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[1]Passed away on October 20, 2007
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EDITORIAL

Spontaneous bacterial peritonitis: A severe complication of liver cirrhosis

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Abstract

This report presents a survey of current knowledge concerning one of the relatively frequent and severe complications of liver cirrhosis and associated ascites-spontaneous bacterial peritonitis. Epidemiology, aetiology, pathogenesis, clinical manifestation, diagnosis and present possibilities of treatment are discussed.

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Key words: Liver cirrhosis; Portal hypertension; Ascites; Spontaneous bacterial peritonitis

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INTRODUCTION

Spontaneous bacterial peritonitis (SBP) is defined as an infection of initially sterile ascitic fluid (AF) without a detectable, surgically treatable source of infection[1]. It is a frequent and severe complication of cirrhotic ascites, first described in the middle of the 1960s[2]. Sometimes spontaneous infection of ascites is divided into three subgroups: (1) Spontaneous bacterial peritonitis is defined as a positive bacterial finding in ascites, together with increased polymorphonuclear leukocytes in ascites (> 250 cells/mm^3)[3]. Microorganisms responsible for SBP are isolated in 60%-70% of cases. (2) Culture-negative neutrocytic ascites (CNNA) - ascites is sterile, bacterial infection is not demonstrable by culturing, only an increased number of polymorphonuclear leukocytes above the limit of 250 cells/mm^3 is revealed. Of course, it is necessary to eliminate another cause of increased leukocytes in ascites, e.g. previous antibiotic therapy, hepatocellular carcinoma, peritoneal carcinomatosis or tuberculosis, pancreatitis or bleeding into ascitic fluid. If the sample of ascites contains blood, SBP diagnosis is made by finding more than one neutrophilic granulocyte per 250 erythrocytes. If left untreated, about one-third of cases can show, after some time, positive bacteriological findings. Both symptoms and mortality in patients with CNNA are similar to the course of disease in patients with diagnosed SBP; 33%-57% of these patients also show a positive blood culture, which provides evidence of a systemic bacterial infection. Infection is also confirmed by the fact that in patients with previous CNNA, there is a more frequent occurrence of SBP and *vice versa*[4]. (3) Monomicrobial non-neutrocytic bacterascites (or only bacterascites) has rarely been described. In this disorder, positive bacterial cultivation is presented without increased leukocytes. It is usually revealed in Child-Pugh class A patients. Recovery from bacterascites can be spontaneous (in 60%-80%), or it can develop into typical SBP. Bacterascites is often quite asymptomatic, and antibiotics should only be used if symptoms appear and cultivation finding is persistent.

As mentioned above, SBP and CNNA are identical, both from the clinical point of view and the therapeutic approach; therefore, the consensus conference of the

International Ascites Club[5] has recommended not to differentiate between these two entities; even in the case of CNNA, SBP is spoken about, and an increased number of neutrophils in ascites is sufficient for the diagnosis. A spontaneous infection complicating ascites may appear even in malignant ascites[6], however, it is found most often in cirrhotic ascites.

Early, mostly retrospective, studies described SBP in about 8% of patients with ascites; later prospective trials revealed SBP in 10%-30% of patients with ascites admitted to hospital[5,7,8]. SBP is found in about 5% of non-selected outpatients[9]. Lethality is very high. Older studies reported 80%-100% lethality connected with SBP[10], which was probably given partly by the generally worse therapeutic possibilities in cirrhotic patients and lack of availability of effective antibiotics, but better results - 20%-40% as reported in later studies[11] - are, to a certain extent, due to early diagnosis and treatment.

However, lethality has not decreased over recent years[12]. Even the long-term prognosis in these patients has been unfavourable. In 40%-70% of patients, SBP relapses within 1 year[11]. One-year survival after previous SBP patients is only 30%-40%, two-year survival is 20% and in patients with a Child-Pugh score > 10 survival is even lower[13].

AETIOLOGY

G^- bacteria are found in 65% of positive culture results- *Escherichia coli* (*E. coli*) and *Klebsiellae* are the most common and second most common agents, respectively[14]. The remaining agents are represented by G^+ cocci[1,7]. There is a difference in bacteria responsible for SBP in hospitalised and non-hospitalised patients. G^- bacteria prevail in the first group of patients, while G^+ bacteria are found in the latter[9].

Experiments modelling hepatic cirrhosis and the accompanying SBP in a rat resulted in discovery of agents in AF that differed from those in humans. It was mainly *Enterococcus faecalis* that was isolated from AF in the rat affected by cirrhosis induced by tetrachloromethane and SBP was of polymicrobial origin in one half of cases[15]. None of the above findings is typical of human patients suffering from SBP.

PATHOGENESIS

Bacteria participating in SBP come from the digestive tract. Extraintestinal bacteria such as those from the respiratory apparatus, urogenital tract or skin are much less frequent. Catheters and other equipment used during invasive procedures represent another possible source of infection. It is currently hypothesised that SBP follows an episode of bacteremia during which, due to the constant exchange of fluids between the peritoneal and intravascular space, AF gets infected[14]. The development of SBP thus depends on the antibacterial capacity of AF (the so-called opsonin activity) which is positively correlated to the content of the total protein in AF and the immunocompetence of the patient. The organism reacts to the infection of AF by activating neutrophilic granulocytes which migrate into the peritoneal cavity and trigger a complex cytokine cascade; the fact being documented, for example, by increased concentrations of interleukin 6 and tumour necrosis factor α in AF.

There are four key elements of SBP pathogenesis: (1) small intestinal bacterial overgrowth, (2) increased intestinal permeability, (3) bacterial translocation, and (4) immunosuppression. These key elements are not separate, but interlinked.

Small intestinal bacterial overgrowth (SIBO)

Considering bacteria commonly found in the gastro-intestinal tract, only some of them frequently participate in translocation. The most frequent bacteria are *E. coli*, *Proteus spp.*, *K. pneumoniae* and other *Enterobacteriaceae*, *Pseudomonas aeruginosa*, *enterococci*, *streptococci* and *staphylococci* - i.e. organisms causing infections in immunocompromised individuals. Bacterial small intestinal overgrowth creates conditions favourable for translocation of the above-mentioned bacteria. Liver cirrhosis is one of the conditions accompanied by SIBO. The main reasons for SIBO in patients affected by liver cirrhosis can be summarised as reduced intestinal passage, abnormalities in bile secretion, hypochlorhydria, abnormalities in IgA production and malnutrition[16]. A total of 20%-60% cirrhotic patients are affected by bacterial overgrowth. SBP is more often diagnosed in alcoholics affected by liver cirrhosis and SIBO as compared to patients affected in the same way but without SIBO[17]. Some authors are doubtful about the importance of SIBO in the pathogenesis of SBP[18]; according to this study, SIBO is associated with the use of drugs decreasing secretion of HCl and this therapeutic approach can increase the risk of SBP. Bacterial translocation found in a healthy individual after ingestion of extremely high numbers of microorganisms[19] can serve as a distant parallel of the relationship between SIBO and bacterial translocation.

Increased intestinal permeability

In severely ill patients, such as those with liver cirrhosis, small intestinal motility is impaired, resulting in bacterial overgrowth and the related translocation of microbes through dysfunctional mucosal barrier. The increased intestinal permeability and thus impaired function of the intestinal barrier are mainly due to portal hypertension. The consequences are dilated vessels in the intestinal mucosa, oedema of the lamina propria mucosae, fibromuscular proliferation, hypertrophy of the lamina muscularis mucosae and compromised integrity of the intestinal mucosa. Portal hypertension can play an important role, as can the toxic effects of alcohol, bile secretion disorders, malnutrition, decreased secretion of growth factors (insulin-like growth factor I), changes in the composition and flow of bile, or higher levels of nitric oxide. Increased intestinal permeability is likely to be proportional to the degree of portal hypertension, but independent of the severity and aetiology of liver disease[20,21].

Bacterial translocation

The term bacterial translocation was first used in 1979[22].

Bacterial translocation is defined as either active or passive penetration of living microorganisms and their toxic products through the mucosal epithelial layer to the lamina propria mucosae. From there, microbes migrate to mesenteric lymph nodes and/or extraintestinal sites. Under normal circumstances, there are only small numbers of bacteria readily destroyed by the immune system in the lamina propria mucosae. Translocation is only possible if their numbers are high, up to 10^8 bacteria in 1 g of faeces[17].

According to clinical significance, there are four degrees of bacterial translocation. Degree 0: Bacteria and/or their components penetrate the mucosa by various mechanisms: active intracellular penetration, diffusion, absorption, endocytosis or phagocytosis by macrophages; Degree 1: Bacteria and/or their components enter the mesenteric lymphatic system and penetrate it centripetally; Degree 2: Bacteria and/or their components are already detectable in the systemic circulation and certain organs. They may also pass directly into small intestinal venules and from there into the portal circulation. Some of the bacteria are probably even capable of intracellular passage through the muscularis propria into the peritoneal cavity; Degree 3: The organism is systemically overwhelmed by bacteria and/or their components, leading to a septic response.

Bacteria escaping both phagocytosis and destruction by the complement system may even get into the bloodstream. *Enterobacteria*, *staphylococci* and *enterococci* are capable of translocation, i.e. passing live across the intestinal epithelium to the mesenteric lymph nodes, blood and other organs, whereas most anaerobic organisms lack this ability. Bacterial translocation may be confirmed by positive culture from the mesenteric lymph nodes. The main mechanisms underlying the translocation are deficient local mucosal immune response, lower phagocytic activity of both macrophages and neutrophils, increased permeability of the intestinal barrier and the above-mentioned intestinal bacterial overgrowth[16,17].

Immunosuppression

Patients with liver cirrhosis suffer from decreased phagocytic activity of neutrophilic granulocytes and the mononuclear phagocytic system, deteriorated humoral immunity and decreased opsonin activity of AF[23]. Neutrophilic granulocytes of patients with hepatic cirrhosis show not only a decrease in the phagocytic activity, but also intracellular destruction of bacteria, deteriorated metabolic activities, frequent apoptosis and considerably reduced chemotaxis. For proper protection against bacteria, neutrophilic granulocytes have first to adhere to the vascular endothelium, then migrate to the endothelial cellular junctions, pass through by diapedesis and migrate further into the target tissue. Neutrophilic granulocytes of cirrhotic patients adhere to the vascular endothelium to a greater extent and their transendothelial migration is thus decreased[24]. Neutrophilic granulocytes show decreased chemotaxis probably due to the presence of inhibitors of chemotaxis in the blood plasma of the cirrhotic.

The opsonin activity of AF correlates with the concentration of immunoglobulins, complement, fibronectin and total protein in AF[5,25]. Patients with a reduced total protein content in AF are prone to the development of SBP.

CLINICAL MANIFESTATION

SBP is particularly revealed in patients with more severe liver functional damage (Child-Pugh classification C), often after bleeding from the upper gastrointestinal tract due to portal hypertension and often recurs. The clinical picture is non-specific. In a lot of cases the infection is quite asymptomatic, common signs - subfebrile states, diffuse abdominal pain - are not very conspicuous. They are frequently manifested only by the occurrence or deepening of symptoms that accompany the course of liver cirrhosis - increased ascites and failure of diuretic therapy, deteriorated encephalopathy, vomiting, *etc*. Therefore, an active search for the ascites infection is necessary.

Diagnostic paracentesis with leukocyte investigation is recommended in all patients with ascites admitted to hospital as well as in cirrhotics (whether in hospital or not) with worsened ascites, who have presented with signs of abdominal or systemic infection (abdominal pain or tenderness, disturbed intestinal passage, fever, acidosis, peripheral leukocytosis) or with encephalopathy or worsened renal functions[5]. An active approach to the SBP diagnosis is extraordinarily important even from the prognostic point of view. If SBP is diagnosed at the first paracentesis carried out in all patients hospitalised with ascites, this infection has no influence on the patient's prognosis. However, if SBP appears over the course of studying these patients, the risk of lethality increases twofold[26]. This can probably be explained by the damaged renal function under a longer-lasting untreated infection.

High lethality is not primarily associated with the severity of the infection, and patients do not die of sepsis. An infection only worsens the changes present in cirrhotic patients, especially blood supply and renal function[27]. Splanchnic vasodilatation occurring in cirrhotics is worsened due to endotoxemia; effective arterial blood volume decreases which can damage renal function and can cause hepatorenal syndrome. Patients who showed a higher level of urea and higher portal pressure at the moment of diagnosis of an infection are threatened with renal failure and the associated high lethality[28].

DIAGNOSIS

Diagnosis is relatively easy. SBP is diagnosed by revealing polymorphonuclear leukocytes in ascites > 250 cells/mm^3 (less accurately at the increase of all leukocytes over 400/mm^3). Reagent papers used for examining leukocytes in urine could be used for the immediate detection of leukocytes in ascites[29] although the sensitivity is not sufficient. A positive cultivation finding is not necessary for diagnosing SBP, it is usually revealed in about 30% of cases. Some trials have mentioned a higher bacteriological

detection if ascites is inoculated on the medium at the bedside as it is carried out for the investigation of blood culture - BactAlert test[30]- however, the advantage of this test has not been demonstrated by other studies[8,31].

In the case of diagnostic doubts, the serum procalcitonin level (the limit of 0.75 ng/mL has 95% sensitivity, and 98% specificity) or the interleukin 6 level in ascites (the limit of 5.0 ng/mL has 100% sensitivity) may be determined[32]. Nowadays, examination of the ascites pH (for the diagnosis of SBP < 7.35) or arterial: ascites pH gradient (> 0.1) as recommended previously is no longer used.

TREATMENT

Therapy should be initiated immediately after revealing increased leukocytes in ascites. Empiric antibiotic therapy, e.g. an intravenous third-generation cephalosporin, preferably cefotaxime in a single dose of 2 g is the recommended antibiotic drug. Mostly, it is sufficient to administer cefotaxime every 8 h as this regimen is as effective as its administration every 6 h[33]. Even a shorter length of application is possible, cefotaxime administered every 8 h for 5 d has the same effects as its 10 d application and from a practical and financial point of view, this regimen seems to be the most suitable. Two 3rd generation cephalosporins may alternatively be used-cefonicid (2 g every 12 h) or ceftriaxone 2 g/d[34]. In uncomplicated SBP (i.e. if diagnosed during preventive examination of ascites without clinical signs of infection) a combination of amoxicillin, clavulanic acid[35], or ofloxacin 400 mg may be administered every 12 h for a period of 7-10 d[36]. Although more than 10 therapeutic studies have been published since 1985, clear proof of the efficacy of these antibiotics supported by evidence-based medicine is still missing, and treatment is based more on clinical experience[37]. Moreover, there are reports on increasing occurrence of enterobacteria resistant to the 3rd generation cephalosporins[38], therefore, a large, well-designed randomised trial is necessary to ensure explicit demonstration of the effectiveness of each antibiotic drug.

