823 cells were treated with integrin-linked kinase (ILK) siRNA or non-silencing siRNA or left untreated. Equal numbers of cells (1×10^7) were injected 48 h later subcutaneously into the armpit of five nude mice in each group. Tumor formation was scored every 3 d. At week 4 after injection, the mice were killed and tumor weight recorded. A: images of tumors excised from the nude mice; B: estimated tumor volume of non-silencing siRNA, untreated or siRNA ILK tumors; C: estimated tumor weights of non-silencing siRNA, untreated or siRNA ILK tumors ($^aP < 0.05$ *vs* control).

sion, microfilament dynamics, angiogenesis and tumor growth *in vivo* by targeted siRNA knockdown of ILK.

Although RNA interference mediated by siRNAs is a powerful technology allowing the silencing of mammalian genes with high specificity and potency, non-specific effects both at the messenger RNA and protein levels can result from siRNA mediated mechanisms, and may represent one of the limitations of this technology[23]. Therefore, we used non-silencing siRNA-transfected cells to control these non-specific effects. We observed significant differences between the ILK-specific siRNA treated group and the non-silencing siRNA treated group in all assays. However, some studies indicated that the inhibition effect of synthetic siRNA may only last a short time. Therefore, following tumorigenesis, ILK expression may be released from RNAi inhibition in tumor tissues. Thus, to get a more stable inhibitory effect, we used a long-acting siRNA whose stability was improved by chemical modification. The expression of ILK was inhibited for about 7 d by this technique[24]. We observed that the suppression of ILK lasted a week and reached a peak 48 h after transfection. Thereafter, the expression of ILK gradually recovered, because of the remaining instability of the ILK siRNA.

We first showed that ILK is important for gastric cancer cells to attach to fibronectin-coated plates. Upon engagement with the extra cellular matrix (ECM), numerous signaling proteins are recruited to the adhesion sites, where cell-matrix contact is established[25]. Fibronectin is one of the major components in the ECM that connect cells through the extracellular domain of integrins. Here, we demonstrate that ILK plays a central role in the transduction of signals when gastric cancer cells engage in ECM-regulated cell attachment. The mechanism of how ILK regulates the dynamic rearrangement of cell-matrix adhesions and cell spreading is not well understood. Some scholars have suggested that ILK regulates cell-matrix adhesion dynamics through Rac-1[26]. In addition, inhibition of PI3K-dependent ILK activity in PTEN-null PC3 prostate cancer cells disrupted the localization of ILK/α-parvin/paxillin complex to focal adhesions, leading to decreased cell adhesion and migration[27]. However, others found that knock-down of human ILK by siRNA increased cell adhesion in diverse gastric carcinoma cell variants, including SNU16, integrin-α5-expressing SNU16, and integrin-α5-expressing SNU620 cells[28]. It appears that the correlation between ILK expression and cell adhesion is very sophisticated, and may depend on cell types and the signaling contexts. The epigenetic control of ILK expression and adhesion properties of gastric carcinoma cells appear to affect each other, through a bidirectional regulatory linkage[28].

Inhibition of ILK kinase activity by an ILK inhibitor is known to impede cell attachment and filamentous actin organization[27]. ILK over-expression induced the distribution of actin filaments onto the cell membrane to form cell motility structures[29]. We found that formation of the actin cytoskeleton and motility structures, which are important for the early events in cell spreading and migration, were severely affected in ILK knockdown gastric cancer cells. ILK may modulate cell spreading, migration and cytoskeletal organization by activating PAK-interactive exchange factor (PIX, also known as ARHGEF6), a guanine-nucleotide exchange factor for Rac1 and Cdc42[28], and by activating cofilin through an interaction with phosphorylated Scr[30]. This study suggests that targeting ILK seems to be linked with microfilament assembly, resulting in a decreased ability of the gastric cancer cells to attach, spread and migrate.

Tumor cell proliferation is another important event

in tumor progression. Knockdown of ILK using siRNA inhibited the proliferation of gastric cancer cells and impaired the growth of gastric cancer xenografts *in vivo*. ILK over-expression or constitutive activation leads to the stimulation of cell-cycle progression, and inhibition of ILK activity in some cancer cells results in inhibition of cyclin D1 expression and G1/S cell-cycle arrest[31-33]. ILK-mediated phosphorylation and consequent inhibition of the activity of GSK-3 may regulate several pathways, leading to stimulation if cyclin D1 expression. Inhibition of GSK-3 activity leads to activation of the AP1 transcription factor, cyclic-AMP-responsive-element-binding protein, and the β-catenin/TCF transcription factor[32,34,35], both of which can stimulate the expression of cyclin D1 and promote cell proliferation.

We also found that ILK is important for gastric cancer cell invasion; knockdown of ILK using siRNA inhibited gastric cancer cell invasion. Increased ILK expression was shown to stimulate the expression and activity of the matrix metallopeptidase 9 (MMP9), through activation of the AP1 transcription factor[36]. Inhibition of ILK activity in highly invasive human glioblastoma cells, resulted in substantial inhibition of invasion into matrigel, and pharmacological inhibition of MMP9 activity also inhibited invasion[36], demonstrating that ILK can promote invasion through up-regulation and activation of MMP9. In gastric cancer, the T allele of the 1562 C/T polymorphism in the MMP9 gene is associated with an invasive tumor phenotype[37] and elevated plasma MMP-9 correlates significantly with lymph node metastasis, lymphatic invasion, venous invasion and poor survival rates[38]. Therefore, it is reasonable to predict that down-regulating ILK and then inhibiting MMP9 hold promise for the treatment of gastric cancer.

Tumor angiogenesis is promoted by the expression and secretion of VEGF from tumor cells, which then binds to the VEGF receptor on the nearby endothelial cells, stimulating their survival, proliferation and migration, which are events required for the formation of new blood vessels. We observed that silencing ILK with siRNA significantly reduced VEGF secretion from gastric cancer cells. What is the mechanism by which ILK regulates VEGF? ILK has been reported to be essential for the regulation of hypoxia inducible factor (HIF)-1α expression, and for the consequent production of VEGF in a PKB/Akt- and mTOR/FRAP-dependent manner[10]. And HIF-1α is a major transcriptional activator of the VEGF gene[39]. A model has been proposed to explain this regulation[10]: phosphorylation of serine 473 of Akt/PKB by activated ILK results in the full activation of PKB/Akt, which promotes the phosphorylation of serine 2448 of mTOR/FRAP. This activates mTOR/FRAP, which raises the levels of HIF-1α protein translation. HIF-1α protein combines with HIF-1β to form an active transcription factor. This heterodimer binds to the VEGF promoter and activates VEGF transcription, translation, and secretion. VEGF binds to its receptor on the nearby endothelial cells and stimulates ILK activity. Furthermore, down-regulation of VEGF expression with siRNA not only impaired tube formation, but also inhibited the synthesis of multiple angiogenic proteins, such as angiogenin, interleukin (IL)-6, IL-8, transforming growth factor β1 and monocyte chemoattractant protein 1[40]. Collectively, these studies demonstrate a crucial role of ILK in the regulation of vascular morphogenesis, and indicate that ILK should be considered as a promising target for anti-angiogenic therapy.

In summary, our results indicate that knockdown of ILK with siRNA is able to inhibit not only gastric cancer cell attachment, proliferation, invasion and tumor angiogenesis *in vitro*, but also tumor growth *in vivo*. These findings also suggest that ILK could be a valid therapeutic target in gastric cancer.

COMMENTS

Background

Gastric cancer is one of the most commonly diagnosed malignant tumors and also is one of the most frequent causes of cancer mortality worldwide. Therefore, an understanding of the molecular mechanisms involved in gastric cancer formation and progression, and the identification of specific gene therapy targets are important for developing more effective approaches for gastric cancer treatment.

Research frontiers

Integrin-linked kinase (ILK), an ankyrin repeat-containing serine/threonine protein kinase, mediates a diversity of cell functions by coupling integrins and growth factors to cascades of downstream signaling events. The expression and activity of ILK are increased in a range of tumors, and small-molecule inhibitors of ILK activity have been identified, and shown to inhibit tumor growth, invasion and angiogenesis. ILK has become a hot topic in tumor research.

Innovations and breakthroughs

To investigate the role of ILK in gastric cancer, the authors specifically knocked down ILK expression using small interfering RNA (siRNA) in the gastric cancer cell line, BGC-823. The authors verified that knockdown of ILK significantly inhibited human gastric cancer cell attachment, proliferation, and invasion, and also disturbed F-actin assembly and reduced vascular endothelial growth factor secretion. Knockdown of ILK also suppressed the growth of gastric cancer cells *in vivo*. This research attempts to systematically understand the role of ILK in gastric cancer progression. The results could improve our understanding of gastric cancer progression.

Applications

The study provides the first evidence that ILK plays an important role in gastric cancer progression. The results indicate that ILK should be considered as a promising target for anti-gastric cancer treatment.

Peer review

The expression and activity of ILK are increased in a range of tumors, including gastric cancer. This study shows that targeting ILK with siRNA suppressed the growth of gastric cancer cells. The results indicate that ILK plays an important role in gastric cancer progression and that ILK could be a therapeutic target for gastric cancer.

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BRIEF ARTICLE

Gemcitabine in elderly patients with advanced pancreatic cancer

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Abstract

AIM: To assess feasibility, tolerability and efficacy of gemcitabine-based chemotherapy in patients ⩾ 75 years old with advanced pancreatic cancer.

METHODS: All consecutive patients ⩾ 75 years old with advanced pancreatic adenocarcinoma were included in this retrospective study. Necessary criteria to receive chemotherapy were: performance status 0-2, adequate biological parameters and no serious comorbidities. Other patients received best supportive care (BSC).

RESULTS: Thirty-eight patients (53% women, median age 78 years, range 75-84) with pancreatic cancer (metastatic: *n* = 20, locally advanced: *n* = 18) were studied. Among them, 30 (79%) were able to receive chemotherapy [median number: 9 infusions (1-45)]. Six patients (23%) had at least one episode of grade 3 neutropenia and one patient developed a grade 3 hemolytic-uremic syndrome. No toxic death occurred. Three patients (11%) had a partial tumor response, 13 (46%) had a stable disease and 12 (43%) had a tumor progression. Median survival was 9.1 mo (metastatic: 6.9 mo, locally advanced: 11.4 mo).

CONCLUSION: Tolerance and efficacy of gemcitabine-based chemotherapy is acceptable in elderly patients in good condition, with similar results to younger patients.

Key words: Elderly; Pancreas; Cancer; Gemcitabine

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INTRODUCTION

Although pancreatic cancer (PC) only accounts for 2% of all cancers, it is the fourth leading cause of cancer death in the United States (US)[1]. Prognosis is very poor, with an estimated incidence of 33 000 per year in the US and a similar death incidence rate[1]. Median survival in patients with advanced PC who receive the best support-

ive care (BSC) is only three to four months. Chemotherapy with gemcitabine has been considered the standard treatment of non-resectable PC since the study by Burris *et al*[2]; it slightly improves both survival and clinical response and is acceptably tolerated. Several drugs have been tested in combination with gemcitabine but with disappointing results. The only combination that showed a slight but significant increase in survival was erlotinib and gemcitabine in a study by Moore *et al*[3].

PC usually occurs in elderly patients. In the US, the incidence rate adjusted by age and for 100 000 is of 64.2 over 65 years old and of 3.7 under 65 years old[4]. In France 37.1% of PC cases occur in patients ≥ 75 years old[5]. Survival rates in this subgroup of patients seem to be shorter than in younger patients[4]. Physicians may hesitate to offer intravenous chemotherapy because of frequent comorbidities and short estimated survival; in addition, the motivation of elderly patients for this type of treatment should be carefully assessed. Nevertheless, it has clearly been shown that elderly patients are underrepresented in cancer trials[6,7]. The efficacy and tolerance of chemotherapy in elderly patients with colorectal cancer has been shown in previous studies[8-12]. Most phase Ⅲ studies of chemotherapy for PC include results of, but do not specifically analyze, the subset of patients ≥ 70-75 years old[2,13-15]. Results by Maréchal *et al*[16] in a pooled analysis of patients ≥ 70 years old who were included in seven prospective phase 2 or phase 3 studies testing various gemcitabine-based first line combinations, suggest that chemotherapy is feasible in the elderly as well as in younger patients with PC. Likewise, Locher *et al*[17] supported the use of gemcitabine in another study in elderly patients.

The aim of this retrospective monocentric study was to assess feasibility, tolerance and efficacy of gemcitabine-based palliative chemotherapy in patients ≥ 75 years old treated for PC.

MATERIALS AND METHODS

Selection of patients

All patients with digestive cancer in our hospital are discussed at the weekly multidisciplinary oncological committee meeting, even if they are only able to receive best supportive care on first intention. For the current study, all patients with pathologically-proven advanced adenocarcinoma of the exocrine pancreas who were ≥ 75 years old and listed in our database were considered. Patients with adenocarcinoma of the ampulla or the biliary tract were excluded. Overall, 40 patients were included for this retrospective analysis. Among them, 2 patients were excluded as they received gemcitabine in another institution (West Indies) and thus follow-up was not possible. Finally, 38 consecutive patients fulfilling these criteria and who were treated in our hospital between March 2000 and June 2006 were retrospectively studied. After clinical and imaging assessment, tumors were classified as locally advanced (stage Ⅲ) or metastatic (stage Ⅳ) according to the UICC classification (UICC).

Criteria required to propose chemotherapy were an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2 and no serious comorbidities. Before starting chemotherapy, pain and biliary obstruction had to be controlled and adequate biological parameters (i.e., neutrophil count > 1500/mL, platelet count > 100 000/mL, serum creatinine < 1.5 × the upper limit of normal value (ULN), alkaline phosphatase < 5 × ULN, and bilirubin < 1.5 × ULN) were required. If one of these criteria was not fulfilled, BSC was decided.

Treatment

Chemotherapy included gemcitabine as a single agent according to the Burris regimen (gemcitabine 1000 mg/m² as a 30-min infusion weekly for 7 out of 8 wk and then for 3 out of 4 wk)[2] or combined with oxaliplatin according to the GemOx regimen (gemcitabine 1000 mg/m² as a 100-min infusion on day 1 and oxaliplatin 100 mg/m² as a 2-h infusion on day 2 every 2 wk)[18].

Patients who received at least one infusion of chemotherapy were placed in the "chemotherapy group". All the other patients received BSC.

Chemotherapy was stopped if there was an unacceptable/life-threatening adverse event, if performance status worsened (i.e., ECOG ≥ 3) and/or if tumor progression occurred according to imaging results. The type of chemotherapy, the number of infusions and the reason why chemotherapy was not administered or was stopped were analysed.

Safety and efficacy evaluation

Baseline assessment included medical history, physical examination with an evaluation of clinical symptoms, and biological analyses (blood cell count, serum creatinine, bilirubin, ASAT, ALAT, alkaline phosphatase). During the treatment period, blood tests, toxicity evaluation and a physical examination were performed before each infusion.

Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 2. Chemotherapy was delayed if the grade of toxicity ≥2; the dose of gemcitabine was reduced by 20% if the toxicity grade was ≥ 3.

Tumor response was assessed by computed tomography scan at three month intervals according to RECIST (Response Evaluation Criteria In Solid Tumors)[19]. Evaluation procedures were performed ahead of schedule if the patient's general condition worsened or severe toxicity occurred. Overall survival (OS) was calculated from the day of diagnosis of non-resectable PC to the date of death. This study was proposed after the agreement of our institution review board.

Statistical analysis

Qualitative data are expressed as numbers and percentages. Quantitative data are expressed as median (range).

Table 1 Characteristics of the 38 patients and their pancreatic cancers

	Metastatic	Locally advanced
Number of patients (%)	20 (53)	18 (47)
Median age (range)	78 (75-84)	78 (75-84)
Gender (M/F)	8/12	10/8
Site of metastases		
-liver	15	0
-other[1]	10	0

[1]Lung, peritoneum, lymph nodes.

Table 2 Characteristics of treatment in the 38 patients according to the stage of pancreatic cancer

	Metastatic	Locally advanced
Number of patients treated by gemcitabine-based chemotherapy (%)	15 (50%)	15 (50%)
Number of patients with BSC on first intention	5	3
-staff decision ECOG ⩾ 2	3	2
-others reasons	2 (Septicaemia, pulmonary embolism	1 (Duodenal stenosis and deep venous thrombosis)
Median number of infusions (range)[1]	*n* = 18 (1-45)	*n* = 7 (2-13)

[1]Data available for 28 patients.

Survival was determined by the Kaplan-Meier method.

RESULTS

General characteristics

Twenty women and 18 men were studied. Median age was 78 years old (75-84). Tumors were metastatic in 20 patients (including tumor relapse after surgical resection in five patients) and locally advanced in 18 patients. Median follow up was 7 mo (1-44) (Table 1). Thirty of the 38 patients (79%) received gemcitabine-based chemotherapy (single agent: $n = 28$, combined with oxaliplatin: $n = 2$) with a median of 9 infusions (1-45). Twenty-four patients (83%) completed at least 2 mo of chemotherapy (i.e., 7 infusions). The relative dose-intensity of gemcitabine was 83%. Chemotherapy was stopped due to tumor progression ($n = 21$), toxicity ($n = 1$) or fatigue ($n = 4$); it was replaced by chemoradiotherapy ($n = 2$) in patients with controlled disease. The eight remaining patients did not receive chemotherapy due to exclusion criteria ($n = 5$) or a life-threatening medical event that occurred after the decision to treat but before the beginning of the treatment ($n = 3$) (Table 2).

Safety evaluation

Tolerance data were available in 26 of 30 patients. Six patients (23%) had at least one episode of grade 3 hematological toxicity (neutropenia). One patient developed grade 3 hemolytic-uremic syndrome, so gemcitabine was discontinued. No grade 4 toxicity or toxic deaths occurred.

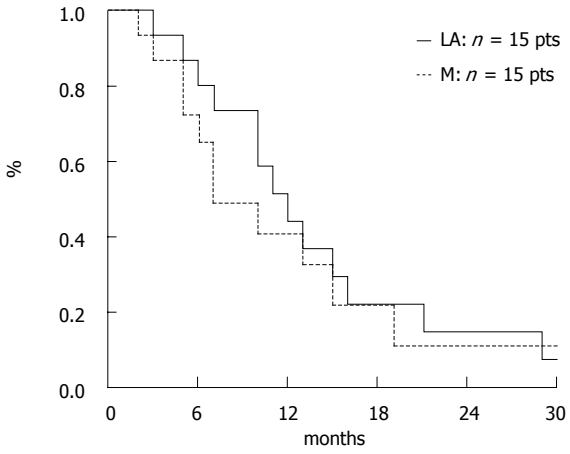

Figure 1 Overall survival of treated patients according to disease stage.

Tumor response rate

Response rate was available in 28 of 30 patients. During the first assessment (at 3 mo), 3 patients (11%) had partial tumor response (PR), disease was stable in 13 (46%) (SD) and 12 (43%) had tumor progression (PD). For 14/15 patients with metastatic PC, 1 PR (7%), 4 SD (29%) and 9 PD (64%) were observed.

Second line treatment included chemotherapy in four patients with progressive disease (GemOx after gemcitabine alone: $n = 3$, and Folfiri after GemOx: $n = 1$), and chemoradiotherapy was proposed in two 75-year old patients with locally advanced tumors who were in very good condition with controlled tumors after 3 mo of chemotherapy. The latter treatment involved irradiation of 50.4 Gy with a continuous infusion (200 mg/m^2) of 5-fluorouracile as a radiosensitizer based on the results of a previous study[20].

Overall survival

Median survival of all patients ($n = 38$) was 8.9 mo and the one-year survival rate was 33.2%. Median survival of the 8 patients who received BSC was 2.95 mo. In patients receiving chemotherapy, median survival was 9.1 mo; this was 6.9 mo in patients with metastatic cancer and 11.4 mo in patients with locally advanced cancer; the 1-year survival rate was 40.6% and 44%, respectively.

Overall survival in patients treated with gemcitabine-based chemotherapy according to disease stage is presented in Figure 1.

DISCUSSION

Although a direct comparison was not performed, this monocentric retrospective study suggests that the safety and efficacy of gemcitabine-based chemotherapy in elderly patients is similar to that in younger patients.

Most eligible patients (79%) received a median of 9 infusions of chemotherapy. Safety was acceptable with grade 3 neutropenia in 23% of patients (with no grade 4), and one case of grade 3 hemolytic-uremic syndrome requiring treatment discontinuation. There were no toxic deaths. These safety results are similar to those in randomised studies including younger patients which report neutropenia as the most frequent type of toxicity with gemcitabine (grade 3-4 toxicities from 9% to 27.6%)[2,3,13-15,18].

In our study, disease control was obtained in 57% of patients (PR: 11% and SD: 46%) who received chemotherapy, which compares favourably to other published randomised studies (41.2% to 52.8%)[2,3,13,21]. The objective response rates in these studies, which include patients with both locally advanced and metastatic cancers, was 7.1% to 17.3%[3,14,15,18]. The survival rate in our study was 9.1 mo in patients who received chemotherapy; this was 6.9 mo in patients in the metastatic subgroup and 11.4 mo in the locally advanced subgroup. The 1-year survival rate of patients with metastatic and locally advanced disease who received chemotherapy was 40.6% and 44%, respectively. In the randomised series with younger patients, median survival rates in the gemcitabine arm were 5.6 and 7.2 mo, respectively[2,3,13-15,18,21].

These results should be cautiously interpreted since methodological biases are inevitable in such a retrospective study. In addition, it was conducted in a tertiary care institution, thus our population should not entirely reflect the "true life" practice for elderly patients with PC. Likewise, our study does not allow distinguishing of the potential influence of performance status (i.e., 0-1 *vs* 2) on both treatment safety and efficacy.

One retrospective phase Ⅱ trial analysed the impact of age (< or ⩾ 65 years) on the efficacy and tolerance to gemcitabine in advanced non-small cell lung cancers. Hematological, non-hematological toxicities and dose reductions, or the mean number of cycles were similar in both age groups[22].

A recent study by Locher *et al*[17] reported 39 patients ⩾ 70 years old with PC treated by a fixed-dose rate of gemcitabine[23]. The authors showed a good efficacy of this treatment with a clinical benefit observed in 20%, a tumor response rate in 10% and a stabilization of the disease in 33% of patients. The median survival was 10 mo and the time to progression was 7 mo. Grade 3-4 neutropenia and alopecia occurred in respectively 38% and 18%. These side-effects were higher than in others trials probably due to the fixed dose rate of gemcitabine[2,3,13-15,18-23]. Maréchal *et al*[16] analyzed 42 patients > 70 years old pooled from seven prospective studies evaluating gemcitabine-based chemotherapy and compared them to 57 younger patients. Two thirds of the elderly patients received gemcitabine alone and one third received gemcitabine-based combinations (mainly gemcitabine-oxaliplatin). The median overall survival (220 d *vs* 240 d), time to progression (104 d *vs* 119 d), response rate (4.8% *vs* 8.9%) and clinical benefit (57.1% *vs* 59.6%) were similar in elderly and non-elderly patients. Tolerance to chemotherapy was acceptable in the elderly group despite a dose reduction or delay in therapy in 62%, a higher figure than that observed in our study. As in our study, neutropenia was the most common cause of grade 3-4 toxicity. Grade 3-4 neutropenia, anaemia and peripheral neuropathy occurred more often in the elderly group than in younger patients (30.9% *vs* 8.8%, 14.3% *vs* 8.8% and 4.8% *vs* 0%, respectively). Age was not an independent prognostic factor in multivariate analysis of the whole population. Multivariate analysis identified ASAT and Karnofsky index as independent prognostic factors in the elderly group[16].

A Japanese study specifically reported results in 25 patients ⩾ 70 years old receiving gemcitabine 800-1000 mg/m² compared to 43 patients receiving BSC. Patients receiving chemotherapy had a more favourable prognosis and acceptable tolerance[24]. Another retrospective study by Nakachi *et al*[25], presented in abstract form at the ASCO GI meeting in 2007, suggested that gemcitabine was effective and well tolerated in selected elderly patients. Thirty-seven patients ⩾ 75 years old were compared to 137 younger patients. Grade 3-4 neutropenia (18.9% *vs* 19 %) and tumor response rates (8.1% *vs* 4.3%) were similar. In contrast, median overall survival was better in the elderly group (8 mo *vs* 5.6 mo, $P = 0.009$).

Recently, the promising schema FOLFIRINOX (5-fluorouracil, irinotecan and oxaliplatin) was shown to be superior to gemcitabine in terms of tumor response and overall survival[26]. However, patients treated in this study were less than 75 years-old and in very good condition (PS 0-1). Moreover, significant toxicity was seen [45.7% of patients experienced a significant (grade 3-4) hematological toxicity with 5.4% of febrile neutropenia] that could be problematic in elderly patients[26]. Further studies are warranted in latter patients using such drugs.

In conclusion, gemcitabine chemotherapy seems to be effective and safe in elderly patients with PC in good condition. The risk/benefit ratio of this treatment should be discussed in a multidisciplinary context and these patients should actively participate in therapeutic decisions. Prospective studies of this specific subgroup of patients with PC are needed.

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COMMENTS

Background

Pancreatic cancer is a severe disease that is often treated using systemic chemotherapy as it is non-resectable in up to 80% of patients at the time of diagnosis. Significant rates of patients with this disease are older than 75 years. However, most phase Ⅲ studies of chemotherapy for pancreatic cancer include elderly patients, but they do not provide a specific analysis of patients ⩾ 70-75 years old. Thus, this specific population is strongly underrepresented in therapeutic trials for digestive cancers and thus guidelines for clinical practice are lacking. In this paper, the results suggest that elderly patients with pancreatic

cancer, when they are in acceptable condition, could receive gemcitabine-based chemotherapy which is safe and seems to be as efficient as in younger patients.

Research frontiers

Tumor response rates, toxicity and duration of tumor control were specifically analyzed in a homogeneous population of 38 elderly patients with pancreatic cancer treated in one center.

Innovations and breakthroughs

This is a homogeneous study of consecutive patients treated by an experienced team in digestive cancers, particularly pancreatic cancer. The authors have shown that toxicity of gemcitabine was manageable, and tumor control and overall survival were encouraging, as they appear to be similar to that of younger patients. The authors hope it will encourage physicians to evaluate and consider chemotherapy in such patients.

Applications

It is time to pave the way of chemotherapy in elderly patients with pancreatic cancer knowing that a significant subset of them may benefit of these treatments. In the future, patients should be better selected for the treatments using molecular markers (i.e., hENT-1 expression and gemcitabine).

Terminology

A locally advanced pancreatic cancer is a tumor involving the arterial axis (celiac trunk, mesenteric artery) and thus is non-resectable despite there being no detectable metastases. This form of cancer should be distinguished from metastatic tumors as the prognosis is different (slightly better, and some patients can return to surgical treatment in cases of good tumor response after chemotherapy), and thus separate analyses are needed.

Peer review

This is an article describing gemcitabine in elderly patients with advanced pancreatic cancer.

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BRIEF ARTICLE

Diagnostic efficacy of gadoxetic acid-enhanced MRI for hepatocellular carcinoma and dysplastic nodule

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Abstract

AIM: To evaluate the relationship between the signal intensity of hepatobiliary phase images on gadoxetic acid-enhanced magnetic resonance imaging (MRI) and histological grade.

METHODS: Fifty-nine patients with 82 hepatocellular lesions were evaluated retrospectively. Hepatobiliary phase images on gadoxetic acid-enhanced MRI were classified into 3 groups: low, iso or high. Angiography-assisted computed tomography (CT) findings were also classified into 3 groups: CT during arterial portography, and CT hepatic arteriography: A: iso, iso or low; B: slightly low, iso or low; and C: low, high. We correlated angiography-assisted CT, hepatobiliary phase findings during gadoxetic acid-enhanced MRI and histological grades. Furthermore, correlations between MRI findings and histological grade for each hemodynamic pattern were performed. Correlations among radiological and pathological findings were statistically evaluated using the chi-square test and Fisher' s exact test.

RESULTS: There was a significant correlation between histological grade and hemodynamic pattern ($P < 0.05$). There was a significant correlation between histological grade and signal intensity in the hepatobiliary phase ($P < 0.05$) in group A lesions. There was no significant correlation between histological grade and signal intensity in the hepatobiliary phase in group B or C lesions ($P > 0.05$).

CONCLUSION: Signal intensity in the hepatobiliary phase correlated with histological grade in the lesions that maintained portal blood flow, but did not correlate in lesions that showed decreased or defective portal blood flow.

Key words: Hepatocellular carcinoma; Gd-EOB-DTPA; Gadoxetic acid; Primovist; Early hepatocellular carcinoma

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INTRODUCTION

Early detection of hepatocellular carcinoma (HCC) is im-

portant in establishing an effective therapeutic strategy[1]. Early stage HCC does not have a hypervascular nature, and the lesions maintain portal blood flow[2,3]. Intranodular portal blood flow can only be evaluated by computed tomography (CT) during arterial portography (CTAP), and the absence or decrease of portal blood flow in the nodule can show that the lesion is malignant[4]. However, dysplastic nodules and some well-differentiated HCC lesions maintain portal blood flow, making differential diagnoses difficult[5]. Gadolinium-ethoxybenzyl diethylenetriaminepentaacetic acid (gadoxetic acid, Primovist®; Bayer-Schering, Osaka, Japan) is a liver-specific contrast medium which is taken into hepatocytes and excreted into bile; therefore a T1 shortening effect in liver parenchyma is obtained. The hepatobiliary phase begins 1.5 min after injection of the contrast medium and continues for 2 h, and the peak liver signal intensity is obtained 20 min after injection of contrast medium[6]. In the hepatobiliary phase, the tumor does not have normal functioning hepatocytes and is hypointense in most cases[7]. However, several investigators reported that some HCC can be iso or hyperintense regardless of overt HCC[8,9]. Gadoxetic acid-enhanced magnetic resonance imaging (MRI) yields a high tumor detection rate[10] and can detect lesions that maintain portal blood flow using angiography-assisted CT[11]. Therefore, gadoxetic acid-enhanced MRI can potentially distinguish the histological grade of hepatocellular lesions. We evaluated the relationships among angiography-assisted CT, hepatobiliary phase findings during gadoxetic acid-enhanced MRI and histological grades, and evaluated the diagnostic efficacy of gadoxetic acid for HCC and dysplastic nodule.

MATERIALS AND METHODS

Subjects

This retrospective study was approved by the Institutional Review Board, and the need for written informed consent was waived. Between January 2008 and June 2009, 460 patients received gadoxetic acid-enhanced MRI. Among them, patients satisfying all of the following criteria were enrolled; (1) gadoxetic acid-enhanced MRI had been performed; (2) angiography-assisted CT had been performed; (3) the duration between gadoxetic acid-enhanced and angiography-assisted CT was less than 60 d; and (4) their lesions had been pathologically confirmed. Patients in whom liver parenchymal enhancement was poor or absent due to severe portal hypertension or tumor invasion to the main portal vein were excluded. The subjects therefore consisted of 59 patients (37 men, 22 women), with 82 nodules. The mean age of the patients was 69 years (range 37-90 years). There were 8 patients with hepatitis B, 36 with hepatitis C, 4 alcoholic patients, 2 non-alcoholic patients with steatohepatitis, 1 with primary biliary cirrhosis and 8 cryptogenic patients. Six lesions were pathologically confirmed by operation. The other lesions were confirmed by ultrasound-guided biopsy (SSA-790A, Aplio XG; Toshiba Medical Systems Corp., Otawara, Japan). Biopsy specimens from the lesion and non-tumor area were obtained with a 20-gauge US-guided fine needle biopsy. Contrast medium (Sonazoid, Daiichi Sankyo, Tokyo, Japan) was used for obscure lesions on ultrasound. All lesions were detected on plain or contrast-enhanced ultrasound. The longest axis of the lesions was 5-66 mm (mean ± SD, 17.4 mm ± 9.5 mm). The longest dimension in 16 lesions was ≤ 10 mm, 21 were ≥10 mm, and 45 were > 15 mm. The long axis was measured on MRI.

Imaging

Angiography-assisted CT was performed with an angiography-combined 16 detector row CT system (Advantx ACT, GE Medical Systems, Milwaukee, WI). Immediately after injecting prostaglandin E2 (Liple®; Mitsubishi Tanabe Pharma, Osaka, Japan) through a catheter, 76 mL of contrast material (Iomeprol 350 mgI/mL; Eisai, Tokyo, Japan), which was diluted twice with physiological saline, was injected at a rate of 2 mL/s. CTAP was obtained 30 s after beginning the injection of contrast material through a catheter in the superior mesenteric artery. The parameters for CT acquisition were: table speed, 13.7 mm/0.5 s; collimation, 10 mm; and reconstruction, 5 mm. CT hepatic arteriography (CTHA) was obtained 6 s after the injection of contrast material through a catheter in the common hepatic or proper hepatic artery. In cases of hepatic artery bifurcation variation, the catheter was first inserted into the right and then the left hepatic artery, or *vice versa*. A total of 10-30 mL of contrast material (Iomeprol 350 mgI/mL) was injected at a rate of 0.8-1.5 mL/s. CTHA was obtained in 3 phases. Immediately after finishing the first phase, the second phase was obtained, and the third phase was obtained 2 min after beginning the injection of contrast material.

MR images were obtained using a 1.5 T superconductive MRI system (Avanto; Siemens, Erlangen, Germany). T1 weighted images (T1WI) included in-phase and opposed-phase images. The T1WI parameters (in-phase and opposed-phase) were: TR/TE, 120/4.76, 2.38 ms; flip angle, 75°; 1 averaging; matrix, 256 × 140; parallel acquisition technique (PAT) factor 2 with generalized autocalibration partially parallel acquisition (GRAPPA) algorithm; slice thickness, 6 mm; slice gap, 1.2 mm; and acquisition time, 13 s. The T2WI parameters were: TR/TE, 3600/99 ms; flip angle, 150°; echo train length, 29; matrix, 256 × 75(%); slice thickness, 6 mm; 1 averaging; PAT factor 2 with GRAPPA algorithm; and acquisition time, 14 s. T2WI was performed while subjects held their breath. 2 or 3 mL/s of gadoxetic acid (0.025 μmol/kg) was injected *via* the antecubital vein followed by 20 or 40 mL of physiological saline. The dynamic study included the arterial phase, portal phase, and 4 min after injecting the contrast material. A 3-dimensional (3-D) volumetric interpolated breath-hold examination (3D-VIBE) was used with the dynamic study. The 3D-VIBE parameters were : TR/TE, 4.28/1.78 ms; flip angle, 15°; matrix, 256 × 85 (%); PAT factor, 2; slice thickness, 3 mm; and acquisition time, 20 s. The monitoring scan technique (Care Bolus method) was used to obtain the optimal arterial phase. The hepato-

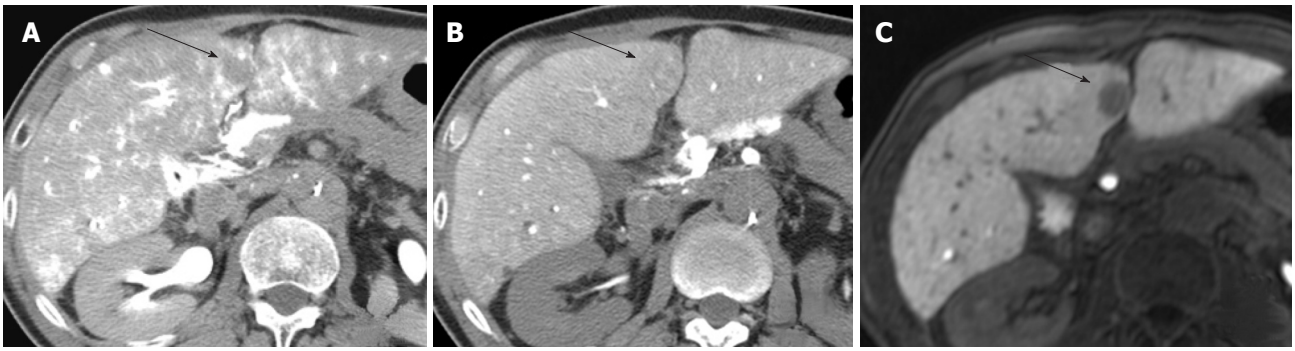

Figure 1 A 69-year-old man with moderately differentiated hepatocellular carcinoma. A: Computed tomography (CT) hepatic arteriography shows hypodensity; B: CT during arterial portography shows isodensity; C: Lesion clearly shows hypointensity in the hepatobiliary phase during gadoxetic acid-enhanced magnetic resonance imaging (arrow).