As mentioned above, the high lethality is associated with damaged renal function due to impaired hypovolemia. Therefore, the efforts of expansion of intravascular volume could be contributory, and some studies have demonstrated that simultaneous application of albumin (as plasma expander) at a dose of 1.5 g/kg during the first 6 h and 1 g/kg on the 3rd d together with antibiotics decreased both the occurrence of renal damage and lethality[39]. This albumin replacement is recommended in patients with even clinical suspicion of SBP and serum creatinine > 1 mg/dL, blood urea nitrogen > 30 mg/dL, or total bilirubin > 4 mg/dL.

Preventive treatment

Three groups of patients are at higher risk of SBP-patients with gastrointestinal bleeding, patients who have survived an episode of SBP and patients with low opsonic activity of ascites.

Preventive administration of antibiotics is recommended in the first two groups. Intravenous ceftriaxone for 7 d or norfloxacin 400 mg twice a day for 7 d should be administered to prevent bacterial infections in patients with cirrhosis and gastrointestinal bleeding. Patients who have survived an episode of SBP should receive long-term prophylaxis with daily norfloxacin (400 mg once a day)[40] or trimethoprim/sulfamethoxazole. In these patients, liver transplantation should be considered as the lethality after passed SBP is higher than that after liver transplantation[5,13]. If transplantation is indicated, antibiotics should be applied until the operation. If transplantation is not indicated, according to literature, they should be applied for the rest of the patient's life. However, in clinical practice, an individual approach to each patient has been recommended.

Preventive application of antibiotics in the last group of patients at higher risk of infection - patients with the ascitic fluid protein < 15 g/L (i.e. with low opsonin activity) has not been generally recommended so far[41]. Long-term use of norfloxacin (or trimethoprim/sulfamethoxazole) can be justified in these patients if at least one of the following is present together with a low level of protein: serum creatinine > 1.2 mg/dL, blood urea nitrogen > 25 mg/dL, serum sodium < 130 mEq/L or Child-Pugh > 9 points with bilirubin > 3 mg/dL[42].

Intraperitoneal re-infusion of concentrated ascites can effectively increase the protein level and opsonic activity of ascites[43]. On the other hand, it is not clear if just this procedure can reduce the risk of SBP in patients with low ascitic fluid protein.

Reports on possible influencing of bacterial translocation (and thus the development of SBP) using prokinetics[44,45] or probiotics[46] have appeared in the literature.

CONCLUSION

Spontaneous infection of ascites is a very frequent and severe complication of ascites occurring in a high number of patients admitted to hospital with ascites. This infection must be actively searched for. Diagnostic paracentesis must be performed with examination of polymorphonuclear leukocytes in ascites in all patients suffering from ascites at the moment of their admission to hospital as well as in all cirrhotics with a sudden increase of ascites or worsening of their general condition.

If the number of polymorphonuclear leukocytes in ascites exceeds 250 cells/mm^3, a positive bacteriological finding is not necessary, SBP is diagnosed and cefotaxime at the minimum dose of 2 g every 8 h for 5 d is administered. If patient has no clinical symptoms and SBP was diagnosed during preventive examination, ofloxacin 400 mg every 12 h for 7-10 d is an alternative. Although more studies are lacking, prevention of the development of simultaneous renal damage due to severe hypovolemia by administration of plasma expander (albumin at a dose of 1.5 g/kg during the first 6 h, and 1 g/kg on the third day) seems to decrease lethality.

Preventive application of antibiotics is indicated in two groups of patients with a high risk of developing SBP. Norfloxacin 400 mg twice a day is recommended

in patients with bleeding from the upper gastrointestinal tract and portal hypertension. The other group involves patients with previous SBP. As secondary prevention, norfloxacin 400 mg once a day is administered. Liver transplantation is recommended and antibiotics are applied until the surgery. Long-term administration (lifelong according to literature) is indicated in patients who are not indicated for transplantation.

REFERENCES

1 **Dore MP**, Casu M, Realdi G, Piana A, Mura I. Helicobacter infection and spontaneous bacterial peritonitis. *J Clin Microbiol* 2002; **40**: 1121

2 **Conn HO**. Spontaneous peritonitis and bacteremia in Laennec's cirrhosis caused by enteric organisms. A relatively common but rarely recognized syndrome. *Ann Intern Med* 1964; **60**: 568-580

3 **Hoefs JC**, Canawati HN, Sapico FL, Hopkins RR, Weiner J, Montgomerie JZ. Spontaneous bacterial peritonitis. *Hepatology* 1982; **2**: 399-407

4 **Runyon BA**, Hoefs JC. Culture-negative neutrocytic ascites: a variant of spontaneous bacterial peritonitis. *Hepatology* 1984; **4**: 1209-1211

5 **Rimola A**, García-Tsao G, Navasa M, Piddock LJ, Planas R, Bernard B, Inadomi JM. Diagnosis, treatment and prophylaxis of spontaneous bacterial peritonitis: a consensus document. International Ascites Club. *J Hepatol* 2000; **32**: 142-153

6 **Makharia GK**, Sharma BC, Bhasin DK, Singh K. Spontaneous bacterial peritonitis in a patient with gastric carcinoma. *J Clin Gastroenterol* 1998; **27**: 269-270

7 **Thanopoulou AC**, Koskinas JS, Hadziyannis SJ. Spontaneous bacterial peritonitis (SBP): clinical, laboratory, and prognostic features. A single-center experience. *Eur J Intern Med* 2002; **13**: 194-198

8 **Lata J**, Fejfar T, Krechler T, Musil T, Husová L, Senkyrík M, Dolina J, Vanasek T. Spontaneous bacterial peritonitis in the Czech Republic: prevalence and aetiology. *Eur J Gastroenterol Hepatol* 2003; **15**: 739-743

9 **Evans LT**, Kim WR, Poterucha JJ, Kamath PS. Spontaneous bacterial peritonitis in asymptomatic outpatients with cirrhotic ascites. *Hepatology* 2003; **37**: 897-901

10 **Hoefs JC**, Runyon BA. Spontaneous bacterial peritonitis. *Dis Mon* 1985; **31**: 1-48

11 **Andreu M**, Sola R, Sitges-Serra A, Alia C, Gallen M, Vila MC, Coll S, Oliver MI. Risk factors for spontaneous bacterial peritonitis in cirrhotic patients with ascites. *Gastroenterology* 1993; **104**: 1133-1138

12 **Khan J**, Pikkarainen P, Karvonen AL, Mäkelä T, Peräaho M, Pehkonen E, Collin P. Ascites: Aetiology, mortality and the prevalence of spontaneous bacterial peritonitis. *Scand J Gastroenterol* 2009; **44**: 970-974

13 **Altman C**, Grangé JD, Amiot X, Pelletier G, Lacaine F, Bodin F, Etienne JP. Survival after a first episode of spontaneous bacterial peritonitis. Prognosis of potential candidates for orthotopic liver transplantation. *J Gastroenterol Hepatol* 1995; **10**: 47-50

14 **Fernández J**, Bauer TM, Navasa M, Rodés J. Diagnosis, treatment and prevention of spontaneous bacterial peritonitis. *Baillieres Best Pract Res Clin Gastroenterol* 2000; **14**: 975-990

15 **Llovet JM**, Bartolí R, March F, Planas R, Viñado B, Cabré E, Arnal J, Coll P, Ausina V, Gassull MA. Translocated intestinal bacteria cause spontaneous bacterial peritonitis in cirrhotic rats: molecular epidemiologic evidence. *J Hepatol* 1998; **28**: 307-313

16 **Ramachandran A**, Balasubramanian KA. Intestinal dysfunction in liver cirrhosis: Its role in spontaneous bacterial peritonitis. *J Gastroenterol Hepatol* 2001; **16**: 607-612

17 **Guarner C**, Runyon BA, Young S, Heck M, Sheikh MY. Intestinal bacterial overgrowth and bacterial translocation in cirrhotic rats with ascites. *J Hepatol* 1997; **26**: 1372-1378

18 **Bauer TM**, Steinbrückner B, Brinkmann FE, Ditzen AK, Schwacha H, Aponte JJ, Pelz K, Kist M, Blum HE. Small intestinal bacterial overgrowth in patients with cirrhosis: prevalence and relation with spontaneous bacterial peritonitis. *Am J Gastroenterol* 2001; **96**: 2962-2967

19 **Wells CL**, Maddaus MA, Simmons RL. Bacterial translocation. In: Marston A, Bulkley GB, Fiddian-Green RG, Haglund UH, editors. Splanchnic ischemia and multiple organ failure. Mosby: St. Louis, 1989: 195-204

20 **Ersöz G**, Aydin A, Erdem S, Yüksel D, Akarca U, Kumanlioglu K. Intestinal permeability in liver cirrhosis. *Eur J Gastroenterol Hepatol* 1999; **11**: 409-412

21 **Goulis J**, Patch D, Burroughs AK. Bacterial infection in the pathogenesis of variceal bleeding. *Lancet* 1999; **353**: 139-142

22 **Berg RD**, Garlington AW. Translocation of certain indigenous bacteria from the gastrointestinal tract to the mesenteric lymph nodes and other organs in a gnotobiotic mouse model. *Infect Immun* 1979; **23**: 403-411

23 **Cereto F**, Molina I, González A, Del Valle O, Esteban R, Guardia J, Genescà J. Role of immunosuppression in the development of quinolone-resistant Escherichia coli spontaneous bacterial peritonitis and in the mortality of E. coli spontaneous bacterial peritonitis. *Aliment Pharmacol Ther* 2003; **17**: 695-701

24 **Fiuza C**, Salcedo M, Clemente G, Tellado JM. Granulocyte colony-stimulating factor improves deficient in vitro neutrophil transendothelial migration in patients with advanced liver disease. *Clin Diagn Lab Immunol* 2002; **9**: 433-439

25 **Deschénes M**, Villeneuve JP. Risk factors for the development of bacterial infections in hospitalized patients with cirrhosis. *Am J Gastroenterol* 1999; **94**: 2193-2197

26 **Jepsen P**, Vilstrup H, Møller JK, Sørensen HT. Prognosis of patients with liver cirrhosis and spontaneous bacterial peritonitis. *Hepatogastroenterology* 2003; **50**: 2133-2136

27 **Coral G**, Mattos A. Renal impairment after spontaneous bacterial peritonititis: incidence a prognosis. *J Gastroent Hepatol* 2002; **17** Suppl: A915

28 **Ruiz-del-Arbol L**, Urman J, Fernández J, González M, Navasa M, Monescillo A, Albillos A, Jiménez W, Arroyo V. Systemic, renal, and hepatic hemodynamic derangement in cirrhotic patients with spontaneous bacterial peritonitis. *Hepatology* 2003; **38**: 1210-1218

29 **Castellote J**, López C, Gornals J, Tremosa G, Fariña ER, Baliellas C, Domingo A, Xiol X. Rapid diagnosis of spontaneous bacterial peritonitis by use of reagent strips. *Hepatology* 2003; **37**: 893-896

30 **Ortiz J**, Soriano G, Coll P, Novella MT, Pericas R, Sàbat M, Sánchez F, Guarner C, Prats G, Vilardell F. Early microbiologic diagnosis of spontaneous bacterial peritonitis with BacT/ALERT. *J Hepatol* 1997; **26**: 839-844

31 **Viguier J**, d'Alteroche L, Bacq Y, Loulergue A, Audurier A, Metman EH. Spontaneous bacterial peritonitits: comparison of two ascitic fluid (AF) culture methods [Abstract]. *Gut* 1999; **45** Suppl V: A217

32 **Viallon A**, Zeni F, Pouzet V, Lambert C, Quenet S, Aubert G, Guyomarch S, Tardy B, Bertrand JC. Serum and ascitic procalcitonin levels in cirrhotic patients with spontaneous bacterial peritonitis: diagnostic value and relationship to pro-inflammatory cytokines. *Intensive Care Med* 2000; **26**: 1082-1088

33 **Rimola A**, Salmerón JM, Clemente G, Rodrigo L, Obrador A, Miranda ML, Guarner C, Planas R, Solá R, Vargas V. Two different dosages of cefotaxime in the treatment of spontaneous bacterial peritonitis in cirrhosis: results of a prospective, randomized, multicenter study. *Hepatology* 1995; **21**: 674-679

34 **França A**, Giordano HM, Sevá-Pereira T, Soares EC. Five days of ceftriaxone to treat spontaneous bacterial peritonitis in cirrhotic patients. *J Gastroenterol* 2002; **37**: 119-122

35 **Ricart E**, Soriano G, Novella MT, Ortiz J, Sàbat M, Kolle L, Sola-Vera J, Miñana J, Dedéu JM, Gómez C, Barrio JL, Guarner C. Amoxicillin-clavulanic acid versus cefotaxime

in the therapy of bacterial infections in cirrhotic patients. *J Hepatol* 2000; **32**: 596-602

36 **Navasa M**, Follo A, Llovet JM, Clemente G, Vargas V, Rimola A, Marco F, Guarner C, Forné M, Planas R, Bañares R, Castells L, Jimenez De Anta MT, Arroyo V, Rodés J. Randomized, comparative study of oral ofloxacin versus intravenous cefotaxime in spontaneous bacterial peritonitis. *Gastroenterology* 1996; **111**: 1011-1017

37 **Soares-Weiser K**, Paul M, Brezis M, Leibovici L. Evidence based case report. Antibiotic treatment for spontaneous bacterial peritonitis. *BMJ* 2002; **324**: 100-102

38 **Dupeyron C**, Campillo B, Mangeney N, Richardet JP, Leluan G. Changes in nature and antibiotic resistance of bacteria causing peritonitis in cirrhotic patients over a 20 year period. *J Clin Pathol* 1998; **51**: 614-616

39 **Sort P**, Navasa M, Arroyo V, Aldeguer X, Planas R, Ruiz-del-Arbol L, Castells L, Vargas V, Soriano G, Guevara M, Ginès P, Rodés J. Effect of intravenous albumin on renal impairment and mortality in patients with cirrhosis and spontaneous bacterial peritonitis. *N Engl J Med* 1999; **341**: 403-409

40 **Garcia-Tsao G**. Current management of the complications of cirrhosis and portal hypertension: variceal hemorrhage, ascites, and spontaneous bacterial peritonitis. *Gastroenterology* 2001; **120**: 726-748

41 **Cohen MJ**, Sahar T, Benenson S, Elinav E, Brezis M, Soares-Weiser K. Antibiotic prophylaxis for spontaneous bacterial peritonitis in cirrhotic patients with ascites, without gastro-intestinal bleeding. *Cochrane Database Syst Rev* 2009; CD004791

42 **Runyon BA**. Management of adult patients with ascites due to cirrhosis: an update. *Hepatology* 2009; **49**: 2087-2107

43 **Lata J**, Kuklínek P, Husová L, Novotný I, Prášek J: Paracentesis vs intraperitoneal reinfusion of concentrated ascites-effect on opsonic activity of ascites. *Eur J Intern Med* 1999; **10**: 209-213

44 **Pardo A**, Bartolí R, Lorenzo-Zúñiga V, Planas R, Viñado B, Riba J, Cabré E, Santos J, Luque T, Ausina V, Gassull MA. Effect of cisapride on intestinal bacterial overgrowth and bacterial translocation in cirrhosis. *Hepatology* 2000; **31**: 858-863

45 **Zhang S**, Ren W, Zhou K, Wang J, Zhu W. The effect of prokinetic drug on small intestinal bacterial overgrowth and endotoxemia in cirrhosis. *J Gastroent Hepatol* 2002; **17** Suppl: A12

46 **Lata J**, Novotný I, Príbramská V, Juránková J, Fric P, Kroupa R, Stibůrek O. The effect of probiotics on gut flora, level of endotoxin and Child-Pugh score in cirrhotic patients: results of a double-blind randomized study. *Eur J Gastroenterol Hepatol* 2007; **19**: 1111-1113

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TOPIC HIGHLIGHT

Dr. Shahid A Khan, *Series Editor*

Liver transplantation for hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is the commonest primary malignancy of the liver. It usually occurs in the setting of chronic liver disease and has a poor prognosis if untreated. Orthotopic liver transplantation (OLT) is a suitable therapeutic option for early, unresectable HCC particularly in the setting of chronic liver disease. Following on from disappointing initial results, the seminal study by Mazzaferro *et al* in 1996 established OLT as a viable treatment for HCC. In this study, the "Milan criteria" were applied achieving a 4-year survival rate similar to OLT for benign disease. Since then various groups have attempted to expand these criteria whilst maintaining long term survival rates. The technique of living donor liver transplantation has evolved over the past decade, particularly in Asia, and published outcome data is comparable to that of OLT. This article will review the evidence, indications, and the future direction of liver transplantation for liver cancer.

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Key words: Hepatocellular carcinoma; Selection criteria; Liver transplantation; Living donors

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the third most common cause of cancer death and the fifth most prevalent cancer in the world[1]. Cirrhosis itself is the major risk factor for the development of HCC[2-6]. Overall, 80%-90% of HCC develop in the context of liver cirrhosis[7]. Hepatitis B infection (HBV) particularly, can cause HCC in the absence of cirrhosis as a result of its direct carcinogenic effect[8]. Hepatitis B is the commonest cause of chronic liver disease in the East, whereas hepatitis C (HCV) and alcohol predominate in the West[9]. This has resulted in widespread geographical variation in HCC prevalence. In particular, in Asia and sub-Saharan Africa the incidence is far higher than in North America and Europe. In our Unit in London, 70% of patients with HCC on the background of liver disease have underlying chronic viral hepatitis; 40% have HCV, 30% HBV and a minority have a combined etiology including viral, steatohepatitis and/or alcoholic chronic liver disease.

The incidence of HCC is increasing in many parts of the world[10]. In the United States, for example, the rate of HCC has increased by 80% over the past 20 years[11]. This may be partly due to the fact that screening for HCC in patients with cirrhosis has become established practice. However, in our Unit over the past 5 years, only 55% of patients with HCC had their cancer detected as part of a screening program. Another likely reason for rising HCC incidence is the increasing burden of chronic viral hepatitis. In addition to HBV and HCV, both fatty liver disease and alcohol-related liver disease continue to rise. As a result, it is predicted that HCC prevalence will increase further over the coming decades.

Without specific treatment the prognosis is poor with median survival of early and advanced HCC being 6-9 mo and 1-2 mo, respectively[12]. Tumor resection in the context of cirrhosis and portal hypertension

leaves a residual cirrhotic liver which remains at risk of developing new lesions and a liver which is at risk of decompensation. Recurrence rates of 50%-70% have been reported after resection of HCC[13]. Many patients have advanced cirrhosis at presentation and are not eligible for resection. In addition, HCC is often multifocal and thus resection is not feasible.

EVOLUTION

The initial liver transplants were performed by Starlz in 1963[14]. Four years elapsed before there was a long term survivor[15]. Over the subsequent decades advances in organ preservation and particularly prevention of organ rejection led to a steady rise in both transplant numbers and outcomes. With the widespread acceptance of OLT, transplantation for HCC was attempted. Unfortunately, the initial results of OLT for HCC showed disappointing short and long term survival rates and high levels of tumor recurrence[16].

Given the huge demand for organs, transplantation needed to be limited to those with a good prognosis, and liver transplantation continued to flourish as the definitive treatment of end stage liver disease. A change in thinking arose from the following two observations. Firstly, finding small unexpected HCC in explanted livers did not affect the outcome of OLT[17]. Secondly, there was no significant difference in terms of overall survival between liver transplantation and resection in cirrhotic patients with hepatocellular carcinoma[18]. Furthermore, in small tumors, < 3 cm, whether uni- or bi-nodular, transplantation resulted in better survival than resection. This culminated in the development of the "Milan criteria" which has cemented OLT as an effective treatment for HCC.

MILAN CRITERIA

The seminal study by Mazzaferro *et al*[19] in 1996 established OLT as a viable treatment for HCC. In this study in Milan, Italy, 48 patients with HCC and cirrhosis were followed and transplanted. Twenty eight patients had preoperative therapy, mainly transarterial chemoembolization (TACE). All patients fulfilled the following restrictive radiographic criteria: single lesion ⩽ 5 cm, up to 3 separate lesions all less than 3 cm, no evidence of vascular invasion, no nodal or distant metastases. The aim of the criteria was to achieve a good prognosis in those who fulfilled them, avoiding a poor prognosis in those who exceeded them. The overall actuarial survival after 26 mo was 75% and recurrence-free survival was 83%. Patients who fulfilled these criteria on the basis of explant pathology had actuarial survival of 85% and recurrence-free survival of 92%. These rates are not dissimilar to the outcome of patients undergoing OLT without HCC. These criteria have become known as the "Milan criteria" and these results have been confirmed throughout the world.

EXPANDED CRITERIA

Since the acceptance of OLT for the treatment of HCC, excellent post-transplant survival has been reported in many centers. With increasing experience of OLT many centers have recognized that many explanted livers have had HCC beyond the size described in the "Milan criteria". These transplanted patients have been followed over time and many have had excellent 1- and 5-year survival. Also, advances in imaging have occurred in the past decade since these criteria were originally published. As a result several groups have attempted to broaden the restrictive limits. A group from the University of California at San Francisco (UCSF) have reported a 5-year survival of 75% using less restrictive criteria derived by retrospectively analyzing 2000 patients undergoing OLT[20]. This was achieved by using the following criteria: a maximum tumor size 6.5 cm or 2 lesions < 4.5 cm diameter with a total tumor diameter < 8 cm. The same group validated the 'San Francisco criteria' on 168 patients based on preoperative imaging assessments[21]. Various other groups have published expanded criteria with results not dissimilar to the original "Milan criteria"[22-28]. The same group in Milan has recently published retrospective data regarding outcome in 1112 patients exceeding the original Milan criteria[29]. In this study, a 71.2% 5-year overall survival could be achieved using the "up-to-7" criteria (HCC with 7 as the sum of the largest tumor (cm) and the number of tumors). It is clear that the larger the tumor size and number, the worse the outcome. Tumor understaging, by preoperative imaging of patients has been one of the major concerns for liberalizing the "Milan criteria"[30-32]. Also, there can be significant tumor progression during the waiting time to transplantation[33]. At the present time, the "Milan criteria" remains the only universally accepted and recurrently validated criteria.

COMPARISON OF OLT TO OTHER TREATMENT STRATEGIES

Various treatment options are available for HCC including the surgical options as outlined above, and local ablative techniques such as radiofrequency ablation (RFA), transarterial chemoembolization (TACE), and percutaneous ethanol injection (PEI). Systemic chemotherapy has been unsuccessful in the studies thus far[34]. The local ablative treatments themselves are used as a palliative procedure or as a bridge to resection or transplantation. The only curative treatments are that of hepatic resection or transplantation.

The published observational studies to date suggest survival following OLT is at least as good as following resection in patients with adequate hepatic reserve[18]. Liver resection can leave residual liver which is of insufficient size to provide adequate function and as stated before, has the possibility of developing further lesions. In patients with well-compensated cirrhosis (Child Pugh A) and HCC, the decision whether to resect or transplant remains controversial. However, in the current realm of organ shortage and long waiting times, the decision to resect in this group appears attractive.

If tumor recurrence were to recur than salvage transplantation can be performed. This strategy has been retrospectively analyzed by different groups with conflicting outcome data. One retrospective study has shown no difference in either outcome or disease-free survival when comparing primary OLT to resection and salvage OLT[35]. In contrast, an observational series has shown primary OLT to have lower operative mortality, recurrence rates and survival rates[36].

REQUIREMENTS FOR LISTING

The United Network for Organ Sharing (UNOS) administers the allocation of organs for transplantation in the United States. UNOS provides a set of specific requirements[37] for listing patients with HCC: (1) Rough evaluation of the number and size of tumors and to rule out extra-hepatic spread by ultrasound, computed tomography (CT) or magnetic resonance imaging (MRI) plus CT of the chest; (2) Prelisting biopsy is not mandatory, however patients must have one of the following: (a) a biopsy confirming HCC; (b) a vascular blush corresponding to the area of suspected HCC; (c) an α fetoprotein (AFP) > 200 mg/mL; (d) an arteriogram confirming a tumor; (e) a history of local ablative therapy (TACE, RFA, PEI); (3) Patients with chronic liver disease and a rising AFP > 500 mg/dL can also be listed even in the absence of discrete tumor on imaging studies; (4) The patient must not be a resection candidate; (5) Reimaging by CT or MRI every 3 mo is required to ensure continued eligibility for OLT.

BRIDGING THERAPY

The "dropout" due to tumor progression whilst waiting for OLT is reported to be at least 20%[20]. This problem has furthered the use of local-regional adjuvant therapy (LAT) whilst awaiting transplantation such as TACE, RFA, and PEI. Unfortunately no randomized, controlled trials have been conducted to assess this approach[38]. As a result, there is no universal consensus as to the optimum bridging therapy prior to transplantation.

The ultimate aim of LAT is to provide complete tumor necrosis in an attempt to halt tumor progression. Analyses of explant specimens subjected to RFA and TACE have shown complete tumor necrosis rates of 47%-66% and 16%-27%, respectively[30,39,40]. A retrospective study looking at tumor necrosis in 61 patients did not find any particular modality of LAT to be superior[41].

Retrospective analyses have shown that multimodality local treatment is associated with a modest survival benefit[42,43]. As a result most units offer dual as well as single modality ablative treatments, in an attempt to reduce tumor progression and recurrence rates. Hepatic resection has also been used as a bridge to transplantation[44]. This is in contrast to the traditional view of resection and transplantation being opposing strategies. Unlike LAT which has been shown in studies to achieve only partial tumor necrosis, resection should achieve the best tumor control. Resection also allows a thorough intra-operative assessment of liver status and tumor burden. A detailed histological analysis can be made of the resected specimen and can give invaluable information about the natural history of the tumor and the presence of microvascular invasion. Resection, however, is associated with increased risk and, as stated earlier, should only be attempted in well-compensated cirrhotic patients.

PROGNOSTIC FACTORS

Conforming to the "Milan criteria", in terms of tumor size and tumor burden, gives rise to 3-4-year recurrence-free survival rates of up to 92%[45-47]. Multivariate analysis has shown these to be the only independent variables predicting patient survival and tumor recurrence. Other biological factors such as tumor grading, microvascular invasion and microsatellites appear to play a role, but within the constraints of the size and number burden. These biological factors can only be accurately assessed post-transplantation or post-resection. The histological grade of the tumor can be assessed by lesional biopsy and several authors have recommended this approach[48-50]. Lesional biopsy does however carry the risk of tumor seeding which has been estimated at approximately 2%[51]. Furthermore, significant histological heterogeneity has been described in large tumors, which limits the utility of needle biopsy[52].

A scoring system incorporating tumor differentiation in addition to tumor size and number has been validated in a 2007 French multicenter study[53]. In this study, tumor histology improved the Milan criteria in predicting post-transplant outcome. These results conflict with a more recent 100 patient study in which tumor differentiation did not predict HCC recurrence[54].

LIVING DONOR TRANSPLANTATION

Living donor liver transplantation (LDLT) has evolved over the past decade, mainly in response to the scarcity of donor livers. Deceased liver donation is particularly scarce in Asia, where organ donation rates are less than 5 donors per million population compared to 10-35 per million in Western countries[55]. This is also compounded by the fact that in most Asian countries HCC is the most common cancer and a frequent indication for OLT. LDLT, in particular right liver transplantation, has dramatically increased the number of potential donors. This can eliminate the problem of long waiting times and 'dropout' whilst waiting for an organ because of disease progression. Furthermore, as there is no direct 'competition' from other potential transplant recipients the restrictive criteria on tumor burden can be relaxed somewhat.

The results from LDLT appear to show good long term survival rates with retrospective studies showing comparable rates to OLT[56-58]. There are, however, published retrospective studies which show a higher rate of tumor recurrence than with conventional OLT[59-61].

The reason for this is not entirely evident. It has been postulated that many candidates are not transplanted because of tumor progression whilst waiting for OLT; these patients, with more aggressive tumor biology, are then removed from the outcome analysis. In LDLT there is no protracted waiting time and hence the patients with aggressive tumors are transplanted.

LDLT has been performed, in some centers, in patients who have a tumor load beyond the Milan criteria with the consent of both the donor and recipient[62]. In addition, LDLT carries a risk to the donor during hepatectomy with morbidity and mortality rates of 14%-21% and 0.25%-1%, respectively[63]. This highlights the need for a universal consensus on the use of LDLT.