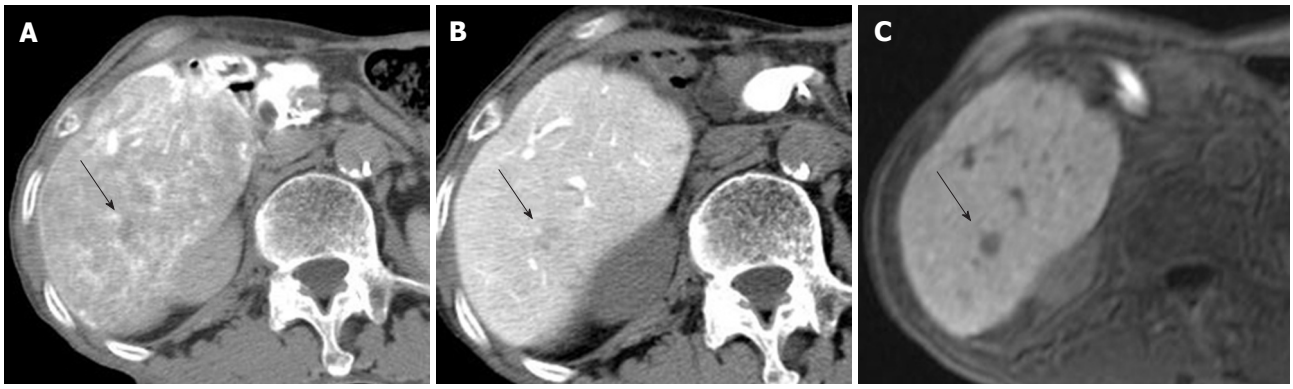

Figure 2 A 72-year-old man with well differentiated hepatocellular carcinoma. A: Computed tomography (CT) hepatic arteriography shows faint hypodensity; B: CT during arterial portography shows faint hypodensity; C: The lesion clearly shows hypointensity in the hepatobiliary phase on gadoxetic acid-enhanced magnetic resonance imaging (arrow).

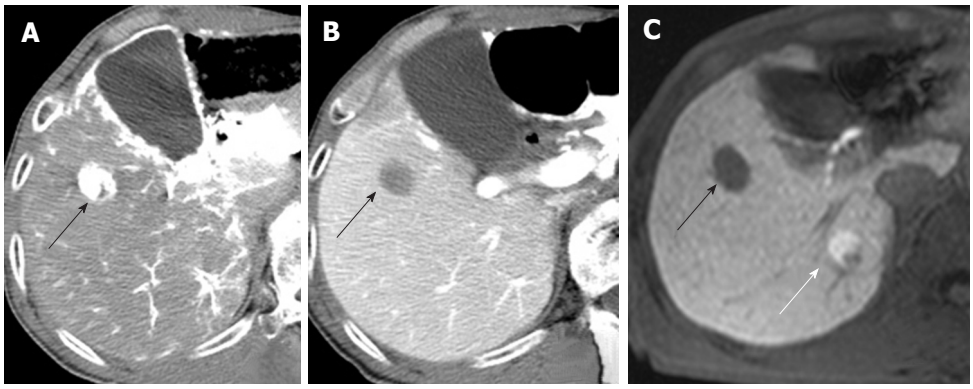

Figure 3 An 80-year-old man with poorly differentiated (black arrow) and well differentiated hepatocellular carcinoma (white arrow). A: Poorly differentiated hepatocellular carcinoma shows hypervascularity on computed tomography (CT) hepatic arteriography; B: Hypodensity on CT during arterial portography; C: Hypointensity in hepatobiliary phase on gadoxetic acid-enhanced magnetic resonance imaging (MRI). The lesion is not visible on CT hepatic arteriography (A) or on CT during arterial portography (B) and shows hyperintensity in the hepatobiliary phase on gadoxetic acid-enhanced MRI.

biliary phase was obtained with 3D-VIBE 20 min after injecting the contrast material.

Evaluation

A radiologist with 18 years of experience, whose specialty was interventional radiology, and a physician with 8 years of experience, whose specialty was liver imaging, evaluated the angiography-assisted CT by consensus. The findings of angiography-assisted CT were classified into 3 groups based on a previous report[12]: A, isodensity on CTAP and isodensity or low density on CTHA; B, slightly low density on CTAP and isodensity or low density on CTHA; and C, low density on CTAP and high density on CTHA (Figures 1-3). Partial hypodensity on CTAP and

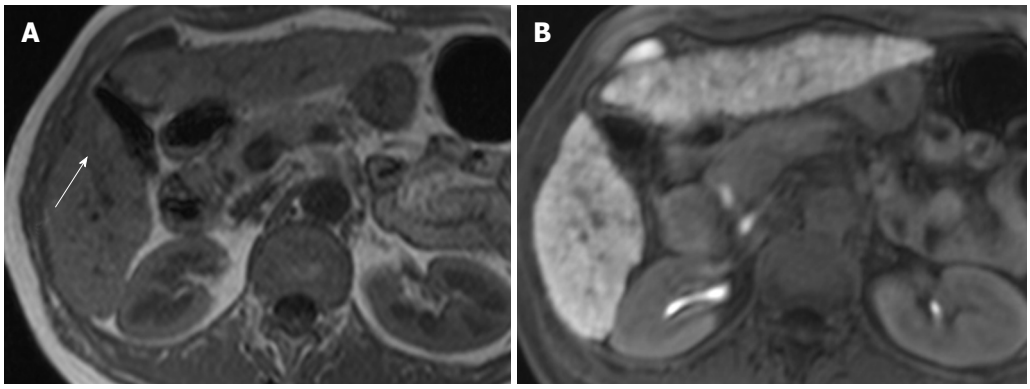

Figure 4 A 70-year-old woman with dysplastic nodule (arrow). A: The lesion shows hyperintensity on T1 weighted images; B: Isointensity in hepatobiliary phase on gadoxetic acid-enhanced magnetic resonance imaging.

Table 1 Correlation of histological grade and hemodynamic pattern

		Hemodynamic pattern			Total
		A	B	C	
Histological grade	DN	2	1	0	3
	Well	16[2]	8[2]	8[1]	32
	Mod.	7[1]	3	26[2]	36
	Poor	1	0	10[2]	11
Total		26	12	44	82

Hemodynamic patterns; A: Isodensity on computed tomography (CT) during arterial portography (CTAP) and isodensity or low density on CT hepatic arteriography (CTHA); B: Slightly low density on CTAP and isodensity or low density on CTHA; C: Low density on CTAP and high density on CTHA.DN: Dysplastic nodule; Well: Well differentiated hepatocellular carcinoma (HCC); Mod: Moderately differentiated HCC; Poor: Poorly differentiated HCC. [1]Significantly lower frequency; [2]Significantly higher frequency.

partial high density on CTHA were included in group C.

Two radiologists with 18 and 8 years of experience respectively, whose specialty was abdominal diagnostic radiology, evaluated MRI by consensus. Lesion signal intensity in the hepatobiliary phase during gadoxetic acid-enhanced MRI compared with the surrounding liver parenchyma was classified as either hypointensity, isointensity, or hyperintensity (Figures 3, 4). The same evaluation was performed for T1WI and T2WI.

We correlated angiography-assisted CT, hepatobiliary phase findings during gadoxetic acid-enhanced MRI and histological grades. Furthermore, correlations between MRI findings and histological grade for each hemodynamic pattern were performed. The signal intensities of T1WI and T2WI also correlated with the signal intensities of the hepatobiliary phase.

Correlations among radiological and pathological findings were statistically evaluated using the chi-square test and Fisher's exact test. $P < 0.05$ was considered statistically significant. Statistical analysis was performed using SPSS version 16.0 (SPSS Inc., Chicago, IL, United States) for Windows.

RESULTS

Correlation of histological grade and hemodynamic pattern

Twenty-six, 12 and 44 lesions were classified into hemodynamic pattern types A, B and C, respectively. Type A included 2 dysplastic nodules, 16 well, 7 moderately and 1 poorly differentiated HCC. Type B included 1 dysplastic nodule, 8 well and 3 moderately differentiated HCC. Type C included 8 well, 26 moderately and 10 poorly differentiated HCC.

There was a significant correlation between histological grade and hemodynamic pattern ($P < 0.05$). Well-differentiated HCC showed hemodynamic patterns of types A and B with significantly high frequency, and that of type C with significantly less frequency. Moderately differentiated HCC showed the hemodynamic pattern of type A significantly less frequently, and that of type C with significantly high frequency. Poorly differentiated HCC showed the hemodynamic pattern of type C with significantly high frequency (Table 1).

Correlations between histological grade and signal intensity in the hepatobiliary phase

There was a significant correlation between histological grade and signal intensity in the hepatobiliary phase ($P < 0.05$). Dysplastic nodules showed isointensity with significantly high frequency and hypointensity with significantly less frequency. Moderately differentiated HCC showed significantly less isointensity frequency (Table 2).

Correlation between histological grade and signal intensity in the hepatobiliary phase in each hemodynamic pattern

Type A lesions: There was a significant correlation between histological grade and signal intensity in the hepatobiliary phase ($P < 0.05$). Dysplastic nodules showed isointensity with significantly high frequency and lower hypointensity (Table 3).

Type B and C lesions: There was no significant correlation between histological grade and signal intensity in the hepatobiliary phase in either hemodynamic pat-

Table 2 Correlation between histological grade and signal intensity in the hepatobiliary phase

		Hepatobiliary phase			Total
		Hyper.	Iso.	Hypo.	
Histological grade	DN	0	2[2]	1[1]	3
	Well	1	3	28	32
	Mod.	1	0[1]	35	36
	Poor	0	0	11	11
Total		2	5	75	82

DN: Dysplastic nodule; Well: Well differentiated hepatocellular carcinoma (HCC); Mod: Moderately differentiated HCC; Poor: Poorly differentiated HCC. Hyper: Hyperintensity; Iso: Isointensity; Hypo: Hypointensity. [1]Significantly lower frequency; [2]Significantly higher frequency.

Table 3 Histological grade and signal intensity in the hepatobiliary phase in lesions which maintained portal blood flow

		Hepatobiliary phase			Total
		Hyperintensity	Isointensity	Hypointensity	
Histological grade	DN	0	2[b]	0[a]	2
	Well	1	0	15	16
	Mod.	0	0	7	7
	Poor	0	0	1	1
Total		1	2	23	26

DN: Dysplastic nodule; Well: Well differentiated hepatocellular carcinoma (HCC); Mod: Moderately differentiated HCC; Poor: Poorly differentiated HCC. [a]Significantly lower frequency, $P < 0.05$; [b]Significantly higher frequency, $P < 0.05$.

Table 4 Histological grade and signal intensity in the hepatobiliary phase in lesions with decreased portal blood flow

		Hepatobiliary phase		Total
		Isointensity	Hypointensity	
Histological grade	DN	0	1	1
	Well	1	7	8
	Mod.	0	3	3
	Poor	0	0	0
Total		1	11	12

DN: Dysplastic nodule; Well: Well differentiated hepatocellular carcinoma (HCC); Mod: Moderately differentiated HCC; Poor: Poorly differentiated HCC.

tern ($P > 0.05$) (Tables 4, 5).

Correlation between signal intensity on pre-contrast T1WI, T2WI and hepatobiliary phase

There was a significant correlation between the signal intensity of the T1-weighted in-phase image and that of the hepatobiliary phase ($P < 0.05$). The all isointense lesions in the hepatobiliary phase showed hyperintensity on T1-weighted in-phase imaging with significantly high frequency. The hyperintense lesions on T1-weighted in-phase imaging showed significantly less frequent hypointensity in the hepatobiliary phase. There were no significant correlations between the signal intensity of T1-weighted opposed-phase imaging and T2WI, or that of the hepatobiliary phase ($P > 0.05$) (Table 6).

Table 5 Histological grade and signal intensity in the hepatobiliary phase in lesions which lacked portal blood flow

		Hepatobiliary phase			Total
		Hyperintensity	Isointensity	Hypointensity	
Histological grade	DN	0	0	0	0
	Well	0	2	6	8
	Mod.	1	0	25	26
	Poor	0	0	10	10
Total		1	2	41	44

DN: Dysplastic nodule; Well: Well differentiated hepatocellular carcinoma (HCC); Mod: Moderately differentiated HCC; Poor: Poorly differentiated HCC.

Table 6 Correlation of signal intensity on pre-contrast T1WI, T2WI and hepatobiliary phase

		Hepatobiliary phase			Total
		Hyperintensity	Isointensity	Hypointensity	
T1WI In-phase	Hyperintensity	1	5[b]	21[a]	27
	Isointensity	0	0	27	27
	Hypointensity	1	0	27	28
T1WI Opposed phase	Hyperintensity	1	2	17	20
	Isointensity	0	3	23	26
	Hypointensity	1	0	35	36
T2WI	Hyperintensity	1	1	42	44
	Isointensity	0	4	27	31
	Hypointensity	1	0	6	7

[a]Significantly lower frequency, $P < 0.05$; [b]Significantly higher frequency, $P < 0.05$.

DISCUSSION

According to previous angiography-assisted CT studies, it is still unclear whether lesions that maintain portal blood flow are dysplastic nodules[13] or well-differentiated HCC[5,14]. In the present study, the lesions that maintained portal blood flow included dysplastic nodules and various types of differentiated HCC. Furthermore, well-differentiated HCC had a significantly high rate of maintenance of portal blood flow. A similar result was previously reported[14], indicating the presence of a high-grade malignant lesion within a small lesion that maintained portal blood flow.

Small HCC up to 1.5 cm in diameter and with an indistinct margin are called early HCC, and their malignant potential is relatively low[2,3,15,16]. The lesions are characterized by a high prevalence of maintained portal blood flow, infrequent intrahepatic metastasis or portal invasion, and generally consist of well-differentiated HCC. This entity is hard to detect and distinguish from dysplastic nodules because of the similarity of radiological findings[5,14,17,18], but it is clinically important to distinguish between these two entities. In the present study, isointense lesions in the hepatobiliary phase were dysplastic nodules or well differentiated HCC. Furthermore, among lesions maintaining portal blood flow, all isointense lesions were dysplastic nodules. However, hypointense lesions all appeared malignant. This finding may indicate that hypointense lesions, in which portal blood flow is maintained, are hepatocellular carcinoma.

Therefore, a combination of angiography-assisted CT and gadoxetic acid-enhanced MRI should improve the accuracy of the diagnosis of hepatocellular lesions.

In the present study, signal intensity in the hepatobiliary phase significantly correlated with histological grade. Dysplastic nodules showed isointensity with significantly high frequency. However, some well differentiated HCC also showed isointensity. In the present study, some hypervascular well differentiated HCC showed isointensity while portal blood flow-maintained well differentiated HCC, with less malignant potential, showed hypointensity. In general, distinguishing between dysplastic nodules and well differentiated HCC in which portal blood flow is maintained is difficult. Dysplastic nodules appeared as hypo- or iso-vascular[5,19,20]. Therefore the evaluation of tumor vascularity is important in distinguishing dysplastic nodules and hypervascular well differentiated HCC. The arterial phase is identifiable on gadoxetic acid, and we consider that elucidating the optimal arterial phase for imaging is essential.

Some HCC showed hyperintensity in the hepatobiliary phase. This finding has been reported previously and appeared in well or moderately differentiated HCC[8,11]. Narita *et al*[8] reported that uptake of gadoxetic acid in HCC was determined by expression of the organic anion transporter 1B3 (OATP1B3). Therefore the degree of expression of OATP1B3 may influence signal intensity in HCC. This is partly because isointense lesions appeared in the hepatobiliary phase. Therefore, we speculated that one of the reasons why isointense lesions in the hepatobiliary phase appeared was due to the expression of OATP1B3, and the pathological appearance was extremely similar to the surrounding liver parenchyma.

All isointense lesions in the hepatobiliary phase were detected on MRI and showed hyperintensity in T1-weighted in-phase images in the present study. Precontrast MRI sequencing has been reported to be able to detect dysplastic nodules and well differentiated HCC[21]. However, dysplastic nodules and well differentiated HCC frequently show hyperintensity[22,23] on T1WI, and the present study emphasized the significance of T1-weighted in-phase images to detect these lesions.

The present study has several limitations. Most nodules were diagnosed by biopsy, and therefore, the classification of the histological grade of differentiation was judged using only the biopsied part of the lesions. Second, it is difficult to differentiate dysplastic nodules from early HCC using biopsy[3] and there were few dysplastic nodules in the present study. Therefore further study is required.

In conclusion, signal intensity in the hepatobiliary phase on gadoxetic acid-enhanced MRI was correlated with histological grade in the lesions that maintained portal blood flow, but did not correlate in the lesions with decreased or no portal blood flow. These findings suggest that lesions in which portal blood flow is maintained, and which appear hypointense in the hepatobiliary phase on gadoxetic acid-enhanced MRI, are most likely to be HCC. These results indicate that gadoxetic acid-enhanced MRI can potentially be a powerful observation tool for HCC. These early-stage malignant lesions should be more easily detected, and enable appropriate work-up. This in turn should lead to improved clinical results of treatment.

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COMMENTS

Background

Dysplastic nodules and some well differentiated hepatocellular carcinoma (HCC) lesions maintain portal blood flow, making differential diagnoses difficult. Gadoxetic acid-enhanced magnetic resonance imaging (MRI) yields a high tumor detection rate and can detect lesions that maintain portal blood flow using angiography-assisted computed tomography (CT).

Research frontiers

According to previous angiography-assisted CT studies, it is still unclear whether lesions that maintain portal blood flow are dysplastic nodules or well differentiated HCC. In this study, the authors demonstrate relationships among angiography-assisted CT, hepatobiliary phase findings during gadoxetic acid-enhanced MRI and histological grades.

Innovation and breakthrough

According to the present study, gadoxetic acid-enhanced MRI should improve the accuracy of the diagnosis of hepatocellular lesions.

Applications

The lesions in which portal blood flow is maintained, and which appear hypointense in the hepatobiliary phase on gadoxetic acid-enhanced MRI, have a greater probability of being HCC. Gadoxetic acid-enhanced MRI can potentially be a powerful observation tool for HCC.

Terminology

Gadolinium-ethoxybenzyl diethylenetriaminepentaacetic acid (gadoxetic acid) is a liver-specific contrast medium for magnetic resonance imaging which is taken into hepatocytes and excreted into bile, producing a T1-shortening effect in liver parenchyma. In the hepatobiliary phase, the tumor does not have normal functioning hepatocytes and is hypointense in most cases.

Peer review

This is a comparison with the pathological and radiological findings of dysplastic liver nodules and HCC. They found a close correlation with the histological grade and the intensity of the radiological views.

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BRIEF ARTICLE

Prediction of nephrotoxicity induced by cisplatin combination chemotherapy in gastric cancer patients

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Abstract

AIM: To evaluate the treatment options for nephrotoxicity due to cisplatin combination chemotherapy.

METHODS: We retrospectively reviewed patients who had received cisplatin combination chemotherapy for gastric cancer between January 2002 and December 2008. We investigated patients who had shown acute renal failure (ARF), and examined their clinical characteristics, laboratory data, use of preventive measures, treatment cycles, the amount of cisplatin administered, recovery period, subsequent treatments, and renal status between the recovered and unrecovered groups.

RESULTS: Forty-one of the 552 patients had serum creatinine (SCR) levels greater than 1.5 mg/dL. We found that pre-ARF SCR, ARF SCR, and ARF glomerular filtration rates were significantly associated with renal status post-ARF between the two groups (P = 0.008, 0.026, 0.026, respectively). On the receiver operating characteristic curve of these values, a 1.75 mg/dL ARF SCR value had 87.5% sensitivity and 84.8% specificity (P = 0.011).

CONCLUSION: Cessation or reduction of chemotherapy should be considered for patients who have an elevation of SCR levels during cisplatin combination chemotherapy.

Key words: Acute renal failure; Cisplatin; Drug toxicities; Nephrotoxicity

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INTRODUCTION

Cisplatin is one of the most commonly used antineoplastic agents for the treatment of solid tumors[1,2]. It is generally used in combination with fluorouracil, docetaxel, paclitaxel, capecitabine or irinotecan for the treatment of gastric cancer[3]. However, cisplatin can induce severe side-effects such as bone-marrow suppression, gastrointestinal toxicity, nephrotoxicity, ototoxicity, and neuropathy. Of these, nephrotoxicity is the major side effect and main obstacle in the therapeutic use of cisplatin[1,4].

Many studies have attempted to determine the pathogenesis of nephrotoxicity caused by cisplatin in order to prevent and reduce patient symptoms. However, prevention cannot be achieved by the traditional manner of decreasing drug dosage, performing specific hydration procedures, and actively screening for renal abnormalities[5,6]. In fact, there are currently no unified recom-

mendations for the treatment of nephrotoxicity. In this study, patients who displayed nephrotoxicity induced by a cisplatin combination regimen for gastric cancer were retrospectively reviewed. The aim of this study was to determine the appropriate therapeutic steps when nephrotoxicity occurs due to cisplatin combination chemotherapy.

MATERIALS AND METHODS

Patient population

We retrospectively examined 552 patients who were diagnosed with gastric cancer, and who received cisplatin combination chemotherapy between January 2002 and December 2008 at the Kosin University Gospel Hospital. Of these patients, 41 who developed nephrotoxicity induced by cisplatin combination chemotherapy were chosen for further analysis; a serum creatinine (SCR) level of 1.5 mg/dL was used as the threshold for nephrotoxicity. Patients were excluded if they had renal disease, hydronephrosis, severe dehydration, SCR > 1.5 mg/dL before the administration of cisplatin, or lack of follow-up care.

Division of patients into recovered and unrecovered groups

Forty-one patients were reviewed in terms of gender, age, body surface area (BSA), combined chemotherapy drugs, stage of gastric cancer, hemoglobin levels, hematocrit, total protein, albumin, electrolytes, blood urea nitrogen, SCR, glomerular filtration rate (GFR), magnesium, phosphate and calcium levels, use of mannitol, furosemide and amifostine, amount of hydration, dose of cisplatin/cycle × BSA, cumulative dose of cisplatin/BSA, recovery period, and course of acute renal failure (ARF). Laboratory data were checked immediately before the administration of chemotherapy drugs; SCR levels greater than 1.5 mg/dL were used as pre-ARF laboratory data. Laboratory data were also collected at peak SCR values after SCR levels increased to greater than 1.5 mg/dL at the time of ARF. Patients were divided into two groups (recovered and unrecovered) according to their post-ARF renal status. The recovered patients were those whose SCRs decreased to less than 1.5 mg/dL after ARF; the unrecovered patients were those whose SCRs were maintained at levels greater than 1.5 mg/dL after ARF. The two groups were compared in terms of the above-mentioned characteristics, before and after collection of the ARF laboratory data, use of protective measures, dose of cisplatin, recovery period, and the course of recovery. With these results, the predictive values for post-ARF renal status were examined. Also, in each group, the relationship between treatment and subsequent renal status in response to treatment was examined. The treatments were then divided into the categories of stop, reduce and continue. The subsequent renal status in response to these treatments was divided into the normal, recovered and unrecovered groups. Normal patients were those whose SCRs did not increase to levels greater than 1.5 mg/dL; definition of the recovered and the unrecovered groups is the same as previously noted. Data collection ceased in June, 2009.

Table 1 Characteristics of 41 patients who developed nephrotoxicity induced by cisplatin combination chemotherapy

Gender	M	36
	F	5
Age	58.36 ± 10.54	
BSA[1]	1.677 ± 0.141	
Operation	None	3
	RSG c B I	15
	RSG c B II	5
	RSG c R-Y	7
	RTG c R-Y	5
	Pal[2]	6
Stage	I A	1
	I B	5
	II	5
	III A	9
	III B	2
	IV	19
Combination drug	5-FU[3]	18
	Docetaxel	10
	5-FU[3] + MMC[4]	5
	Paclitaxel	3
	TS-1	2
	Irinotecan	2
	Capecitabine	1

[1]Body surface area; [2]Palliative operation including gastrojejuno bypass, open and closure; [3]5-flourouracil; [4]Mitomycin.

Statistical analysis

Statistical analysis was performed using SPSS Statistics 17.0 for Windows. We collected the laboratory data, which were checked immediately before the SCR increased to > 1.5 mg/dL, for use as the pre-ARF laboratory data. Laboratory data were also checked at peak SCR values after levels increased to > 1.5 mg/dL at the time of ARF. The data on administration of the anticancer drug were reported in number and percentage with some overlap. Other data were reported as mean and standard deviation, and compared using the unpaired Student's *t* test. The predictive value of the post-ARF renal status was examined by receiver operating characteristic (ROC) analysis. The $\chi 2$ test was used to examine the relationship between treatment and subsequent renal status in response to treatment. *P* values less than 0.05 were considered statistically significant.

RESULTS

Patient characteristics

Five hundred and fifty-two patients were diagnosed with gastric cancer and received cisplatin combination chemotherapy between January 2002 and December 2008. The patients received several different cisplatin combination drugs, including 5-flourouracil (5-FU) in 193 patients (34.96%), docetaxel in 113 (20.47%), TS-1 in 86 (15.58%), paclitaxel in 71 (12.86%), capecitabine in 30 (5.43%), irinotecan in 29 (5.25%), mitomycin in 23 (4.17%), and

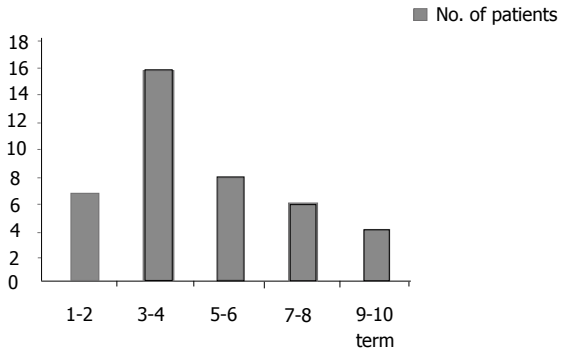

Figure 1 The term during which nephrotoxicity occurred due to cisplatin combination chemotherapy.

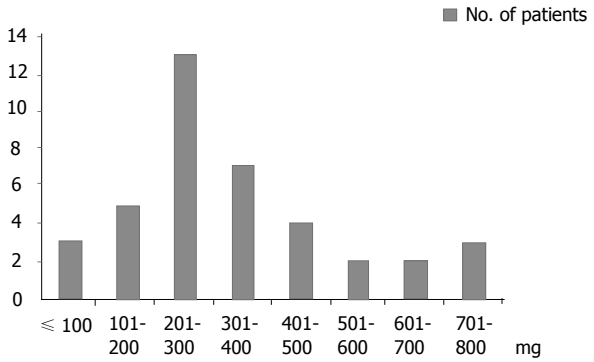

Figure 2 The cumulative dose of cisplatin/body surface area at which nephrotoxicity occurred due to cisplatin combination chemotherapy.

Table 2 Pre-acute renal failure (ARF) laboratory data[1] corresponding to renal status post-ARF[2]

			Renal status of post-ARF		*P* value[3]
Variable	Normal range	Unit	Recovered (*n* = 33)	Unrecovered (*n* = 8)	
Hb	14.0–16.7	g/dL	10.33 ± 1.28 (*n* = 33)	9.75 ± 0.95 (*n* = 8)	0.238
HT	14.7–50.7	%	29.88 ± 3.33 (*n* = 33)	28.56 ± 2.78 (*n* = 8)	0.307
Protein	6.3–8.3	g/dL	6.792 ± 0.69 (*n* = 26)	6.429 ± 0.39 (*n* = 7)	0.191
Albumin	3.5–5.0	g/dL	3.97 ± 0.54 (*n* = 26)	3.87 ± 0.38 (*n* = 7)	0.657
BUN	5–23	mg/dL	18.07 ± 5.35 (*n* = 33)	15.63 ± 4.03 (*n* = 8)	0.236
SCR	0.3-1.5	mg/dL	1.17 ± 0.20 (*n* = 33)	1.38 ± 0.13 (*n* = 8)	0.008
GFR	120-130	mL/min	68.03 ± 13.31 (*n* = 33)	59.13 ± 6.64 (*n* = 8)	0.076
Na	136–150	meg/L	139.24 ± 3.19 (*n* = 33)	140.13 ± 4.09 (*n* = 8)	0.510
Cl	98–110	meg/L	105.31 ± 4.42 (*n* = 24)	105.57 ± 3.65 (*n* = 7)	0.887
K	3.5–5.3	meg/L	4.49 ± 0.46 (*n* = 8)	4.66 ± 0.65 (*n* = 8)	0.284
P	3.0–4.5	mg/dL	3.97 ± 0.81 (*n* = 20)	3.93 ± 1.16 (*n* = 6)	0.940
Mg	1.6–2.6	mg/dL	2.08 ± 0.26 (*n* = 24)	2.03 ± 0.29 (*n* = 7)	0.635
Ca	8.0-10.0	mg/dL	9.15 ± 0.53 (*n* = 24)	8.86 ± 0.30 (*n* = 7)	0.170

[1]Pre-acute renal failure (ARF) Laboratory data were checked immediately before the administration of the chemotherapy drug that caused the serum creatinine (SCR) to increase to > 1.5 mg/dL. [2]We divided 41 patients into two groups; the recovered group included patients whose SCRs had decreased below 1.5 mg/dL after ARF, the unrecovered group included patients whose SCRs remained greater than 1.5 mg/dL. [3]Unpaired Student's *t*–test. BUN: Blood urea nitrogen; GFR: Glomerular filtration rate.

others in 4 patients (0.72%), with some overlap. In our investigation, 5-FU was the most frequently used anticancer drug in combination with cisplatin for gastric cancer chemotherapy. Table 1 lists the characteristics of the 41 patients who had an SCR > 1.5 mg/dL after receiving cisplatin combination chemotherapy for gastric cancer. There were 36 males and 5 females, with an average age of 58.36 years, and an average BSA of 1.677. 5-FU made up the largest proportion of the combined drug regimens (18 patients, 43.9%), and there were more stage Ⅳ patients than any other stage classification (19 patients, 46.3%).

The chemotherapy cycle during which nephrotoxicity occurred

Of 41 patients, nephrotoxicity occurred more frequently during the 3rd-4th cycle (16 patients), and 7 patients experienced nephrotoxicity during the 1st-2nd cycle. The most common cumulative dose of cisplatin/BSA at which nephrotoxicity occurred was 200-300 mg, while the second most common cumulative dose was 300-400 mg, and these were correlated with the greatest number of cycles and the dose of cisplatin/cycle × BSA (Figures 1, 2).

The recovery period for nephrotoxicity induced by cisplatin combination chemotherapy

The average length of recovery time among the patients was 15 d, and was less than 7 d for 27 patients, 8-14 d for 1 patient, 15-30 d for 2 patients, and more than 30 d for 3 patients. These results showed that approximately 70% of recovered patients reached this state within 2 wk.

Comparison between recovered and unrecovered patients

Patients were divided into two groups based on their post-ARF renal status: the recovered patients and the unrecovered patients. The average age of patients in the unrecovered group (51.88 ± 6.01) was lower than that of the recovered group (59.94 ± 10.86), with a *P* value ($P = 0.051$) near 0.05. In the analysis of the laboratory data (Tables 2, 3), the pre-ARF SCR, ARF SCR, and ARF GFR were significantly associated with the renal status post-ARF ($P = 0.008$, 0.026, 0.026, respectively). The ROC curve was constructed using these values (Figure 3). On the ROC curve, an ARF SCR value of 1.75 mg/dL

Table 3 Acute renal failure (ARF) laboratory data[1] corresponding to renal status post-ARF[2]

Variable	Normal range	Unit	Renal status post-ARF		*P* value[3]
			Recovered (*n* = 33)	Unrecovered (*n* = 8)	
Hb	14.0–16.7	g/dL	10.28 ± 1.62 (*n* = 31)	10.238 ± 1.30 (*n* = 8)	0.941
HT	14.7–50.7	%	29.52 ± 4.70 (*n* = 32)	29.93 ± 3.41 (*n* = 8)	0.820
Protein	6.3–8.3	g/dL	6.72 ± 0.78 (*n* = 24)	7.00 ± 0.95 (*n* = 3)	0.564
Albumin	3.5–5.0	g/dL	3.95 ± 0.61 (*n* = 24)	4.03 ± 0.65 (*n* = 3)	0.818
BUN	5–23	mg/dL	23.78 ± 13.60 (*n* = 33)	23.0 ± 4.87 (*n* = 8)	0.876
SCR	0.3-1.5	mg/dL	1.75 ± 0.48 (*n* = 33)	2.21 ± 0.61 (*n* = 8)	0.026
GFR	120-130	mL/min	43.30 ± 7.81 (*n* = 33)	36.0 ± 8.98 (*n* = 8)	0.026
Na	136–150	meg/L	136.60 ± 4.36 (*n* = 32)	137.63 ± 2.72 (*n* = 8)	0.529
Cl	98–110	meg/L	101.49 ± 6.59 (*n* = 24)	99.50 ± 2.65 (*n* = 4)	0.564
K	3.5–5.3	meg/L	4.22 ± 0.93 (*n* = 32)	4.46 ± 0.60 (*n* = 8)	0.491
P	3.0–4.5	mg/dL	4.13 ± 0.87 (*n* = 18)	3.93 ± 0.43 (*n* = 4)	0.659
Mg	1.6–2.6	mg/dL	1.83 ± 0.42 (*n* = 22)	1.833 ± 0.47 (*n* = 3)	0.995
Ca	8.0-10.0	mg/dL	9.06 ± 0.51 (*n* = 23)	9.40 ± 0.80 (*n* = 4)	0.269

[1]Laboratory data were checked at the time at which the value of serum creatinine (SCR) was highest after increasing the SCR > 1.5 mg/dL. [2]We divided 41 patients into two groups; recovered consisted of patients whose SCRs had decreased below 1.5 mg/dL after acute renal failure, unrecovered consisted of patients whose SCRs remained greater than 1.5 mg/dL. [3]Unpaired Student's *t*-test. BUN: Blood urea nitrogen; GFR: Glomerular filtration rate.

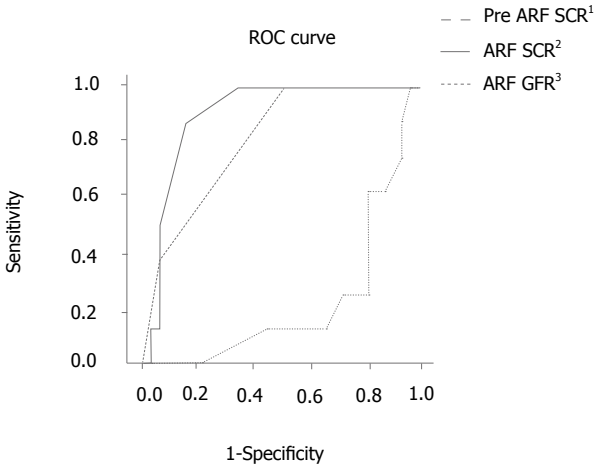

Figure 3 Receiver operating characteristic curves of pre acute renal failure serum creatinines and acute renal failure glomerular filtration rate. [1]Pre-acute renal failure serum creatinine (Pre-ARF SCRs) were checked immediately before the administration of the chemotherapy drug that caused the SCR to increase to > 1.5 mg/dL; [2]ARF SCRs were checked at the time at which the value of SCR was highest after increasing the SCR > 1.5 mg/dL; 3ARF glomerular filtration rate (GFRs) were checked at the time at which the value of SCR was highest after increasing the SCR > 1.5 mg/dL.

showed 87.5% sensitivity and 84.8% specificity. The use of amifostine, mannitol, and furosemide was not significantly different between the two groups ($P = 0.203$, $P = 0.587$, $P = 0.542$, respectively), as nearly all the patients who were followed-up, received a routine formula of hydration and diuretics. The time during which nephrotoxicity occurred and the cumulative dose of cisplatin in each group was assessed and compared (Table 4). The time during which nephrotoxicity occurred was greater in the unrecovered group than in the recovered group (6.63 cycles ± 2.62 cycles *vs* 4.24 cycles ± 2.09 cycles, respectively, $P = 0.009$), and the cumulative dose of cisplatin/BSA was also significantly greater in the unrecovered group compared to the recovered group (497.75 ± 222.61 *vs* 302.85 ± 152.73, respectively, $P = 0.005$).

Table 4 Comparison between the cycle during which nephrotoxicity occurred and the amount of accumulated cisplatin according to renal status post-acute renal failure (ARF)

	Renal status post-ARF		*P* value[3]
	Recovered[1] (*n* = 33)	Unrecovered[2] (*n* = 8)	
The cycle nephrotoxicity occurred	4.24 ± 2.09	6.63 ± 2.62	0.009
The cumulative dose of cisplatin/BSA, mg	302.85 ± 152.73	497.75 ± 222.61	0.005

[1]Recovered were patients whose serum creatinine (SCR)s had decreased below 1.5 mg/dL after acute renal failure. [2]Unrecovered were patients whose SCRs remained greater than 1.5 mg/dL. [3]Unpaired Student's *t*-test. ARF: Acute renal failure; BSA: Body surface area.

Relationship between treatment and renal status after ARF

In the recovered group, the relationship between treatment and renal status following ARF was examined. Table 5 shows that more recovered patients were present in the group that stopped therapy; their SCRs returned to normal. Meanwhile, there were more unrecovered patients in the group that continued treatment; their SCRs remained above 1.5 mg/dL. The relationship between treatment and renal status was significant ($P = 0.011$). Seven normal and recovered patients stopped treatment, including two patients who changed their chemotherapy regimens, two patients who ceased chemotherapy due to metastasis to other organs, two patients who ceased chemotherapy due to poor quality of life (weight loss, anorexia), and one patient who terminated cisplatin in their combination regimen.

The relationship between subsequent treatment and renal status was also examined in unrecovered patients, but it was not statistically significant (Table 6). Two patients stopped receiving cisplatin combination chemotherapy and were switched to another regimen. As a result, their SCRs returned to values less than 1.5 mg/dL after 150 and 181 d, respectively.

Table 5 Subsequent renal status corresponding to subsequent treatment in the recovered group

		Subsequent renal status				*P* value[4]
		Normal[1]	Recovered[2]	Unrecovered[3]	Total	
Subsequent treatment	Stop	5	2	0	7	
	Reduce	2	4	0	6	
	Continue	5	8	7	20	
	Total	12	14	7	33	
						0.011

[1]Normal were patients whose serum creatinine (SCR)s had not increased greater than 1.5 mg/dL after subsequent treatment. [2]Recovered were patients whose SCRs had decreased below 1.5 mg/dL after acute renal failure. [3]Unrecovered were patients whose SCRs remained greater than 1.5 mg/dL. [4]Linear by linear association.

Table 6 Subsequent renal status corresponding to subsequent treatment in the unrecovered group

		Subsequent renal status				*P* value[4]
		Normal[1]	Recovered[2]	Unrecovered[3]	Total	
Subsequent treatment	Stop	0	2	2	4	
	Reduce	0	0	3	3	
	Continue	0	0	1	1	
	Total	0	2	6	8	
						0.170

[1]Normal were patients whose serum creatinine (SCR)s had not increased greater than 1.5 mg/dL after subsequent treatment. [2]Recovered were patients whose SCRs had decreased below 1.5 mg/dL after acute renal failure. [3]Unrecovered were patients whose SCRs remained greater than 1.5 mg/dL. [4]Linear by linear association.

DISCUSSION

Nephrotoxicity induced by cisplatin

Cisplatin is the single most active antitumor agent in the treatment of solid tumors, including gastric cancer. Nevertheless, the use of cisplatin has been restricted because of its side effects, especially nephrotoxicity[1,2]. It has been reported that approximately 25% of patients who received a single dose of cisplatin developed reversible azotemia[7]. In addition, irreversible renal failure can occur when large doses are administered, or with repeated cycles of treatment[8]. In this study, the incidence of nephrotoxicity due to cisplatin combination chemotherapy was 7.43% (41/552). Since patients who had an SCR > 1.5 mg/dL as a measure of nephrotoxicity were selected, these results probably underestimated the incidence of nephrotoxicity. In this study, 5-FU was the most frequently used anticancer drug combined with cisplatin for gastric cancer chemotherapy; the 5-FU/cisplatin regimen is also the most traditional adjuvant chemotherapy for gastric cancer in South Korea.

Criteria for nephrotoxicity

Nephrotoxicity is evaluated by GFR and creatinine clearance values using the Modification of Diet in Renal Disease (MDRD) formula or the Cockcroft and Gault formula, as well as SCR values[9-11]. Only SCR was used for the selection of patients with nephrotoxicity, although the use of a single cutoff to define an elevated SCR is not appropriate[12,13]. The National Kidney Foundation (NKF) recommended that clinicians should not use serum creatinine concentration as the sole means of assessing the level of kidney function[14]. The Renal Insufficiency and Cancer Medications study group suggested that renal function should be evaluated in all cancer patients, including those with normal SCR levels, using either the Cockcroft-Gault formula or the MDRD formula[15]. In this context, the definition of nephrotoxicity in this study as > 1.5 mg/dL is a limitation. In 41 patients, the averages of the pre-nephrotoxic ARF SCR and GFR using MDRD were 1.21 mg/dL ± 0.20 mg/dL and 66.29 mg/dL ± 12.74 mL/min, respectively. Thus, their renal status prior to ARF was already stage 2 according to the clinical guidelines published by the Working Group of the NKF. However, in the case of ARF or acute renal injury (AKI), SCR can be used as a criterion for the definition of ARF or AKI[13,14]. RIFLE and AKIN defined an increase in SCR > 1.5 fold from baseline as a risk or stage 1[16,17]. In this methodology, the SCR can be used as one of the predictive values for renal status after a nephrotoxic event.

The purpose of this study was not to detect and evaluate renal toxicity due to cisplatin. Rather, this study was focused on choosing the appropriate next step after nephrotoxicity occurs due to cisplatin combination chemotherapy. Our data showed that the pre-ARF SCR, ARF SCR, and ARF GFR values were significantly associated with renal status post-ARF (P = 0.008, 0.026, 0.026, respectively). When the ROC curves of these values were assessed, an ARF SCR of 1.75 mg/dL showed 87.5% sensitivity and 84.8% specificity (Figure 3). This indicated that if a patient with nephrotoxicity experiences an SCR > 1.75 mg/dL, then that patient's renal status can progress to severe renal failure. Thus, an ARF SCR value of 1.75 mg/dL can be considered as a predictive measure for renal status post-ARF.

The mechanism of nephrotoxicity

Cisplatin accumulates in the kidneys, and the nephrotoxic effect of cisplatin is proportional to the amount of drug accumulated[3,5,18]. It is known that cisplatin accumulates in the mitochondrial DNA more than in the nucleus or other organelles[2,6]. In a rodent study, the mitochondrial DNA decreased by up to 63% 3–4 d after cisplatin injection[19,20]. Thus, repetitive cisplatin administration lowers the GFR in a dose-related manner[2,21]. In this respect, the dose-related toxicity of cisplatin correlated with the results from this study in terms of the number of cycles before nephrotoxicity occurred. Moreover, the cumulative dose of cisplatin/BSA was greater in the unrecovered group compared to the recovered group (Table 4), suggesting that the earlier nephrotoxicity occurs and the lower the cumulative dose of cisplatin combination chemotherapy, the more quickly the patient will recover (Table 4).

Figures 1 and 2 show that most of the nephrotoxicity occurred in the 3rd-4th cycles of treatment, and the

most common cumulative dose of cisplatin/BSA was 200-300 mg. However, in seven patients, nephrotoxicity occurred in the 1st-2nd cycle. Thus, it appears that the threshold of nephrotoxicity varies according to the individual. Furthermore, renal function should be evaluated, and chemotherapy must be carefully considered before administering cisplatin combination chemotherapy[15,22].

Subsequent chemotherapy and renal status

Upon analysis of the relationship between chemotherapy and renal status in the recovery group, it was found that continuing chemotherapy imparts an increased risk of severe renal failure, compared to ceasing treatment or decreasing the dosage of cisplatin combination chemotherapy (Table 5, $P = 0.011$). In the unrecovered group, all of the cases in which chemotherapy was not stopped remained unrecovered according to their renal status. There were only two patients who stopped receiving cisplatin combination chemotherapy and began another regimen. Their SCRs returned to values less than 1.5 mg/dL, although their recovery took a long time; 150 and 181 d, respectively. Therefore, if a nephrotoxic patient's SCR is > 1.5 mg/dL, chemotherapy should be stopped, the drug dosage should be reduced, or the regimen should be changed.

Other side effects of cisplatin

A common complication resulting from cisplatin treatment is electrolyte wasting, or hypomagnesemia[23,24]. The laboratory data of all the patients in this study were not checked routinely as this was not a prospective study. However, hyponatremia, hypokalemia, hypocalcemia, and hypomagnesemia were found in some patients (Tables 2, 3). Electrolyte imbalances are common in these types of patients, but are not severe[18]. Severe electrolyte imbalance can induce ototoxicity and neurotoxicity, or it can aggravate nephrotoxicity. Such conditions should be corrected by supplementation[18,25,26].

Protective measures

The most commonly used protective measure against renal toxicity is to establish solute diuresis[18,27]. Nearly all of the patients received a routine formula of hydration and diuretics which included hydrations of 1-2 L before and after administration of chemotherapy, diuretics after hydration, and sometimes amifostine. Despite the many recent physiopathological advances in the understanding of the mechanism of anticancer drug nephrotoxicity, especially that of cisplatin, prevention still relies on decreases in drug dosage, hydration measures, and active screening for renal abnormalities as part of the usual pre-therapeutic biological work-up in patients treated with anticancer drugs[6,18]. The European Society of Clinical Pharmacy Special Interest Group on Cancer Care suggested that hydration should be maintained for at least 3 d after the chemotherapy course, and by IV or oral route when feasible[6]. However, there are no specific recommendations or convincing data on the renal protective effect of cisplatin administration in fractionated doses[28].

Combined nephrotoxic drugs

In this study, the nephrotoxicity of combined anticancer drugs was not considered. Mitomycin is known to have renal toxicity. In fact, it has been reported that the onset of renal insufficiency induced by mitomycin administration occurs after an average time of 10-11 mo[29]. However, since the kidney is not a major route of mitomycin excretion, it is not suggested that the dose be adjusted in patients with renal insufficiency[29]. Paclitaxel and irinotecan are also known to cause potential nephrotoxicity, but the need for dosage adjustment has not been confirmed. A comparative prospective study of renal toxicity induced by combined drugs is needed[29,30]. This study has several limitations. Nevertheless, we believe that this issue is important and worthy of further prospective studies.

The author reviewed patients who were diagnosed with gastric cancer, who received cisplatin combination chemotherapy, and who displayed nephrotoxicity. The results show that the patients who experienced a SCR > 1.75 mg/dL after receiving cisplatin combination chemotherapy had a greater risk of chronic renal failure than did patients with a SCR < 1.75 mg/dL. Secondly, in subsequent chemotherapy regimens in patients who experienced SCR > 1.5 mg/dL, the patients who continued cisplatin combination chemotherapy had a greater tendency to experience severe chronic renal disease. Therefore, these results suggest that when a patient experiences a SCR > 1.5 mg/dL after receiving cisplatin combination chemotherapy, the chemotherapy should be stopped, reduced, or the regimen should be changed, and when a patient experiences a SCR > 1.75 mg/dL after receiving cisplatin combination chemotherapy, the chemotherapy should be stopped or changed. More prospective and comparative studies are needed on this subject.

COMMENTS

Background

Cisplatin is one of the most commonly used drugs in the chemotherapy of solid tumors. The major adverse effect of cisplatin is nephrotoxicity, with an incidence of up to 25%. Cisplatin accumulates in the kidneys, and the nephrotoxic effect of cisplatin is proportional to the accumulated drug dose. It is known that cisplatin accumulates in the mitochondrial DNA more than it does in the nucleus or other organelles. Thus, repeated cisplatin administration lowers the glomerular filtration rate (GFR) in a dose-related manner. The aim of this study was to determine the appropriate therapeutic steps when nephrotoxicity occurs due to cisplatin combination chemotherapy in gastric cancer.

Research frontiers

Nephrotoxicity is evaluated by the GFR and creatinine clearance (CrCl) using the Modification of Diet in Renal Disease formula or the Cockcroft and Gault formula, and not only by serum creatinine (SCR). However, in the case of acute renal failure (ARF) or acute renal injury (AKI), SCR can be used as a criterion for the definition of ARF or AKI. The authors suggest that the SCR can be used as one of the predictive values for renal status after a nephrotoxic event. The purpose of this study was not to detect and evaluate the renal toxicity of cisplatin. This study focused on choosing the next step after nephrotoxicity due to cisplatin combination chemotherapy.

Innovations and breakthroughs

Forty-one out of 552 patients, who received cisplatin combination chemotherapy, had SCR levels greater than 1.5 mg/dL. These patients were divided into two groups according to post-ARF renal status, the recovered patients and unrecovered patients. The two groups were compared in terms of the above-mentioned characteristics, before and after ARF laboratory data, use of protective measures, dose of cisplatin, recovery period, and the course of recovery. With these results, the predictive values for the post-ARF renal status were examined. The authors found that pre-AFR SCR, ARF SCR, and ARF GFR were significantly associated with renal status post-ARF in the two groups (P = 0.008, 0.026, 0.026, respectively). In the receiver operating characteristic curve of these values, a 1.75 mg/dL ARF SCR value showed 87.5% sensitivity and 84.8% specificity. This indicated that if a patient with nephrotoxicity experienced an SCR > 1.75 mg/dL, then the patient's renal status can progress to severe renal failure. Thus, an ARF SCR value of 1.75 mg/dL can be considered as a predictive value for renal status post-ARF. In addition, in each group, the relationship between subsequent treatment and renal status in response to treatment was examined. In the recovered group, the relationship between subsequent treatment and renal status following ARF was determined. The results showed that more recovered patients were present in the group who stopped therapy; their SCRs had returned to normal. Meanwhile, in patients who continued treatment, more unrecovered patients whose SCRs were maintained above 1.5 mg/dL were present. The relationship showed a significant difference (P = 0.011). Therefore, if a nephrotoxic patient's SCR is > 1.5 mg/dL, chemotherapy should be stopped, the drug dosage should be reduced, or the regimen should be changed.

Applications

In cisplatin combination chemotherapy in gastric cancer patients, when a patient has experienced a SCR level greater than 1.5 mg/dL, cessation or reduction of chemotherapy should be considered. Furthermore, when a patient experiences a SCR greater than 1.75 mg/dL, chemotherapy should be stopped or changed.

Terminology

The recovered patients consisted of those whose SCRs had decreased to less than 1.5 mg/dL after ARF. The unrecovered patients consisted of those whose SCRs were maintained at greater than 1.5 mg/dL. Subsequent treatment is the next chemotherapy regimen after cisplatin-induced nephrotoxicity, which was divided into stop, reduce and continue. Subsequent renal status is the renal status (recovered or unrecovered) corresponding to subsequent treatment.

Peer review

Despite the many recent physiopathological advances in the understanding of the mechanism of anticancer drug nephrotoxicity, especially that of cisplatin, prevention still relies on a drug dosage decrease, hydration measures, and active screening for renal abnormalities as part of the usual pre-therapeutic biological work-up in patients treated with anticancer drugs. In addition, there are no specific recommendations or convincing data about the renal protective effect of the administration of cisplatin and the subsequent step of nephrotoxicity.

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BRIEF ARTICLE

Stomach cancer screening and preventive behaviors in relatives of gastric cancer patients

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Abstract

AIM: To investigate gastric cancer screening and preventive behaviors among the relatives of patients with gastric cancer [i.e., gastric cancer relatives (GCRs)].

METHODS: We examined the Korean National Health and Nutrition Examination Survey 2005 (KNHANES Ⅲ) database and compared the gastric cancer screening and preventive behaviors of GCRs (n = 261) with those of non-GCRs (n = 454) and controls without a family history of cancer (n = 2842).

RESULTS: The GCRs were more likely to undergo gastric cancer screening compared with the control group (39.2% *vs* 32.3%, adjusted odds ratio: 1.43, CI: 1.05-1.95), although the absolute screening rate was low. Dietary patterns and smoking rates did not differ significantly between the groups, and a high proportion of GCRs reported inappropriate dietary habits (i.e., approximately 95% consumed excessive sodium, 30% were deficient in vitamin C, and 85% were deficient in dietary fiber).

CONCLUSION: The gastric cancer screening and preventive behaviors of GCRs have yet to be improved. To increase awareness among GCRs, systematic family education programs should be implemented.

Key words: Family history of cancer; Cancer relatives; Gastric cancer screening; Preventive behaviors; Cancer prevention

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INTRODUCTION

Gastric cancer is the most common cancer, and the third leading cause of death from cancer, in Korea[1]. It is also the fourth most prevalent cancer in the world[2], although recent trends show stabilization of incidence rates and a continued decrease in cancer death rates[3].

Prevention of gastric cancer can be broadly divided

into primary and secondary prevention. Primary prevention is essentially behavioral modification, which seeks to control the etiological agents of gastric cancer[4]. Several modifiable risk factors contribute to the development of gastric cancer. Infection with *Helicobacter pylori* (*H. pylori*) is a well-established risk factor[5], and the potential of preventing gastric cancer by eradicating *H. pylori* infection has been emphasized in the recent studies[6,7]. Salt intake levels of at least 10 g/d (4000 mg Na) significantly increase the risk of gastric cancer[8]. Fresh fruits and vegetables contain sufficient amounts of vitamin C and dietary fiber, which strongly reduce the risk of gastric cancer[4,9]. A previous study found that subjects in the bottom third of the distribution of vitamin C intake had a 2.5-fold higher risk of developing gastric cancer[10]. Additionally, there is a significant dose-dependent relationship between smoking and gastric cancer[11].

Secondary prevention relies on early detection, which can be achieved through regular cancer screenings[12]. This form of prevention is a priority in Korea, which has one of the highest incidence rates of stomach cancer in the world. The Korean National Cancer Screening Program (KNCSP) recommends that individuals aged 40 years or older undergo biennial gastric cancer screening (Table 1). Although the effect of mass screening remains controversial, it may help by identifying cancer at an early stage[13,14]. According to a study in Korea, the proportion of early gastric cancer (EGC) was 96% in a repeated screening group and 71% in an infrequent screening group, among patients with newly diagnosed gastric cancer[15]. The 5-year survival rate of EGC is greater than 90%[16].

A positive family history of gastric cancer is one of the most important factors, increasing the risk of developing the disease by three-fold[17,18]. There is evidence that there may be a synergistic interaction between family history and *H. pylori* infection in the development of gastric cancer[18]. In addition, patients with a family history tend to have larger or more deeply infiltrated tumors[15]. As many risk factors of gastric cancer are modifiable, it is meaningful to investigate gastric cancer screening and preventive behaviors among high risk groups, such as the relatives of patients with gastric cancer [i.e. gastric cancer relatives (GCRs)] such that early detection and prevention can be achieved. The main purpose of this study is to investigate the current status of gastric cancer screening and preventive behaviors in GCRs.

MATERIALS AND METHODS

Study design

We performed a cross-sectional study of Koreans (n = 3557) who were at least 40 years old, with the aim of investigating the gastric cancer screening rates and preventive behaviors of GCRs compared with those of the general population. To differentiate the impact of family history of gastric cancer from that of other cancers, we studied subjects with a family history of cancer other than gastric cancer [i.e., non-GCRs (NGCRs)] and subjects without a family history of any cancer (controls).

Data source

We analyzed data from the 2005 Korea National Health and Nutrition Examination Survey (KNHANES Ⅲ), which was conducted by the Korea Centers for Disease Control to evaluate the health and nutrition status of the Korean population. The KNHANES Ⅲ categorized the nation into 600 regions at the first stage, selecting 20 households from each region at the second stage. Data collected from the samples were adjusted to represent the entire population of Korea. The questionnaire consisted of four parts: a health interview survey, a health behavior survey, a health examination survey, and a nutrition survey. Information about family histories of cancer were obtained from the health examination survey, cancer screening behaviors and smoking behaviors were assessed using the health behavior survey, and 1 d food intake (i.e. for the last 24 h prior to the survey) was evaluated using the nutrition survey.

Study subjects

The completion rate of the health examination survey in KNHANES Ⅲ was 70.2%. Of the 7597 subjects who responded to the health examination survey, we excluded respondents under the age of 40 years (n = 4008), former and current patients with stomach cancer (n = 23), and those who did not complete questions about their family history (n = 9). Data from the remaining 3557 respondents were analyzed (Figure 1). The following questions from the health examination survey supplement were used to categorize the subjects into three groups: (1) "Has your father, mother, brother or sister ever been clinically diagnosed with any form of cancer?" (responses included "yes" or "no") and (2) "If you responded 'yes', write the type of the cancer." These questions were asked three times to identify exactly which family member, if any, had a history of cancer. According to the answers, respondents were categorized into the following three groups: (1) GCRs; (2) NGCRs; and (3) controls. We defined "cancer family history" as subjects whose parents or siblings had a history of cancer.

We compared the screening patterns for other common cancers (i.e., breast, cervical, and colon cancers) with those of gastric cancer. We excluded subjects with a history of breast, cervical, or colon cancer, respectively. Only females were included in the analysis of breast and cervical cancer. Only subjects 50 years and older were included in the analysis of colon cancer.

Variables

Factors known or thought to affect gastric cancer screening behavior were used as covariates, including socioeconomic factors (e.g., sex, age, education level, marital status, and income), health-related behaviors (e.g., smoking and alcohol consumption), and psychological factors (e.g.,

Table 1 The national cancer screening program in Korea

Cancer	Target population	Frequency	Test or procedure	Co-payment[1] (US $)
Stomach	40 and over (adults)	Every 2 yr	Endoscopy or Upper Gastrointestinal Series	7
Colorectal	50 and over (adults)	Every 1 yr[2]	Fecal Occult Blood Test[3]	0.5
Breast	40 and over (women)	Every 2 yr	Mammography and Clinical breast exam	3.5
Cervix	30 and over (women)	Every 2 yr	Pap smear	0

[1]Co-payments only applied to people with a higher income (i.e. upper 50%), and account for 20% of the total price. No co-payment is applied to the low-income population (i.e., lower 50%). There is no co-payment for cervical cancer screening regardless of income level; [2]Colorectal screening is provided every 2 years to most of the target population, with the exception of low-income people or manual laborers; [3]Colonoscopy or barium enema are performed if the fecal occult blood test is positive.

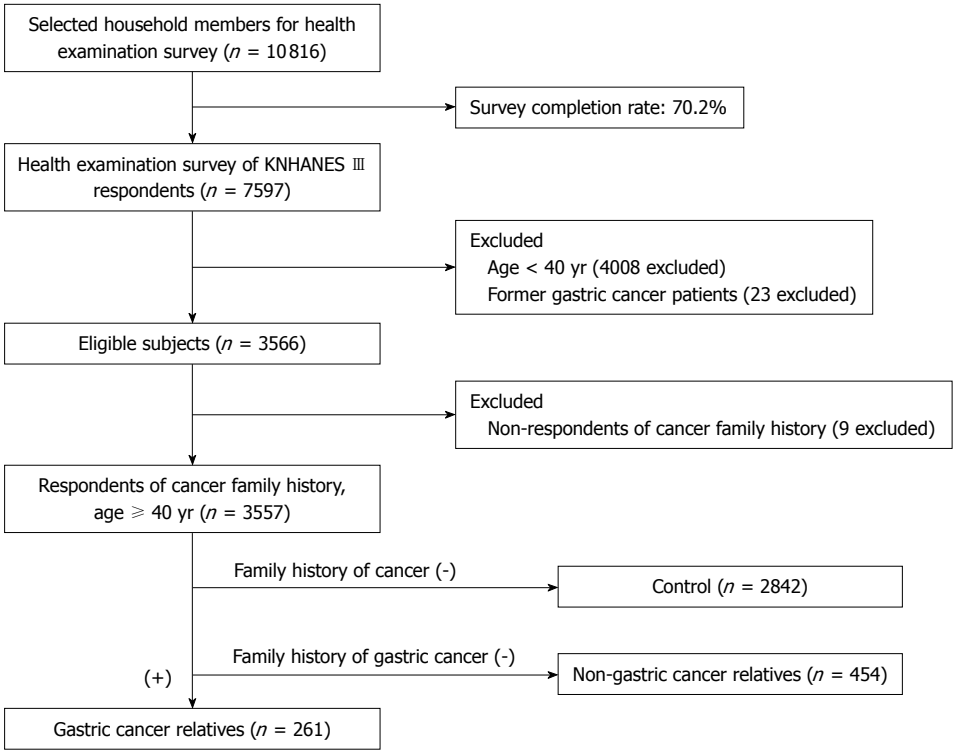

Figure 1 Selection of gastric cancer relatives and controls.

self-reported health status)[19].

Gastric cancer screening behaviors were assessed *via* the question, "When was the last time you were screened for gastric cancer (i.e., gastroscopy or upper gastrointestinal series)?" Responses included, "Less than 1 year ago", "1 year to 2 years ago", "More than 2 years ago" and "Never". In accordance with the KNCSP guidelines, we distinguished screened and unscreened subjects based on whether they had undergone gastric cancer screening within the previous 2 years, and whether they had received a mammography or ultrasonography for breast cancer, a pap smear for cervical cancer, or a colonoscopy or barium enema for colon cancer within the past 2, 2, or 5 years, respectively (Table 1).

The 1 d intakes of sodium, vitamin C, and dietary fiber were calculated using the subjects' responses to the interviewer-administered 24-h dietary recall, a tool that has been used in American surveys because of the accurate and complete self-reported information that it provides[20]. In our analyses, sodium, vitamin C, and dietary fiber were dichotomized according to current dietary recommendations, with a maximum recommended sodium intake of 2000 mg[21], a minimum recommended vitamin C intake of 60 mg[22], and a minimum recommended dietary fiber intake of 20 g[23].

Statistical analysis

The STATA program (version 10.0) was used to analyze the data. The chi-squared test was used to analyze the general characteristics, cancer screening, and preventive behaviors of each group. Adjusted means and adjusted rates of each group were analyzed *via* analysis of covariance, after adjustment for age, sex, education, marital status, smoking status, alcohol consumption, income, and self-reported health status. Crude odds ratios were analyzed *via* simple logistic regression, while adjusted odds ratios (aORs) were analyzed *via* multiple logistic regression, after adjustment for factors that affect gastric cancer screen-

Table 2 Characteristics of gastric cancer relatives and controls *n* (%)

	Controls (*n* = 2842)	Non-gastric cancer relatives (*n* = 454)	Gastric cancer relatives (*n* = 261)	*P*[1]
Sex				
Male	1258 (44.3)	190 (41.9)	105 (40.2)	0.321
Female	1584 (55.7)	264 (58.2)	156 (59.8)	
Age (yr)				
40-49	1024 (36.0)	211 (46.5)	104 (39.9)	< 0.001
50-59	710 (25.0)	118 (26.0)	85 (32.6)	
60-69	665 (23.4)	85 (18.7)	51 (19.5)	
≥ 70	443 (15.6)	40 (8.8)	21 (8.1)	
Education				
Elementary	1136 (40.0)	116 (25.6)	79 (30.3)	< 0.001
Middle to high school	1271 (44.7)	246 (54.2)	146 (55.9)	
University and higher	435 (15.3)	92 (20.3)	36 (13.8)	
Marital status				
Married	2188 (77.0)	387 (85.4)	211 (80.8)	< 0.001
Single	653 (23.0)	66 (14.6)	50 (19.2)	
Smoking status				
Non-smoker	1588 (57.3)	260 (59.8)	152 (59.6)	0.657
Ex-smoker	582 (21.0)	86 (19.8)	45 (17.7)	
Current smoker	602 (21.7)	89 (20.5)	58 (22.8)	
Alcohol drinking				
None	1524 (55.0)	241 (55.4)	145 (56.9)	0.841
More than once a month	1248 (45.0)	194 (44.6)	110 (43.1)	
Income (US $/mo)[2]				
< 1000	801 (28.2)	87 (19.2)	48 (18.4)	< 0.001
1000-5000	1825 (64.2)	315 (69.4)	192 (73.6)	
≥ 5000	216 (7.6)	52 (11.5)	21 (8.1)	
Self-reported health status				
Good	873 (30.8)	149 (33.0)	82 (31.7)	0.502
Intermediate	1017 (35.9)	171 (37.8)	96 (37.1)	
Bad	946 (33.4)	132 (29.2)	81 (31.3)	
Stress				
Low	1822 (65.7)	294 (67.6)	168 (65.9)	0.703
Moderate	770 (27.8)	120 (27.6)	73 (28.6)	
High	180 (6.5)	21 (4.8)	14 (5.5)	

[1]*P* values were calculated by using a χ^2 test; [2]1 US $ = 1000 won.

ing behaviors, as mentioned above. Association analysis weights were used to minimize selection bias.

RESULTS

Characteristics of subjects

The socioeconomic environment, health behaviors, and psychological factors of the subjects are shown in Table 2. Of the 3557 subjects in the study population, 715 had a family history of cancer and 261 had a family history of gastric cancer. The factors listed in Table 2 were used as variables in subsequent multivariate logistic regression analyses.

Gastric cancer screening behavior

Our analysis of gastric cancer screening rates revealed that GCRs were significantly more likely than the control group to undergo gastric cancer screening (39.2% *vs* 32.3%, aOR: 1.43, CI: 1.05-1.95). The gastric cancer screening rate of NGCRs was not significantly different from that of the control group (37.2% *vs* 32.3%, aOR: 1.08, CI: 0.83-1.41) (Table 3).

The rate of gastric cancer screening was higher among younger than older GCRs (42.4% *vs* 31.0%), and higher among younger GCRs than among controls (aOR 1.53 *vs* 1.08) Similarly, GCRs with a high income were screened more often than were GCRs with middle or low incomes (68.4% *vs* 41.8% and 17.0%, respectively), or controls (aOR: 2.70 *vs* 1.56 and 0.70, respectively). Gastric cancer screening did not vary according to education level (Table 4).

Other cancer screening behaviors

The prevalence rates of cancer screening were slightly higher in GCRs and NGCRs compared with control subjects, although these differences were not consistently significant. Female NGCRs were more likely to undergo breast cancer screening (40.8% *vs* 29.6%, aOR: 1.42, CI: 1.02-2.00) and cervical cancer screening (53.9% *vs* 39.9%, aOR: 1.51, CI: 1.04-2.20) when compared with controls. Female GCRs were slightly more likely to undergo breast cancer screening compared with the control group (40.9% *vs* 29.6%, aOR: 1.40, CI: 0.95-2.08), although this difference was insignificant. The groups did not differ with regards to colon cancer screening.

Gastric cancer preventive behaviors

Gastric cancer preventive behaviors were similar among the three groups (Table 5). Sodium consumption was elevated in all three groups. The proportion of individuals with excessive sodium intake (i.e. more than 2000 mg per day) was more than 90% in all three groups, even in GCRs (94.6%). There was a tendency toward higher intake of vitamin C in GCRs and NGCRs compared with the control group [mean ± SE (mg): 110.0 ± 6.2 and 114.1 ± 4.9 *vs* 98.5 ± 1.6, respectively], but this difference was not statistically significant after adjustment. Approximately 30% of the subjects in each groups consumed less than 60 mg vitamin C per day. The average consumption of dietary fiber was not significantly different among the groups. The proportion of individuals with a deficient intake of dietary fiber (< 20 g/d) was approximately 85% in all three groups. The current smoking rate was similar in the three groups.