Akin to the published "expanded criteria" in OLT, several groups have attempted to determine an "expanded criteria" in LDLT. Using a scoring system including the measurement of "protein induced by vitamin K absence or antagonist-Ⅱ" (PIVKA-Ⅱ), a Japanese group have achieved a 5-year recurrence rate of only 4.9%[24,64]. PIVKA-Ⅱ, also known as des-carboxyprothrombin, is an abnormal prothrombin protein found in the serum of patients with HCC and in patients with vitamin K deficiency or on warfarin therapy. It has been suggested as an alternative marker to AFP for the surveillance of HCC, but is not currently used as standard practice internationally. This study reported an 86.7% survival rate in patients with ≤ 10 tumors, all ≤ 5 cm (on pre-transplant imaging) and with PIVKA-Ⅱ values ≤ 400 mAU/mL. Without the application of PIVKA-Ⅱ, patients with ≤ 10 tumors, all ≤ 5 cm had similar 5-year recurrence rates as those conforming to the Milan criteria (7.3% *vs* 9.7%)[24,64].

CONCLUSION

Liver transplantation remains the definitive treatment for HCC complicating cirrhosis. Since the publication of the "Milan criteria", OLT has progressed into a universally accepted treatment for HCC. The results of OLT for HCC continue to improve with time. The retrospective studies to date suggest outcome for OLT for HCC is only marginally worse than for end stage liver disease itself. Over the past decade LDLT has evolved but at present needs more stringent universal guidance on its usage. However, the results of LDLT for HCC seem slightly worse than for OLT but this may be a result of selection bias. The use of single or combined local ablative therapy in the period prior to OLT appears to be accepted. As operative techniques have improved, resection as a bridge to transplantation now appears a viable option in compensated cirrhotic patients. Resection also offers the ability to gain invaluable information about the tumor.

Undoubtedly further work will be published on expanded criteria for transplantation. The various studies to date have shown that acceptable long term outcomes are possible with OLT for HCC outside the "Milan criteria". With time a new universal consensus will be required to standardize these 'expanded' criteria.

REFERENCES

1 **World Health Organization**. Mortality database. Available from: URL: http://www.who.int/whosis/en

2 **Lau WY**. Primary hepatocellular carcinoma. In: Blumgart LH, Fong Y, editors. Surgery of the liver and biliary tract. Volume II. London: WB Saunders, 2000: 1423-1450

3 **Lau WY**. Primary liver tumors. *Semin Surg Oncol* 2000; **19**: 135-144

4 **Lau WY**. Management of hepatocellular carcinoma. *J R Coll Surg Edinb* 2002; **47**: 389-399

5 **Lai EC**, Lau WY. The continuing challenge of hepatic cancer in Asia. *Surgeon* 2005; **3**: 210-215

6 **Llovet JM**, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003; **362**: 1907-1917

7 **Zaman SN**, Melia WM, Johnson RD, Portmann BC, Johnson PJ, Williams R. Risk factors in development of hepatocellular carcinoma in cirrhosis: prospective study of 613 patients. *Lancet* 1985; **1**: 1357-1360

8 **Liu CJ**, Kao JH. Hepatitis B virus-related hepatocellular carcinoma: epidemiology and pathogenic role of viral factors. *J Chin Med Assoc* 2007; **70**: 141-145

9 **Okuda K**. Hepatocellular carcinoma. *J Hepatol* 2000; **32**: 225-237

10 **Caldwell S**, Park SH. The epidemiology of hepatocellular cancer: from the perspectives of public health problem to tumor biology. *J Gastroenterol* 2009; **44** Suppl 19: 96-101

11 **Montalto G**, Cervello M, Giannitrapani L, Dantona F, Terranova A, Castagnetta LA. Epidemiology, risk factors, and natural history of hepatocellular carcinoma. *Ann N Y Acad Sci* 2002; **963**: 13-20

12 **Bosch FX**, Ribes J, Díaz M, Cléries R. Primary liver cancer: worldwide incidence and trends. *Gastroenterology* 2004; **127**: S5-S16

13 **Figueras J**, Jaurrieta E, Valls C, Benasco C, Rafecas A, Xiol X, Fabregat J, Casanovas T, Torras J, Baliellas C, Ibaüñez L, Moreno P, Casais L. Survival after liver transplantation in cirrhotic patients with and without hepatocellular carcinoma: a comparative study. *Hepatology* 1997; **25**: 1485-1489

14 **Starzl TE**, Marchioro TL, Faris TD. Liver transplantation. *Ann Intern Med* 1966; **64**: 473-477

15 **Starzl TE**, Groth CG, Brettschneider L, Penn I, Fulginiti VA, Moon JB, Blanchard H, Martin AJ Jr, Porter KA. Orthotopic homotransplantation of the human liver. *Ann Surg* 1968; **168**: 392-415

16 **Williams R**, Smith M, Shilkin KB, Herbertson B, Joysey V, Calne RY. Liver transplantation in man: the frequency of rejection, biliary tract complications, and recurrence of malignancy based on an analysis of 26 cases. *Gastroenterology* 1973; **64**: 1026-1048

17 **Iwatsuki S**, Starzl TE, Sheahan DG, Yokoyama I, Demetris AJ, Todo S, Tzakis AG, Van Thiel DH, Carr B, Selby R. Hepatic resection versus transplantation for hepatocellular carcinoma. *Ann Surg* 1991; **214**: 221-228; discussion 228-229

18 **Bismuth H**, Chiche L, Adam R, Castaing D, Diamond T, Dennison A. Liver resection versus transplantation for hepatocellular carcinoma in cirrhotic patients. *Ann Surg* 1993; **218**: 145-151

19 **Mazzaferro V**, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, Montalto F, Ammatuna M, Morabito A, Gennari L. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996; **334**: 693-699

20 **Yao FY**, Bass NM, Nikolai B, Davern TJ, Kerlan R, Wu V, Ascher NL, Roberts JP. Liver transplantation for hepatocellular carcinoma: analysis of survival according to the intention-to-treat principle and dropout from the waiting list. *Liver Transpl* 2002; **8**: 873-883

21 **Yao FY**, Xiao L, Bass NM, Kerlan R, Ascher NL, Roberts JP. Liver transplantation for hepatocellular carcinoma: validation of the UCSF-expanded criteria based on preoperative imaging. *Am J Transplant* 2007; **7**: 2587-2596

22 **Lee SG**, Hwang S, Moon DB, Ahn CS, Kim KH, Sung KB, Ko GY, Park KM, Ha TY, Song GW. Expanded indication criteria of living donor liver transplantation for hepatocellular carcinoma at one large-volume center. *Liver Transpl* 2008; **14**: 935-945

23 **Duffy JP**, Vardanian A, Benjamin E, Watson M, Farmer DG, Ghobrial RM, Lipshutz G, Yersiz H, Lu DS, Lassman C, Tong MJ, Hiatt JR, Busuttil RW. Liver transplantation criteria for hepatocellular carcinoma should be expanded: a 22-year experience with 467 patients at UCLA. *Ann Surg* 2007; **246**: 502-509; discussion 509-511

24 **Ito T**, Takada Y, Ueda M, Haga H, Maetani Y, Oike F, Ogawa K, Sakamoto S, Ogura Y, Egawa H, Tanaka K, Uemoto S. Expansion of selection criteria for patients with hepatocellular carcinoma in living donor liver transplantation. *Liver Transpl* 2007; **13**: 1637-1644

25 **Soejima Y**, Taketomi A, Yoshizumi T, Uchiyama H, Aishima S, Terashi T, Shimada M, Maehara Y. Extended indication for living donor liver transplantation in patients with hepatocellular carcinoma. *Transplantation* 2007; **83**: 893-899

26 **Sugawara Y**, Tamura S, Makuuchi M. Living donor liver transplantation for hepatocellular carcinoma: Tokyo University series. *Dig Dis* 2007; **25**: 310-312

27 **Jonas S**, Mittler J, Pascher A, Schumacher G, Theruvath T, Benckert C, Rudolph B, Neuhaus P. Living donor liver transplantation of the right lobe for hepatocellular carcinoma in cirrhosis in a European center. *Liver Transpl* 2007; **13**: 896-903

28 **Llovet JM**, Schwartz M, Mazzaferro V. Resection and liver transplantation for hepatocellular carcinoma. *Semin Liver Dis* 2005; **25**: 181-200

29 **Mazzaferro V**, Llovet JM, Miceli R, Bhoori S, Schiavo M, Mariani L, Camerini T, Roayaie S, Schwartz ME, Grazi GL, Adam R, Neuhaus P, Salizzoni M, Bruix J, Forner A, De Carlis L, Cillo U, Burroughs AK, Troisi R, Rossi M, Gerunda GE, Lerut J, Belghiti J, Boin I, Gugenheim J, Rochling F, Van Hoek B, Majno P. Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. *Lancet Oncol* 2009; **10**: 35-43

30 **Lu DS**, Yu NC, Raman SS, Lassman C, Tong MJ, Britten C, Durazo F, Saab S, Han S, Finn R, Hiatt JR, Busuttil RW. Percutaneous radiofrequency ablation of hepatocellular carcinoma as a bridge to liver transplantation. *Hepatology* 2005; **41**: 1130-1137

31 **Maluf DG**, Stravitz RT, Williams B, Cotterell AH, Mas VR, Heuman D, Luketic V, Shiffman ML, Sterling R, Posner MP, Fisher RA. Multimodality therapy and liver transplantation in patients with cirrhosis and hepatocellular carcinoma: 6 years, single-center experience. *Transplant Proc* 2007; **39**: 153-159

32 **Martin AP**, Goldstein RM, Dempster J, Netto GJ, Katabi N, Derrick HC, Altrabulsi B, Jennings LW, Ueno T, Chinnakotla S, Dawson S 3rd, Randall HB, Levy MF, Onaca N, Sanchez EQ, Orr D, Klintmalm GB. Radiofrequency thermal ablation of hepatocellular carcinoma before liver transplantation--a clinical and histological examination. *Clin Transplant* 2006; **20**: 695-705

33 **Schwartz M**, Roayaie S, Uva P. Treatment of HCC in patients awaiting liver transplantation. *Am J Transplant* 2007; **7**: 1875-1881

34 **Yau T**, Chan P, Epstein R, Poon RT. Evolution of systemic therapy of advanced hepatocellular carcinoma. *World J Gastroenterol* 2008; **14**: 6437-6441

35 **Belghiti J**, Cortes A, Abdalla EK, Régimbeau JM, Prakash K, Durand F, Sommacale D, Dondero F, Lesurtel M, Sauvanet A, Farges O, Kianmanesh R. Resection prior to liver transplantation for hepatocellular carcinoma. *Ann Surg* 2003; **238**: 885-892; discussion 892-893

36 **Adam R**, Azoulay D, Castaing D, Eshkenazy R, Pascal G, Hashizume K, Samuel D, Bismuth H. Liver resection as a bridge to transplantation for hepatocellular carcinoma on cirrhosis: a reasonable strategy? *Ann Surg* 2003; **238**: 508-518; discussion 518-519

37 http://www.optn.org/PoliciesandBylaws2/policies/pdfs/policy_8.pdf

38 **Lubienski A**. Hepatocellular carcinoma: interventional bridging to liver transplantation. *Transplantation* 2005; **80**: S113-S119

39 **Mazzaferro V**, Battiston C, Perrone S, Pulvirenti A, Regalia E, Romito R, Sarli D, Schiavo M, Garbagnati F, Marchianò A, Spreafico C, Camerini T, Mariani L, Miceli R, Andreola S. Radiofrequency ablation of small hepatocellular carcinoma in cirrhotic patients awaiting liver transplantation: a prospective study. *Ann Surg* 2004; **240**: 900-909

40 **Pompili M**, Mirante VG, Rondinara G, Fassati LR, Piscaglia F, Agnes S, Covino M, Ravaioli M, Fagiuoli S, Gasbarrini G, Rapaccini GL. Percutaneous ablation procedures in cirrhotic patients with hepatocellular carcinoma submitted to liver transplantation: Assessment of efficacy at explant analysis and of safety for tumor recurrence. *Liver Transpl* 2005; **11**: 1117-1126

41 **Morisco F**, Stigliano R, Godfrey A, Leandro G, Patch D, Davidson B, Rolles K, Dhillon A, Dhillon AP, Quaglia A, Burroughs AK. Efficacy of loco-regional ablation therapy of HCC in a population of liver transplanted patients. *Dig Dis Sci* 2008; **53**: 1131-1137

42 **Yao FY**, Kinkhabwala M, LaBerge JM, Bass NM, Brown R Jr, Kerlan R, Venook A, Ascher NL, Emond JC, Roberts JP. The impact of pre-operative loco-regional therapy on outcome after liver transplantation for hepatocellular carcinoma. *Am J Transplant* 2005; **5**: 795-804

43 **Freeman RB Jr**, Steffick DE, Guidinger MK, Farmer DG, Berg CL, Merion RM. Liver and intestine transplantation in the United States, 1997-2006. *Am J Transplant* 2008; **8**: 958-976

44 **Belghiti J**. Resection and liver transplantation for HCC. *J Gastroenterol* 2009; **44** Suppl 19: 132-135

45 **Cheng SJ**, Pratt DS, Freeman RB Jr, Kaplan MM, Wong JB. Living-donor versus cadaveric liver transplantation for non-resectable small hepatocellular carcinoma and compensated cirrhosis: a decision analysis. *Transplantation* 2001; **72**: 861-868

46 **Rossi M**, Merli M, Lai Q, Gentili F, Mennini G, Bussotti A, Pugliese F, Della Pietra F, Poli L, Novelli G, Giusto M, Ginanni Corradini S, Iappelli M, Onetti Muda A, Di Tondo U, Gossetti F, Attili AF, Berloco PB. Outcome after liver transplantation in patients with cirrhosis and hepatocellular carcinoma. *Transplant Proc* 2007; **39**: 1895-1897

47 **Pelletier SJ**, Fu S, Thyagarajan V, Romero-Marrero C, Batheja MJ, Punch JD, Magee JC, Lok AS, Fontana RJ, Marrero JA. An intention-to-treat analysis of liver transplantation for hepatocellular carcinoma using organ procurement transplant network data. *Liver Transpl* 2009; **15**: 859-868

48 **Cillo U**, Vitale A, Bassanello M, Boccagni P, Brolese A, Zanus G, Burra P, Fagiuoli S, Farinati F, Rugge M, D'Amico DF. Liver transplantation for the treatment of moderately or well-differentiated hepatocellular carcinoma. *Ann Surg* 2004; **239**: 150-159

49 **Sutcliffe R**, Maguire D, Portmann B, Rela M, Heaton N. Selection of patients with hepatocellular carcinoma for liver transplantation. *Br J Surg* 2006; **93**: 11-18

50 **Vauthey JN**, Ajani JA. Liver transplantation and hepatocellular carcinoma biology: beginning of the end of the era of educated guesses. *J Clin Oncol* 2003; **21**: 4265-4267

51 **Stigliano R**, Marelli L, Yu D, Davies N, Patch D, Burroughs AK. Seeding following percutaneous diagnostic and therapeutic approaches for hepatocellular carcinoma. What is the risk and the outcome? Seeding risk for percutaneous approach of HCC. *Cancer Treat Rev* 2007; **33**: 437-447

52 **An FQ**, Matsuda M, Fujii H, Tang RF, Amemiya H, Dai YM, Matsumoto Y. Tumor heterogeneity in small hepatocellular carcinoma: analysis of tumor cell proliferation, expression and mutation of p53 AND beta-catenin. *Int J Cancer* 2001; **93**: 468-474

53 **Decaens T**, Roudot-Thoraval F, Badran H, Meyer C, Durand F, Adam R, Boillot O, Bresson-Hadni S, Gugenheim J, Dharancy S, Bernard PH, Compagnon P, Calmus Y, Hardwigsen J, Ducerf

C, Pageaux GP, Hilleret MN, Chazouilleres O, Cherqui D, Duvoux C. Liver transplantation for hepatocellular carcinoma: validation of a new prognostic score predicting disease-free survival. *J Hepatol* 2007; **46**: S25

54 **Marelli L**, Grasso A, Pleguezuelo M, Martines H, Stigliano R, Dhillon AP, Patch D, Davidson BR, Sharma D, Rolles K, Burroughs AK. Tumour size and differentiation in predicting recurrence of hepatocellular carcinoma after liver transplantation: external validation of a new prognostic score. *Ann Surg Oncol* 2008; **15**: 3503-3511

55 **de Villa VH**, Lo CM, Chen CL. Ethics and rationale of living-donor liver transplantation in Asia. *Transplantation* 2003; **75**: S2-S5

56 **Lo CM**, Fan ST, Liu CL, Chan SC, Wong J. The role and limitation of living donor liver transplantation for hepatocellular carcinoma. *Liver Transpl* 2004; **10**: 440-447

57 **Hwang S**, Lee SG, Joh JW, Suh KS, Kim DG. Liver transplantation for adult patients with hepatocellular carcinoma in Korea: comparison between cadaveric donor and living donor liver transplantations. *Liver Transpl* 2005; **11**: 1265-1272

58 **Thuluvath PJ**, Yoo HY. Graft and patient survival after adult live donor liver transplantation compared to a matched cohort who received a deceased donor transplantation. *Liver Transpl* 2004; **10**: 1263-1268

59 **Kulik L**, Abecassis M. Living donor liver transplantation for hepatocellular carcinoma. *Gastroenterology* 2004; **127**: S277-S282

60 **Fisher RA**, Kulik LM, Freise CE, Lok AS, Shearon TH, Brown RS Jr, Ghobrial RM, Fair JH, Olthoff KM, Kam I, Berg CL. Hepatocellular carcinoma recurrence and death following living and deceased donor liver transplantation. *Am J Transplant* 2007; **7**: 1601-1608

61 **Lo CM**, Fan ST, Liu CL, Chan SC, Ng IO, Wong J. Living donor versus deceased donor liver transplantation for early irresectable hepatocellular carcinoma. *Br J Surg* 2007; **94**: 78-86

62 **Gondolesi GE**, Roayaie S, Muñoz L, Kim-Schluger L, Schiano T, Fishbein TM, Emre S, Miller CM, Schwartz ME. Adult living donor liver transplantation for patients with hepatocellular carcinoma: extending UNOS priority criteria. *Ann Surg* 2004; **239**: 142-149

63 **Poon RT**, Fan ST, Lo CM, Liu CL, Wong J. Difference in tumor invasiveness in cirrhotic patients with hepatocellular carcinoma fulfilling the Milan criteria treated by resection and transplantation: impact on long-term survival. *Ann Surg* 2007; **245**: 51-58

64 **Takada Y**, Ito T, Ueda M, Sakamoto S, Haga H, Maetani Y, Ogawa K, Ogura Y, Oike F, Egawa H, Uemoto S. Living donor liver transplantation for patients with HCC exceeding the Milan criteria: a proposal of expanded criteria. *Dig Dis* 2007; **25**: 299-302

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REVIEW

Rheumatic manifestations of inflammatory bowel disease

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Abstract

This article reviews the literature concerning rheumatic manifestations of inflammatory bowel disease (IBD), including common immune-mediated pathways, frequency, clinical course and therapy. Musculoskeletal complications are frequent and well-recognized manifestations in IBD, and affect up to 33% of patients with IBD. The strong link between the bowel and the osteo-articular system is suggested by many clinical and experimental observations, notably in HLA-B27 transgenic rats. The autoimmune pathogenic mechanisms shared by IBD and spondyloarthropathies include genetic susceptibility to abnormal antigen presentation, aberrant recognition of self, the presence of autoantibodies against specific antigens shared by the colon and other extra-colonic tissues, and increased intestinal permeability. The response against microorganisms may have an important role through molecular mimicry and other mechanisms. Rheumatic manifestations of IBD have been divided into peripheral arthritis, and axial involvement, including sacroiliitis, with or without spondylitis, similar to idiopathic ankylosing spondylitis. Other periarticular features can occur, including enthesopathy, tendonitis, clubbing, periostitis, and granulomatous lesions of joints and bones. Osteoporosis and osteomalacia secondary to IBD and iatrogenic complications can also occur. The management of the rheumatic manifestations of IBD consists of physical therapy in combination with local injection of corticosteroids and nonsteroidal anti-inflammatory drugs; caution is in order however, because of their possible harmful effects on intestinal integrity, permeability, and even on gut inflammation. Sulfasalazine, methotrexate, azathioprine, cyclosporine and leflunomide should be used for selected indications. In some cases, tumor necrosis factor-α blocking agents should be considered as first-line therapy.

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Key words: Inflammatory bowel disease; Spondylitis; Rheumatic diseases

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INTRODUCTION

The association between arthritis and inflammatory bowel disease (IBD) was originally described in 1929[1], but it was not until the 1950s when peripheral arthritis associated with IBD was distinguished from rheumatoid arthritis, and in the 1960s, the concept of spondyloarthropathy (SpA) was established[2-4]. Here, we present a review of all pertinent literature from Medline regarding rheumatic complications of IBD. We include original and review articles, as well as relevant case reports published from 1929 to 2009.

With time, clinical and experimental evidence of the close relationship between IBD and some rheumatic diseases, particularly seronegative SpAs, has grown. In fact, IBD is considered part of the concept of SpA[5].

Some studies have shown that up to 70% of patients with SpA have inflammatory intestinal lesions, and up to 26% of patients with SpA who undergo ileocolonoscopy

have intestinal abnormalities compatible with Crohn's disease (CD). In fact, 6%-10% of patients with SpA develop IBD on follow-up; besides, studies with serial ileocolonoscopy have shown strong correlation between the presence of gut and joint inflammation in SpA[6-10]. Therefore, some authors have suggested that joint abnormalities are the initial manifestation of IBD, and after several years, these patients later may develop florid intestinal abnormalities. On the other hand, some studies that have evaluated extraintestinal manifestations of patients with IBD have shown that 36%-46% of patients have at least one extraintestinal manifestation, and rheumatic abnormalities are the most frequent (22%-33%)[11,12]. Besides, rheumatic manifestations are significantly more common in patients with disease confined to the colon. For instance, some patient series with ulcerative colitis (UC) have reported a prevalence of joint involvement of 62%[13]. Several studies have evaluated the prevalence of seronegative SpA in patients with IBD: 18%-45% of patients with IBD fulfill the criteria for SpA; 3%-9.9% fulfill diagnostic criteria for ankylosing spondylitis (AS); around 14% develop one or more clinical manifestations of SpA without fulfilling diagnostic criteria, and some of these patients (up to 24%) have asymptomatic sacroiliitis[14-18].

IMMUNOPATHOGENESIS OF GUT AND JOINT INFLAMMATION

Immunologic alterations shared by patients with IBD and SpA

There have been interesting studies about the immunopathogenesis of IBD and SpA, which have shown alterations in key molecules that regulate the immune response in the gut of patients with SpA, and some of them are almost the same as those found in CD[19]. For instance E-cadherin, a transmembrane glycoprotein that mediates the intercellular adhesion of epithelial cells is expressed highly in the gut of patients with IBD[20] and in patients with SpA[21], and subclinical acute and chronic gut inflammation, even in those without macroscopic lesions, which indicates that the alteration in the regulation of these proteins may be an early event in the development of gut inflammation. Besides, E-cadherin is a ligand of α4β7 integrin in intraepithelial T cells. Two studies have shown increased expression of this integrin in T cell cultures from the gut mucosa of patients with AS[22] and CD[23]. Increased expression of this integrin has been found in patients without histological signs of gut inflammation, which suggests that there are common abnormalities in patients with SpA and IBD that precede clinical inflammation.

Other alterations that are common in both groups of diseases are those described in $CD4^+$ T cells. Currently, four types of $CD4^+$ T cells have been described: Th1 cells are identified as interferon γ (IFN-γ) producers; Th2 cells produce primarily interleukin (IL)-4, IL-5, IL-10 and IL-13; Th17 cells produce mainly IL-17A and they also produce IL-17F, IL-21, IL-22, granulocyte-monocyte colony-stimulator factor (GM-CSF), CCL-20, and potentially tumor necrosis factor (TNF) and IL-6. These cytokines have pro-inflammatory properties and they act on a broad range of cell types to induce the expression of several cytokines (TNF, IL-1β, IL-6, GM-CSF, G-CSF), chemokines (CXCL1, CXCL8, CXCL10), and metalloproteinases. On the other hand, regulatory T cells (Tregs) have a suppressor function; they influence the immune system by cell-to-cell contact and also *via* regulatory mechanisms that are still not fully elucidated. It is known that they are able to produce IL-10 and transforming growth factor (TGF)-β, and it is interesting that Th17 and Treg developmental programs are reciprocally interconnected. Upon T-cell receptor stimulation, a naïve T cell can be driven to express Foxp3 and become a Treg cell in the presence of TGF-β, but in the presence of TGF-β plus IL-6 or IL-21, the Treg developmental pathway is abrogated, and instead, T cells develop into Th17 cells[24]. Initial studies have shown Th1 predominance in intestinal mucosa of patients with IBD and SpA, however, recent studies have suggested that, in both groups of patients, Th17 cells may have an important role in initiation and perpetuation of autoimmune inflammation. One study that involved 499 patients with CD and 216 with UC has shown increased IL-17F mRNA expression in intestinal biopsies of patients compared to controls[25]. A recent study has shown an increased proportion of Th17 cells in patients with SpA[26] and increased IL-17, IL-6, TGF-β and IFN-γ levels in synovial fluid of patients with SpA, when compared with patients with rheumatoid arthritis[27]. Also, some authors have suggested that dysfunction of Tregs participates in the immunopathogenesis of these diseases, and they have proposed their use as therapeutic agents in IBD[24,28].

TNF-α is a pro-inflammatory cytokine that is produced mainly by macrophages and activated T cells. It is a key molecule in chronic inflammation of SpA and IBD. In the latter, the interaction between antigen-presenting cells (APCs) and intestinal bacterial flora contributes to the development of uncontrolled $CD4^+$ cell activation, which leads to the release of pro-inflammatory cytokines such as TNF-α, IL-6, IL-12, IL-23 and IL-17. In SpA, models of transgenic rats and clinical observations have suggested that pathogenic microorganisms and their interaction with APCs also have a crucial role in the initiation and perpetuation of the altered immune response that leads to joint and enthesis inflammation. Additionally, increased intestinal permeability has been found in patients with SpA and IBD. This fact could alter the innate immune response to bacterial antigens[29]. These discoveries have had important therapeutic implications for these groups of patients.

Other relevant molecules involved in the pathogenesis of both groups of diseases are the toll-like receptors (TLRs) that play an important role in the innate immune response against pathogenic microorganisms. Several studies have shown increased expression of TLR-4 and TLR-2 in APCs of patients with SpA[30,31] and in intestinal

biopsies of patients with UC and CD[32-34]. The alterations in function and regulation of these molecules may have an important role in the initiation and perpetuation of chronic inflammation in these diseases[35]. There are studies that have correlated several mutations and genetic variations of these receptors with susceptibility for these diseases. The results are controversial, probably due to the heterogeneity of the patients and the ethnic groups that have been included in the studies[36-39].

Other immunopathogenic abnormalities in IBD and SpA

Abnormal responses to certain microorganisms have been described in patients with IBD or SpA; for instance, increased levels of the same serotypes of *Klebsiella pneumoniae* have been found more frequently in patients with IBD and SpA, compared with healthy controls and controls with celiac disease[40]. Besides, several studies have shown increased levels of antibodies against *Klebsiella* antigens, and against collagens type Ⅰ, Ⅲ, Ⅳ and Ⅴ in patients with CD or AS. There are molecular similarities between *Klebsiella* nitrogenase and HLA-B27 genetic markers and between *Klebsiella* pullulanase and collagen fibers types Ⅰ, Ⅲ and Ⅳ. Therefore, several authors have proposed that there may be molecular mimicry between *Klebsiella* and human molecules that could participate in the initiation and perpetuation of these diseases[41], i.e. anti-*Klebsiella* IgA cross-reacts with HLA-B27 antigen, and antibodies to enteric bacteria are able to lyse lymphocytes from HLA-B27 patients with AS.

The participation of the HLA-B27 molecule in the immunopathogenesis of SpA has been well known since 1973 when Brewerton and Schlosstein detected its high prevalence in patients with AS, psoriatic arthritis (PsA), reactive arthritis and anterior uveitis[42]. Its functional relevance was demonstrated elegantly in 1990 by Hammer *et al*[43]; their group developed a transgenic rat with the human HLA-B27 molecule. These rats developed a multisystemic inflammatory disease that had several clinical and histopathological similarities to SpA and IBD[43]. An important finding was that these rats did not develop joint or gut inflammation when they were in a bacteria-free environment, which supports the theory of the participation of microorganisms in the pathogenesis of these diseases[44]. Some studies involving patients with IBD have shown an association of HLA-B27 molecule with the presence of sacroiliitis, spondylitis, enthesitis, peripheral arthritis, erythema nodosum, uveitis and oral ulcers[14].

Other genes have been associated with the clinical presentation of several arthropathies associated with IBD[45]. The Oxford Group has found an association of type 1 arthropathy (pauci-articular, self limited, with relapses) with HLA-DRB1*0103, HLA-B35 and HLA-B24, while type 2 arthropathy (polyarticular, progressive) is associated with HLA-B44 in British patients[46]. One study that evaluated Spanish patients has found an association of the mini-haplotype TNF a6B5 with the presence of joint manifestations in patients with UC[47]. Another study has found that a polymorphism of the gene CARD15 is associated with the presence of gut inflammation in patients with SpA, and the same polymorphism is over-represented in patients with CD[48].