DISCUSSION

To our knowledge, this is the first study of gastric cancer screening and preventive behaviors among GCRs. The strengths of this study are the use of a nationally representative sample and the inclusion of three comparison groups to more clearly reveal relationships. Our findings suggest that GCRs undergo gastric cancer screening more often than others, although the gastric cancer screening rate among GCRs was still relatively low (39.2%). The rates of breast cancer, cervical cancer, and colon cancer screening were not significantly higher in GCRs than in the

Table 3 Prevalence of cancer screening *n* (%)

	Controls (*n* = 2842)	Non-gastric cancer relatives (*n* = 454)	Gastric cancer relatives (*n* = 261)
Stomach cancer screening (within 2 yr)			
Crude rate	894 (32.3)	162 (37.2)	100 (39.2)
Adjusted rate (%, 95% CI)[2]	32.2 (30.5, 34.0)	35.2 (30.8, 39.8)	38.1 (32.3, 44.2)
Crude OR (95% CI)	1 (referent)	1.16 (0.89, 1.50)	1.47 (1.08, 2.00)[a]
Adjusted OR (95% CI)[1]	1 (referent)	1.08 (0.83, 1.41)	1.43 (1.05, 1.95)[a]
Breast cancer screening (within 2 yr)			
Crude rate	456 (29.6)	102 (40.8)	61 (40.9)
Adjusted rate (%, 95% CI)[2]	28.9 (26.7, 31.4)	36.5 (30.7, 42.8)	38.5 (31.0, 46.7)
Crude OR (95% CI)	1 (referent)	1.68 (1.21, 2.33)[a]	1.53 (1.05, 2.23)[a]
Adjusted OR (95% CI)[1]	1 (referent)	1.42 (1.02, 2.00)[a]	1.40 (0.95, 2.08)
Cervical cancer screening (within 2 yr)			
Crude rate	596 (39.9)	133 (53.9)	71 (47.7)
Adjusted rate (%, 95% CI)[2]	39.1 (36.5, 41.8)	47.3 (40.7, 54.1)	43.2 (35.1, 51.7)
Crude OR (95% CI)	1 (referent)	1.90 (1.35, 2.68)[a]	1.33 (0.90, 1.97)
Adjusted OR (95% CI)[1]	1 (referent)	1.51 (1.04, 2.20)[a]	1.14 (0.76, 1.71)
Colon cancer screening (within 5 yr)			
Crude rate	309 (17.5)	55 (23.6)	33 (21.4)
Adjusted rate (%, 95% CI)[2]	17.2 (15.4, 19.0)	22.6 (17.7, 28.5)	20.2 (14.6, 27.3)
Crude OR (95% CI)	1 (referent)	1.17 (0.78, 1.74)	1.48 (0.85, 2.56)
Adjusted OR (95% CI)[1]	1 (referent)	1.10 (0.73, 1.67)	1.41 (0.83, 2.41)

[a]$P < 0.05$. [1]Calculated *via* multiple logistic regression and adjusted for age, sex, education, marital status, smoking, alcohol consumption, income, and self-reported health status; [2]Calculated *via* analysis of covariance adjusted for age, sex, education, marital status, smoking, alcohol consumption, income, and self-reported health status. OR: Odds ratio.

Table 4 Gastric cancer screening prevalence by education level, age and income subgroups

	Controls (*n* = 2842)	Non-gastric cancer relatives (*n* = 454)	Gastric cancer relatives (*n* = 261)
Education level			
Elementary			
n (%)	322 (29.2)	37 (32.5)	27 (35.1)
aOR (95% CI)[1]	1 (referent)	0.93 (0.57, 1.51)	1.59 (0.85, 2.96)
Middle and higher			
n (%)	572 (34.3)	125 (38.9)	73 (41)
aOR (95% CI)[1]	1 (referent)	1.13 (0.82, 1.55)	1.36 (0.97, 1.92)
Age group (yr)			
40-59			
n (%)	585 (34.5)	118 (37.7)	78 (42.4)
aOR (95% CI)[1]	1 (referent)	1.11 (0.82, 1.49)	1.53 (1.07, 2.17)[a]
≥ 60			
n (%)	309 (28.8)	44 (36.1)	22 (31)
aOR (95% CI)[1]	1 (referent)	0.99 (0.57, 1.69)	1.08 (0.59, 1.98)
Income (US $/mo)			
< 1000			
n (%)	214 (27.1)	22 (26.2)	8 (17)
aOR (95% CI)[1]	1 (referent)	0.86 (0.47, 1.57)	0.70 (0.28, 1.77)
1000-5000			
n (%)	580 (32.8)	113 (37.7)	79 (41.8)
aOR (95% CI)[1]	1 (referent)	1.16 (0.84, 1.60)	1.56 (1.10, 2.21)[a]
≥ 5000			
n (%)	100 (47.4)	27 (53.0)	13 (68.4)
aOR (95% CI)[1]	1 (referent)	1.02 (0.46, 2.26)	2.70 (0.82, 8.88)

[a]$P < 0.05$. [1]Adjusted odds ratios (aOR) were calculated *via* multiple logistic regression.

control group. The dietary patterns and smoking behaviors of GCRs were similar to those of the other two groups.

The finding that GCRs undergo more frequent gastric cancer screening is consistent with previous reports for other cancers. Female relatives of patients with breast cancer are more likely to undergo mammogram screening than are females without a family history of breast cancer[24]. Similarly, men with a family history of prostate cancer are more likely to undergo prostate cancer screening. These findings suggest that a family history of cancer creates a greater sense of vulnerability and is an important factor in the decision to undergo screening[25]. Nonetheless, the screening rates for cancers other than gastric cancer were not different from those of the controls, suggesting that gastric cancer screening behaviors in GCRs is incidental and opportunistic, and not necessarily the result of a formal, systematic training on the importance of cancer screening in general. In addition, it is supposed that GCRs are motivated to undergo gastric cancer screening because of worries about possible cancer development rather than recognition of the benefits of screening. This hypothesis is also explained by the fact that individuals' awareness of the benefits of screening, which is thought to be the result of educational campaigns, was no higher in GCRs than in the control group (63.9% *vs* 64.4%, Table 6). The diagnosis of cancer in a first-degree relative may spur a person into action, as suggested by the Health Belief Model[26], which might explain the increased rate of gastric cancer screening in GCRs.

More importantly, the absolute screening rate in GCRs was only 39.2%, indicating that more than half of the GCRs had not yet undergone regular gastric screening. Endoscopic mass screening for gastric cancer is effective in identifying cancer at an early stage and is cost-effective, especially in moderate- to high-risk popu-

Table 5 Gastric cancer-preventive behaviors

	Controls (n = 2842)		Non-gastric cancer relatives (n = 454)		Gastric cancer relatives (n = 261)	
	Crude	Adjusted	Crude	Adjusted	Crude	Adjusted
Na (mg)						
Mean intake (SE)[2]	5582 (66)	5602 (64)	5574 (166)	5522 (162)	5516 (213)	5483 (212)
P value			0.86	0.99	0.76	0.50
High sodium intake (> 2000 mg)						
Rate, *n* (%)[2]	2625 (92.4)	93.8	429 (94.5)	94.9	247 (94.6)	95.1
Odds ratio[1]	1 (referent)	1 (referent)	1.67 (1.04, 2.67)[a]	1.56 (0.95, 2.57)	1.34 (0.71, 2.54)	1.17 (0.61, 2.26)
Vitamin C (mg)						
Mean intake (SE)[2]	98.5 (1.6)	100.0 (1.6)	114.1 (4.9)	109.7 (4.1)	110.0 (6.2)	107.2 (5.4)
P value			0.03[a]	0.15	0.07	0.19
Low vitamin C intake (< 60 mg)						
Rate, *n* (%)[2]	922 (32.4)	30.7	129 (28.4)	30.5	78 (29.9)	30.8
Odds ratio[1]	1 (referent)	1 (referent)	0.79 (0.59, 1.06)	0.91 (0.67, 1.23)	0.79 (0.55, 1.13)	0.89 (0.62, 1.28)
Dietary fiber (g)						
Mean intake (SE)[2]	8.0 (0.1)	8.0 (0.1)	8.4 (0.2)	8.3 (0.2)	8.3 (0.3)	8.3 (0.3)
P-value			0.20	0.30	0.08	0.14
Low fiber intake (< 20 g)						
Rate, *n* (%)[2]	2447 (86.1)	87	383 (84.4)	86.9	225 (86.2)	87.4
Odds ratio[1]	1 (referent)	1 (referent)	0.86 (0.44, 1.66)	0.86 (0.44, 1.68)	1.17 (0.50, 2.72)	1.20 (0.50, 2.85)
Current smoking status						
Rate, *n* (%)[2]	602 (21.7)	12.9	89 (20.5)	13.2	58 (22.8)	15.4
Odds ratio[1]	1 (referent)	1 (referent)	0.95 (0.69, 1.29)	1.04 (0.73, 1.47)	1.10 (0.78, 1.55)	1.18 (0.76, 1.83)

[a]$P < 0.05$. [1]Adjusted odds ratios were calculated *via* multiple logistic regression and adjusted for age, sex, education, marital status, smoking, alcohol consumption, income, and self-reported health status; [2]Adjusted means and adjusted rates were calculated *via* analysis of covariance adjusted for age, sex, education, marital status, smoking, alcohol consumption, income, and self-reported health status.

Table 6 Perception of the benefits of screening *n* (%)

	Controls (n = 2842)	Non-gastric cancer relatives (n = 454)	Gastric cancer relatives (n = 261)
Beneficial	1783 (64.4)	292 (67.1)	163 (63.9)
Not beneficial	292 (10.5)	47 (10.8)	36 (14.1)
Have never received	696 (25.1)	96 (22.1)	56 (22.0)

lations[14,27]. In Korea, gastric cancer screening is provided as a part of the national cancer screening program, with virtually no economic barrier (Table 1). Therefore, the gastric cancer screening rate should theoretically be high, even in the general population, and GCRs should undergo at least biennial screening, barring a contraindication. Proper educational programs are needed to emphasize the benefits of screening to GCRs, especially those who are older and earn a lower income.

Although GCRs underwent gastric cancer screening more often than other people, their dietary habits and smoking behaviors were not significantly different from those of the control group. Many members of the GCR group had inappropriate dietary habits, with 94.6% consuming excessive sodium, 29.9% deficient in vitamin C, and 86.2% deficient in dietary fiber. This finding was consistent with a previous study of breast cancer relatives, which found that female relatives were more likely to undertake medical actions but not lifestyle preventive behaviors[28]. However, another study suggested that relatives were motivated to change their consumption of fruits, vegetables, and fat once they understood that their behavior could increase their risk of cancer[29]. It is possible that a large proportion of the subjects did not completely understand the extent to which unhealthy behaviors increase the risk of gastric cancer. Healthy lifestyle changes are most successful when individuals believe that the changes will reduce their risk of adverse conditions[30]. For example, perceived vulnerability was a primary motivator for efforts to quit smoking among family members of lung cancer patients[31]. These findings suggest that GCRs should be made aware of the elevated risk of gastric cancer due to unhealthy behaviors. However, a survey has shown that the general Korean public did not clearly understand the risk factors for gastric cancer[32]. Therefore, family education programs should be developed to ensure that GCRs are aware of the risk factors for gastric cancer and the importance of regular screening and preventive behaviors. As the cancer diagnosis and treatment provide a teachable moment for family members as well as the patients themselves[26,31], hospital-based education programs involving both patients and family members could be considered as a potential method to deliver educational messages about gastric cancer screening and other preventive behaviors to them. In a similar example, a family-based health education and counseling intervention program was effective in changing health behaviors of children with a family history of cardiovascular diseases[33]. Another promising method of intervention is clinical treatment that is combined with computerized family-history tools, such as Family Healthware[34], which provides tailored preventive health messages focused on health behaviors and screening, not only for patients, but also for their doctors to of-

fer appropriate recommendations.

This study had several limitations. First, we were unable to assess the prevalence of *H. pylori* existence in the subjects because of the retrospective nature of the study. *H. pylori* eradication is recommended for patients who are first degree relatives of patients with gastric cancer[6,7,35]. Second, the survey did not assess whether the subjects were aware of the causes of gastric cancer or the recommended biennial gastric cancer screening. Third, the statistical significance may have been limited by the relatively small number of GCRs. Fourth, as the design of this study is cross-sectional, we have no information regarding the gastric screening adherence at follow-up. Thus, further research is needed to determine how many subjects actually continue to undergo a 2-year screening procedure. Fifth, only 70.2% of the selected household members responded to the health examination survey. Therefore, it cannot be excluded that the other 29.8% of the household members who did not participate in the survey were less interested in health. As a result, preventive behaviors could be even worse than the findings of this study. Finally, the survey was based on self-reported data, which can potentially increase the risk of inaccuracy. However, the validity of self-reported cancer screening histories and interviewer-administered 24-h dietary recall have been shown to be accurate and reliable[20,36], although few studies have examined the validity of self-reported upper endoscopy history, which is still used in national surveys.

In conclusion, GCRs were found to be more likely to undergo gastric cancer screening compared with the control group. However, this behavior may be incidental, opportunistic, and motivated by concern rather than a true recognition of the benefits of screening by systematic education. The overall gastric cancer screening rate was relatively low in GCRs. The GCRs did not differ from controls with regards to the 1 d intake of sodium, vitamin C, and dietary fiber and a high proportion of GCRs reported inappropriate dietary habits. In addition, the smoking rate was similar in GCRs and controls. To promote awareness about gastric cancer screening and prevention in GCRs, family education programs should be developed and implemented in a systematic manner.

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COMMENTS

Background

These days, increasing emphasis is placed on early detection and prevention of cancers, as it is difficult to cure them when they develop. Gastric cancer is one of the cancers that are modifiable through lifestyle preventive behaviors and regular cancer screening. Currently, only a few Asian countries, including Korea, Japan, and Matsu Island in Taiwan, are conducting population-based screening for gastric cancer.

Research frontiers

Regular screening and health behaviors of high-risk groups have been always emphasized. However, it has not been unequivocally addressed as to how regularly or strictly gastric cancer relatives (GCRs), one of the high-risk groups for gastric cancer, are practicing these measures. In this study, the authors demonstrated that GCRs had much room for improvement in their cancer screening and preventive behaviors.

Innovations and breakthroughs

There have been previous reports that highlighted the low gastric cancer screening rate in the general public. This is the first study to use a nationally representative sample and report that GCRs were more likely to undergo gastric cancer screening, even though their lifestyle preventive behaviors did not show significant differences compared to controls. Furthermore, this study suggests that GCRs were not fully aware of the importance of screening and the potential impacts of risk factors for gastric cancer.

Applications

This study highlights the necessity of targeted intervention for GCRs and also proposes a future strategy through systematic family education programs.

Peer review

This is an interesting, well-written study.

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BRIEF ARTICLE

Virological response to adefovir monotherapy and the risk of adefovir resistance

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Abstract

AIM: To evaluate virological response to adefovir (ADV) monotherapy and emergence of ADV-resistant mutations in lamivudine (LAM)-resistant chronic hepatitis B patients.

METHODS: Seventy-seven patients with documented LAM resistance who were treated with 10 mg/d ADV for > 96 wk were analyzed for ADV resistance.

RESULTS: At week 48 and 96, eight (10%) and 14 (18%) of 77 LAM-resistant patients developed the ADV-resistant strain (rtA181V/T and/or rtN236T mutations), respectively. Hepatitis B virus (HBV) DNA levels during therapy were significantly higher in patients who developed ADV resistance than in those who did not. Incidence of ADV resistance at week 96 was 11%, 8% and 6% among patients with complete virological response (HBV DNA level < 60 IU/mL); 0%, 5% and 19% among patients with partial virological response (HBV DNA level ⩾ 60 to 2000 IU/mL); and 32%, 34% and 33% among patients with inadequate virological response (HBV DNA levels > 2000 IU/mL) at week 12, week 24 and week 48, respectively. HBV DNA levels > 2000 IU/mL at week 24 showed best performance characteristics in predicting ADV resistance.

CONCLUSION: Development of ADV resistance mutations was associated with HBV DNA levels, which could identify patients with LAM resistance who are likely to respond to ADV monotherapy.

Key words: Hepatitis B virus; Viral DNA; Adefovir; Lamivudine; Drug resistance

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INTRODUCTION

Lamivudine (LAM) has been the most popular antiviral agent for the treatment of chronic hepatitis B for many years, but its efficacy is hampered by the high incidence of drug resistance[1]. Adefovir dipivoxil (ADV) is a nucleotide analog that exhibits activity against wild-type and LAM-resistant hepatitis B virus (HBV)[2-4]. Early

studies have demonstrated potent viral suppression of LAM-resistant HBV by either switching to or adding ADV to LAM[5]. However, ADV-resistant strains have been reported after either switching to or adding ADV in patients with LAM resistance, and several recent clinical studies have found that combined LAM with ADV is associated with improvements in virological response and lower rates of ADV resistance than sequential ADV monotherapy[6-8]. Thus, recent guidelines suggest adding ADV to LAM as a better approach than sequential monotherapy for patients with LAM resistance[9-12].

Although ADV add-on therapy represents a new paradigm that is highly effective at restoring viral suppression and preventing the emergence of resistance in patients with LAM resistance[13], the higher cost of add-on therapy may allow ADV monotherapy to retain its role in selected patients, particularly in developing countries[14]. In clinical practice, some patients with LAM resistance do respond to ADV monotherapy[15]. Identification of patients who may be sufficiently treated with ADV monotherapy alone may reduce medical costs in areas where resources are limited.

HBV DNA levels on treatment may help select patients who are likely to respond to ADV monotherapy. Maintenance of undetectable HBV DNA levels during treatment with nucleoside/nucleotide analogs has been suggested as a desirable endpoint[10]. Recently proposed "on-treatment strategy" for patients receiving oral nucleoside/nucleotide therapy is also based on HBV DNA levels[16]. On-treatment monitoring strategies are based on the nature of virological response during treatment, and HBV DNA levels during treatment may be a valuable predictor of treatment response to ADV monotherapy.

The aim of this study was to establish whether HBV DNA levels during treatment are associated with the emergence of genotypic ADV resistance. We studied HBV DNA levels at weeks 12, 24 and 48 after the start of ADV monotherapy among LAM-resistant patients who had received ADV monotherapy for > 96 wk and assessed genotypic resistance to ADV at weeks 48 and 96.

MATERIALS AND METHODS

Patients

Data were collected retrospectively from 85 LAM-resistant chronic hepatitis B patients who started ADV monotherapy between March 2004 and May 2006, and maintained ADV monotherapy for at least 96 wk at the Samsung Medical Center, Seoul, Korea. All 85 patients were treated with LAM for chronic HBV infection and experienced virological breakthrough (VB), which was defined as an increase in the level of HBV DNA of at least 1 $\log_{10}$ IU/mL from the lowest point during treatment. Serum samples were collected every 3 mo during treatment and kept at -80℃ until mutation analyses were performed. Eight patients were excluded from analyses for the following reasons: (1) serum samples were not available for six patients; and (2) an ADV-resistant strain (rtA181V/T) was present at baseline in two patients. Finally, a total of 77 patients were included in this study. This study was approved by the Institutional Review Board at Samsung Medical Center.

Blood tests

Routine biochemical tests were performed by standard procedures every 12 wk during therapy. Hepatitis B surface antigen, hepatitis B e antigen (HBeAg), and hepatitis B e antibody were tested by electrochemiluminescence immunoassay (Elecsys; Roche Diagnostics, Indianapolis, IN, USA). LAM resistance was tested with direct sequencing (ABI 3130 Genetic Analyzer; Applied Biosystems, Foster City, CA, United States).

HBV DNA assay

HBV DNA was quantified using the COBAS TaqMan HBV test (detection limit of 12 IU/mL, Roche Molecular Systems, Branchburg, NJ, USA) at the onset of ADV treatment (baseline), and at weeks 12, 24 and 48 using stored serum samples. Virological response was defined as complete virological response (CVR, HBV DNA level < 60 IU/mL); partial virological response (PVR, HBV DNA levels ⩾ 60 to 2000 IU/mL); and inadequate virological response (IVR, HBV DNA levels > 2000 IU/mL)[13]. VB was defined as an increase in the level of HBV DNA of at least 1 $\log_{10}$ IU/mL from the lowest point during treatment[13].

Detection of ADV resistance

Genotype resistance to ADV was determined at baseline and at weeks 48 and 96 by direct sequencing after amplification of polymerase chain reaction (PCR) products. To detect mutations, PCR amplification was performed using the following primers: external primers were RTF (5'-tat gtt gcc cgt ttg tcc tc-3', position 460-479) and RTR (5'-tga cat act ttc caa tca ata gg-3', position 970-992); internal primers were RTNF (5'-aaa acc ttc gga cgg aaa ct-3', position 574-593) and RTNR (5'-tgc ggt aaa gta ccc caa ct-3', position 895-914). The PCR-amplified DNA was purified by using a QIAquick PCR purification kit (QIAGEN, Hilden, Germany). Purified DNA was treated with an ABI Prism BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems). The primers used for direct sequencing were RTNS and RTNR. DNA sequencing was performed in both directions by an Applied Biosystems 3130 Genetic Analyzer (Applied Biosystems). In this study, ADV resistance was defined as the presence of mutations that confer resistance to ADV, which were rtN236T and/or rtA181V/T[17,18].

Statistical analysis

Differences between patient groups were tested using *t* tests or Mann-Whitney *U* tests, as appropriate, for numeric variables, and χ^2 tests or Fisher's exact tests, as appropriate, for categorical variables. For statistical analysis, HBV DNA levels < 12 IU/mL were considered to be 12 IU/mL. Sensitivity, specificity, positive predictive value, and negative predictive value of HBV DNA levels

Table 1 Baseline characteristics of patients

Variables	
No. of patients	77
Age (yr, mean ± SD)	49.3 ± 11.7
Female: Male (*n*, % male)	18: 59 (77%)
HBeAg positive (*n*, % positive)	21: 56 (73%)
ALT (U/L, median, range)	119 (25–926)
AST (U/L, median, range)	77 (30–483)
Albumin (g/dL, median, range)	4.0 (2.6–4.6)
Total bilirubin (mg/dL, median, range)	1.0 (0.3–4.0)
Prothrombin time (INR, median, range)	1.1 (0.9–1.7)
Baseline creatinine level (mg/dL, mean ± SD)	0.90 ± 0.13
Creatinine level at weeks 96 (mg/dL, mean ± SD)	0.94 ± 0.16
LAM resistance mutation profile (*n*, %)	
rtM204 I ± rtL180M	48 (62%)
rtM204V ± rtL180M	29 (38%)

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; INR: International normalized ratio; LAM: Lamivudine.

Table 2 Patterns of ADV-resistant mutations at weeks 48 and 96 after ADV monotherapy in LAM-resistant patients

Variables	Week 48	Week 96
rtA181T	5	5
rtA181V	2	5
rtA181V + rtN236T	1	2
rtA181T + rtN236T	0	2
Total *n* (%)	8 (10)	14 (18)

LAM: Lamivudine; ADV: Adefovir dipivoxil; rtA181V/T and/or rtN236T: ADV-resistant strain.

Table 3 HBV DNA levels during ADV monotherapy according to emergence of ADV-resistant mutations in LAM-resistant patients

HBV DNA level	ADV resistance at week 96 (*n* = 14)	No ADV resistance at week 96 (*n* = 63)	*P* value
Baseline (log10 IU/mL, mean ± SD)	7.1 ± 0.7	6.8 ± 1.0	0.245
Month 3 (log10 IU/mL, mean ± SD)	4.2 ± 1.6	3.1 ± 1.6	0.027
Month 6 (log10 IU/mL, mean ± SD)	4.1 ± 1.4	2.7 ± 1.6	0.002
Month 12 (log10 IU/mL, mean ± SD)	3.9 ± 1.3	2.4 ± 1.5	0.002

LAM: Lamivudine; ADV: Adefovir dipivoxil; HBV: Hepatitis B virus.

were calculated for the prediction of genotypic resistance to ADV at week 96. Receiver operator curve (ROC) analysis was performed to compare the performance of HBV DNA levels at each time points. Statistical analysis was conducted using PASW Statistics 17.0 (SPSS, Inc., Chicago, IL, United States) and $P < 0.05$ was considered significant.

RESULTS

Genotypic ADV resistance at weeks 48 and 96

Baseline characteristics of enrolled patients are shown in Table 1. At week 48, 8 (10%) of 77 LAM-resistant patients had developed the rtA181V/T and/or rtN236T mutations. At week 96, 14 (18%) of 77 LAM-resistant patients had developed rtA181V/T and/or rtN236T mutations (Table 2).

Table 4 Incidence of ADV resistance according to HBV DNA levels

HBV DNA level	Patient number	ADV resistance at week 48 *n* (%)	ADV resistance at week 96 *n* (%)
Week 12[a]			
< 60 IU/mL	18	2 (11)	2 (11)
≥ 60 to < 2000 IU/mL	21	0 (0)	0 (0)
> 2000 IU/mL	38	6 (16)	12 (32)
Week 24[b]			
< 60 IU/mL	26	2 (8)	2 (8)
≥ 60 to < 2000 IU/mL	19	1 (5)	1 (5)
> 2000 IU/mL	32	5 (16)	11 (34)
Week 48[c]			
< 60 IU/mL	34	2 (6)	2 (6)
≥ 60 to < 2000 IU/mL	16	0 (0)	3 (19)
> 2000 IU/mL	27	6 (22)	9 (33)

[a]*P* value; week 48 = 0.162, week 96 = 0.007. [b]*P* value; week 48 = 0.431, week 96 = 0.008. [c]*P* value; week 48 = 0.036, week 96 = 0.022. ADV: Adefovir dipivoxil; HBV: Hepatitis B virus.

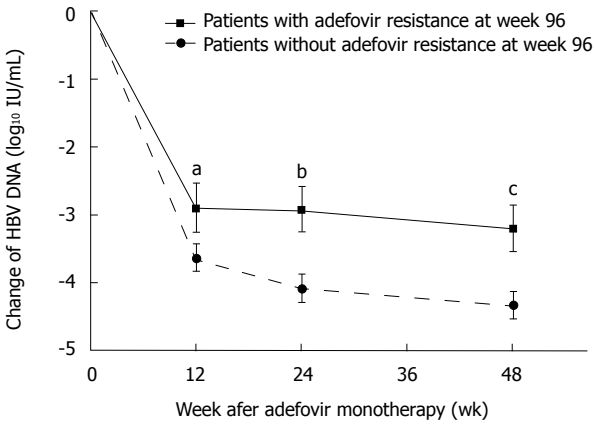

Figure 1 Hepatitis B virus DNA levels after adefovir monotherapy. There were significant differences in the degree of Hepatitis B virus (HBV) DNA reduction between patients who developed adefovir resistance and those who did not (*P* value; [a]week 12 = 0.027, [b]week 24 = 0.002, [c]week 48 = 0.002).

HBV DNA levels on treatment and emergence of ADV resistance

HBV DNA levels during treatment were significantly lower in patients who did not develop ADV resistance than in those who did, while pretreatment HBV DNA levels were not significantly different (Table 3). The degree of reduction of HBV DNA level was also significantly greater among patients who developed ADV resistance (Figure 1). There was a significant difference in the incidence of ADV resistance at week 96 according to HBV DNA levels at week 12 ($P = 0.007$), at week 24 ($P = 0.008$), and at week 48 ($P = 0.022$) (Table 4). Only 8% and 6% of patients with CVR at weeks 24 and 48 developed ADV resistance at week 96, whereas, > 30%

Table 5 Virological response in predicting ADV resistance at week 96

Variables	Adefovir resistance *n* (%)	Sensitivity (%, 95% CI)	Specificity (%, 95% CI)	Positive predictive value (%, 95% CI)	Negative predictive value (%, 95% CI)
HBV DNA level ≥ 60 IU/mL at Week 12 (n = 59)	12 (20)	85 (56–97)	25 (15–38)	20 (11–33)	89 (63-98)
HBV DNA level > 2,000 IU/mL at Week 12 (n = 38)	12 (32)	85 (56–97)	58 (45–70)	31 (18–48)	84 (81–99)
HBV DNA level ≥ 60 IU/mL at Week 24 (n = 51)	12 (24)	85 (56–97)	38 (26-51)	23 (13–38)	92 (73–98)
HBV DNA level > 2000 IU/mL at Week 24 (n = 32)	11 (34)	78 (48–94)	66 (53–77)	34 (19–53)	93 (80–98)
HBV DNA level ≥ 60 IU/mL at Week 48 (n = 43)	12 (28)	85 (56–97)	51 (38–63)	28 (16–44)	72 (56–84)
HBV DNA level > 2000 IU/mL at Week 48 (n = 27)	9 (33)	64 (35–86)	71 (58–81)	33 (17–53)	90 (77–96)

ADV: Adefovir dipivoxil; HBV: Hepatitis B virus.

of patients with IVR at weeks 12, 24 and 48 developed ADV resistance at week 96.

The HBV DNA levels at weeks 12, 24 and 48 were tested for sensitivity, specificity, positive predictive value and negative predictive value for the prediction of ADV resistance at week 96 (Table 5). ROC curve analysis showed that the area under the curve in predicting ADV resistance was lowest at HBV DNA level ≥ 60 IU/mL at week 12 (area = 0.556, P = 0.51) and highest at HBV DNA level > 2000 IU/mL at week 24 (area = 0.726, P = 0.008).

DISCUSSION

In this study, we found that on-treatment serum HBV DNA levels were associated with genotypic ADV resistance. Patients who developed ADV resistance showed higher HBV DNA levels at weeks 12, 24 and 48 after the start of ADV monotherapy. The incidence of ADV resistance was lowest among patients with CVR, and highest among patients with IVR (Table 4). These data suggest that risk of ADV resistance is low among patients who achieve CVR at weeks 12, 24 and 48 after ADV monotherapy, and they may continue ADV monotherapy. IVR at weeks 12, 24 and 48 was associated with development of ADV resistance, and ADV monotherapy should not be continued. Patients with PVR at weeks 12 and 24 showed low incidence of ADV resistance, and may continue ADV monotherapy with careful follow-up, but patients with PVR at weeks 48 may not continue ADV monotherapy because of significant risk of ADV resistance at week 96. The best predictor of ADV resistance was IVR at week 24 (Table 5).

The findings of this study are in line with the recently proposed "on-treatment strategy" for patients receiving oral nucleoside/nucleotide therapy[13]. Keeffe *et al*[13] have suggested that management strategies should be changed for patients with IVR response at week 24. Shin *et al*[19] also have described the importance of HBV DNA levels on treatment among patients with LAM resistance who received ADV monotherapy. They have reported that patients who had HBV DNA levels < 200 IU/mL at week 48 were unlikely to develop VB and genotype mutations. Chen *et al*[20] have reported that ADV resistance was associated with higher HBV DNA levels and lower HBV DNA reduction during the first 6 mo of ADV treatment, compared to patients who did not develop ADV resistance. Gallego *et al*[21] also have shown that initial virological response (reduction ≥ 4 $\log_{10}$ IU/mL) in HBV DNA at 6 mo is an important factor for predicting treatment outcome. These studies, as well as the present study, suggest that HBV DNA level during treatment is a valuable parameter for making early decisions regarding the continuation of ADV monotherapy or switching to another therapy in patients who show LAM resistance[19].

For patients with LAM resistance, adding ADV is a better approach than switching to ADV, as has been demonstrated by several studies[6-12]. However, because of the higher cost of add-on therapy, in areas with limited resources, ADV monotherapy may still be considered. If so, these data suggest that ADV monotherapy may be tried for up to 24 wk, depending on virological response. Patients who show favorable virological response may continue ADV monotherapy.

The cumulative probability of ADV resistance in our series was 10% and 18% at weeks 48 and 96, respectively. However, in this study, only patients who maintained ADV monotherapy for at least 96 wk were enrolled, which indicates that patients who were good responders to ADV were preferentially selected for the study. Thus, the incidence of ADV resistance in this study does not reflect true genotypic resistance rates among LAM-resistant patients who received ADV monotherapy. We included patients only for those who had received at least 96 wk of ADV monotherapy, because the aim of this study was to determine which patients could continue ADV monotherapy.

In conclusion, the results of this study demonstrate the importance of HBV DNA levels during treatment as an indicator of future ADV resistance. The development of ADV-resistant mutations was closely associated with HBV DNA levels during therapy. The risk of developing ADV-resistant mutations in patients who experi-

enced IVR at week 24 was high. These findings suggest that ADV monotherapy is a viable alternative for LAM-resistant patients with good on-treatment virological response, in areas with limited resources.

COMMENTS

Background

A major concern with adefovir (ADV) treatment in lamivudine (LAM)-resistant patients is the selection of ADV-resistant mutations.

Research frontiers

Recent studies suggest that combination therapy with ADV and LAM is better than ADV monotherapy in preventing development of ADV resistance among LAM-experienced patients. However cost is of concern, in areas with limited resources.

Innovations and breakthroughs

Recent reports have highlighted the importance of hepatitis B virus (HBV) DNA levels during antiviral therapy. On-treatment monitoring strategies are based on the nature of virological response during treatment. This study showed that HBV DNA levels during treatment were also useful in predicting ADV resistance in LAM-resistant patients, thus helps to identify patients that might respond to ADV monotherapy.

Applications

This study suggests that ADV monotherapy could be a viable alternative for LAM-resistant patients with good on-treatment virological response to ADV. ADV monotherapy may still be alternative, cost-effective approach especially in areas with limited resources.

Terminology

Genotypic resistance refers to the detection of mutations that have been shown in *in vitro* studies to confer resistance to the drug that is being administered. Antiviral-resistant mutations can be detected at months and sometimes years before biochemical breakthrough. Thus, early detection and intervention can prevent hepatitis flares and hepatic decompensation.

Peer review

This is a retrospective study that evaluated the virological response to ADV monotherapy. This was a good study.

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BRIEF ARTICLE

Histological origin of pseudomyxoma peritonei in Chinese women: Clinicopathology and immunohistochemistry

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Abstract

AIM: To investigate the histological origin of pseudomyxoma peritonei (PMP) in Chinese women.

METHODS: The clinical and pathological data were reviewed for 35 women with PMP, and specimens of the peritoneal, appendiceal and ovarian lesions of each patient were examined using the PV-6000 immunohistochemistry method. Antibodies included cytokeratin (CK)7, CK20, mucin (MUC)-1, MUC-2, carbohydrate antigen (CA)-125, estrogen receptor (ER), and progesterone receptor (PR).

RESULTS: Abundant colloidal mucinous tumors were observed in the peritoneum in all 35 cases. Thirty-one patients had a history of appendectomy, 28 of whom had mucinous lesions. There was one patient with appendicitis, one whose appendix showed no apparent pathological changes, and one with unknown surgical pathology. Ovarian mucinous tumors were found in 24 patients. The tumors were bilateral in 13 patients, on the right-side in nine, and on the left side in two. Twenty patients had combined appendiceal and ovarian lesions; 16 of whom had undergone initial surgery for appendiceal lesions. Four patients had undergone initial surgery for ovarian lesions, and relapse occurred in these patients at 1, 11, 32 and 85 mo after initial surgery. Appendiceal mucinous tumors were found in each of these four patients. Thirty-three of the 35 patients showed peritoneal lesions that were positive for CK20 and MUC-2, but negative for CK7, MUC-1, CA125, ER and PR. The expression patterns in the appendix and the ovary were similar to those of the peritoneal lesions. In one of the remaining two cases, CK20, CK7 and MUC-2 were positive, and MUC-1, CA125, ER and PR were negative. The ovaries were not resected. The appendix of one patient was removed at another hospital, and no specimen was evaluated. In the other case, the appendix appeared to be normal during surgery, and was not resected. Peritoneal and ovarian lesions were negative for CK20, MUC-2, CK7, MUC-1, CA125, ER and PR.

CONCLUSION: Most PMP originated from the appendix. Among women with PMP, the ovarian tumors were implanted rather than primary. For patients with PMP, appendectomy should be performed routinely. The ovaries, especially the right ovaries should be explored.