On the other hand, there is evidence to suggest that, for patients with IBD, tobacco use and history of appendectomy are risk factors for developing extraintestinal manifestations, particularly seronegative SpA and dermatological manifestations such as erythema nodosum and pyoderma gangrenosum[49].

In summary, the autoimmune pathogenic mechanisms shared by IBD and SpA include genetic susceptibility to abnormal antigen presentation, aberrant recognition of self, the presence of autoantibodies against specific antigens shared by the colon and other extra-colonic tissues, and increased intestinal permeability. The response against microorganisms may have an important role through molecular mimicry and other mechanisms.

CLINICAL PRESENTATION OF THE RHEUMATIC MANIFESTATIONS OF IBD

The rheumatic manifestations of IBD have been divided into peripheral arthritis, and axial involvement, including sacroiliitis, with or without spondylitis, similar to the presentation of classic AS. There may be other periarticular manifestations such as enthesopathy, which is the involvement of tendinous insertions, tendonitis, periostitis, clubbing and granulomatous lesions in joints and bones. There may also be osteoporosis and osteomalacia secondary to IBD and to its treatment[50].

Peripheral arthritis

The frequency of peripheral arthritis in IBD ranges from 17% to 20%, and it is more common in CD[51]. In a retrospective study that included 1459 patients with IBD, peripheral arthritis was present in 6% of patients with UC and in 10% of patients with CD[52]. Another study has shown arthritis in 10%, enthesitis in 7%, and a history of arthritis in 29% in a group of patients with IBD[53].

The Oxford Group[52] has classified the peripheral arthritis associated with IBD (also called peripheral enteropathic arthropathy) without axial involvement, as pauci-articular or polyarticular arthropathy (Table 1). In the pauci-articular form, joint symptoms are usually acute and self-limited; the joint involvement is asymmetric and migratory, with participation of both large and small joints, and lower limbs are more affected. Many episodes last from 6 to 10 wk but relapses are frequent and they tend to coexist with IBD relapses; these patients have high frequencies of other extraintestinal manifestations such as erythema nodosum and uveitis. It is interesting that in 31% of these patients the arthropathy may appear even 3 years before the diagnosis of IBD is made. On the other hand, the polyarticular form tends to have a chronic course and it may be destructive; its course is independent of IBD exacerbations and the coexistence of other extraintestinal manifestations is rare, except for uveitis[50,54]. Enthesopathy, particularly affecting the Achilles tendon or plantar fascia insertion are common manifestations.

Table 1 Classification of peripheral arthropathy associated with IBD

Type 1 (Pauci-articular)	Type 2 (Polyarticular)
Less than five joints	Five or more joints
Asymmetric involvement	Persistent inflammation for months or even years
Lower limbs more affected	May be erosive
Self limited episodes that last < 10 wk	Affects both large and small joints
Usually concomitant IBD relapse	It can be symmetric or asymmetric
High frequency of other extraintestinal manifestations	Clinical course is independent of IBD activity
	Associated with uveitis

IBD: Inflammatory bowel disease.

Table 2 AS prevalence studies in patients with IBD

Author and reference number	*n*	Origin of patients	Diagnostic criteria	Prevalence (%)				
				AS	SpA	Sacroiliitis (Rx or MRI)	Inflammatory low back pain	Articular manifestations
de Vlam *et al*[53]	103	Referral center	ESSG	10	35	30	30	39
Scarpa *et al*[13]	79	Referral center	New York	25			18	43
Salvarani *et al*[17]	160	Open population	ESSG	2 (CD) 5.1 (UC)	18.1			32.5
Palm *et al*[58]	654	Open population	ESSG	6 (CD) 2.6 (UC) 3.7 Total	22	2	19	
Wordsworth *et al*[60]	42	Referral center	ESSG	25 (CD)		36		

Rx: Radiography; MRI: Magnetic resonance image; ESSG: European Spondyloarthropathy Study Group.

In most of the cases, intestinal symptoms precede or coexist with joint manifestations, but in some patients, arthritis precedes the gut manifestations, even by several years. In a prospective study that included 123 patients with SpA, 6% of the patients developed CD from 2 to 9 years after the first joint symptom. For UC, some studies have described a temporal relationship between arthritis and gut inflammation relapses[50]; there have been case reports of patients that have developed arthritis at the same time as ileal pouchitis after total proctocolectomy for UC activity. However, other studies have shown that colectomy has almost no effect on joint inflammation[50,55]. The most consistent finding is that colon involvement increases the susceptibility to peripheral arthritis.

There have been isolated case reports about patients that have presented with progressive, destructive monoarthritis with granulomatous synovial inflammation, and CD has been diagnosed when extension studies have been carried out as part of the diagnostic workup[56].

AS and other forms of axial involvement

Axial involvement is part of CD and UC, although it is more common in CD (5%-22%) than in UC (2%-6%), and in general, the prevalence is 10%-20% for sacroiliitis and 7%-12% for AS[51,57,58]. A study that included patients from a referral center has shown that 30% of the patients with IBD had inflammatory low back pain, 33% had abnormal Shober index, and 30% had unilateral or bilateral grade I or II sacroiliitis. In fact, 35% of the patients fulfilled the European Spondyloarthropathy Study Group criteria for SpA[53,59], and 10% fulfilled the criteria for AS. Other studies from referral centers have shown similar results and higher prevalence than for population studies, which probably reflects referral bias[13,17,53,58,60] (Table 2).

The clinical picture is virtually the same as the one for patients with AS without extra-articular manifestations. Axial symptoms usually precede gut symptoms; clinical course is totally independent of the intestinal manifestations and intestinal surgery does not alter the course of SpA. Ankylosing spondylitis associated with IBD can develop at any age, whereas idiopathic AS usually starts before 40 years of age. In idiopathic AS, males are more affected than females (ratio 2.5:1), while in AS associated with IBD, the male to female ratio is 1:1[61].

LABORATORY AND OTHER DIAGNOSTIC WORKUP

The most common laboratory abnormalities are: anemia due to chronic inflammation and intestinal bleeding, leukocytosis, thrombocytosis, elevated acute phase reactants such as C reactive protein, and erythrocyte sedimentation rate. Perinuclear anti-neutrophil cytoplasm antibodies are found in up to 60% of patients with UC and in some patients with CD, and they seem to be directed against lactoferrin autoantigens[62]. Antinuclear antibodies and rheumatoid factor are usually absent. Synovial fluid may be inflammatory but sterile.

Anti-*Saccharomyces cerevisiae* antibodies (ASCAs) were first described in patients with CD (both IgG and IgA isotypes). Several studies have confirmed their relevance as markers of CD. One multicenter study has shown that their presence in patients with IBD, who are negative for ANCA, has a positive predictive value of 94.2% for CD,

and it is associated with severe clinical manifestations of this disease[63]. Another recent study has shown that the prevalence of ASCAs in patients with CD is 80.6%, and they have a sensitivity of 81%, specificity of 78%, positive predictive value of 45.5%, and negative predictive value of 95% when used as a single marker in patients with IBD[64]. One study that evaluated 87 SpA patients positive for HLA-B27 has found increased levels of IgA but not IgG ASCAs, particularly in patients with AS; although patients with increased levels of the antibody had no gastrointestinal symptoms. It remains unclear if these antibodies are associated with the development of IBD in patients with SpA, but this finding supports the etiopathogenic association of AS with IBD[65].

Sacroiliitis and spondylitis in IBD are associated with the presence of HLA-B27, although in lower frequencies than in AS (33% *vs* 71%). Besides, patients with AS without HLA-B27 have a higher risk of developing IBD than those with HLA-B27[53]. The Oxford study[52] has shown an association of pauci-articular arthritis with the presence of HLA-B27 (27% *vs* 7% in controls), HLA-B35 (32% *vs* 15%) and HLA-DRB1*0103 (33% *vs* 3%), while the polyarticular form was associated with HLA-B44 (62% *vs* 30%)[45]. Another study has shown that the presence of the shared epitope is associated with synovitis in patients with IBD without sacroiliitis[50,66].

Radiological studies of peripheral joints have shown changes of acute arthritis such as increased volume of soft tissue. Erosions are uncommon in patients with self-limited arthritis. Patients with persistent arthritis may develop joint erosion and, if the hips are affected, there may be also loss of joint space. Axial radiographs show typical AS changes, although some studies have suggested that asymmetric sacroiliitis frequency may be higher[67].

OTHER RHEUMATIC MANIFESTATIONS ASSOCIATED WITH IBD

Less frequently, IBD has been associated with Sjögren's syndrome[68], rheumatoid arthritis[69], inflammatory myopathy and Takayasu arteritis[70,71], although these come from isolated case reports and no confirmed clinical or pathophysiological association can be made. There has been one study that has evaluated the prevalence of fibromyalgia and spread of musculoskeletal pain in patients with IBD, which has found equal frequencies to those in the general population[72]. In addition, it is advisable to evaluate IBD patients for osteoporosis if they have received high-dose steroids for long periods, and to implement prophylactic measures such as calcium and vitamin D supplements[73].

TREATMENT OF RHEUMATIC MANIFESTATIONS OF IBD

Treatment depends on the severity of the clinical picture. Patients with mild oligoarthritis usually respond to relative rest, physiotherapy and intra-articular steroid injections[74]. Most of the patients respond to nonsteroidal anti-inflammatory drugs because they control the symptoms and joint and enthesis inflammation, but they do not stop joint destruction, and they may have important side effects including exacerbation of IBD[75,76] and produce small intestine and colon ulcers[77]. Hence, they are recommended for patients with mild exacerbations, to control symptoms in arthritis flares, but their use must be limited to the minimal effective dose and time.

Sulfasalazine and 5-aminosalicylic acid are often recognized as useful for IBD, and are efficacious for moderate peripheral arthritis in SpA and IBD-associated arthritis, particularly in UC[78,79]. Their usefulness in CD is less clear. Some studies have shown promising results, others, no benefit at all, to control CD-associated arthritis[80-82]. These drugs have no effect in the progression of aggressive arthritis and their utility in axial involvement is marginal; also, they do not prevent the appearance of IBD in patients with SpA[83]. Their beneficial effect may be explained by their anti-inflammatory effects in the intestinal wall, by normalizing its permeability and preventing antigen entrance through an abnormal intestinal wall[84].

Immunosuppression with methotrexate, azathioprine, 6-mercaptopurine, cyclosporine and leflunomide has been successful in some patients with peripheral arthritis and other extraintestinal manifestations, although there are no controlled studies that have demonstrated their efficacy[74,85-88], and the evidence of their efficacy comes from uncontrolled studies or case reports.

The description of the pathogenic mechanisms of SpAs and IBD have relevant therapeutic implications for both groups of diseases. The initial observation that intestinal manifestations of patients with both SpA and IBD improve with TNF-α blocking agents has led to the use of these medications in patients with IBD, with favorable results, particularly with infliximab and adalimumab in CD[89]. Currently, infliximab is a first-line treatment for patients with active AS associated with IBD, and other TNF-α blocking agents such as certolizumab that have shown good results for patients with IBD, are promising[90,91].

Also, considering the pathogenic relationship between gut and joint inflammation in SpA, there are other agents with potential benefits in both diseases. Some of these include IL-10, IL-11, IL-6, intercellular adhesion molecule 1, mitogen-activated protein kinase and integrin (α4 and α4β7) blockade, as well as TLR modulation[92-95].

Some groups have proposed the use of probiotics for the treatment of patients with IBD and arthritis or AS. Their use is based on modulation of intestinal flora by bacteria and their products. The objective is to treat patients with persistent arthralgia in the early phases of the disease, before chronic damage is established, to improve the quality of life and have a positive influence on the natural course of the disease. Some interesting studies have demonstrated that these compounds improve experimental colitis in murine models and in patients with IBD. One of these models has shown that the anti-

inflammatory effect depends on the probiotic DNA, in an IFN-mediated response induced by TLR9. Further studies should evaluate these and other mechanisms in animal models[96,97].

CONCLUSION

Rheumatic manifestations of IBD include peripheral arthritis, enthesitis and axial involvement, and they are present in up to 62% of patients with IBD. There is an important immunopathogenic relationship between gut and joint abnormalities in patients with IBD. TLR variants and abnormalities, altered function and balance of T-cell subpopulations and their production of pro-inflammatory cytokines, as well as integrin and E-cadherin dysfunction are partially responsible for such a relationship, and research in these areas will help to clarify the pathogenesis of these complex diseases. Medical treatment of rheumatic manifestations of IBD includes sulfasalazine and mesalamine, immunomodulators and TNF-α inhibitors. New treatments for IBD directed against pro-inflammatory cytokines and the regulation of the immune response may have long-term benefits for rheumatic manifestations.