Key words: Pseudomyxoma peritonei; Peritoneum; Tumor origin; Ovary; Appendix; Immunohistochemistry

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Guo AT, Song X, Wei LX, Zhao P. Histological origin of pseudomyxoma peritonei in Chinese women: Clinicopathology and immunohistochemistry. *World J Gastroenterol* 2011; 17(30): 3531-3537 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i30/3531.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i30.3531

INTRODUCTION

Pseudomyxoma peritonei (PMP) is a rare disease that was

first described in association with a mucinous tumor of the ovary in 1884[1]. It is characterized by dissemination of voluminous jelly-like mucus on the peritoneal surface. Benign or malignant epithelial cells may be found in the mucinous deposit. Its etiology and pathological origin are not yet fully understood to date. It is reported in the literature that PMP often originates from the appendix[2-10]; however, a third to a half of PMP cases are accompanied by ovarian mucinous tumors in women. It has been controversial over the years whether the ovarian lesions in patients with PMP are primary or metastatic or implanted from the appendix. Most studies have indicated that PMP associated with ruptured mucinous lesions originates from the appendix. However, some authors consider that PMP originates from the ovaries[1,11-14].

Cytokeratin (CK)7 is a specific marker of primary ovarian epithelial tumor, which is rarely expressed in epithelial cells of the gastrointestinal tract[4,6]. CK20 is mainly expressed in the epithelium of the gastrointestinal tract[4,6]. Mucin (MUC)2 is a specific marker of goblet cells in the gastrointestinal tract[4,6]. MUC1 is often expressed in epithelial tumors of the breast and female reproductive system[6]. Carbohydrate antigen (CA)125 is a membrane surface glycoprotein that is associated with ovarian cancer cells, which is expressed in the epithelium in most cases of ovarian cancer[6]. In female patients, different levels of estrogen receptor (ER) and progesterone receptor (PR) expression are present in most primary ovarian epithelial tumors.

We reviewed 35 cases of PMP diagnosed at out hospital since the establishment of our hospital, and collected the clinical and pathological data of all the female patients. We carried out immunohistochemical studies to explore the causes and pathological origin of PMP in women, and to provide guidance for clinical diagnosis and treatment of PMP. To the best of our knowledge, this is the first large sample study on the origin of PMP in Chinese women.

MATERIALS AND METHODS

Clinical data

Our hospital treated 83 patients with PMP from 1962 to January 2010, including 38 women (45.8%) and 45 men (54.2%). Three of the 38 women with PMP did not undergo tumor resection or cytoreductive surgery, and underwent pathological examination only via puncture or biopsy, and were therefore, excluded from analysis. The clinical data for the remaining 35 female patients were reviewed, including age of onset, main symptoms and physical signs, imaging findings, intraoperative findings, surgical approach, pathological diagnosis, postoperative adjuvant therapy, recurrence, number of surgical procedures, and survival. The survival rate of patients was analyzed using the Kaplan-Meier method.

Methods

Pathological sections were obtained from all patients to the best of our ability, and all pathological sections obtained were reviewed. For each patient, tissue blocks of lesions of the peritoneum, appendix, and ovary were selected for immunohistochemical staining. Three-micrometer sections of the paraffin-embedded tissue were deparaffinized, rehydrated in a graded series of alcohol and microwave-treated for 10 min in a citrate buffer (pH 6.0). Endogenous peroxidase activity was blocked using 0.3% hydrogen peroxide. The tissues were processed in an automatic immunohistochemical staining machine using the standard protocols (Lab Vision Autostainer; Lab Vision Co., Fremont, CA, USA) with DAKO Real™ EnVision™ Detection System (K5007, DAKO). We used the the following primary antibodies: CK7 (clone OV.TL-12/30, Dako; dilution 1:15 000), CK20 (clone Ks20.8, Dako; dilution 1:2000), MUC-1 (clone Ma 695, Novocastra; dilution 1:200) , MUC-2 (clone ccp58, Novocastra; dilution 1:100), CA125 (clone OC125, Dako; dilution 1:500), ER (clone 6F11, Novocastra; dilution 1:100), and PR (clone 16, Novocastra; dilution 1:200). All antibodies were incubated for 1 h at room temperature. The sections were visualized with 3-3'-diaminobenzidine and tissues were counterstained with Mayer's hematoxylin. We used colon mucinous carcinoma tissue as a positive control for MUC2 and CK20, ovarian mucinous carcinoma tissue as positive control for MUC1, CA125 and CK7, and endometrial carcinoma tissue as a positive control for ER and PR. The same tissues without labeling by primary antibody were used as negative controls. Reactions were interpreted as positive, based on the presence of cytoplasmic staining for MUC2, CK7 and CK20 or cytoplasmic and membranous staining for MUC1 and CA125. For descriptive purposes, the staining was scored semi-quantitatively based on the percentage of positive cells: 1, negative; 2, < 10%; 3, 10-50%; and 4, > 50%. For comparative purposes, scores of 2-4 were considered to be positive.

RESULTS

Clinical characteristics

The 35 female patients were 24-76 years of age, with a mean age of 52.5 years. Most patients complained of abdominal distension and abdominal pain, and physical examination showed abdominal distension with ascites.

During surgery, a large volume of jelly-like mucous substances was seen in the abdomen. Multiple mucous lesions could be observed on the surface of the peritoneum and visceral organs. Thirty-one patients (88.6%) underwent appendectomy. A space-occupying lesion of the gallbladder was seen in one patient, whose appendix was not resected because intraoperative exploration of the appendix did not show any obvious abnormalities. The remaining three patients did not have a history of appendectomy, and the appendix was not explored during surgery.

Twenty-seven patients underwent ovarian resection, and mucinous ovarian tumors were observed in 24. In the remaining eight patients, no obvious abnormalities of the ovaries were observed during surgery, and the ovaries were not resected.

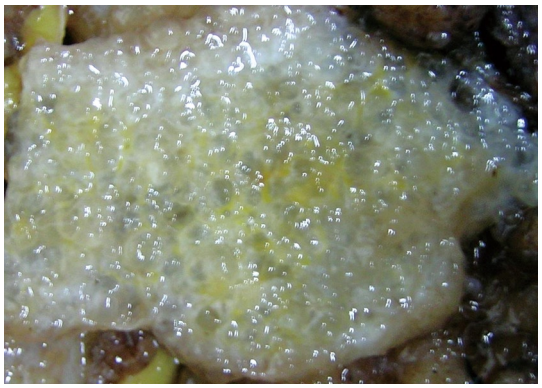

Figure 1 Upon sectioning, the peritoneal lesions were full of a jelly-like mucous substance.

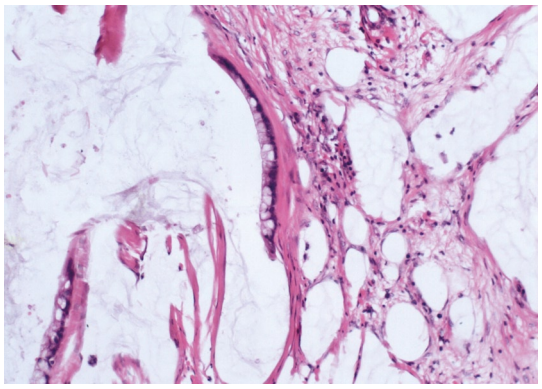

Figure 2 Microscopically, some mucous glandular structures were floating in a large number of mucous lakes (HE stain, × 200).

Lesions of the appendix and ovary were present in 20 patients (57.1%); 16 of whom underwent initial surgery for appendiceal lesions and four for ovarian lesions. None of these four patients underwent appendectomy, and the appendix was not explored during surgery. However, mucinous tumors recurred 1, 11, 32 and 85 mo after initial surgery, respectively, and appendiceal mucinous tumors were found in them. The other four patients with ovarian mucinous tumors did not undergo appendectomy, and the appendix was not explored during surgery in three patients, and a space-occupying lesion of the gallbladder was present in the remaining patient. The ovary was negative for tumor in 10 patients with appendiceal mucinous tumors.

Twenty-five patients (71.43%) underwent at least two surgical procedures, and all 35 patients received varying degrees of abdominal and systemic chemotherapy after surgery. Eleven patients died during follow-up (survival was 3-312 mo); five patients were lost to follow-up after 2-12 mo; and 19 patients survived with tumors (3-129 mo).

Pathological characteristics

In all the patients, the intra-abdominal masses consisted of multiple nodules or grape-like masses; most of which had a smooth and shiny surface. Upon sectioning, the nodules were full of a jelly-like mucous substance (Figure 1). Microscopically, some mucous glandular structures were floating in a large number of mucous lakes, whose epithelium showed varying degrees of differentiation; mostly highly differentiated (Figure 2). Part of the epithelium was poorly differentiated. Mucinous tumors or tumor-like lesions were observed in 28 of 31 patients who underwent appendectomy (Figure 3A). Among the three patients who underwent surgery at other hospitals, the surgical pathology result was a chronic inflammatory mass of the appendix in one patient, no significant changes of the appendix in one patient, and unclear in the remaining patient. Ovarian mucinous tumors were found in 24 of the 27 patients who underwent resection of the ovary (Figure 3B). The tumors were bilateral in 13 cases, on the right side in nine, and on the left side in two (Table 1). The morphological changes in lesions of the appendix and ovary were similar to those of the peritoneal lesions.

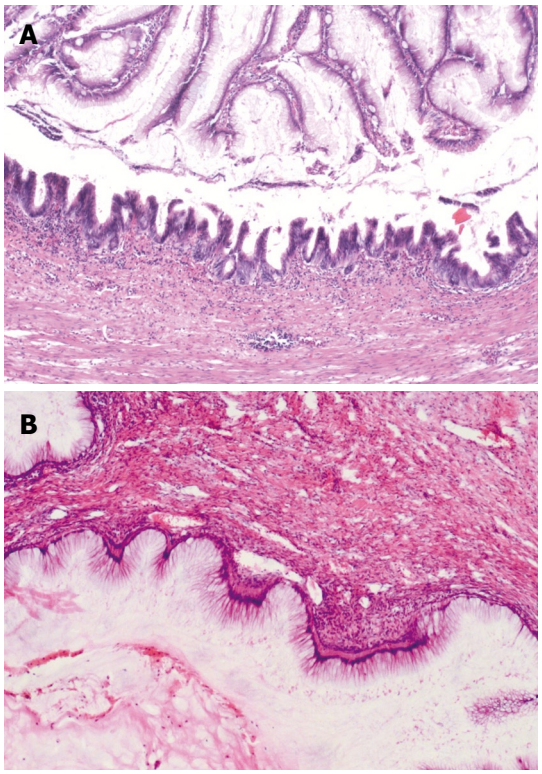

Figure 3 Mucinous tumors were observed in the appendix (A) (HE stain, × 100) and ovary (B) (HE stain, × 100).

Immunohistochemistry results

CK20 and MUC-2 were positive in 33 of the 35 patients with peritoneal lesions (94.3%) (Figure 4A), but CK7, MUC-1 and CA125 were negative. The staining results of the appendix and/or ovaries (Figure 4B and C) were consistent with those of peritoneal lesions. In one patient, peritoneal tumors expressed CK20, CK7 and MUC-2, but not MUC-1 and CA125. That patient underwent appendectomy at another hospital, and no specimen was examined. No ovarian tumors were seen during surgery; consequently, the ovaries were not resected. The peritoneal and ovarian lesions were negative for CK20, MUC-2, CK7,

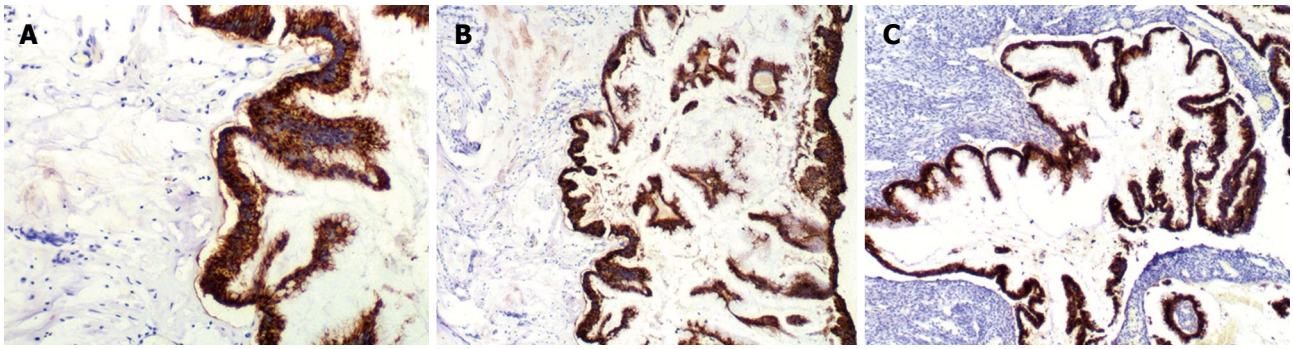

Figure 4 Mucin-2 was positive in peritoneal (A) (× 200), appendiceal (B) (× 100) and ovarian (C) (× 100) lesions.

Table 1 Pathological findings in 35 women with pseudomyxoma peritonei

		Ovary (*n*)					Other organs	Total (*n*)
		Bilateral	Right-side	Left-side	NSC	UR		
Appendix (*n*)	Mucocele	1	0	0	0	0	No	1
	MA	3	5	1	0	3	No	12
	MAC	4	3	1	3	4	No	15
	Appendicitis	1	0	0	0	0	No	1
	UP	1	0	0	0	0	No	1
	NSC	0	0	0	0	1	No	1
	UR	1	0	0	0	0	Gallbladder	1
	UDS	2	1	0	0	0	No	3
Total (*n*)		13	9	2	3	8		35

MA: Mucinous adenoma; MAC: Mucinous adenocarcinoma; UP: Unknown pathology; NSC: No significant changes; UR: Unresected; UDS: Unexplored during surgery.

MUC-1 and CA125 in one patient with a space-occupying lesion of the gallbladder. Additionally, ER and PR status was examined in the peritoneal, appendiceal, and ovarian lesions of the 24 patients with ovarian mucinous lesions, and the results were negative.

Survival

The survival of patients ranged between 2 and 312 mo. The average survival of patients was 47 mo, as calculated using the Kaplan-Meier survival curve. The 3-, 5- and 10-year survival was 54.3%, 23.8% and 13.6%, respectively (Figure 5).

DISCUSSION

PMP is a rare disease with a large volume of extensively implanted gelatinous mucous substance on the surface of the peritoneum or omentum majus[1,2]. The jelly-like substance is called pseudo-mucin, whose chemical properties are different from those of the mucous proteins. PMP was first discovered by Werth[1] in 1884, and its incidence is approximately 2/10 000 in all the patients who are undergoing laparotomy. This disease, with a mean age of 60 years (range: 30-88 years) and a male:female ratio 1:3.4, is characterized by unrelenting pain of gradual onset, abdominal distension, and mucous ascites in literatures. Ultimately, the tumor may occupy the majority of the abdominal cavity and "jelly belly" syndrome may occur. Definitive clinical diagnosis is very difficult to make, and almost all cases are diagnosed with assistance of laparotomy. Detection of jelly-like ascites through abdominal puncture has high diagnostic value for this disease. Our patients with PMP were 24-76 years of age, (mean age, 52.5 years), and the female:male ratio was 1:1.2.

The causes of PMP have remained controversial for many years. It has been reported that the primary tumor of PMP is present in many organs, of which the most common are the appendix and ovaries, followed by the Fallopian tube, pancreas, and intestine. The primary foci are hard to detect in some cases[2,3]. Most patients with PMP either suffer from appendiceal mucinous diseases (including cystadenoma and cystadenocarcinoma) or have a history of recent appendectomy[4-10]. In women, PMP may be accompanied by ovarian mucinous tumors, which often occur bilaterally. If the tumor occurs unilaterally, it more often affects the right ovary[9,10]. Clinically, about a third to a half of women with PMP have concurrent ovarian and appendiceal mucinous tumors.

Our hospital has treated 83 cases of PMP (45 men and 38 women) since the establishment of our hospital. The number of male patients was greater than female patients, which is different from that reported in the literature. Thirty-one of the 35 female patients in our study underwent appendectomy, and appendiceal mucinous tu-

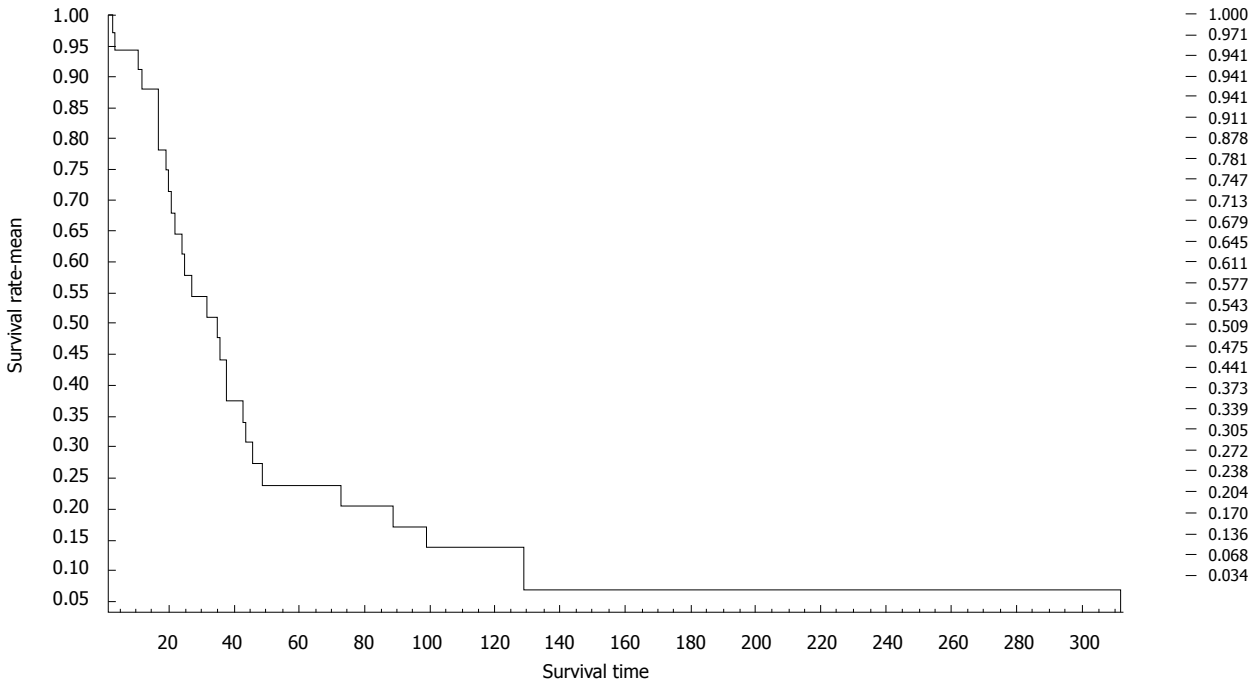

Figure 5 Survival of 35 women with pseudomyxoma peritonei.

mors were seen in 28 of these. Three patients underwent surgery at other hospitals, and the pathological results of two were obtained. The pathological results of the remaining patient were unknown. For the two patients with pathological results, the pathological diagnosis was chronic inflammatory mass of the appendix in one patient, and no significant change in the appendix in the other. The remaining four patients did not have a history of appendectomy, and in three of them, the appendix was not explored during cytoreductive surgery for peritoneal tumor.

Twenty-four of the 35 patients had ovarian mucinous lesions, which were bilateral in 13 cases, on the right side in nine, and on the left side in two. Concurrent appendiceal and ovarian lesions occurred in 20 cases. It is controversial whether the ovarian lesions in these patients were primary or secondary to the appendiceal lesions.

In recent years, the techniques of immunohistochemistry and molecular biology have become more sophisticated, and have greatly enhanced our ability to identify the origin of this disease. In 1991, Young *et al*[5] analyzed 22 cases of ovarian mucinous tumors and PMP-induced appendiceal mucinous tumors. Their results showed that PMP and ovarian lesions both originated from the appendix. Subsequently, Ferreira[6] and Ronnett[7] have made the same observation. Using immunohistochemical methods, Dong *et al*[10] in China have analyzed CK7, CK20 and CA125 expression in peritoneal, ovarian and appendiceal tumors in women with PMP. These investigators drew the same conclusion. Szych *et al*[8] have analyzed the *k-ras* mutations and chromosome 18q, 17p, 5q and 6q alleles in patients with PMP. Their results support the conclusion that the ovarian lesions originate from the appendix in patients with PMP.

It has been reported in the literature that CK7 is a specific marker of primary ovarian epithelial tumors, which is rarely expressed in gastrointestinal epithelial cells[4,6]; however, our experience has shown that CK7 is not specific enough, and sometimes CK7 is positive in typical gastrointestinal adenocarcinoma. CK20 is mainly expressed in the epithelial cells of the gastrointestinal tract[4,6], but it can also be positive in intestinal-type ovarian mucinous tumors. MUC2 is a highly specific marker of intestinal goblet cells[4,6]. MUC1 is often expressed in epithelial tumors of the breast and female reproductive systems[6], but it is negative in epithelial cells of the gastrointestinal tract. CA125 is a surface membrane glycoprotein that is associated with ovarian cancer cells, which is positive in epithelial cells of most ovarian cancers[6]. In women, different levels of ER and PR expression are present in most of the primary ovarian epithelial tumors. Application of the aforementioned antibodies in the detection of tissue antigens is helpful in differentiating whether the tumor originates in the appendix or the ovaries.

Thirty-three of the 35 patients in our study had CK20- and MUC-2-positive peritoneal lesions, but CK7, MUC-1, CA125, ER and PR were negative. The expression pattern of the appendix and the ovary was similar to that of the peritoneal lesions. In one of the remaining two cases, CK20, CK7 and MUC-2 were positive, and MUC-1, CA125, ER and PR were negative. The ovaries were not resected because there were no abnormal intraoperative findings. This patient's appendix was removed at another hospital, and no specimen was examined. In the other case, the appendix appeared to be normal during surgery and was not resected. Peritoneal and ovarian lesions were negative for CK20, MUC-2, CK7, MUC-1, CA125, ER and PR. The above results suggest that PMP

and ovarian lesions were implanted and metastatic appendiceal tumors in 34 of the 35 cases.

It has been reported in the literature that the appendix can be normal in patients with PMP and ovarian mucinous tumors[11]. Lee *et al*[11] have studied 196 patients with borderline ovarian mucinous tumors; of whom, only 11 had PMP. These investigators stated that the apparent absence of appendiceal lesions could have been explained by a variety of circumstances, but that the appendix was not truly normal. First, the appendix may have been left unresected because the surgeon might have observed a normal-appearing appendix during surgery. Second, even if the appendix was resected, the sample collection might not have been sufficient and complete. Finally, even if a sufficient sample was collected, tiny foci of ruptured wall might have been missed due to failure to cut serial sections. In cases with apparent absence of appendiceal lesions, we believe that lesions may have been missed.

In our study, concurrent appendiceal and ovarian lesions occurred in 20 patients, of which, 16 underwent initial surgery for appendiceal lesions, and four for ovarian tumors. Abdominal recurrence occurred at 1-85 mo after surgery, and lesions of appendiceal mucinous tumors were found in all four patients. For one patient who underwent surgery that involved the right ovary, the surgeon explored the appendix during surgery, but the appendix was not resected because no obvious abnormalities were observed by the naked eye. Mucinous tumors were found throughout the abdominal cavity in that patient at 39 mo after surgery, and the resected appendix was confirmed to contain mucinous cystadenoma.

PMP caused by pancreatic mucinous tumor occasionally has been reported in the literature[15]. In our study, bilateral ovarian mucinous tumors were seen in one patient, accompanied by a space-occupying lesion of the gallbladder. The gallbladder was not resected due to the difficulty of the surgery. Immunohistochemical studies of the peritoneal and ovarian lesions showed that CK20, MUC-2, CK7, MUC-1, CA125, ER and PR were negative, which suggested that the ovarian tumor might be metastatic. In the literature, mucinous tumors of the gallbladder have not been reported to cause PMP, and this needs further study.

Treatment and prognosis of PMP

Treatment for PMP is complete resection of the tumors, supplemented by intraperitoneal and systemic chemotherapy. The disease often relapses, and recurrence occurs in 60%-76% of patients after surgery. Multiple surgical resections are often required, and sometimes > 20 operations are performed. Extent and invasiveness of PMP are the important causes of post-surgical recurrence. Although this disease may progress slowly, it is often fatal. The reported median survival was 5.9 years, and the 3-, 5- and 10-year survival was 81.1%-83%, 50.0%-81%, and 18.2%-32%, respectively. Common causes of death are systemic infection secondary to wound infection, bowel obstruction, hernia, and pleural pseudomyxoma caused by tumor passing through the diaphragm. Twenty-five of the 35 patients in our study underwent two or more operations, and 11 patients died. The 5- and 10-year survival was 23.8% and 13.6%, respectively, which was lower than the survival reported in the literature. Therefore, we suggest close follow-up of patients with a diagnosis of PMP; especially those patients whose appendix has not been resected or explored during the initial surgery.

In summary, we believe that PMP often originates in the appendix, and that mucinous ovarian lesions are implanted or metastatic appendiceal tumors. Therefore, appendectomy should be performed routinely for patients in whom PMP is considered during laparotomy. The pathologist should carefully examine the gross specimen of the appendix, and collect as many tissue blocks as possible. Serial sections should be made for suspicious tissue blocks in order to search for small lesions. Additionally, because the incidence of ovarian involvement with implanted tumor is high in women with PMP, adnexa should be explored bilaterally during surgery; especially the right-side adnexa. Patients with PMP should be followed up closely; especially those whose appendix has not been resected or explored during initial surgery.

COMMENTS

Background

Pseudomyxoma peritonei (PMP) is a rare disease that is characterized by dissemination of voluminous jelly-like mucus on the peritoneal surface. Its etiology and pathological origin are not yet fully understood. It has been reported that PMP often originates from the appendix; however, a third to a half of PMP cases are accompanied by ovarian mucinous tumors in women.

Research frontiers

It has been controversial over the years whether the ovarian lesions in patients with PMP are primary or metastatic or implanted from the appendix. Most studies have indicated that PMP associated with ruptured mucinous lesions originates from the appendix. However, some authors consider that PMP originates from the ovaries. In this study, the authors demonstrated that most PMP in Chinese women originated from the appendix, and the ovarian tumors were implanted but not primary.

Innovations and breakthroughs

Recent reports have highlighted the origin of PMP in women. Unfortunately, few reports have observed the origin of PMP in Chinese women, and most of them were case reports or small studies. To the best of our knowledge, this study is the first large sample study on the origin of PMP in Chinese women.

Applications

By understanding the appendiceal origin of PMP in women, appendectomy should be performed routinely for patients in whom PMP is considered during laparotomy. The pathologist should carefully examine the gross specimen of the appendix, and collect as many tissue blocks as possible. Serial sections should be made for suspicious tissue blocks in order to search for small lesions. Additionally, because the incidence of ovarian involvement with implanted tumor is high in women with PMP, the adnexae should be explored bilaterally during surgery, especially the right-side adnexa.

Terminology

PMP is a disease with a large volume of extensively implanted gelatinous mucous substance on the surface of the peritoneum. The term comes from the jelly-like substance, whose chemical properties are different from those of the mucous proteins, and called pseudo-mucin.

Peer review

This study considers the investigation of the histological origin of PMP in Chinese women, using immunohistochemical methods for detection of several mucin and tumor markers. The authors found that most PMP originated from

the appendix, and among women, the PMP was predominately originated from implanted ovarian tumors. The study was set up correctly. The aim of the study was fulfilled. The figures give a good overview of the results. The methods used and the results are not described sufficiently well.

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BRIEF ARTICLE

Propofol *vs* traditional sedative agents for endoscopic retrograde cholangiopancreatography: A meta-analysis

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Abstract

AIM: To investigate the efficacy and safety of propofol sedation for endoscopic retrograde cholangiopancreatography (ERCP).

METHODS: Databases including PubMed, Embase, and the Cochrane Central Register of Controlled Trials updated as of October 2010 were searched. Main outcome measures were ERCP procedure duration, recovery time, incidence of hypotension and hypoxia.

RESULTS: Six trials with a total of 663 patients were included. The pooled mean difference in ERCP procedure duration between the propofol and traditional sedative agents was -8.05 (95% CI: -16.74 to 0.63), with no significant difference between the groups. The pooled mean difference in the recovery time was -18.69 (95% CI: -25.44 to -11.93), which showed a significant reduction with use of propofol sedation. Compared with traditional sedative agents, the pooled OR with propofol sedation for ERCP causing hypotension or hypoxia was 1.69 (95% CI: 0.82-3.50) and 0.90 (95% CI: 0.55-1.49), respectively, which indicated no significant difference between the groups.

CONCLUSION: Propofol sedation during ERCP leads to shorter recovery time without an increase of cardiopulmonary side effects. Propofol sedation can provide adequate sedation during ERCP.

Key words: Endoscopic retrograde cholangiopancreatography; Propofol; Sedative agents; Meta-analysis; Outcomes

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INTRODUCTION

Endoscopic retrograde cholangiopancreatography (ERCP), the most complex gastrointestinal procedure since its introduction in 1968[1], is a highly effective tool to diagnose or treat a variety of biliopancreatic diseases. It is generally recognized that ERCP is a lengthy and potentially uncomfortable procedure that should be performed under

at least conscious sedation[2]. Over the past two decades, propofol, a short-acting agent with rapid metabolism *in vivo* has been used frequently worldwide as a sedative agent for standard endoscopic procedures[3]. However, propofol may lead to deep sedation or even dangerous adverse events that require cardiopulmonary support[4]. Previous studies and several meta-analyses[5,6] have demonstrated that, compared with the traditional sedative agents, propofol sedation is associated with a lower risk of complications in gastrointestinal endoscopy. To date, several studies have compared the effectiveness of propofol with conventional sedation during ERCP. However, the results of individual studies have been inconclusive. Thus, we propose that pooling all available studies together systematically may provide a better understanding of the procedure. Here, we performed a meta-analysis to assess the safety and efficacy of propofol sedation for ERCP, including all randomized controlled trials (RCTs).

MATERIALS AND METHODS

Searching strategy

Related articles in all languages were identified and selected by searching multiple electronic databases including PubMed, Embase, and the Cochrane Central Register of Controlled Trials updated to October 2010, and all bibliographies were identified in the reference lists to identify eligible studies. Due to the relatively small number of articles in this field, we did not use an automated RCT filter in the searching strategy. Key words including ERCP, propofol and diprivan, were used to identify as many articles as possible. Internet search engines, Google Scholar and Yahoo, were also searched with relevant keywords. Major proceedings of international meetings were handsearched.

Inclusion and exclusion criteria

The primary objective of this meta-analysis was to determine the safety and efficacy of propofol sedation for ERCP by comparing with traditional sedative agents such as meperidine, midazolam, scopolamine, and/or pentazocine. Only RCTs in adult patients aged > 18 years who underwent ERCP, published as full articles or meeting abstracts in peer-reviewed journals were considered. Studies were included if they provided the sedation-related outcomes: patient monitoring and complications (i.e., hypoxia or hypotension), procedure-related outcomes (i.e., ERCP duration, sedation and recovery time). All the studies that used propofol plus other agents simultaneously in the same group were excluded. We also excluded studies that could not provide actual frequencies of the complications rather than percentages of complications or percentage decline in complications.

Data extraction and validity assessment

Two authors (Bo LL and Bai Y) selected the studies, extracted the data, and assessed study quality using a predesigned form. This process resulted in high inter-observer agreement (K = 0.86). Disagreement was resolved by consensus or discussion with the third author (Deng XM). Extracted information includes study design, interventions, outcomes, and adverse effects. When necessary, authors were contacted for data not reported or not fully clarified in the original article.

Included studies were assessed for methodological quality on a scale validated by Jadad *et al*[7] and scored from 0 to 5: randomization (0-2 points), blinding (0-2 points), and full accounting of all patients (0-1 point); a higher score indicating better quality. All the included studies had a score of at least 1 because randomization was a requirement for inclusion.

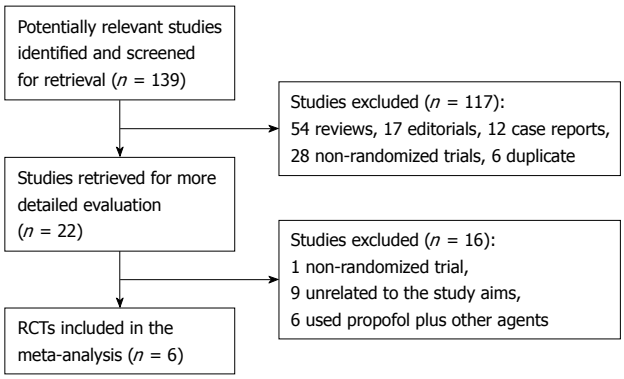

Figure 1 Flow diagram of included and excluded trials.

Statistical analysis

All statistical analysis was performed using Review Manager (RevMan version 5.0), the Cochrane Collaboration's software for preparing and maintaining Cochrane systematic reviews. Meta-analysis was performed using fixed-effect or random-effect methods, depending on the absence or presence of significant heterogeneity. We used the χ^2 test to assess heterogeneity between trials and the I^2 statistic to assess the extent of inconsistency. $P < 0.10$ was defined as significant heterogeneity. Results were expressed as OR or mean difference with 95% CI. $P < 0.05$ was considered statistically significant. Potential publication bias was examined by funnel plot.

RESULTS

Selected RCTs

Figure 1 shows the process of study selection. Our initial searching strategy yielded 139 citations in Embase, PubMed, and Cochrane library (updated to October 12, 2010), of which 117 were excluded on the basis of the title or abstract. Of the remaining 22 articles, we excluded one study that was not randomized, nine unrelated to the study aims, and six having used some other agents plus propofol in the same group or in the control.

Finally, six RCTs[8-13], with a total of 663 subjects, 331 who received propofol, and 332 who received traditional agents for sedation, fulfilled our inclusion criteria.

Table 1 Characteristics of studies included in the meta-analysis

Included studies	Country	Administrator	Procedure	Sedation	Sample size	Hypoxia (SaO_2 < 90%)	Hypotension (SBP < 90 mmHg)	Procedure duration (min)	Recovery time (min)	Jadad score
Chen *et al*[11], 2005	China	ICU physician	ERCP	Propofol	35	2	7	49.22 ± 24.51	5.20 ± 1.94	2
				Meperidine + scopolamine	35	3	0	69.59 ± 25.16	63.94 ± 78.02	
Jung *et al*[10], 2000	Germany	Anesthesiologist	ERCP	Propofol	40		1			2
				Midazolam	40		0			
Kongkam *et al*[13], 2008	Thailand	ACLS trained gastroenterologist	ERCP	Propofol	67	15	6	39.79 ± 32.49	17.24 ± 5.99	5
				Meperidine + midazolam	67	21	6	41.82 ± 21.85	34.25 ± 16.06	
Krugliak *et al*[12], 2000	Israel	Anesthesiologist	ERCP	Propofol	15				13.1 ± 5.8	5
				Midazolam	17				58.4 ± 29.4	
Riphaus *et al*[8], 2005	Germany	ICU physician	ERCP	Propofol	75	8	6		22 ± 7	5
				Meperidine + midazolam	75	7	4		31 ± 8	
Wehrmann *et al*[9], 1999	Germany	Physician unspecified	ERCP	Propofol	99	11	7	27 ± 16	19 ± 8	4
				Midazolam + pentazocine	98	8	2	32 ± 14	29 ± 8	

SBP: Systolic blood pressure; ERCP: Endoscopic retrograde cholangiopancreatography.

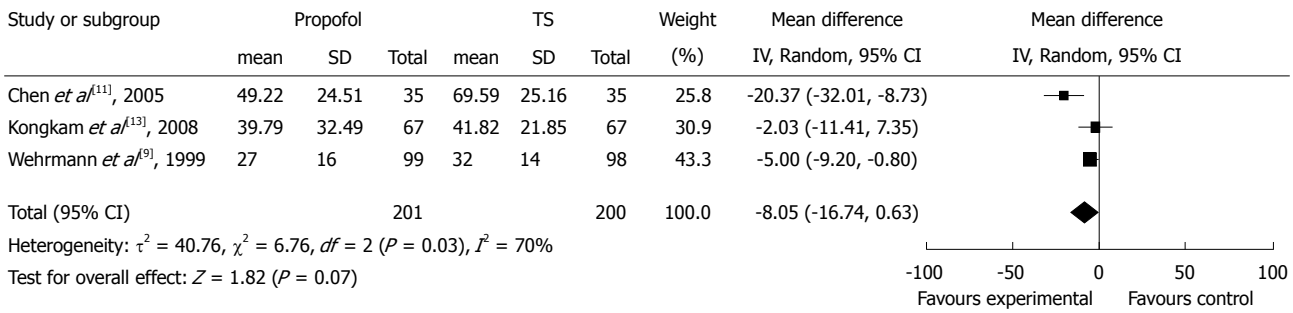

Figure 2 Forest plot of meta-analysis of propofol *vs* traditional sedative agents in endoscopic retrograde cholangiopancreatography procedure duration. IV: Inverse variance; TS: Traditional sedation.

Among them, three trials were reported from Germany[8-10] (Riphaus, 2005 #30), one from China[11], one from Israel[12], and one from Thailand[13]. All eligible articles were reported in the form of full-text articles.

Characteristics of the selected studies

The characteristics of the six included studies are summarized in Table 1. The median number of enrolled patients was 107 (range, 32-197). The indication for ERCP in these trials was generally biliary diseases. All of them were randomized controlled single-center trials. Four of them[8,10,12,13] reported the method of randomization with a Jadad score of $\geqslant$ 3, which suggested a good study design or high quality of report.

Meta-analysis results

Procedure time: The duration of ERCP procedure between propofol and control groups was measured in three studies. Although all of them showed a trend towards duration reduction in the propofol group, the pooled mean difference between the propofol and control groups was -8.05 (95% CI: -16.74 to 0.63), which suggested a statistically non-significant difference between the two groups. The χ^2 and I^2 were 6.76 ($P < 0.10$) and 70%, which indicated heterogeneity among the studies (Figure 2).

Recovery time: Five studies with 583 patients reported recovery time. All of them found a shorter mean recovery time using propofol with pooled weighted mean difference (WMD) of -18.69 (95% CI: -25.44 to -11.93), which indicated a statistically significant difference between the two groups. The χ^2 and I^2 were 46.9 ($P < 0.10$) and 91%, which suggested heterogeneity among the studies. Sensitivity analysis omitting two studies[11,12] with a high risk of bias did not alter the findings, pooled WMD -11.61 (95% CI: -15.45 to -7.78) (Figure 3).

Complications: The complications of hypotension and hypoxia were recorded in most of the studies. However, amnesia was recorded in only three studies. Systolic blood pressure < 75% of baseline and heart rate < 75% of baseline were recorded in only two studies. Due to the limited number of studies, and different criteria for amnesia recognition, only hypotension and hypoxia were

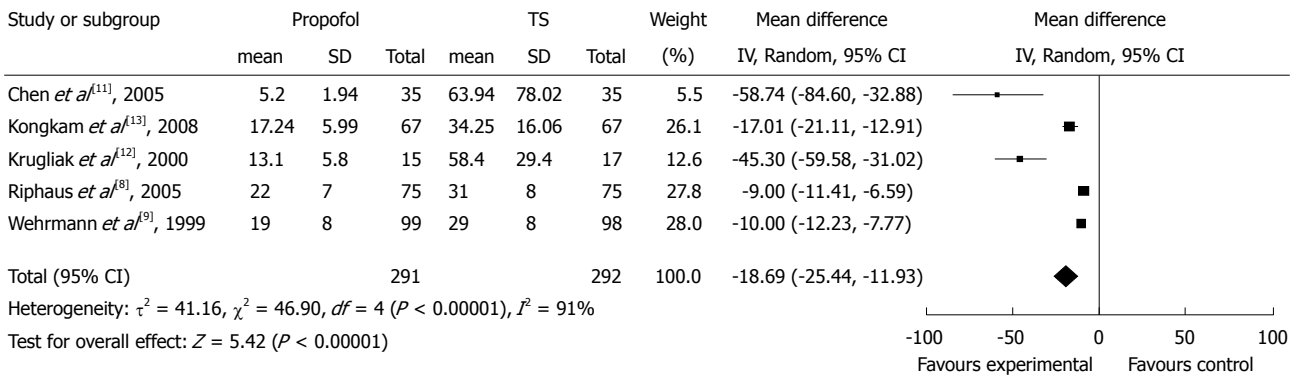

Figure 3 Forest plot of meta-analysis of propofol *vs* traditional sedative agents in endoscopic retrograde cholangiopancreatography recovery time. IV: Inverse variance; TS: Traditional sedation.

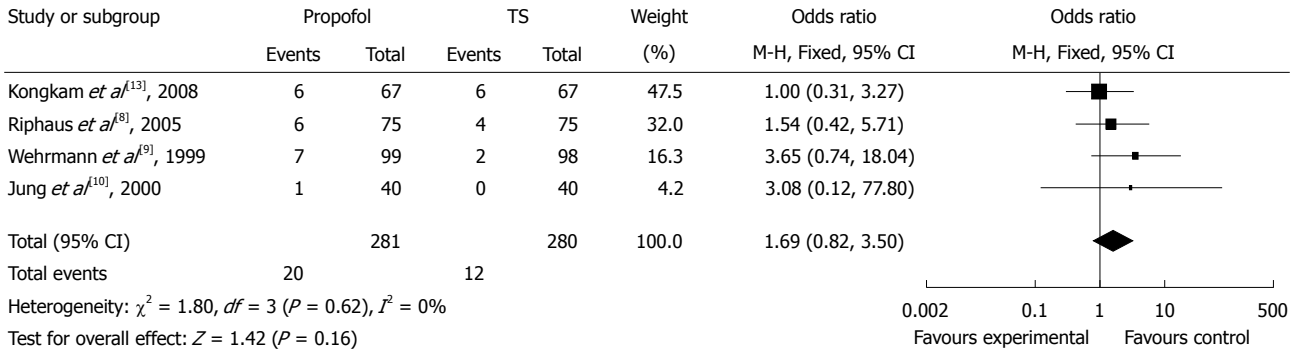

Figure 4 Forest plot of meta-analysis of propofol *vs* traditional sedative agents in occurrence of hypotension during endoscopic retrograde cholangiopancreatography. M-H: Mantel-Haenszel; TS: Traditional sedation.

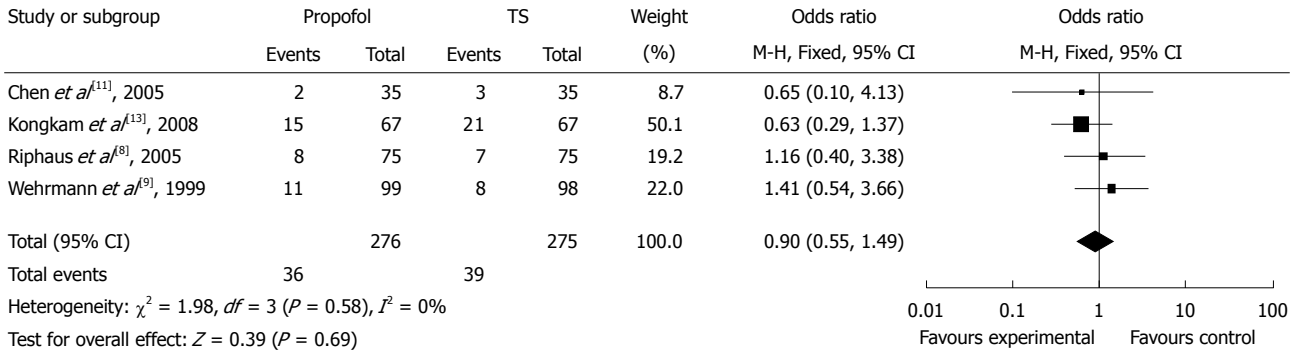

Figure 5 Forest plot of meta-analysis of propofol *vs* traditional sedative agents in occurrence of hypoxia during endoscopic retrograde cholangiopancreatography. M-H: Mantel-Haenszel; TS: Traditional sedation.

eligible for inclusion in the present meta-analysis.

Of the four studies, the OR of hypotension in three studies was in favor of traditional agents, with one showing no difference. The meta-analysis demonstrated that hypotension occurred in 4.29% of controls (12/280) *vs* 7.12% (20/281) of the propofol group. Compared with traditional agents for sedation, the pooled OR of developing hypotension using propofol was 1.69 (95% CI: 0.82-3.50), which indicated no statistically significant difference between the two groups (Figure 4).

In evaluating the OR between propofol and traditional sedation agents causing hypoxia, two studies favored propofol, whereas two studies favored traditional sedation agents. The meta-analysis demonstrated that hypoxia occurred in 14.19% of controls (39/275) *vs* 13.04% (36/276) of the propofol group. Overall, the pooled OR of developing hypoxia using propofol was 0.90 (95% CI: 0.55-1.49), which suggested no statistically significant difference between the two groups (Figure 5).

Publication bias: Funnel plot analysis was conducted using the occurrence of hypotension as the index. The graphical funnel plot of the five studies appeared to be asymmetrical (Figure 6).

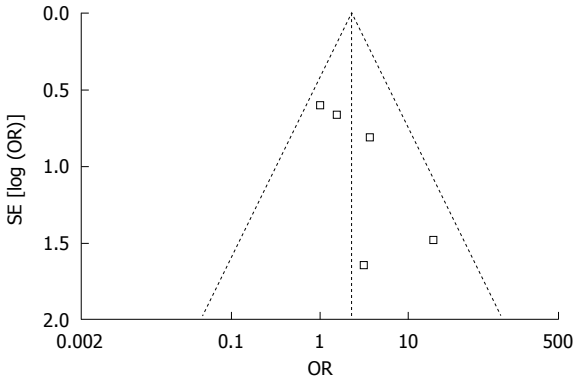

Figure 6 Funnel plot of trials of propofol sedation during endoscopic retrograde cholangiopancreatography. OR: Odds ratio.

DISCUSSION

By summarizing the current best evidence, this meta-analysis conclusively revealed that there are clear benefits of propofol sedation during ERCP regarding the recovery time, without an increase in hypotension and hypoxia occurrence.

Propofol is widely used to induce and maintain anesthesia. It is also used to induce moderate to deep sedation for other procedures, and its advantages include rapid onset, rapid recovery time, and absence of nausea or vomiting[14]. During the past decade, with the growing interest in sedation for gastrointestinal endoscopy worldwide, the use of propofol sedation during endoscopy has been increased[15]. In a previous meta-analysis[6], propofol sedation for colonoscopy was associated with significantly fewer adverse effects. Our present meta-analysis of propofol in ERCP indicated that propofol was not inferior to traditional sedation agents.

Our present meta-analysis showed that the recovery time with propofol sedation was significantly reduced when compared with that with traditional sedation. We also confirmed that the incidence of hypotension and hypoxia during ERCP with propofol sedation was comparable to traditional sedation. It has been reported that propofol for sedation during colonoscopy for generally healthy individuals can lead to a faster recovery time without an increase in side effects[6]. Our results in ERCP also found a significant reduction in recovery time. Qadeer *et al*[6] also concluded that propofol is not inferior to other agents when used for ERCP/endoscopic ultrasound sedation (EUS) in terms of complications of hypoxia and hypotension. However, their meta-analysis of propofol sedation in ERCP included only three studies; since then, three new RCTs have been published[8,11,13]. Estimation based on the three trials, involving only 304 patients, was underpowered to detect the risk of hypoxia or hypotension. Several differences should also be highly noted. First, our present meta-analysis focused specifically on ERCP, whereas the previous meta-analysis focused on colonoscopy. Second, the procedure duration and recovery time were compared in the present meta-analysis, whereas the previous analysis only estimated the risk of hypoxia and hypotension caused by propofol sedation during ERCP/EUS.

Guidelines and a position statement[16] published jointly by four American gastroenterology and hepatology societies regarding non-anesthesiologist administration of propofol for gastrointestinal endoscopy state that, non-anesthesiologist administration of propofol is more cost-effective than standard sedation with benzodiazepines and opioids. Propofol has the potential to induce general anesthesia, and there is no pharmacological antagonist to reverse its effect. Although propofol sedation appears to be a promising strategy during ERCP, its side effects should never be underestimated. With respect to its potential side effects, the administrator should be aware of the risk of hypotension and respiratory depression[4]. Further studies with standardized end-points are also needed to compare propofol administration by anesthesiologists to that by non-anesthesiologists.

The objectives of a meta-analysis include increasing power to detect an overall therapeutic effect by estimating the degree of benefit associated with a particular study treatment[17]. In the case of propofol sedation during ERCP, the current meta-analysis pooled all available data from published RCTs, which substantially reduced the type II error. However, the present meta-analysis also has several limitations that need to be taken into account in interpreting the results.

First, this meta-analysis is a study-level but not an individual patient-level meta-analysis. It is known that study-level analysis can lead to biased assessments, and use of aggregated summary values has some limitations for explaining the heterogeneity[18]. Second, the administrator of propofol sedation was not the same in all the included studies: two studies by anesthesiologists[10,12], two by ICU physicians[8,11], one by ACLS trained gastroenterologists[13], and one by an unspecified physician[9]. This may be considered as a source of heterogeneity. However, due to the limited number of included studies and differently recorded data, subgroup analysis was not carried out. Third, we originally intended to analyze other complications (e.g., arrhythmias, antegrade amnesia, and apnea), assessment of the procedure by the patients (i.e. satisfaction, pain or discomfort), and assessment of the procedure by physicians (i.e., satisfaction with sedation and patient cooperation). However, due to the limited number of studies that reported relevant outcomes, and the different methods in reporting outcomes, it was not appropriate to combine them together for the present meta-analysis. It should be emphasized that future studies should take into a comprehensive consideration of uniform outcome reporting.

A high incidence of hypotension was noticed among all original studies except one[13]. Although the present meta-analysis found no significant statistical difference between two sedative agents, there was a trend toward a higher incidence of hypotension with propofol sedation. This result may have been caused by the relatively small

numbers included in each study, leading to a high possibility of type Ⅱ error, which could weaken the conclusions. Further studies with a large number of patients are warranted to clarify the safety of propofol sedation during ERCP.

In conclusion, propofol sedation during ERCP can lead to a shorter recovery time without an increase of cardiopulmonary side effects. Propofol sedation seems to be an effective method for providing adequate sedation during ERCP.

ACKNOWLEDGMENTS

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COMMENTS

Background

Endoscopic retrograde cholangiopancreatography (ERCP), a highly effective tool to diagnose or treat a variety of biliopancreatic diseases, is considered as the most complex gastrointestinal procedure. It is generally recognized that ERCP is a lengthy and potentially uncomfortable procedure that should be performed under at least conscious sedation.

Research frontiers

Propofol, a short-acting agent with rapid metabolism *in vivo*, has been used frequently worldwide as a sedative agent for standard endoscopic procedures. However, propofol may lead to deep sedation or even dangerous adverse events that require cardiopulmonary support.

Innovations and breakthroughs

The current meta-analysis summarized all available studies to support the propofol sedation for ERCP. The authors found from this meta-analysis that propofol can lead to a shorter recovery time without an increase of cardiopulmonary side effects. Propofol sedation can provide adequate sedation during ERCP.

Applications

This study generated the best evidence to support the clinical use of propofol for ERCP sedation.

Terminology

Propofol: a short-acting, intravenously administered hypnotic agent. It is used in the induction and maintenance of general anesthesia, sedation for mechanically ventilated adults, and procedural sedation. ERCP: a technique that combines the use of endoscopy and fluoroscopy to diagnose and treat certain problems of the biliary or pancreatic ductal systems.

Peer review

The authors made a meta-analysis comparing the effect and adverse effects of propofol for ERCP sedation. Nowadays, propofol is more frequently used especially to sedate patients undergoing ERCP. Clearly, there is a need to update on the propofol effect and safety. The great effort provided by the authors is appreciated.

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BRIEF ARTICLE

Contrast-enhanced multiple-phase imaging features in hepatic epithelioid hemangioendothelioma

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Abstract

AIM: To investigate and review the contrast-enhanced multiple-phase computed tomography (CEMP CT) and magnetic resonance imaging (MRI) findings in patients with pathologically confirmed hepatic epithelioid hemangioendothelioma (HEHE).

METHODS: Findings from imaging examinations in 8 patients (5 women and 3 men) with pathologically confirmed HEHE were retrospectively reviewed (CT images obtained from 7 patients and MR images obtained from 6 patients). The age of presentation varied from 27 years to 60 years (average age 39.8 years).

RESULTS: There were two types of HEHE: multifocal type (n = 7) and diffuse type (n = 1). In the multifocal-type cases, there were 74 lesions on CT and 28 lesions on MRI with 7 lesions found with diffusion weighted imaging; 18 (24.3%) of 74 lesions on plain CT and 26 (92.9%) of 28 lesions on pre-contrast MRI showed the target sign. On CEMP CT, 28 (37.8%) of 74 lesions appeared with the target sign and a progressive-enhancement rim and 9 (12.2%) of 74 lesions displayed progressive enhancement, maintaining a state of persistent enhancement. On CEMP MRI, 27 (96.4%) of 28 lesions appeared with the target sign with a progressive-enhancement rim and 28 (100%) of 28 lesions displayed progressive-enhancement, maintaining a state of persistent enhancement. In the diffuse-type cases, an enlarged liver was observed with a large nodule appearing with persistent enhancement on CEMP CT and MRI.

CONCLUSION: The most important imaging features of HEHE are the target sign and/or progressive enhancement with persistent enhancement on CEMP CT and MRI. MRI is advantageous over CT in displaying these imaging features.

Key words: Liver; Neoplasm; Epithelioid hemangioendothelioma; Computed tomography; Magnetic resonance imaging

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INTRODUCTION

Hepatic epithelioid hemangioendothelioma (HEHE) is

a rare vascular tumor in adults with a variable but often long clinical course, which is intermediate between hemangioma and angiosarcoma in clinical biological behavior[1]. This tumor is histologically characterized by an epithelial appearance and endothelial nature in tumor cells[1]. Clinical, imaging and pathologic diagnosis of HEHE is difficult[2-4], however, its correct diagnosis is very important because long-term survival (5-10 years) of HEHE is possible[5]. Treatment modalities include hepatic resection, orthotopic liver transplantation (even in cases with known metastases), radiotherapy, chemotherapy and use of interferon alpha-2[3,6].

No more than 200 cases of HEHE have been reported since its first description[1-19] and most of them were of sporadic cases and small case series[1,2,8,10,12-15]. The contrast-enhanced multiple-phase computed tomography (CEMP CT) and magnetic resonance imaging (MRI) findings of HEHE have not been well addressed. In this paper, we highlight the predominant imaging features of this type of tumor, focusing on the target sign and/or progressive enhancement with persistent enhancement on CEMP CT and MRI, which have not been extensively described previously in the English-language literature, to the best of our knowledge.

MATERIALS AND METHODS

Subjects

CT (n = 7), MRI (n = 6), clinical (n = 8) and pathological (n = 8) features of 8 cases of HEHE were retrospectively reviewed at our institution from 2004 to 2009. This study was approved by the Institutional Research and Ethics Board of our institution. Among the 8 cases, there were 3 males and 5 females, with ages ranging from 27 to 57 years (mean, 39.8 years). The duration of symptoms ranged from 10 d to 2 years.

The clinical signs and symptoms included epigastric pain (n = 2), discomfort (n = 3), weight loss (n = 3), weakness (n = 2), hepatomegaly (n = 5), splenomegaly (n = 4) and ascites (n = 1). Two patients without any complaints were incidentally found by a routine physical examination. One of 8 cases was accompanied by lung epithelioid hemangioendothelioma. Laboratory tests showed abnormal liver function in two cases, with mild elevation of serum bilirubin, alkaline phosphatase and aspartate aminotransferase levels. HBsAg was positive in two patients. Tumor marker levels, including α-fetoprotein, carcinoembryonic antigen (CEA) and cancer antigen 19-9, were negative in all patients except for an increased level of CEA in one patient.

Radiological examination

CT imaging was performed in seven patients using Siemens Somatom Sensation 16-row CT scanners with 5-mm axial sections from the dome of the diaphragm to the last plane of the liver. All patients were examined in a fasting state with plain scanning at first, and then non-ionic contrast medium (Omnipaque 300 g/L, GE Healthcare, USA) 80 mL per bolus injection was given *via* antecubital vein for enhanced scanning. Images were obtained separately at the arterial phase (25-35 s after injection), portal venous phase (65-75 s after injection) and equilibrium phase (100-110 s after injection).

MR scanning was performed using a 1.5 T or 3.0 T magnet (Signa, GE Healthcare, USA) with an eight-channel torso-array coil. Axial T1-weighted images (T1WI) and T2-weighted images (T2WI) were obtained from all six patients, and additional contrast-enhanced T1WI (Omniscan, GE Healthcare, USA, 0.1 mmol/kg body weight) images were obtained from four patients. Dynamic breath-hold T1WI acquisitions were obtained at 15-27 s, 40-52 s, 70-82 s and 130-142 s after contrast enhancement. The imaging parameters for T1WI and T2WI were as follows: repetition time/echo time (TR/TE) of 205/3.2 ms and 6000/102.5 ms. The matrix was 256 × 256, the standard field-of-view was 400 mm and slice thickness was 4.0 mm with no interslice gap. Additional diffusion weighted single-shot echo-planar imaging was performed in two patients using the following parameters: TR/TE = 1300/52.5 ms, 7-8 mm thickness, water selective excitation for fat suppression, matrix size = 128 × 128, field of view = 36 cm × 36 cm, number of excitations = 6.0, slice thickness/gap = 5 mm/1.0 mm, 20 axial slices, scan time = 2 min 24 s, b value = 0 and 600 s/mm^2, under breath-hold.

Image analysis

All CT and MR images were reviewed separately by two radiologists who were blinded to the identity of the patient and clinical outcome. Discordance between the two was resolved by consensus.

Pathological examination

Histologic specimens of HEHE were obtained by percutaneous needle biopsy in five patients and by exploratory laparotomy and nodule biopsy in three patients. HEHE was diagnosed on the basis of light microscopic examinations of histologic specimens. HE staining and immunohistochemical staining for at least one endothelial marker, i.e., factor Ⅷ-related antigen, CD34, or CD31, were performed on all tumors to confirm the endothelial origin[2,8]. All HEHE specimen analyses were confirmed by an experienced pathologist for diagnostic accuracy.

RESULTS

Imaging findings

There were two types of HEHE in the 8 cases of our study: multifocal type (n = 7) and diffuse type (n = 1). In the 7 multifocal type cases, a total of 74 lesions were found with CT, 28 lesions with MRI and 7 with diffusion weighted imaging (DWI). Eighteen (24.3%) of 74 lesions on plain CT showed a low density with peripheral isodensity (Figure 1A), which looked like a "target" with an inner low density/intensity and a peripheral hyperdensity/intensity or isodensity/intensity (target sign).

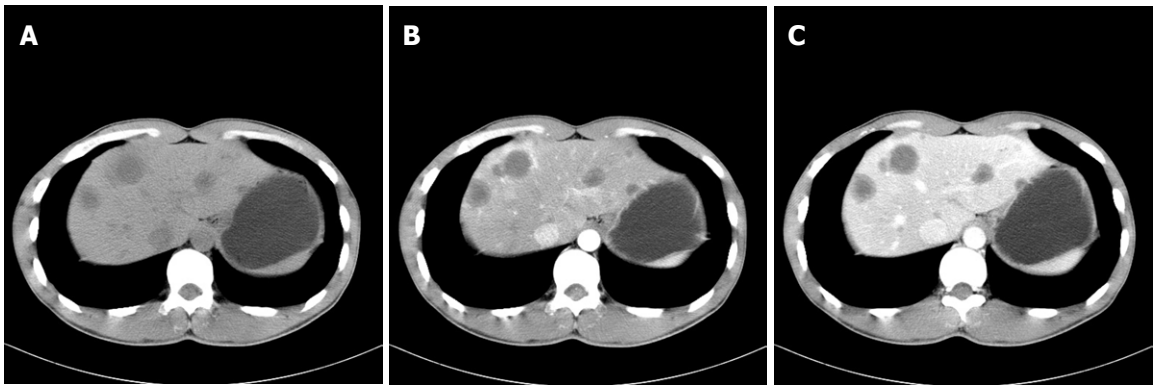

Figure 1 Multifocal hepatic epithelioid hemangioendothelioma in a 27-year-old male. (A) Unenhanced axial computed tomography scan of liver shows multiple discrete masses, which are round with a low density and peripheral iso-density. (B-C) Partial lesions show peripheral ring-like enhancement in the arterial phase (B) with even stronger enhancement in the portal venous phase (C).

On CEMP CT images, 28 (37.8%) of 74 lesions showed peripheral ring-like enhancement in the arterial phase with even stronger enhancement in the portal venous and equilibrium phases, appearing as a target sign with a progressive-enhancement rim (Figures 1B, C). Nine (12.2%) of 74 lesions displayed progressive enhancement, with 8 (10.8%) lesions showing progressive enhancement in the center and 1 (1.4%) lesion showing lamellar progressive enhancement on CEMP CT, maintaining the state of persistent enhancement.

Twenty-six (92.9%) of 28 lesions showed hypointensity relative to normal liver parenchyma with peripheral faint hyperintensity on T1WI (Figure 2A) and hyperintensity with peripheral hypointensity and an area of evident hyperintensity in the center on T2WI (Figure 2B), appearing as the target sign. Six (85.7%) of 7 lesions showed hyperintensity with a peripheral hypointense rim on DWI (Figure 2C) and 1 (14.3%) showed lamellar hyperintensity on DWI.

On CEMP MRI, 27 (96.4%) of 28 lesions displayed peripheral ring-like enhancement in the arterial phase, and even stronger enhancement in the portal venous and equilibrium phases, appearing as a target sign with a progressive-enhancement rim; 28 (100%) of 28 lesions displayed progressive-enhancement in the arterial, portal venous and equilibrium phases and became isointense to liver parenchyma in the delayed phase, with 27 (96.4%) lesions showing progressive enhancement in the center of the target (Figures 2D-G, Figures 3A-E) and 1 (3.6%) lesion showing lamellar progressive enhancement on CEMP MRI (Figures 3F-J), maintaining the state of persistent enhancement.

One diffuse case manifested an enlarged liver with a large nodule appearing slightly hyperdense relative to normal liver parenchyma on plain CT (Figure 4A), isointensity on T1WI (Figure 4E) and hypointensity on T2WI (Figure 4F), with slight enhancement in the arterial phase (Figure 4B), evident enhancement in the portal venous phase (Figures 4C and G) and isodense/intense in the equilibrium phase (Figures 4D and H), which manifested persistent enhancement on CEMP CT and MRI, associated with splenomegaly and ascites (Figure 4F). All the imaging features are summarized in Table 1.

Pathologic results

All tumors were consistent with a diagnosis of HEHE on pathological review. Grossly, the tumors were solid, firm and hyperemic in the outer portions. Histologically, the tumors were composed of dendritic and epithelioid cells. Signet ring–like structures appeared in the tumor cells with intracytoplasmic lumina, occasionally containing red blood cells (Figure 5). The tumors consisted of large amounts of mucinous and dense stroma in the center and rich cellular zones in the periphery. Immunohistochemically, tumors were positive for factor Ⅷ -related antigen in 3 patients (Figure 6A), CD34 in 5 patients (Figure 6B) and CD31 in 4 patients (Figure 6C). Tumors were negative for epithelial markers (cytokeratin and CEA).

DISCUSSION

HEHE is a rare tumor of vascular origin, first defined as a specific entity by Weiss and Enzinger in 1982[5]. Because of the prolonged course and nonspecific clinical manifestations, the age of the patients varies greatly at the time of HEHE detection by biopsy or imaging studies. The incidence of this neoplasm is higher in females than in males (a female to male ratio of 3:2), with a peak incidence occurring between 30 and 40 years of age[2]. Most patients survive 5-10 years after diagnosis[4]. This study covered 5 females and 3 males and their average age was 39.8 years, which was comparable with other reports in the literature.

Clinical manifestation is variable, with most showing nonspecific symptoms such as right upper quadrant pain and weight loss. Physical examination findings are uncommon but may include hepatomegaly, a palpable mass, or jaundice. Some patients present with hemoperitoneum[20] and Budd-Chiari syndrome due to hepatic vein invasion[9]; others present with incidental findings[2]. Liver function tests reveal mild abnormalities in most patients. Tumor marker levels are negative apart from el-

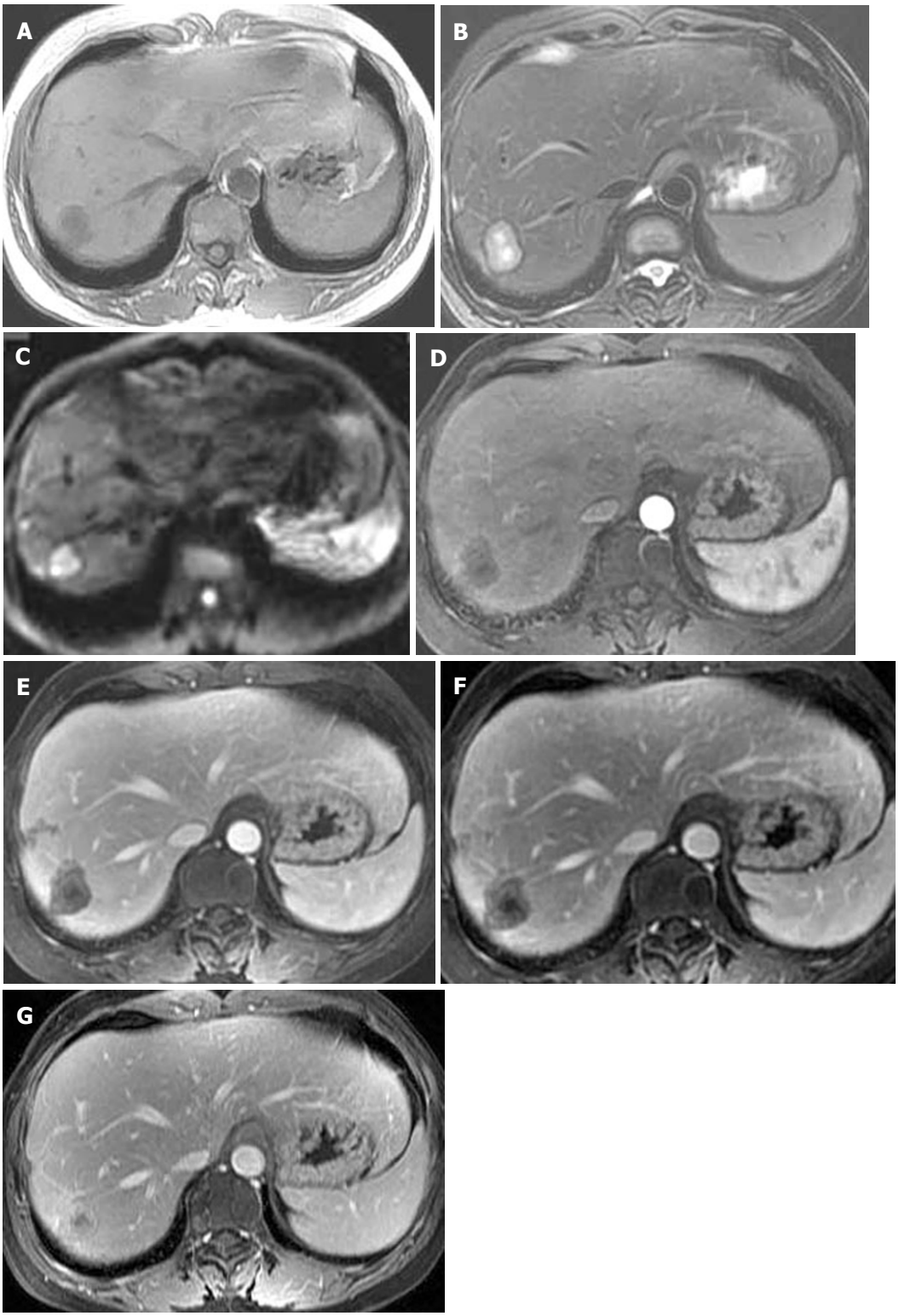

Figure 2 Multifocal hepatic epithelioid hemangioendothelioma in a 48-year-old female. Precontrast axial magnetic resonance imaging scan of the liver shows multiple lesions of low signal intensity with peripheral faint hyperintensity on T1WI (A), high signal intensity with peripheral hypointensity and an area of evident hyperintensity in the center of one lesion on T2WI (B), and hyperintensity with peripheral hypointensity on diffusion weighted imaging (C). Lesions show peripheral ring-like enhancement in the arterial phase (D), and heterogeneously progressive reinforcement in the portal venous phase (E), equilibrium phase (F) and it approaches isointensity to liver parenchyma in the delayed phase (G). There is an association with an area of unenhanced necrosis in the center, which looks like a conspicuous "target" with an inner low intensity, in-between high intensity and outer lower intensity layers.

evated CEA levels in a small number of patients, which is in conformity with our series. No risk factors or specific causes of HEHE were identified. Two patients in our study were HBsAg positive, which is similar to the rate reported in a few studies[10]. However, more cases are needed to clarify the relationship between HBV infection and the occurrence of HEHE.