REFERENCES

1 **Bargen JA**, Jackman RJ, Kerr JG. Complications and sequelae of chronic ulcerative colitis. *Ann Intern Med* 1929; **3**: 335-352

2 **Bywaters EG**, Ansell BM. Arthritis associated with ulcerative colitis; a clinical and pathological study. *Ann Rheum Dis* 1958; **17**: 169-183

3 **Ford DK**, Vallis DG. The clinical course of arthritis associated with ulcerative colitis and regional ileitis. *Arthritis Rheum* 1959; **2**: 526-536

4 **Wright V**, Watkinson G. The arthritis of ulcerative colitis. *Medicine* (Baltimore) 1959; **38**: 243-262

5 **Wright V**. Seronegative polyarthritis: a unified concept. *Arthritis Rheum* 1978; **21**: 619-633

6 **Mielants H**, Veys EM, Cuvelier C, de Vos M. Ileocolonoscopic findings in seronegative spondylarthropathies. *Br J Rheumatol* 1988; **27** Suppl 2: 95-105

7 **Mielants H**, Veys EM, De Vos M, Cuvelier C, Goemaere S, De Clercq L, Schatteman L, Elewaut D. The evolution of spondyloarthropathies in relation to gut histology. I. Clinical aspects. *J Rheumatol* 1995; **22**: 2266-2272

8 **De Vos M**, Mielants H, Cuvelier C, Elewaut A, Veys E. Long-term evolution of gut inflammation in patients with spondyloarthropathy. *Gastroenterology* 1996; **110**: 1696-1703

9 **Mielants H**, Veys EM, Cuvelier C, De Vos M, Goemaere S, De Clercq L, Schatteman L, Gyselbrecht L, Elewaut D. The evolution of spondyloarthropathies in relation to gut histology. III. Relation between gut and joint. *J Rheumatol* 1995; **22**: 2279-2284

10 **Leirisalo-Repo M**, Turunen U, Stenman S, Helenius P, Seppälä K. High frequency of silent inflammatory bowel disease in spondylarthropathy. *Arthritis Rheum* 1994; **37**: 23-31

11 **Repiso A**, Alcántara M, Muñoz-Rosas C, Rodríguez-Merlo R, Pérez-Grueso MJ, Carrobles JM, Martínez-Potenciano JL. Extraintestinal manifestations of Crohn's disease: prevalence and related factors. *Rev Esp Enferm Dig* 2006; **98**: 510-517

12 **Paredes JM**, Barrachina MM, Román J, Moreno-Osset E. [Joint disease in inflammatory bowel disease] *Gastroenterol Hepatol* 2005; **28**: 240-249

13 **Scarpa R**, del Puente A, D'Arienzo A, di Girolamo C, della Valle G, Panarese A, Lubrano E, Oriente P. The arthritis of ulcerative colitis: clinical and genetic aspects. *J Rheumatol* 1992; **19**: 373-377

14 **Turkcapar N**, Toruner M, Soykan I, Aydintug OT, Cetinkaya H, Duzgun N, Ozden A, Duman M. The prevalence of extraintestinal manifestations and HLA association in patients with inflammatory bowel disease. *Rheumatol Int* 2006; **26**: 663-668

15 **Podswiadek M**, Punzi L, Stramare R, D'Incà R, Ferronato A, Lo Nigro A, Sturniolo GC. [The prevalence of radiographic sacroiliitis in patients affected by inflammatory bowel disease with inflammatory low back pain] *Reumatismo* 2004; **56**: 110-113

16 **Steer S**, Jones H, Hibbert J, Kondeatis E, Vaughan R, Sanderson J, Gibson T. Low back pain, sacroiliitis, and the relationship with HLA-B27 in Crohn's disease. *J Rheumatol* 2003; **30**: 518-522

17 **Salvarani C**, Vlachonikolis IG, van der Heijde DM, Fornaciari G, Macchioni P, Beltrami M, Olivieri I, Di Gennaro F, Politi P, Stockbrügger RW, Russel MG. Musculoskeletal manifestations in a population-based cohort of inflammatory bowel disease patients. *Scand J Gastroenterol* 2001; **36**: 1307-1313

18 **Queiro R**, Maiz O, Intxausti J, de Dios JR, Belzunegui J, González C, Figueroa M. Subclinical sacroiliitis in inflammatory bowel disease: a clinical and follow-up study. *Clin Rheumatol* 2000; **19**: 445-449

19 **Baeten D**, De Keyser F, Van Damme N, Veys EM, Mielants H. Influence of the gut and cytokine patterns in spondyloarthropathy. *Clin Exp Rheumatol* 2002; **20**: S38-S42

20 **Demetter P**, De Vos M, Van Damme N, Baeten D, Elewaut D, Vermeulen S, Mareel M, Bullock G, Mielants H, Verbruggen G, De Keyser F, Veys EM, Cuvelier CA. Focal up-regulation of E-cadherin-catenin complex in inflamed bowel mucosa but reduced expression in ulcer-associated cell lineage. *Am J Clin Pathol* 2000; **114**: 364-370

21 **Demetter P**, Baeten D, De Keyser F, De Vos M, Van Damme N, Verbruggen G, Vermeulen S, Mareel M, Elewaut D, Mielants H, Veys EM, Cuvelier CA. Subclinical gut inflammation in spondyloarthropathy patients is associated with upregulation of the E-cadherin/catenin complex. *Ann Rheum Dis* 2000; **59**: 211-216

22 **Van Damme N**, Elewaut D, Baeten D, Demetter P, Cuvelier C, Verbruggen G, Mielants H, Veys EM, De Vos M, De Keyser F. Gut mucosal T cell lines from ankylosing spondylitis patients are enriched with alphaEbeta7 integrin. *Clin Exp Rheumatol* 2001; **19**: 681-687

23 **Elewaut D**, De Keyser F, Cuvelier C, Lazarovits AI, Mielants H, Verbruggen G, Sas S, Devos M, Veys EM. Distinctive activated cellular subsets in colon from patients with Crohn's disease and ulcerative colitis. *Scand J Gastroenterol* 1998; **33**: 743-748

24 **Korn T**, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. *Annu Rev Immunol* 2009; **27**: 485-517

25 **Seiderer J**, Elben I, Diegelmann J, Glas J, Stallhofer J, Tillack C, Pfennig S, Jürgens M, Schmechel S, Konrad A, Göke B, Ochsenkühn T, Müller-Myhsok B, Lohse P, Brand S. Role of the novel Th17 cytokine IL-17F in inflammatory bowel disease (IBD): upregulated colonic IL-17F expression in active Crohn's disease and analysis of the IL17F p.His161Arg polymorphism in IBD. *Inflamm Bowel Dis* 2008; **14**: 437-445

26 **Jandus C**, Bioley G, Rivals JP, Dudler J, Speiser D, Romero P. Increased numbers of circulating polyfunctional Th17 memory cells in patients with seronegative spondylarthritides. *Arthritis Rheum* 2008; **58**: 2307-2317

27 **Singh R**, Aggarwal A, Misra R. Th1/Th17 cytokine profiles in patients with reactive arthritis/undifferentiated spondyloarthropathy. *J Rheumatol* 2007; **34**: 2285-2290

28 **Sanchez-Munoz F**, Dominguez-Lopez A, Yamamoto-Furusho JK. Role of cytokines in inflammatory bowel disease. *World J Gastroenterol* 2008; **14**: 4280-4288

29 **Mielants H**, Veys EM, De Vos M, Cuvelier C. Increased intestinal permeability in ankylosing spondylitis. *Gut* 1992; **33**: 1150

30 **De Rycke L**, Vandooren B, Kruithof E, De Keyser F, Veys EM, Baeten D. Tumor necrosis factor alpha blockade treatment down-modulates the increased systemic and local expression of Toll-like receptor 2 and Toll-like receptor 4 in spondylarthropathy. *Arthritis Rheum* 2005; **52**: 2146-2158

31 **Yang ZX**, Liang Y, Zhu Y, Li C, Zhang LZ, Zeng XM, Zhong RQ. Increased expression of Toll-like receptor 4 in peripheral blood leucocytes and serum levels of some cytokines in patients with ankylosing spondylitis. *Clin Exp Immunol* 2007; **149**: 48-55

32 **Muratov V**, Ulfgren AK, Engström M, Elvin K, Winqvist O, Löfberg R, Lundahl J. Decreased numbers of FoxP3-positive and TLR-2-positive cells in intestinal mucosa are associated with improvement in patients with active inflammatory bowel disease following selective leukocyte apheresis. *J Gastroenterol* 2008; **43**: 277-282

33 **Frolova L**, Drastich P, Rossmann P, Klimesova K, Tlaskalova-Hogenova H. Expression of Toll-like receptor 2 (TLR2), TLR4, and CD14 in biopsy samples of patients with inflammatory bowel diseases: upregulated expression of TLR2 in terminal ileum of patients with ulcerative colitis. *J Histochem Cytochem* 2008; **56**: 267-274

34 **Szebeni B**, Veres G, Dezsõfi A, Rusai K, Vannay A, Mraz M, Majorova E, Arató A. Increased expression of Toll-like receptor (TLR) 2 and TLR4 in the colonic mucosa of children with inflammatory bowel disease. *Clin Exp Immunol* 2008; **151**: 34-41

35 **Arseneau KO**, Tamagawa H, Pizarro TT, Cominelli F. Innate and adaptive immune responses related to IBD pathogenesis. *Curr Gastroenterol Rep* 2007; **9**: 508-512

36 **Snelgrove T**, Lim S, Greenwood C, Peddle L, Hamilton S, Inman R, Rahman P. Association of toll-like receptor 4 variants and ankylosing spondylitis: a case-control study. *J Rheumatol* 2007; **34**: 368-370

37 **Adam R**, Sturrock RD, Gracie JA. TLR4 mutations (Asp299Gly and Thr399Ile) are not associated with ankylosing spondylitis. *Ann Rheum Dis* 2006; **65**: 1099-1101

38 **Baumgart DC**, Buning C, Geerdts L, Schmidt HH, Genschel J, Fiedler T, Gentz E, Molnar T, Nagy F, Lonovics J, Lochs H, Wiedenmann B, Nickel R, Witt H, Dignass A. The c.1-260C>T promoter variant of CD14 but not the c.896A>G (p.D299G) variant of toll-like receptor 4 (TLR4) genes is associated with inflammatory bowel disease. *Digestion* 2007; **76**: 196-202

39 **Yamamoto-Furusho JK**, Podolsky DK. Innate immunity in inflammatory bowel disease. *World J Gastroenterol* 2007; **13**: 5577-5580

40 **Tiwana H**, Walmsley RS, Wilson C, Yiannakou JY, Ciclitira PJ, Wakefield AJ, Ebringer A. Characterization of the humoral immune response to Klebsiella species in inflammatory bowel disease and ankylosing spondylitis. *Br J Rheumatol* 1998; **37**: 525-531

41 **Ebringer A**, Rashid T, Tiwana H, Wilson C. A possible link between Crohn's disease and ankylosing spondylitis via Klebsiella infections. *Clin Rheumatol* 2007; **26**: 289-297

42 **Reveille JD**, Ball EJ, Khan MA. HLA-B27 and genetic predisposing factors in spondyloarthropathies. *Curr Opin Rheumatol* 2001; **13**: 265-272

43 **Hammer RE**, Maika SD, Richardson JA, Tang JP, Taurog JD. Spontaneous inflammatory disease in transgenic rats expressing HLA-B27 and human beta 2m: an animal model of HLA-B27-associated human disorders. *Cell* 1990; **63**: 1099-1112

44 **Taurog JD**, Richardson JA, Croft JT, Simmons WA, Zhou M, Fernández-Sueiro JL, Balish E, Hammer RE. The germfree state prevents development of gut and joint inflammatory disease in HLA-B27 transgenic rats. *J Exp Med* 1994; **180**: 2359-2364

45 **Yamamoto-Furusho JK**. Genetic factors associated with the development of inflammatory bowel disease. *World J Gastroenterol* 2007; **13**: 5594-5597

46 **Orchard TR**, Thiyagaraja S, Welsh KI, Wordsworth BP, Hill Gaston JS, Jewell DP. Clinical phenotype is related to HLA genotype in the peripheral arthropathies of inflammatory bowel disease. *Gastroenterology* 2000; **118**: 274-278

47 **Núñez C**, Alecsandru DM, Mendoza JL, Urcelay E, Díaz-Rubio M, de la Concha EG, Martinez A. Genetic markers linked to rheumatoid arthritis are also strongly associated with articular manifestations in ulcerative colitis patients. *Hum Immunol* 2006; **67**: 324-330

48 **Laukens D**, Peeters H, Marichal D, Vander Cruyssen B, Mielants H, Elewaut D, Demetter P, Cuvelier C, Van Den Berghe M, Rottiers P, Veys EM, Remaut E, Steidler L, De Keyser F, De Vos M. CARD15 gene polymorphisms in patients with spondyloarthropathies identify a specific phenotype previously related to Crohn's disease. *Ann Rheum Dis* 2005; **64**: 930-935

49 **Manguso F**, Sanges M, Staiano T, Gargiulo S, Nastro P, Gargano D, Somma P, Mansueto G, Peluso R, Scarpa R, D'Armiento FP, Astarita C, Ayala F, Renda A, Mazzacca G, D'Arienzo A. Cigarette smoking and appendectomy are risk factors for extraintestinal manifestations in ulcerative colitis. *Am J Gastroenterol* 2004; **99**: 327-334

50 **Holden W**, Orchard T, Wordsworth P. Enteropathic arthritis. *Rheum Dis Clin North Am* 2003; **29**: 513-530, viii

51 **Gravallese EM**, Kantrowitz FG. Arthritic manifestations of inflammatory bowel disease. *Am J Gastroenterol* 1988; **83**: 703-709

52 **Orchard TR**, Wordsworth BP, Jewell DP. Peripheral arthropathies in inflammatory bowel disease: their articular distribution and natural history. *Gut* 1998; **42**: 387-391

53 **de Vlam K**, Mielants H, Cuvelier C, De Keyser F, Veys EM, De Vos M. Spondyloarthropathy is underestimated in inflammatory bowel disease: prevalence and HLA association. *J Rheumatol* 2000; **27**: 2860-2865

54 **Mielants H**, Veys EM, Goethals K, Van Der Straeten C, Ackerman C. Destructive lesions of small joints in seronegative spondylarthropathies: relation to gut inflammation. *Clin Exp Rheumatol* 1990; **8**: 23-27

55 **Balbir-Gurman A**, Schapira D, Nahir M. Arthritis related to ileal pouchitis following total proctocolectomy for ulcerative colitis. *Semin Arthritis Rheum* 2001; **30**: 242-248

56 **Frayha R**, Stevens MB, Bayless TM. Destructive monarthritis and granulomatous synovitis as the presenting manifestations of Crohn's disease. *Johns Hopkins Med J* 1975; **137**: 151-155

57 **Dekker-Saeys BJ**, Meuwissen SG, Van Den Berg-Loonen EM, De Haas WH, Agenant D, Tytgat GN. Ankylosing spondylitis and inflammatory bowel disease. II. Prevalence of peripheral arthritis, sacroiliitis, and ankylosing spondylitis in patients suffering from inflammatory bowel disease. *Ann Rheum Dis* 1978; **37**: 33-35

58 **Palm O**, Moum B, Ongre A, Gran JT. Prevalence of ankylosing spondylitis and other spondyloarthropathies among patients with inflammatory bowel disease: a population study (the IBSEN study). *J Rheumatol* 2002; **29**: 511-515

59 **Dougados M**, van der Linden S, Juhlin R, Huitfeldt B, Amor B, Calin A, Cats A, Dijkmans B, Olivieri I, Pasero G. The European Spondylarthropathy Study Group preliminary criteria for the classification of spondylarthropathy. *Arthritis Rheum* 1991; **34**: 1218-1227

60 **Wordsworth P**. Arthritis and inflammatory bowel disease. *Curr Rheumatol Rep* 2000; **2**: 87-88

61 **De Keyser F**, Elewaut D, De Vos M, De Vlam K, Cuvelier C, Mielants H, Veys EM. Bowel inflammation and the spondyloarthropathies. *Rheum Dis Clin North Am* 1998; **24**: 785-813, ix-x

62 **Török HP**, Glas J, Gruber R, Brumberger V, Strasser C, Kellner H, Märker-Hermann E, Folwaczny C. Inflammatory bowel disease-specific autoantibodies in HLA-B27-associated spondyloarthropathies: increased prevalence of ASCA and pANCA. *Digestion* 2004; **70**: 49-54