Pathologically, there are three types of growth patterns in the gross appearance of HEHE: multiple nodules, diffuse nodules and a single mass[3,8,11]. Histologically, the tumors are composed of dendritic and epithelioid cells. Immunohistochemically, tumors are positive for factor Ⅷ-related antigen, CD34 or CD31, demonstrating the endothelial origin of these tumors[2,8]. Two types of HEHE were found in our cases, i.e., multifocal and diffuse, and the immunohistochemical results of our series were consistent with other observations. HEHE may have been present in some patients with pulmonary

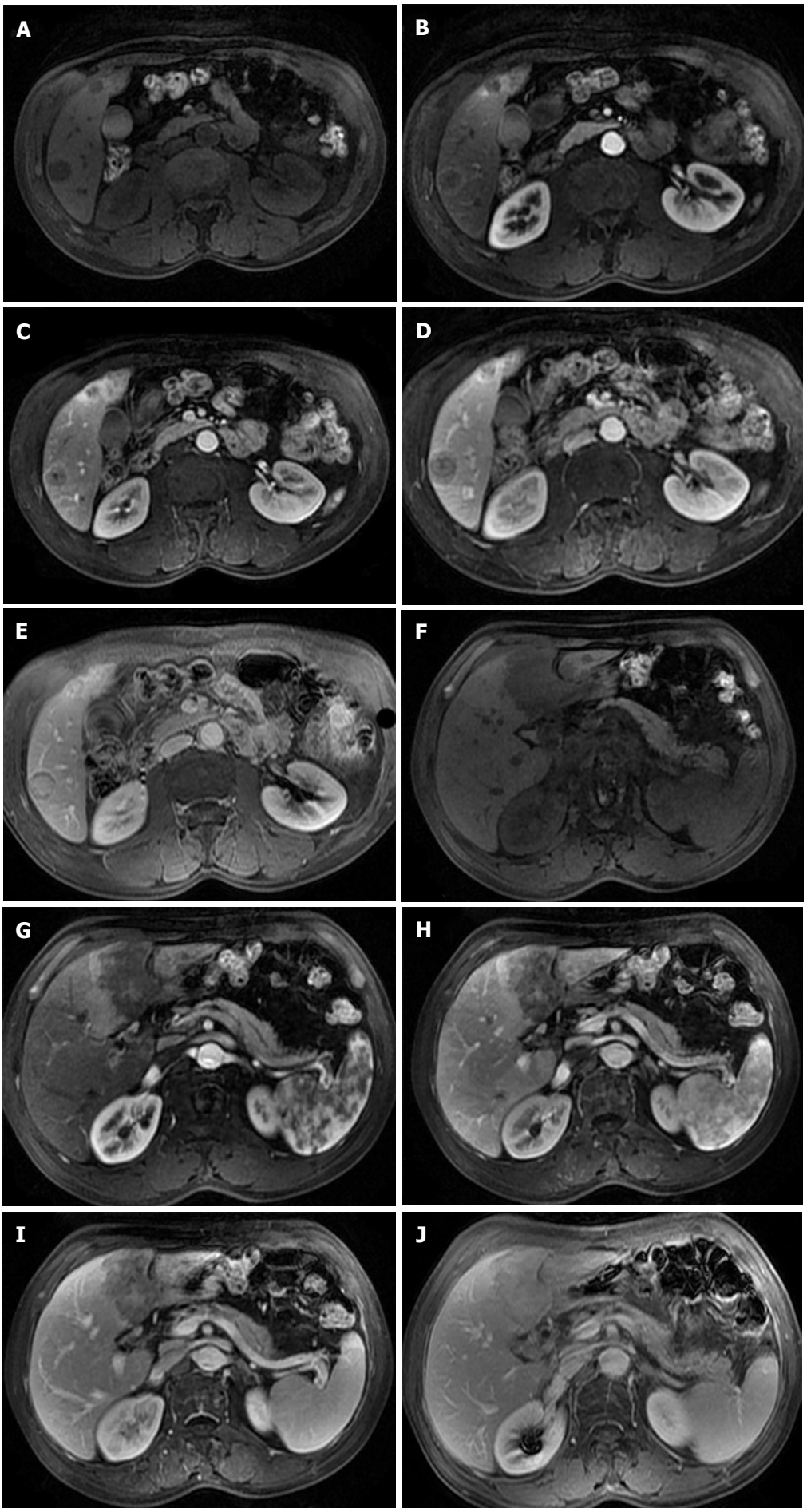

Figure 3 Multifocal hepatic epithelioid hemangioendothelioma in a 60-year-old man. Contrast-enhanced multiple-phase magnetic resonance imaging shows a progressive-enhancement target sign in the lesions in segment Ⅵ of the liver (A-E) and lamellar lesions with progressive enhancement in the left lobe of the liver (F-J).

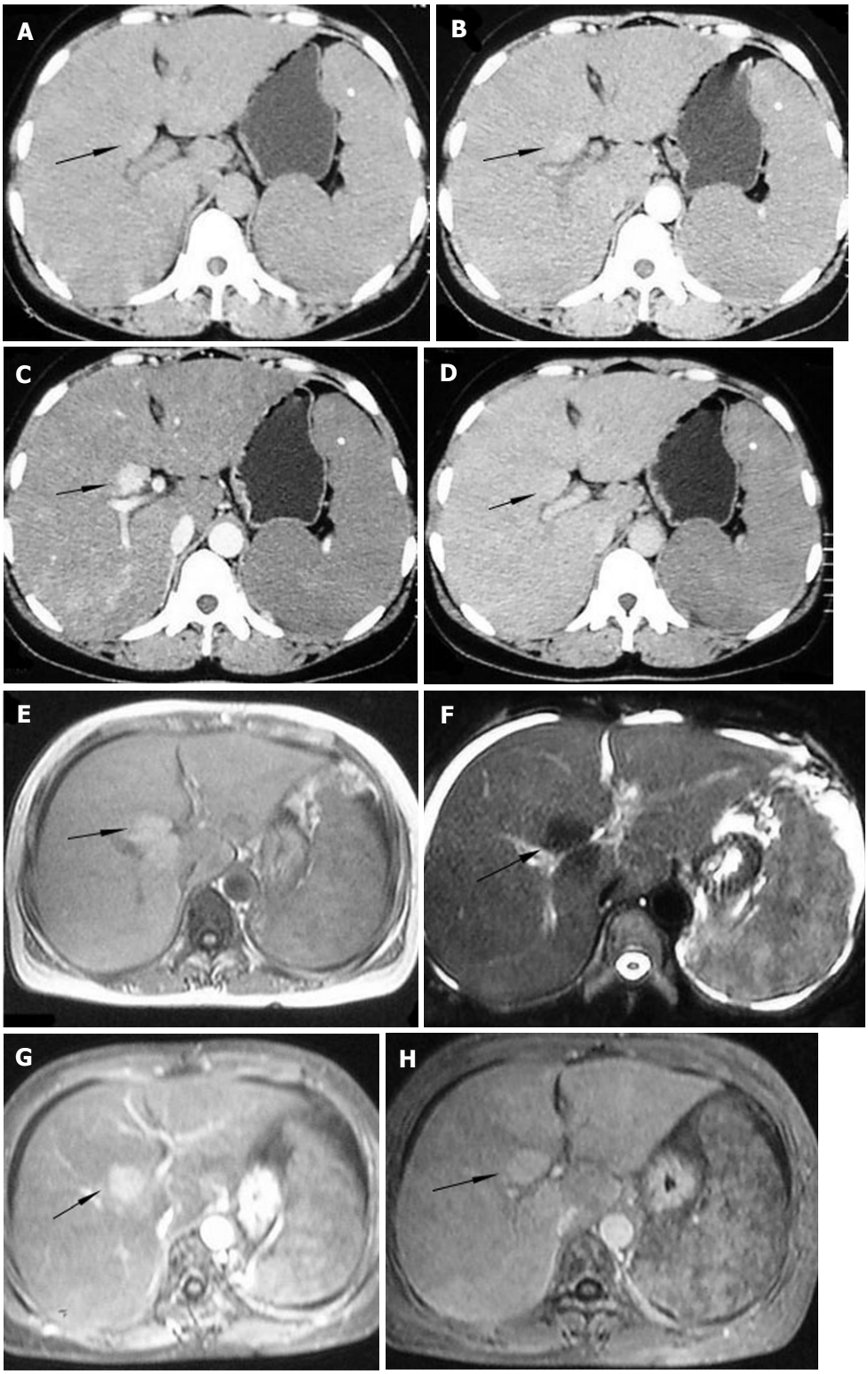

Figure 4 Diffuse hepatic epithelioid hemangioendothelioma in a 48-year-old woman. Plain computed tomography and magnetic resonance imaging manifests an obviously enlarged liver with a large nodule (black arrow) appearing slightly hyperdense relative to normal liver parenchyma (A), isointense on T1WI (E) and hypointense on T2WI (F), with slight enhancement in the arterial phase (B), evident enhancement in the portal venous phase (C and G) and iso-density/intensity in the equilibrium phase (D and H), associated with splenomegaly and ascites (F).

EHE or soft tissue EHE, but was considered metastatic[5,21-23]. In our series, there was one case of HEHE associated with pulmonary EHE.

A few radiological findings in HEHE have been described[1,2,8,10,12-15], however, CEMP CT and MRI findings have not been well addressed. According to the literature on multifocal-pattern HEHE[1,2,8,12,13], plain CT usually shows multiple discrete low-attenuation lesions and extensive confluent masses. Contrast-enhanced CT findings include marginal enhancement during the arterial phase[12], becoming isodense to liver parenchyma on contrast-enhanced scans[8], and a halo or target pattern of enhancement with larger lesions[1,13]. Lin *et al*[10] found that about 38 (48.1%) of 79 lesions showed the "halo" sign on contrast-enhanced CT. We had similar findings with CT, but 24.3% of the lesions showed the target sign on plain CT, 37.8% lesions showed the target sign with a progressive-enhancement rim and 12.2% le-

Table 1 Radiological findings of hepatic epithelioid hemangioendothelioma (HEHE)

Cases	Sex	Age (yr)	Total lesions (*n*)	Plain CT findings of HEHE	Pre-enhanced MRI of HEHE	CEMP imaging findings of HEHE		Radiological findings of extra-hepatic lesions
						Lesions of target sign with peripheral progressive enhancement (*n*)	Lesions of central or lamellar progressive enhancement (*n*)	
Case 1 multifocal-type (Figure 1)	Male	27	42	All discrete, peripheral, low-attenuation lesions with 1 coalescence and 13 target sign	No MRI obtained	42	0	No extra-hepatic lesions
Case 2 multifocal-type	Female	30	14	All discrete, peripheral, low-attenuation lesions with 1 coalescence and 5 target sign	All lesions showing hypointense on T1WI and hyperintense on T2WI with 14 target sign	3 (CT), 14 (MRI)	3 (CT), 14 (MRI)	No extra-hepatic lesions
Case 3 multifocal-type	Female	28	2	All discrete, peripheral, low-attenuation lesions	No MRI obtained	2 (CT), 2 (MRI)	2 (CT), 2 (MRI)	No extra-hepatic lesions
Case 4 multifocal-type	Female	53	4	All discrete, peripheral, low-attenuation lesions	No MRI obtained	4 (CT)	0	No extra-hepatic lesions
Case 5 multifocal-type (Figure 2)	Female	48	10	All discrete, peripheral, low-attenuation lesions with 1 coalescence	All lesions showing hypointense on T1WI and hyperintense on T2WI with 10 target sign, and 5 hyperintense with peripheral hypointense on DWI	10 (CT), 10 (MRI)	4 (CT), 10 (MRI)	No extra-hepatic lesions
Case 6 multifocal-type	Female	56	2	All discrete, peripheral, low-attenuation lesions, with compensatory hypertrophy in the left lobe of liver	No MRI obtained	0	2 (CT)	Pleural effusion in both sides
Case 7 multifocal-type (Figure 3)	Male	60	2	No CT obtained	Two lesions showing hypointense on T1WI and hyperintense on T2WI, and 1 hyperintense with peripheral hypointense on DWI	1 (MRI)	2 (MRI)	No extra-hepatic lesions
Case 8 diffuse – type (Figure 4)	Female	48	Diffuse	Diffuse hepatomegaly with a slightly hyperdense nodule	Diffuse hepatomegaly with a nodule appearing isointense on T1WI and hypointense on T2WI	Diffuse hepatomegaly with a nodule appearing persistent enhancement		Splenomegaly and ascites

HEHE: Hepatic epithelioid hemangioendothelioma; CEMP: Contrast-enhanced multiple-phases; *n*: Number; CT: Computed tomography; MRI: Magnetic resonance imaging; DWI: Diffusion weighted imaging.

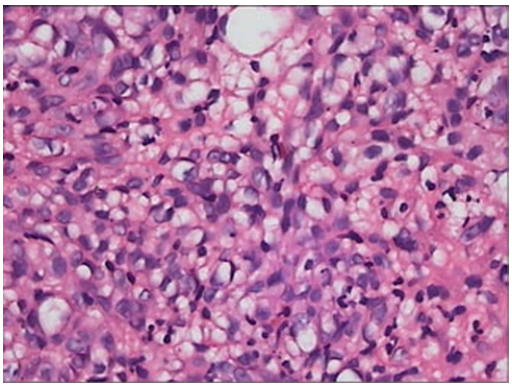

Figure 5 Photomicrography shows epithelioid cells with intracytoplasmic lumina, occasionally containing red blood cells, appearing as signet ring–like structures (hematoxylin and eosin, HE × 200).

sions displayed progressive enhancement on CEMP CT, maintaining the state of persistent enhancement. The CT findings in our cases were not compatible with other reports in the literature.

According to the literature on multifocal pattern HEHE[1,2,8,12,13], precontrast MR imaging revealed hypointense lesions relative to normal liver parenchyma on unenhanced T1-weighted images, heterogeneously increased signal intensity on T2-weighted images and hyperintensity with peripheral hypointensity on DWI[7,8]. Some lesions have a peripheral halo or a target-type enhancement pattern on enhanced MR imaging, with occasional observation of a thin peripheral hypointense rim[2,8,12]. Lin *et al*[10] found that 9 (23.1%) of 39 lesions presented the characteristic "halo" sign on contrast-enhanced MRI. In our series, we had similar findings with precontrast MRI and DWI, but 92.9% of the lesions showed the target sign on precontrast MRI and 14.3% of the lesions showed lamellar hyperintensity on DWI, which is in contrast to the previous literature. In our series, 96.4% of the lesions appeared with the target sign and a progressive-enhancement rim and 100% of the lesions displayed progressive-enhancement, maintaining the state of persistent enhancement. The MRI findings in our cases were not compatible with the previous lit-

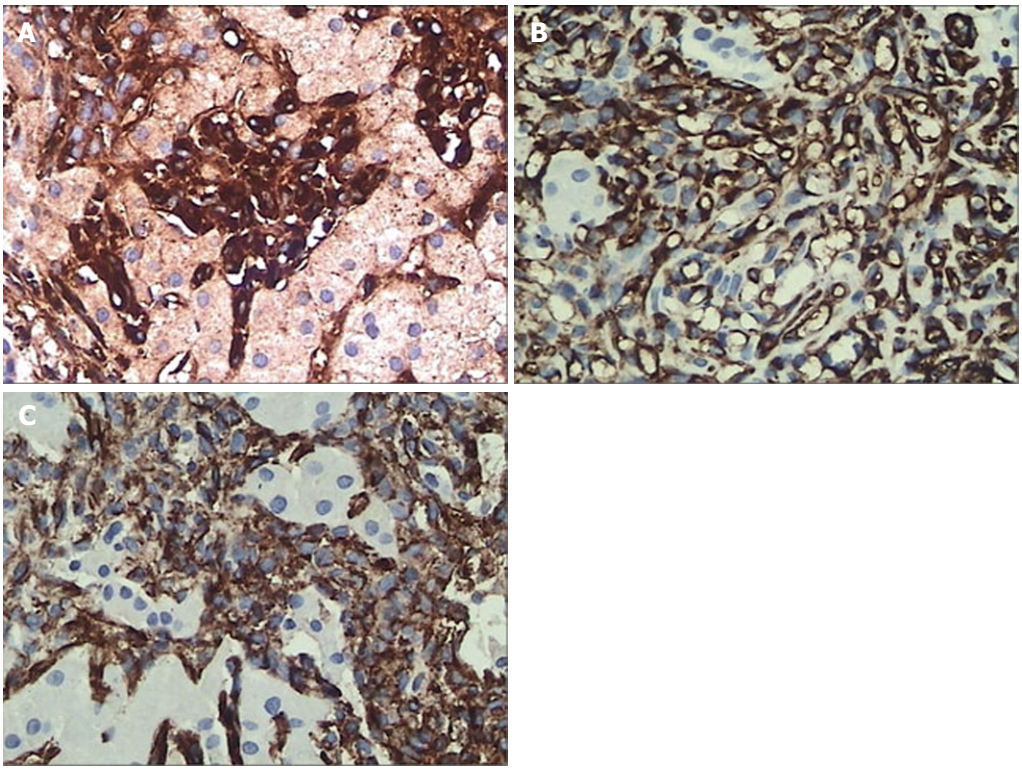

Figure 6 Immunohistochemical staining shows that the tumor cells are positive for factor Ⅷ-related antigen (A), CD34 (B) and CD31 (C) (× 250).

erature. The MRI features of progressive reinforcement on CEMP MRI have not been reported previously according to our literature search.

It could be considered that the distinctive image appearance of the tumor is correlated with the pathologic characteristics in many ways. Histologically, the tumors consisted of large amounts of mucinous and dense stroma in the center and rich cellular zones in the periphery. These findings might account for the central low density and peripheral isodensity on plain CT images, hypointensity with peripheral faint hyperintensity on T1WI and hyperintensity with peripheral hypointensity on T2WI and DWI. The actively proliferating, increased cellular periphery of the nodules may account for the peripheral progressive-enhancement target sign on CEMP CT. The tumor also produced a fibrous myxoid stroma that was most dense in the center of the nodules, which may attribute to the heterogeneously progressive reinforcement on CEMP MRI. Tumor infiltration and occlusion of hepatic sinusoids and small vessels caused a narrow avascular zone between the tumor nodules and liver parenchyma. This may be the reason for the halo appearance on CT or MRI.

According to the reported studies on diffuse-pattern HEHE, a multifocal nodular pattern of infiltration is usually considered as the early stage of a diffuse pattern[1,8,13]. Local lesions may increase in size and coalesce, thus forming the diffuse pattern. The diffuse lesions contain many lowly attenuating, round or irregular spots, which may be associated with calcified foci and dilated bile ducts in the lesions. The lesions were slightly enhanced on dynamic CT scans and became iso-attenuated to non-tumorous liver on subsequent scans, but spots of lower attenuation remained inside or showed marked contrast enhancement during and after intra-arterial contrast material injection and disappeared within 1 min after the contrast material injection. In our series, the diffuse case was also associated with splenomegaly and ascites, but had different imaging findings manifesting an obviously enlarged liver with a large nodule. The manifestation of diffuse-pattern HEHE appearing as an obviously enlarged liver was only reported by Lorber *et al*[11]. Necropsy showed that the liver was grossly enlarged without cirrhosis, and contained a very discrete red area (0.2-2.5 cm in diameter). However, according to our search, the MRI findings of diffuse-pattern HEHE with a large nodule and the imaging feature of a large nodule manifesting persistent enhancement on CEMP CT and MRI have not been reported previously.

Because the histologic specimens of this diffuse-pattern HEHE were obtained by percutaneous needle biopsy of many sites in the liver, but not with exploratory laparotomy, it is hard to analyze the correlation between the imaging findings of the large nodule in a diffuse case with the pathologic findings. We suppose that the large nodule in the diffuse case might consist of large amounts of tumor cells, manifesting persistent enhancement on CEMP CT and MRI, and the nodule appearing slightly hyper-dense on plain CT, isointense on T1WI and hypointense on T2WI might correlate with hemorrhage in the nodule.

According to the isolated case report literature of single-pattern HEHE, it only accounted for about 18%[3]. Jeong *et al*[14] and Hsieh *et al*[15] found that the single lesion is usually ovoid, with a low density and calcification in segment 7 of the liver in the pre-contrast phase of

liver dynamic CT. The mass exhibited central enhancement in the arterial phase, heterogeneous peripheral enhancement in the portal phase, and then peripheral enhancement was washed out in the delayed phase, or with central enhancement in the delayed phase on CT. According to these reports, the single nodular pattern of HEHE typically preferentially involved the right lobe of the liver, and the pattern of contrast enhancement was different and more studies are needed in the future. To our regret, there was no single-pattern HEHE in our cases.

For differential diagnosis, the most important imaging features of a target sign and progressive enhancement could differentiate HEHE from intrahepatic multiple metastatic tumors, cavernous hemangioma and primary hepatic angiosarcoma.

In conclusion, MRI is more advantageous over CT in displaying the imaging features of a target sign and progressive enhancement. Although the incidence of HEHE is low and the diagnosis can only be confirmed by pathological examination, it should be considered in the differential diagnosis list of intrahepatic nodules appearing with a target sign and/or progressive enhancement with persistent enhancement on CEMP CT and MRI, which demonstrates a vasoformative nature, especially in multiple lesions in middle-aged women.

COMMENTS

Background

Hepatic epithelioid hemangioendothelioma (HEHE) is a rare vascular tumor. Clinical assessment, imaging and pathologic diagnosis of HEHE is difficult, however, its correct diagnosis is very important because long-term survival of HEHE is possible.

Research frontiers

No more than 200 cases of HEHE have been reported since its first description. The contrast-enhanced multiple-phase computed tomography (CEMP CT) and magnetic resonance imaging (MRI) findings of HEHE have not been well addressed. In this study, the authors highlight the predominant imaging features of this tumor, which have not been described previously in the English-language literature.

Innovations and breakthroughs

Most of the radiologic studies on HEHE were sporadic case reports and small case series reports. In this study, the authors evaluated and described target sign and/or progressive enhancement with persistent enhancement in CEMP CT and MRI of HEHE. MRI is advantageous over CT in displaying these imaging features. Furthermore, the authors described a diffuse-type HEHE, manifesting diffuse hepatomegaly with a slightly hyperdense nodule appearing with persistent enhancement on CEMP CT and MRI.

Applications

By demonstrating imaging features of HEHE on CEMP CT and MRI, this study may represent a future strategy for correct diagnosis of HEHE and therapeutic intervention in the treatment of HEHE.

Terminology

A target sign is an image that looks like a "target" with inner density/intensity and peripheral hyper-density/intensity or iso-density/intensity. On CEMP CT and MRI, it appears as peripheral ring-like and progressive enhancement. In some typical cases, it may show hyperintensity with peripheral hypointensity and an area of evident hyperintensity in the center on T2WI or an area of unenhanced necrosis in the center.

Peer review

The authors investigated and described CEMP CT and MRI findings in patients with pathologically confirmed HEHE. It revealed the most important imaging features of HEHE may be a target sign and/or progressive enhancement with persistent enhancement on CEMP CT and MRI. MRI is advantageous over CT in displaying these imaging features. The results are interesting and may represent the imaging features in HEHE.

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CASE REPORT

Simultaneous bile duct and portal venous branch ligation in two-stage hepatectomy

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Key words: Two-stage hepatectomy; Bile duct ligation; Portal vein ligation; Synchronous liver metastases

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Abstract

Hepatectomy is an effective surgical treatment for multiple bilobar liver metastases from colon cancer; however, one of the primary obstacles to completing surgical resection for these cases is an insufficient volume of the future remnant liver, which may cause postoperative liver failure. To induce atrophy of the unilateral lobe and hypertrophy of the future remnant liver, procedures to occlude the portal vein have been conventionally used prior to major hepatectomy. We report a case of a 50-year-old woman in whom two-stage hepatectomy was performed in combination with intraoperative ligation of the portal vein and the bile duct of the right hepatic lobe. This procedure was designed to promote the atrophic effect on the right hepatic lobe more effectively than the conventional technique, and to the best of our knowledge, it was used for the first time in the present case. Despite successful induction of liver volume shift as well as the following procedure, the patient died of subsequent liver failure after developing recurrent tumors. We discuss the first case in which simultaneous ligation of the portal vein and the biliary system was successfully applied as part of the first step of two-stage hepatectomy.

INTRODUCTION

Hepatectomy has been established as an effective treatment method for liver metastases from colon cancer, but there are some limits to this procedure when indicated in cases of synchronous multiple bilobar liver metastases. One of the reasons that limit resectability for these cases is an insufficient volume of the future remnant liver, which poses a risk of postoperative liver failure. To address this concern and improve the resectability, ligation or embolization of the portal vein is used in clinical settings. In addition, according to several animal experiments, ligation of the bile duct effectively induces atrophy of the ipsilateral liver.

For the present case of synchronous multiple bilobar liver metastases from cecal cancer, we first performed a right hemicolectomy and a segment 3 resection combined with microwave coagulation therapy (MCT) of the metastatic tumors situated in the left hepatic lobe, together with simultaneous ligation of the right portal vein and right-sided bile duct. Second, we performed an extended right hepatic lobectomy. Here, we discuss the effectiveness of intraoperative simultaneous ligation of the unilateral portal

Table 1 Laboratory findings on admission

Laboratory finding	Values
Leukocytes	8300/μL
Hemoglobin	9.7 g/dL
Hematocrit	30.70%
Platelets	408 000/μL
Alkaline phosphatase	595 IU/L
Lactate dehydrogenase	1295 IU/L
Aspartate aminotransferase	49 IU/L
Alanine aminotransferase	47 IU/L
Total-bilirubin	0.4 mg/dL
Direct-bilirubin	0.1 mg/dL
CEA	22 120 ng/dL
CA 19-9	16 354 U/mL

CEA: Carcinoembryonic antigen; CA: Carbohydrate antigen.

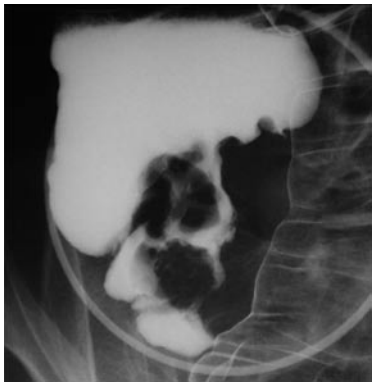

Figure 1 Barium enema findings. A filling defect caused by a tumor in the cecum was observed.

vein and bile duct applied to two-stage hepatectomy, as a therapeutic strategy for multiple bilobar liver metastases.

CASE REPORT

A 50-year-old woman began to have back pain and nausea around December 2004 and was referred to our hospital. Her liver was palpable at three finger widths below the right costal arch. The palpebral conjunctiva was anemic but bulbar conjunctiva was not icteric. Blood tests suggested that she had anemia (hemoglobin and hematocrit levels were 9.7 g/dL and 30.7%, respectively) and liver dysfunction (lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase were 1295 IU/L, 49 IU/L, 47 IU/L and 595 IU/L, respectively). The serum levels of carcinoembryonic antigen (CEA) and carbohydrate antigen (CA) 19-9 were 22 120 ng/mL and 16 354 U/mL, respectively (Table 1). Barium enema showed a filling defect in the cecum. The results of biopsy indicated moderately differentiated adenocarcinoma (Figure 1). Contrast-enhanced computer tomography (CT) of the abdomen revealed many metastatic nodules with 2-6 cm diameter in the right hepatic lobe and three nodules with 3-3.5 cm diameter in the left hepatic lobe. A preoperative volume rate of the right hepatic lobe was 56.3% (Figure 2). There were no other metastases. Before implementation of two-stage hepatectomy, preoperative chemotherapy (TS-1: tegafur, gimeracil, and oteracil) was initiated at a starting dose of 80 mg/d. Four weeks later, the liver metastases shrank markedly and the serum levels of CEA and CA 19-9 decreased to 12 680 ng/dL and 8640 U/mL, respectively.

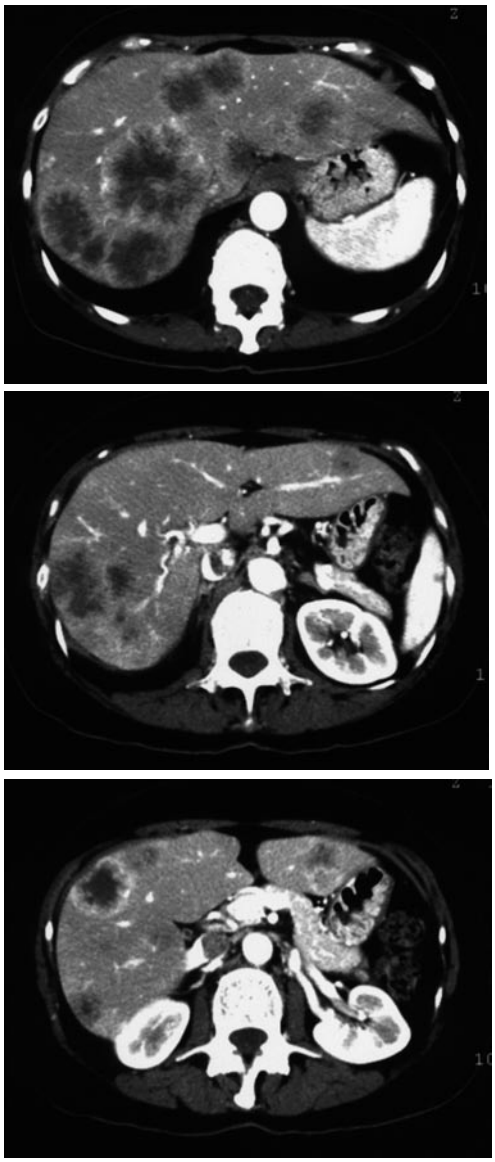

Figure 2 Preoperative findings from contrast-enhanced computer tomography of the abdomen. Multiple metastatic nodules with 2-6 cm diameter were observed in the right hepatic lobe and three nodules measuring 3-3.5 cm diameter in the left hepatic lobe. A preoperative volume rate of the right hepatic lobe was 56.3%.

Written informed consent was obtained from the patient and her family prior to the new surgical procedure. In March 2005, a right hemicolectomy was performed. At the same time, the metastatic tumor in segment 3 was resected, and the metastatic tumors in segments 2 and 4 were coagulated using MCT (90 W, 60 s). Furthermore, the right bile duct and right branch of the portal vein were ligated simultaneously (Figure 3). Intraoperative cholangiography was performed after ligation to confirm complete occlusion of the right bile duct and no constriction of the contralateral bile duct. Contrast-

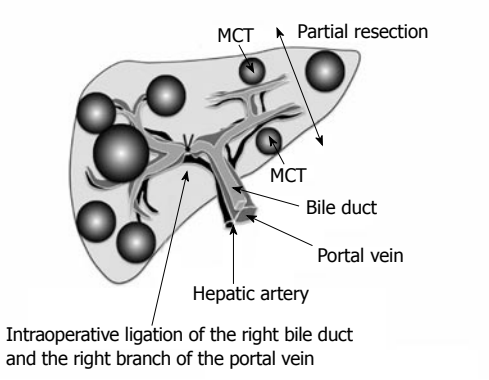

Figure 3 Initial operation scheme. Right hemicolectomy was performed. At the same time, the metastatic tumor in segment 3 was resected and the metastatic tumors in segments 2 and 4 were coagulated using MCT (90 W, 60 s). Furthermore, the right bile duct and right branch of the portal vein were ligated simultaneously. MCT: Microwave coagulation therapy.

enhanced CT of the abdomen performed at 1 wk after surgery showed coagulated areas in the left hepatic lobe and dilation of the right bile duct. Ligation of the right branch of the portal vein resulted in a complementary increase in the right hepatic arterial blood flow. The volume rate of the right hepatic lobe was 55.0%, which was similar to the preoperative rate (Figure 4). However, abdominal CT performed at 1 mo after surgery showed that the volume rate of the right hepatic lobe decreased to 44.3%, along with compensatory hypertrophy of the left hepatic lobe. This liver volume shift was approximately 12% from the right to the left lobe. No viable tumors were observed in the left hepatic lobe (Figure 5).

An extended right hepatic lobectomy was performed in May 2005. There were 10 nodules with 1-4 cm diameter in the right hepatic lobe. The resection was performed using a suture of the right bile duct and the right branch of the portal vein. The weight of the resected specimen was 555 g (Figure 6). There was no complication in the postoperative course. However, in October 2005, two recurrent nodules with 3.2 cm and 1.8 cm diameter were found in the remaining liver. She did not accept repeat resection, therefore, percutaneous radiofrequency ablation was performed.

In November 2005, the biweekly administration of oxaliplatin (80 mg), levofolinate calcium (125 mg), and 5-fluorouracil (1250 mg) was started, leading to some improvement for a while; specifically, a decrease in the serum levels of CEA and CA 19-9 to 36.3 ng/dL and 96 U/mL, respectively. Nevertheless, further recurrence was observed in the remaining liver. At 1 year and 8 mo after the initial operation, the patient developed liver failure and died (Figure 7). There was no other distant metastasis.

DISCUSSION

Liver metastasis occurs in 40%-70% of patients with colon cancer, and 15%-30% of these patients develop multiple liver metastases in both lobes[1]. However, in comparison to other cancers, we can expect long-term survival by using a combined therapy of resection and chemotherapy. In particular, hepatectomy is widely used as the most effective surgical treatment, and the 5-year survival rate after a curative resection is 24%-44%[2]. Even for multiple liver metastases in both lobes previously considered unresectable, complete resection is feasible in some cases by adopting preoperative portal vein embolization in addition to chemotherapy. In 2004, Jaeck *et al*[3] reported a method called two-stage hepatectomy; applicable to cases of multiple bilobar liver metastases. In the first stage of the operation, they conducted a partial hepatic resection or ablation for the unilateral lobe, and at the same time embolized the portal vein in order to induce hypertrophy of the future remnant lobe. In the

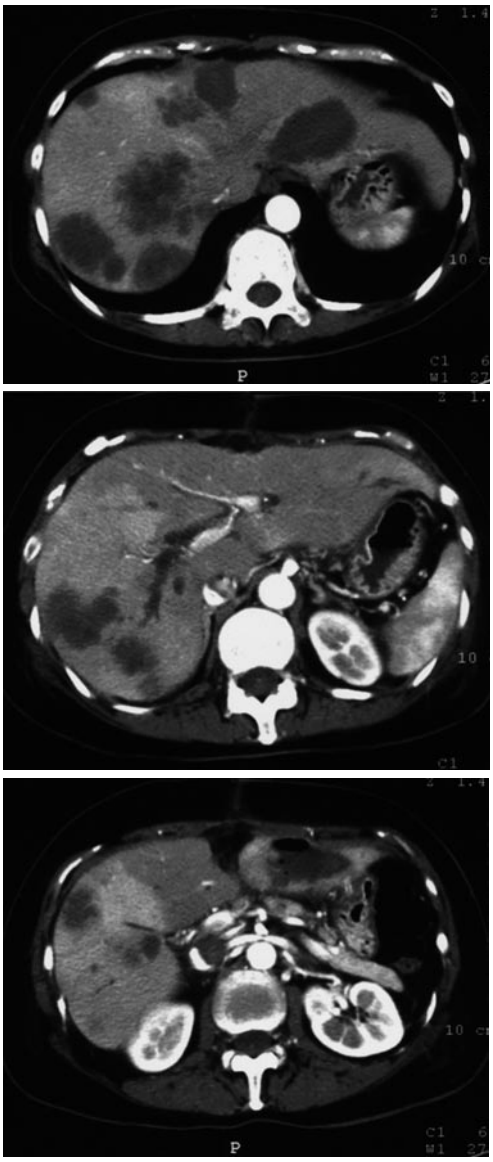

Figure 4 Findings from contrast-enhanced computer tomography of the abdomen at 1 wk after surgery. Two coagulated areas in the left hepatic lobe and dilation of the right bile duct were observed. Ligation of the right branch of the portal vein resulted in a complementary increase in the right hepatic arterial blood flow. The volume rate of the right hepatic lobe was 55.0%, which was similar to the preoperative rate.

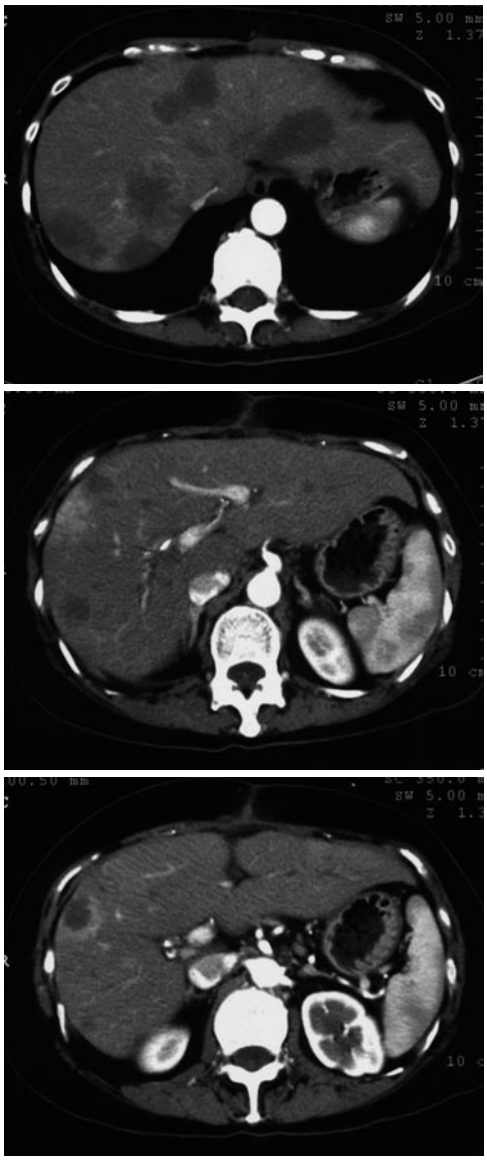

Figure 5 Findings from contrast-enhanced computer tomography of the abdomen at 1 mo after surgery. The volume rate of the right hepatic lobe decreased to 44.3% and the volume of left hepatic lobe underwent compensatory hypertrophy. This liver volume shift was approximately 12% from the right to the left lobe. There were no viable tumors in the left hepatic lobe.

second stage of the operation, hepatic lobectomy was performed in the atrophic lobe. They reported that the 1-year survival rate was 70% and the 3-year survival rate was 54.4%[3]. Moreover, for two-stage hepatectomy conducted by Togo *et al*[4], the 1-year survival rate was 90% and the 3-year survival rate was 45%. The treatment strategy includes combined therapy of systemic chemotherapy, arterial infusion therapy and ablation therapy. Such a multidisciplinary therapy allows resection of marginal or so-called unresectable tumors and contributes to improvement in the prognosis.

Ligation of the portal vein of the unilateral lobe is well-known to induce atrophy of the unilateral hepatic lobe and hypertrophy of the future remnant liver. Honjo *et al*[5] clinically applied this technique to a case of liver metastasis in 1961. Such a method was adopted for cases

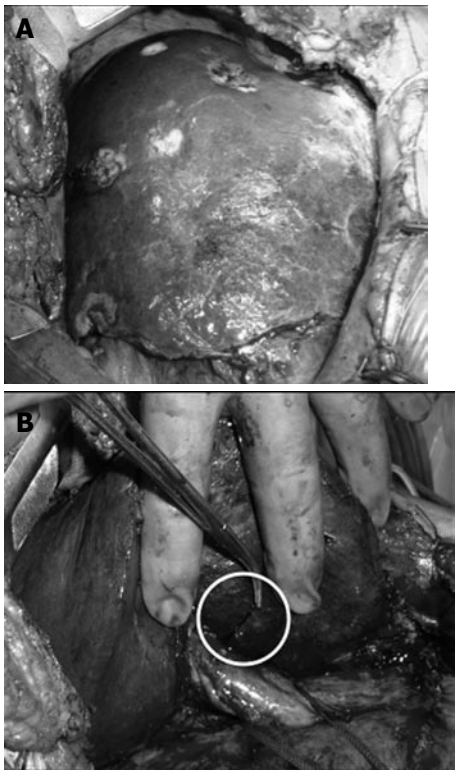

Figure 6 Intraoperative findings from the secondary operation. A: There were 10 nodules with 1-4 cm diameter in the right hepatic lobe; B: Resection was performed using the suture of the right bile duct and the right branch of the portal vein.

of major resection initially considered to have a high risk of postoperative liver failure based on preoperative prognosis[6-9]. Reportedly, at 3-4 mo after occlusion of the right portal vein, 9.5%-14.5% of the liver volume was transferred from the right to the left lobe[10]. In addition, according to a literature review of animal experiments, in 1920, Rous *et al*[11] reported that atrophy of the ligated lobe and hypertrophy of the future remnant lobe were induced in mice after bile duct ligation. Subsequently, Tanaka *et al*[12] have reported that the atrophic rate was enhanced after portal vein embolization in a rabbit that had undergone bile duct ligation. It was also pointed out that occlusion of the bile duct and portal vein could reduce biligenesis and occurrence of complications[12].

We applied these findings to the initial surgery in the present case and used simultaneous ligation of the bile duct and portal vein, with an aim of promoting an atrophic effect in the right hepatic lobe. As a result, 12.0% of the liver volume was transferred from the right to the left hepatic lobe at 1 mo after ligation, and the amount of this volume shift could reverse the primary unresectablity. The possible explanation that we consider for the benefit of our simultaneous ligation strategy is that it can overcome several factors that may diminish the atrophic effect of the conventional technique. The bile duct not being ligated may allow the portal blood flow to remain, due to backflow from the hepatic vein and the use of an arterioportal shunt. In our clinical experience of four cases in

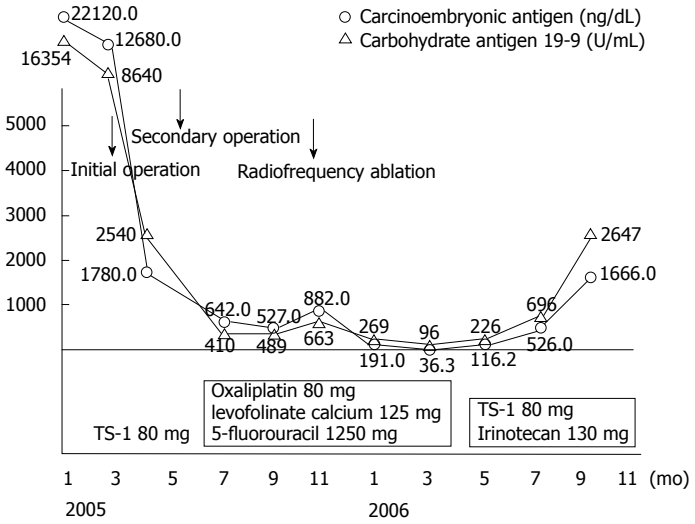

Figure 7 Postoprative course. The serum levels of carcinoembryonic antigen and carbohydrate antigen 19-9 decreased to 36.3 ng/dL and 96 U/mL, respectively. But further recurrence was observed in the remaining liver, and at 1 year and 8 mo after the initial operation, the patient developed liver failure and died. TS-1: Tegafur, gimeracil, and oteracil.

Table 2 Atrophy achieved in our previous four PVL cases

Case No.	Age	Sex	Primary disease
Case 1	55 years	Female	Multiple liver metastasis
Case 2	61 years	Male	Multiple liver metastasis
Case 3	61 years	Male	Hepatocellular carcinoma
Case 4	58 years	Male	Hepatocellular carcinoma

PVL: Portal vein ligation; RHL: Right hepatic lobe.

Table 3 Atrophy achieved in our previous four PVL cases

Case No.	Preoperative volume rate of RHL	The volume rate of RHL 1 mo after PVL
Case 1	74.80%	68.50%
Case 2	68.30%	62.50%
Case 3	58.60%	55.50%
Case 4	58.90%	52.40%

PVL: Portal vein ligation; RHL: Right hepatic lobe.

which the right portal vein alone was ligated, the volume shift at 1 mo after the procedure was only 5.4% ± 1.6% (Tables 2, 3), which suggests that ligation of the bile duct made a substantial contribution to inducing atrophy of the ipsilateral liver in the present case.

Regarding the limitations of our simultaneous ligation strategy, it should be noted that this technique should be performed with careful consideration of the risks of obstructive cholangitis and involution of the contralateral bile duct. Intraoperative cholangiography may help avoid these occurrences. With respect to the risks of the aforementioned complications, the safety of our procedure remains to be confirmed in a future study.

To the best of our knowledge, there has not been any reported case in which simultaneous ligation of the portal vein and bile duct has been used in two-stage hepatectomy. There was no postoperative biliary tract infection in our patient. Unfortunately, the patient developed further recurrent tumors after hepatectomy and died of subsequent liver failure. Yet, it can be concluded that the simultaneous ligation strategy could be used to accelerate atrophy of the hemiliver to be resected, and thereby provide us with a further possibility of addressing the primary unresectability of metastatic bilobar liver tumors.

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CASE REPORT

Endoscopic naso-pancreatic drainage for the treatment of pancreatic fistula occurring after LDLT

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Abstract

Pancreatic fistula is a quite rare complication in patients who undergo living donor liver transplantation (LDLT). However, in the cases that show pancreatic fistula, the limited volume of the graft and the resultant inadequate liver function may complicate the management of the fistula. As a result, the pancreatic fistula may result in the death of the patient. We present 2 cases in which endoscopic treatment was effective against pancreatic fistulas that developed after LDLT. In case 1, a 61-year-old woman underwent LDLT for primary biliary cirrhosis. Because of a portal venous thrombus caused by a splenorenal shunt, the patient underwent portal vein reconstruction, and a splenorenal shunt was ligated on postoperative day (POD) 7. The main pancreatic duct was injured during the manipulation to achieve hemostasis, thereby necessitating open drainage. However, discharge of pancreatic fluid continued even after POD 300. Endoscopic naso-pancreatic drainage (ENPD) was performed, and this procedure resulted in a remarkable decrease in drain output. The refractory pancreatic fistula healed on day 40 after ENPD. In case 2, a 58-year-old man underwent LDLT for cirrhosis caused by the hepatitis C virus. When the portal vein was exposed during thrombectomy, the pancreatic head was injured, which led to the formation of a pancreatic fistula. Conservative therapy was ineffective; therefore, ENPD was performed. The pancreatic fistula healed on day 38 after ENPD. The findings in these 2 cases show that endoscopic drainage of the main pancreatic duct is a less invasive and effective treatment for pancreatic fistulas that develop after LDLT.

Key words: Pancreatic fistula; Endoscopic treatment; Living donor liver transplantation; Complications

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Nagatsu A, Taniguchi M, Shimamura T, Suzuki T, Yamashita K, Kawakami H, Abo D, Kamiyama T, Furukawa H, Todo S. Endoscopic naso-pancreatic drainage for the treatment of pancreatic fistula occurring after LDLT. *World J Gastroenterol* 2011; 17(30): 3560-3564 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i30/3560.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i30.3560

INTRODUCTION

The incidence of complications associated with living donor liver transplantation (LDLT) is known to be greater than that associated with deceased donor liver transplantation (DDLT)[1-4]. In the patients who undergo LDLT, the incidence of complications, including mild complications, during the perioperative period can be as high as 82.8%. In particular, the rate of development of biliary complications after LDLT is twice that after DDLT[1]. Moreover, patients who undergo LDLT often have inadequate liver function because of the limited volume of the graft. Therefore, the management of complications is very difficult, and the mortality rate in critical cases is high.

Compared to other abdominal surgery, pancreatic fistula is a quite rare complication after LDLT[1-5], but it is theoretically possible because LDLT involves surgery in the area surrounding the portal vein, the pancreas, and the spleen. Pancreatic fistula causes hemorrhage, abscess, etc., and may result in the death of the patient[6-8]. Only 1 previous study has reported 2 cases of leakage of pancreatic fluid after liver transplantation. In those cases, leakage of a mixture of pancreatic fluid and bile was observed at the anastomosis site of the bile duct after DDLT[9]. However, there is no clear consensus on the management of pancreatic fistula after liver transplantation. Generally, conservative therapy is the first line of treatment, and surgery is performed only when the patient does not respond to conservative therapy[6-8]. Recently, however, endoscopic treatment has attracted more attention because it is less invasive than surgical treatment. We present 2 cases in which endoscopic treatment was effective against refractory pancreatic fistulas that developed after LDLT.

CASE REPORT

Case 1

A 61-year-old woman underwent LDLT for primary biliary cirrhosis; the graft was obtained from the left liver lobe of her son. Abdominal computed tomography (CT) performed on postoperative day (POD) 7 revealed a portal vein thrombus; therefore, urgent exploratory laparotomy was performed. The thrombus was believed to have been induced by reduced portal blood flow, which was caused by splenorenal steal by an artificial shunt. After removing the thrombus, portal vein reconstruction was performed by using the right external iliac vein, and this procedure was followed by splenectomy. To increase the portal blood flow, a splenorenal shunt was ligated. The main pancreatic duct on the dorsal side of the pancreas was injured at the time of hemostatic manipulation; however, this injury was not identified immediately. CT performed on POD 13 revealed a hematoma at the lower edge of the pancreas. CT on POD 21 revealed that the hematoma under the pancreas had decreased in size (Figure 1). The amylase level of the drainage fluid was 22 690 IU/L; therefore, the hematoma at the inferior edge of the pancreas was considered to have ruptured because of a pancreatic leak. Another CT examination revealed fluid collection in the mesentery on the ventral side of the upper pole of the left kidney; therefore, open drainage was performed. To this end, a drain was placed at the tail of the pancreas and administration of octreotide was started. However, the leakage of pancreatic fluid from the drain did not stop. After several days, the patient's general status stabilized, but surgical treatment for pancreatic fistula was still unsafe because of inadequate liver function. Therefore, the patient was discharged on POD 128 with the drain in place and was followed up. The patient was in a stable state at discharge. However, on POD 318, she was readmitted to our department because of fever. Examinations revealed that the drain at the tail of the pancreas had deviated and that the patient had developed liver necrosis, supposedly because of contact with the drain. On POD 320, we repositioned the drain by using a fluoroscope. A contrast test performed at that time revealed that the main pancreatic duct was completely disrupted (Figure 2). The patient was diagnosed with refractory pancreatic fistula, and an endoscopic naso-pancreatic drainage (ENPD) tube was inserted to the proximal side of the leakage on POD 331 (Figure 3); this procedure resulted in a remarkable decrease in drain output (Figures 4 and 5). The ENPD tube was removed on POD 368, and the drains at the tail of the pancreas were removed on POD 371. The patient was discharged on POD 375 without abnormal fluid collection around the pancreas (Figure 6). The patient is well without the recurrence of pancreatic fistula up to this time.

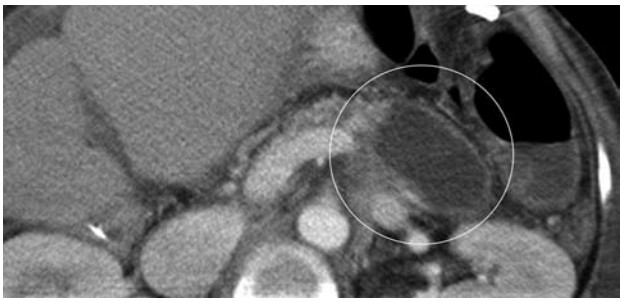

Figure 1 Computed tomography performed on postoperative day 13 showing fluid collection (circle) at the lower edge of the pancreas. The hematoma was considered to be ruptured.

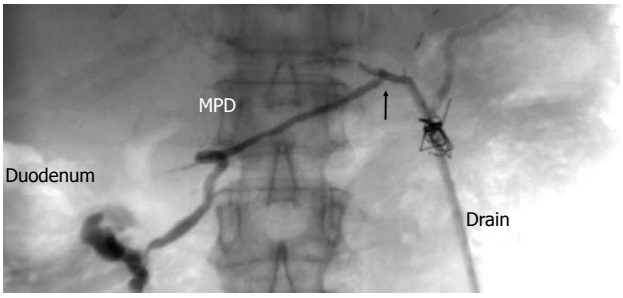

Figure 2 Pancreatographic examination of the drain at the tail of the pancreas (Drain) in case 1 reveals the disrupted (arrow) main pancreatic duct with flow of contrast into the duodenum. MPD: Main pancreatic duct.

Case 2

A 58-year-old man underwent LDLT for cirrhosis C; the graft was obtained from the right liver lobe of his daughter. Because the portal vein was occluded by a thrombus,

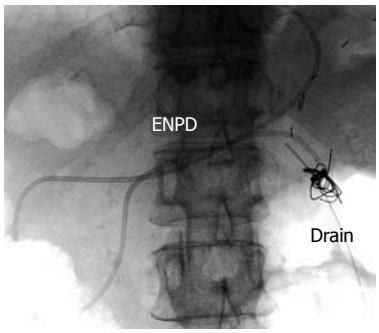

Figure 3 A radiograph showing postprocedure endoscopic naso-pancreatic drainage in case 1. Excellent drainage of the pancreatic duct is noted. ENPD: Endoscopic naso-pancreatic drainage.

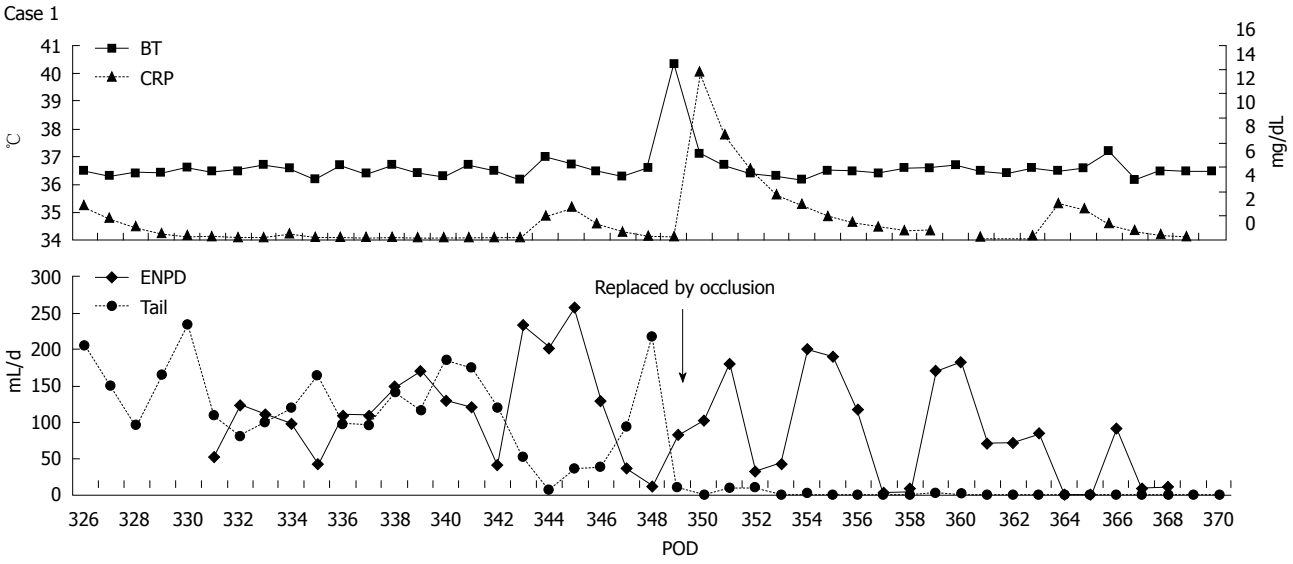

Figure 4 Upper chart shows the body temperature and serum C-reactive protein level. Lower one shows daily output of the endoscopic naso-pancreatic drainage tube and the drain at the tail of the pancreas (Tail) in case 1. The patient had an episode of fever caused by the occlusion of the endoscopic naso-pancreatic drainage (ENPD) tube. After the tube was replaced, the pancreatic fistula healed completely. BT: Body temperature; CRP: C-reactive protein; POD: Postoperative day.

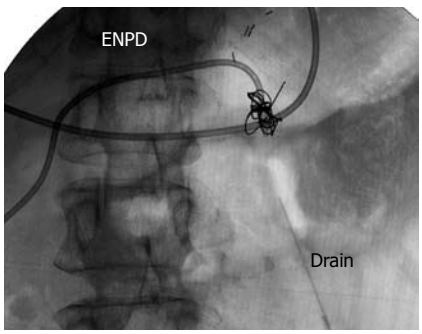

Figure 5 Contrast examination from the drain at the tail of the pancreas (Drain) in case 1 on postoperative day 363 reveals the closure of fistula. ENPD: Endoscopic naso-pancreatic drainage.

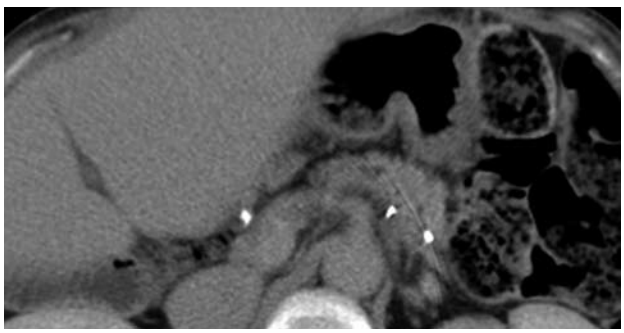

Figure 6 Computed tomography performed 2 d after removal of the drain in case 1 showing no fluid collection around the pancreas.

the portal and splenic veins were stripped off from the surrounding tissue and were exposed in order to remove the thrombus. However, the upper edge of the pancreatic head was injured during this process. The amylase level measured at the upper edge of the pancreatic drain was high on POD 1; therefore, the patient received octreotide on POD 2. On POD 5, the patient showed high fever and acute peritonitis. Therefore, an emergency exploratory laparotomy was performed. Because fluid collection was observed around the pancreas, drains were placed at both the right and left edges of the pancreas. On POD 21, the total output from the drain was 460 mL/d, and the amylase level of the drainage fluid was 166 700 IU/L. The patient was

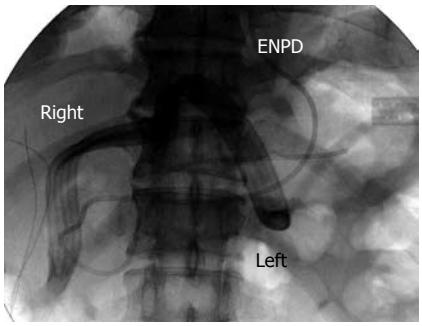

Figure 7 A radiograph showing postprocedure endoscopic naso-pancreatic drainage in case 2. The image shows drains placed at both the right and left edges of the pancreas (Right and Left) and endoscopic naso-pancreatic drainage (ENPD).

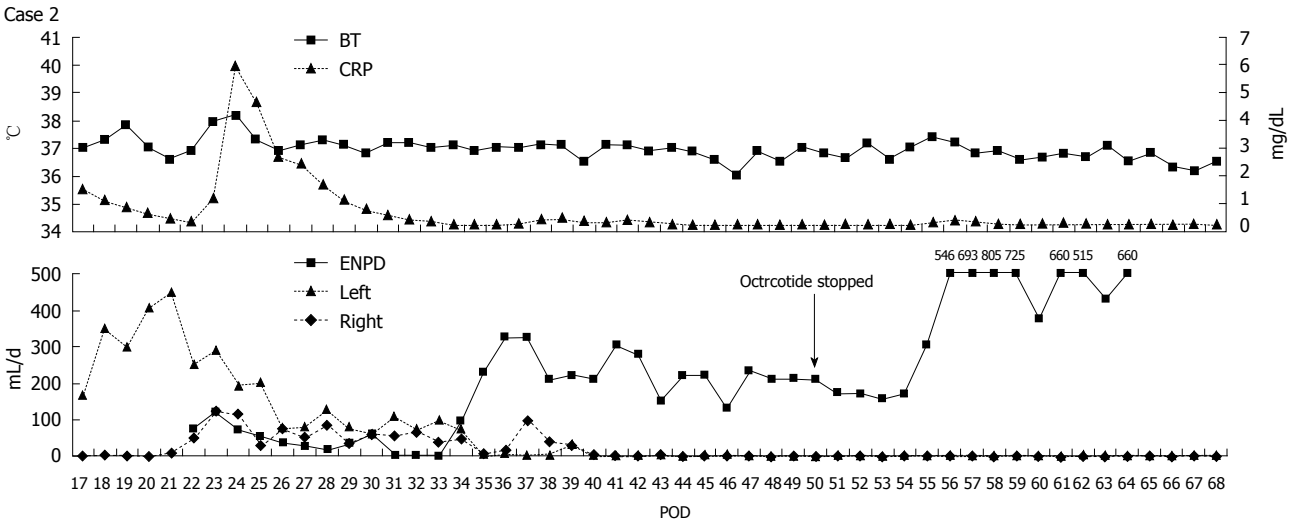

Figure 8 Upper chart shows the body temperature and serum C-reactive protein level. Lower one shows daily output of endoscopic naso-pancreatic drainage (ENPD) tube and the drains at both the edges of the pancreas (Right and Left) in case 2. The pancreatic fistula healed completely on post-ENPD day 38. BT: Body temperature; CRP: C-reactive protein; POD: Postoperative day.

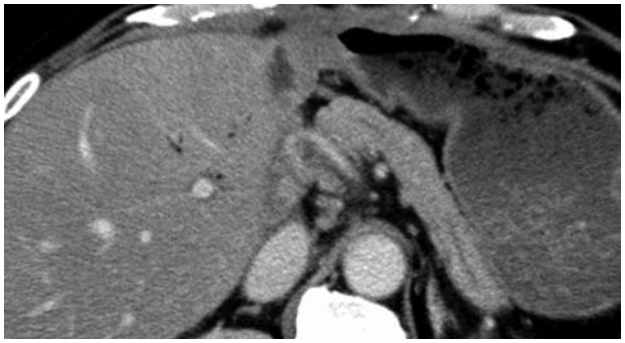

Figure 9 Computed tomography performed 14 d after removal of the drains in case 2 showing no fluid collection around the pancreas.

diagnosed with high-output pancreatic fistula, and ENPD was performed on POD 22 (Figure 7). The drain output decreased very rapidly (Figure 8); therefore, the patient was allowed to consume solid foods on POD 49, and octreotide administration was stopped on POD 50. The ENPD tube was removed on POD 65, and the drains placed at the right and left edges of the pancreas were removed on POD 68 and POD 70, respectively. The patient was discharged on POD 100 without abnormal fluid collection around the pancreas (Figure 9). The patient is well and receiving regular out-patient treatment and is showing no recurrence of pancreatic fistula.

DISCUSSION

Pancreatic fistula is primarily treated by conservative therapy, which includes rapid total infusion or enteral nutrition along with administration of octreotide. The recovery rate after conservative therapy ranges from 44% to 85%[6-8]; thus, a number of cases are not resolved by conservative treatment. Surgical treatment has been performed in such cases. However, surgical treatment is highly invasive and may lead to various complications. Further, surgical treatment is associated with high mortality rates, with the mortality rate being as high as 23%-67% in the cases showing early peritonitis after the operation[9]. Endoscopic drainage of the main pancreatic duct *via* the ampulla of Vater, which was first reported in 1991[10], has drawn considerable attention. Boerma *et al*[11] (2006) reported an excellent recovery rate (87%) after endoscopic treatment of 15 cases of pancreatic fistula. In addition, other studies

have reported recovery rates of about 58%-100% in the cases of pancreatic fistulas that do not respond to conservative therapy and involve endoscopic treatment[12-20]. To date, only 1 death caused by acute pancreatitis has been reported. However, since this death may also have been caused by inadequate drainage, a direct relationship between the death and endoscopic treatment could not be confirmed[13]. Unlike LDLT, endoscopic treatment for pancreatic fistula allows greater accessibility to the ampulla of Vater. Further, endoscopic treatment is less invasive than surgical treatment; therefore, it can easily replace conservative therapy if sufficient drainage is achieved. Thus, patients who undergo endoscopic treatment for pancreatic fistula can be expected to make an early recovery. Irrespective of their merits and demerits, both ENPD and endoscopic pancreatic stenting (EPS) have been referred to in the reports. ENPD causes a sense of discomfort in the pharynx; however, this technique enables easy diagnosis of occlusion and dropout because it allows monitoring of the pancreatic fluid. In contrast, in EPS the diagnosis of occlusion and dropout is difficult; however, this technique causes no sense of discomfort in the pharynx. We selected ENPD to enable safe monitoring of 2 channels of drainage: the endoscopic retrograde pancreatic drain as well as the intraperitoneal drain. In case 1, the drain tube had to be replaced because of the fever caused by occlusion; therefore, the choice of ENPD was considered to be reasonable. The patient in case 1 could have recovered earlier if the endoscopic treatment for pancreatic fistula had been initiated earlier. In each case, the patient recovered within approximately 40 d after ENPD. Further, the treatment had no influence on the patients' general status. Endoscopic treatment is considered to be safe for treating pancreatic fistulas that develop after LDLT. New endoscopic techniques, such as ultrasonography (US)-guided drainage, have also been used to treat refractory cases that do not respond to drainage via the ampulla of Vater; however, only few reports have described these techniques. These new techniques may also be less invasive than surgical treatment[21,22].

In conclusion, we described 2 cases of pancreatic fistula after LDLT that were not responsive to conservative therapy. In each case, the patient recovered within approximately 40 d after ENPD. Thus, endoscopic treatment for pancreatic fistula after LDLT should be adopted because of its high recovery rate and low invasiveness.

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Events Calendar 2011

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AGA Clinical Congress of Gastroenterology and Hepatology: Best Practices in 2011 Miami, FL 33101, United States

January 20-22, 2011
Gastrointestinal Cancers Symposium 2011, San Francisco, CA 94143, United States

January 27-28, 2011
Falk Workshop, Liver and Immunology, Medical University, Franz-Josef-Strauss-Allee 11, 93053 Regensburg, Germany

January 28-29, 2011
9. Gastro Forum München, Munich, Germany

February 4-5, 2011
13th Duesseldorf International Endoscopy Symposium, Duesseldorf, Germany

February 13-27, 2011
Gastroenterology: New Zealand CME Cruise Conference, Sydney, NSW, Australia

February 17-20, 2011
APASL 2011-The 21st Conference of the Asian Pacific Association for the Study of the Liver
Bangkok, Thailand

February 22, 2011-March 04, 2011
Canadian Digestive Diseases Week 2011, Vancouver, BC, Canada

February 24-26, 2011
Inflammatory Bowel Diseases 2011-6th Congress of the European Crohn's and Colitis Organisation, Dublin, Ireland

February 24-26, 2011
2nd International Congress on Abdominal Obesity, Buenos Aires, Brazil

February 24-26, 2011
International Colorectal Disease Symposium 2011, Hong Kong, China

February 26-March 1, 2011
Canadian Digestive Diseases Week, Westin Bayshore, Vancouver, British Columbia, Canada

February 28-March 1, 2011
Childhood & Adolescent Obesity: A whole-system strategic approach, Abu Dhabi, United Arab Emirates

March 3-5, 2011
42nd Annual Topics in Internal Medicine, Gainesville, FL 32614, United States

March 7-11, 2011
Infectious Diseases: Adult Issues in the Outpatient and Inpatient Settings, Sarasota, FL 34234, United States

March 14-17, 2011
British Society of Gastroenterology Annual Meeting 2011, Birmingham, England, United Kingdom

March 17-19, 2011
41. Kongress der Deutschen Gesellschaft für Endoskopie und Bildgebende Verfahren e.V., Munich, Germany

March 17-20, 2011
Mayo Clinic Gastroenterology & Hepatology 2011, Jacksonville, FL 34234, United States

March 18, 2011
UC Davis Health Informatics: Change Management and Health Informatics, The Keys to Health Reform, Sacramento, CA 94143, United States

March 25-27, 2011
MedicReS IC 2011 Good Medical Research, Istanbul, Turkey

March 26-27, 2011
26th Annual New Treatments in Chronic Liver Disease, San Diego, CA 94143, United States

April 6-7, 2011
IBS-A Global Perspective, Pfister Hotel, 424 East Wisconsin Avenue, Milwaukee, WI 53202, United States

April 7-9, 2011
International and Interdisciplinary Conference Excellence in Female Surgery, Florence, Italy

April 15-16, 2011
Falk Symposium 177, Endoscopy Live Berlin 2011 Intestinal Disease Meeting, Stauffenbergstr. 26, 10785 Berlin, Germany

April 18-22, 2011
Pediatric Emergency Medicine: Detection, Diagnosis and Developing Treatment Plans, Sarasota, FL 34234, United States

April 20-23, 2011
9th International Gastric Cancer Congress, COEX, World Trade Center, Samseong-dong, Gangnam-gu, Seoul 135-731, South Korea

April 25-27, 2011
The Second International Conference of the Saudi Society of Pediatric Gastroenterology, Hepatology & Nutrition, Riyadh, Saudi Arabia

April 25-29, 2011
Neurology Updates for Primary Care, Sarasota, FL 34230-6947, United States

April 28-30, 2011
4th Central European Congress of Surgery, Budapest, Hungary

May 7-10, 2011
Digestive Disease Week, Chicago, IL 60446, United States

May 12-13, 2011
2nd National Conference Clinical Advances in Cystic Fibrosis, London, England, United Kingdom

May 19-22, 2011
1st World Congress on Controversies in the Management of Viral Hepatitis (C-Hep), Palau de Congressos de Catalunya, Av. Diagonal, 661-671 Barcelona 08028, Spain

May 21-24, 2011
22nd European Society of Gastrointestinal and Abdominal Radiology Annual Meeting and Postgraduate Course, Venise, Italy

May 25-28, 2011
4th Congress of the Gastroenterology Association of Bosnia and Herzegovina with international participation, Hotel Holiday Inn, Sarajevo, Bosnia and Herzegovina

June 11-12, 2011
The International Digestive Disease Forum 2011, Hong Kong, China

June 13-16, 2011
Surgery and Disillusion XXIV SPIGC, II ESYS, Napoli, Italy

June 14-16, 2011
International Scientific Conference on Probiotics and Prebiotics-IPC2011, Kosice, Slovakia

June 22-25, 2011
ESMO Conference: 13th World Congress on Gastrointestinal Cancer, Barcelona, Spain

June 29-2, 2011
XI Congreso Interamericano de Pediatria "Monterrey 2011", Monterrey, Mexico

September 2-3, 2011 Falk Symposium 178, Diverticular Disease, A Fresh Approach to a Neglected Disease, Gürzenich Cologne, Martinstr. 29-37, 50667 Cologne, Germany

September 10-11, 2011
New Advances in Inflammatory Bowel Disease, La Jolla, CA 92093, United States

September 10-14, 2011
ICE 2011-International Congress of Endoscopy, Los Angeles Convention Center, 1201 South Figueroa Street Los Angeles, CA 90015, United States

September 30-October 1, 2011
Falk Symposium 179, Revisiting IBD Management: Dogmas to be Challenged, Sheraton Brussels Hotel, Place Rogier 3, 1210 Brussels, Belgium

October 19-29, 2011
Cardiology & Gastroenterology | Tahiti 10 night CME Cruise, Papeete, French Polynesia

October 22-26, 2011
19th United European Gastroenterology Week, Stockholm, Sweden

October 28-November 2, 2011
ACG Annual Scientific Meeting & Postgraduate Course, Washington, DC 20001, United States

November 11-12, 2011
Falk Symposium 180, IBD 2011: Progress and Future for Lifelong Management, ANA Interconti Hotel, 1-12-33 Akasaka, Minato-ku, Tokyo 107-0052, Japan

December 1-4, 2011
2011 Advances in Inflammatory Bowel Diseases/Crohn's & Colitis Foundation's Clinical & Research Conference, Hollywood, FL 34234, United States

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

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

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