63 **Desplat-Jégo S**, Johanet C, Escande A, Goetz J, Fabien N,

Olsson N, Ballot E, Sarles J, Baudon JJ, Grimaud JC, Veyrac M, Chamouard P, Humbel RL. Update on Anti-Saccharomyces cerevisiae antibodies, anti-nuclear associated anti-neutrophil antibodies and antibodies to exocrine pancreas detected by indirect immunofluorescence as biomarkers in chronic inflammatory bowel diseases: results of a multicenter study. *World J Gastroenterol* 2007; **13**: 2312-2318

64 **Mokrowiecka A**, Daniel P, Słomka M, Majak P, Malecka-Panas E. Clinical utility of serological markers in inflammatory bowel disease. *Hepatogastroenterology* 2009; **56**: 162-166

65 **Riente L**, Chimenti D, Pratesi F, Delle Sedie A, Tommasi S, Tommasi C, Bombardieri S, Migliorini P. Antibodies to tissue transglutaminase and Saccharomyces cerevisiae in ankylosing spondylitis and psoriatic arthritis. *J Rheumatol* 2004; **31**: 920-924

66 **Urlep D**, Mamula P, Baldassano R. Extraintestinal manifestations of inflammatory bowel disease. *Minerva Gastroenterol Dietol* 2005; **51**: 147-163

67 **Helliwell PS**, Hickling P, Wright V. Do the radiological changes of classic ankylosing spondylitis differ from the changes found in the spondylitis associated with inflammatory bowel disease, psoriasis, and reactive arthritis? *Ann Rheum Dis* 1998; **57**: 135-140

68 **Rhew EY**, Ramsey-Goldman R, Buchman AL. Sjogren's syndrome in association with Crohn's disease. *J Clin Gastroenterol* 2003; **37**: 312-314

69 **Toussirot E**, Wendling D. Crohn's disease associated with seropositive rheumatoid arthritis. *Clin Exp Rheumatol* 1997; **15**: 307-311

70 **Aoyagi S**, Akashi H, Kawara T, Ishihara K, Tanaka A, Kanaya S, Koga Y, Ishikawa R. Aortic root replacement for Takayasu arteritis associated with ulcerative colitis and ankylosing spondylitis--report of a case. *Jpn Circ J* 1998; **62**: 64-68

71 **Hilário MO**, Terreri MT, Prismich G, Len C, Kihara EN, Goldenberg J, Solé D. Association of ankylosing spondylitis, Crohn's disease and Takayasu's arteritis in a child. *Clin Exp Rheumatol* 1998; **16**: 92-94

72 **Palm O**, Moum B, Jahnsen J, Gran JT. Fibromyalgia and chronic widespread pain in patients with inflammatory bowel disease: a cross sectional population survey. *J Rheumatol* 2001; **28**: 590-594

73 **Rodríguez-Bores L**, Barahona-Garrido J, Yamamoto-Furusho JK. Basic and clinical aspects of osteoporosis in inflammatory bowel disease. *World J Gastroenterol* 2007; **13**: 6156-6165

74 **Juillerat P**, Mottet C, Froehlich F, Felley C, Vader JP, Burnand B, Gonvers JJ, Michetti P. Extraintestinal manifestations of Crohn's disease. *Digestion* 2005; **71**: 31-36

75 **Kaufmann HJ**, Taubin HL. Nonsteroidal anti-inflammatory drugs activate quiescent inflammatory bowel disease. *Ann Intern Med* 1987; **107**: 513-516

76 **Bjarnason I**, Hayllar J, MacPherson AJ, Russell AS. Side effects of nonsteroidal anti-inflammatory drugs on the small and large intestine in humans. *Gastroenterology* 1993; **104**: 1832-1847

77 **Kaufman HL**, Fischer AH, Carroll M, Becker JM. Colonic ulceration associated with nonsteroidal anti-inflammatory drugs. Report of three cases. *Dis Colon Rectum* 1996; **39**: 705-710

78 **Dissanayake AS**, Truelove SC. A controlled therapeutic trial of long-term maintenance treatment of ulcerative colitis with sulphazalazine (Salazopyrin). *Gut* 1973; **14**: 923-926

79 **Dick AP**, Grayson MJ, Carpenter RG, Petrie A. Controlled trial of sulphasalazine in the treatment of ulcerative colitis. *Gut* 1964; **5**: 437-442

80 **Van Hees PA**, Van Lier HJ, Van Elteren PH, Driessen M, Van Hogezand RA, Ten Velde GP, Bakker JH, Van Tongeren JH. Effect of sulphasalazine in patients with active Crohn's disease: a controlled double-blind study. *Gut* 1981; **22**: 404-409

81 **Malchow H**, Ewe K, Brandes JW, Goebell H, Ehms H, Sommer H, Jesdinsky H. European Cooperative Crohn's Disease Study (ECCDS): results of drug treatment. *Gastroenterology* 1984; **86**: 249-266

82 **Wenckert A**, Kristensen M, Eklund AE, Barany F, Jarnum S, Worning H, Folkenborg O, Holtz A, Bonnevie O, Riis P. The long-term prophylactic effect of salazosulphapyridine (Salazopyrin) in primarily resected patients with Crohn's disease. A controlled double-blind trial. *Scand J Gastroenterol* 1978; **13**: 161-167

83 **Ferraz MB**, Tugwell P, Goldsmith CH, Atra E. Meta-analysis of sulfasalazine in ankylosing spondylitis. *J Rheumatol* 1990; **17**: 1482-1486

84 **Mielants H**, Veys EM, Cuvelier C, De Vos M. Course of gut inflammation in spondylarthropathies and therapeutic consequences. *Baillieres Clin Rheumatol* 1996; **10**: 147-164

85 **Padovan M**, Castellino G, Govoni M, Trotta F. The treatment of the rheumatological manifestations of the inflammatory bowel diseases. *Rheumatol Int* 2006; **26**: 953-958

86 **De Keyser F**, Van Damme N, De Vos M, Mielants H, Veys EM. Opportunities for immune modulation in the spondyloarthropathies with special reference to gut inflammation. *Inflamm Res* 2000; **49**: 47-54

87 **Chen J**, Liu C. Methotrexate for ankylosing spondylitis. *Cochrane Database Syst Rev* 2004; CD004524

88 **Haibel H**, Rudwaleit M, Braun J, Sieper J. Six months open label trial of leflunomide in active ankylosing spondylitis. *Ann Rheum Dis* 2005; **64**: 124-126

89 **Van den Bosch F**, Kruithof E, De Vos M, De Keyser F, Mielants H. Crohn's disease associated with spondyloarthropathy: effect of TNF-alpha blockade with infliximab on articular symptoms. *Lancet* 2000; **356**: 1821-1822

90 **Rudwaleit M**, Baeten D. Ankylosing spondylitis and bowel disease. *Best Pract Res Clin Rheumatol* 2006; **20**: 451-471

91 **Behm BW**, Bickston SJ. Tumor necrosis factor-alpha antibody for maintenance of remission in Crohn's disease. *Cochrane Database Syst Rev* 2008; CD006893

92 **Van Assche G**, Vermeire S, Rutgeerts P. Focus on mechanisms of inflammation in inflammatory bowel disease sites of inhibition: current and future therapies. *Gastroenterol Clin North Am* 2006; **35**: 743-756

93 **D'Haens G**, Daperno M. Advances in biologic therapy for ulcerative colitis and Crohn's disease. *Curr Gastroenterol Rep* 2006; **8**: 506-512

94 **Nakamura K**, Honda K, Mizutani T, Akiho H, Harada N. Novel strategies for the treatment of inflammatory bowel disease: Selective inhibition of cytokines and adhesion molecules. *World J Gastroenterol* 2006; **12**: 4628-4635

95 **Cario E**. Therapeutic impact of toll-like receptors on inflammatory bowel diseases: a multiple-edged sword. *Inflamm Bowel Dis* 2008; **14**: 411-421

96 **Karimi O**, Peña AS. Indications and challenges of probiotics, prebiotics, and synbiotics in the management of arthralgias and spondyloarthropathies in inflammatory bowel disease. *J Clin Gastroenterol* 2008; **42** Suppl 3 Pt 1: S136-S141

97 **Lee J**, Rachmilewitz D, Raz E. Homeostatic effects of TLR9 signaling in experimental colitis. *Ann N Y Acad Sci* 2006; **1072**: 351-355

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ORIGINAL ARTICLE

Acoustic radiation force imaging sonoelastography for noninvasive staging of liver fibrosis

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Abstract

AIM: To investigate the diagnostic accuracy of acoustic radiation force impulse (ARFI) imaging as a noninvasive method for the assessment of liver fibrosis in chronic hepatitis C (CHC) patients.

METHODS: We performed a prospective blind comparison of ARFI elastography, APRI index and FibroMax in a consecutive series of patients who underwent liver biopsy for CHC in University Hospital Bucharest. Histopathological staging of liver fibrosis according to the METAVIR scoring system served as the reference. A total of 74 patients underwent ARFI elastography, APRI index, FibroMax and successful liver biopsy.

RESULTS: The noninvasive tests had a good correlation with the liver biopsy results. The most powerful test in predicting fibrosis was ARFI elastography. The diagnostic accuracy of ARFI elastography, expressed as area under receiver operating characteristic curve (AUROC) had a validity of 90.2% (95% CI AUROC = 0.831-0.972, $P < 0.001$) for the diagnosis of significant fibrosis ($F \geqslant 2$). ARFI sonoelastography predicted even better F3 or F4 fibrosis (AUROC = 0.993, 95% CI = 0.979-1).

CONCLUSION: ARFI elastography had very good accuracy for the assessment of liver fibrosis and was superior to other noninvasive methods (APRI Index, FibroMax) for staging liver fibrosis.

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Key words: Elasticity imaging techniques; Hepatitis C; Liver biopsy; Liver fibrosis

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INTRODUCTION

The prognosis and clinical management of chronic liver diseases are highly dependent on the extent of liver fibrosis. This is particularly true in patients with chronic hepatitis C (CHC), which is the leading cause of cirrhosis in many countries. CHC progresses to cirrhosis through a sequence of morphological changes that reflect increasing fibrosis and architectural distortion[1]. Staging systems of varying complexity based on these morphological changes have been used in patient management as a guide to prognosis, and in clinical investigations as an estimate of natural history and response to therapy.

Liver biopsy is the current reference examination for the assessment of liver fibrosis. It is important to remember that a needle biopsy is merely a sample of the entire liver, and that scarring in chronic liver disease is typically irregularly distributed in the liver. Needle liver biopsy removes only about 1/50 000th of the liver and carries substantial interpreter errors[2]. Liver biopsy is an invasive procedure with certain unavoidable risks and complications. Therefore, the development of noninvasive tests to assess hepatic inflammation and fibrosis has been an active area of research. Furthermore, as new antifibrotic therapies are studied, there is a growing need to develop noninvasive tests for fibrosis for use in clinical trials.

Several noninvasive methods have been proposed to stage liver fibrosis, including biochemical tests and imaging techniques. The biochemical tests consist of sophisticated indices and scores, or a large number of serological markers of liver fibrosis. However, the value of these diagnostic methods remains debated.

Among the imaging methods, transient elastography is a new technique that rapidly and noninvasively measures mean tissue stiffness. It is largely accepted that hepatic stiffness is related to the degree of fibrosis. Most clinical studies have been performed with FibroScan. Recently, acoustic radiation force impulse (ARFI) imaging sonoelastography has been proposed as an alternative method to assess liver elasticity. To the best of our knowledge, no study has compared ARFI sonoelastography and biochemical tests (APRI index, FibroMax) for the assessment of liver fibrosis.

The aim of our study was to evaluate the value of ARFI elastography for the diagnosis of liver fibrosis and to compare the accuracy of ARFI elastography, APRI index and FibroMax for staging liver fibrosis in patients who underwent liver biopsy for CHC.

MATERIALS AND METHODS

Study population

The study was a single-centered, prospective, blind comparison of ARFI sonoelastography, APRI index and FibroMax in 100 patients with CHC who underwent liver biopsy at the University Hospital Bucharest between January 2007 and June 2008. Patients with CHC were selected out of 215 patients who attended the University Hospital Bucharest for hepatic cytolysis.

Inclusion criteria were the presence of HCV RNA in serum without previous antiviral treatment and hepatic cytolysis [alanine aminotransferase (ALT) > 1.5 × normal]. Patients with other forms of chronic liver disease and those with ascites were excluded from the study.

Hepatitis C virus (HCV) infection was defined by the presence in the serum of anti-HCV antibodies using the third generation ELISA. HCV infection was confirmed by performing the COBAS TaqMan HCV test (Roche Molecular Systems, Inc., NJ, USA). This test is an *in vitro* amplification of the HCV nucleic acid, which uses the High Pure System Viral nucleic acid kit (Roche Diagnostics Corp, IN, USA) for manual preparation and the COBAS TaqMan 48 analyzer (Roche Diagnostics Corp, IN, USA) for automatic amplification and detection. The RNA titer was expressed in IU/mL. The detection limit was > 15 IU/mL with a positive rate of 95%.

Patients who fulfilled the inclusion criteria were enrolled and underwent blood tests for APRI, FibroMax measurement, and ultrasound elastography on the day before the liver biopsy.

The study was approved by the local ethics committees and all individuals provided written informed consent prior to enrollment in the study.

Biochemical evaluation of the liver

Liver function tests were performed prior to the liver biopsy. Blood samples were obtained under fasting conditions and routine liver function tests [ALT and aspartate transaminase (AST), total proteins, serum albumin and γ globulins, γ-glutamyl transferase (GGT), total bilirubin, alkaline phosphatase, international normalized ratio of prothrombin time] were performed. All were measured using Dade Behring reactants and the Dimension RXL analyzer (Dade Behring, FL, USA).

APRI index

AST levels and platelet counts were measured with a Dimension RXL analyzer and a Hematology System analyzer (Beckman-Coulter, Inc., CA, USA). The APRI index was calculated as follows: AST (/Upper Limit of Normal) × 100/platelet count (10^9/L). The upper normal limit of AST was considered as 38 U/L.

FibroMax

FibroMax (Biopredictive, France) is a new noninvasive panel of markers reported to predict advanced fibrosis. It contains five tests: FibroTest (to assess the degree of fibrosis); ActiTest (to assess the degree of activity, inflammation and necrosis); SteatoTest (to diagnose hepatic steatosis); NashTest (to diagnose nonalcoholic steatohepatitis); and AshTest (to diagnose severe alcoholic steatohepatitis). FibroMax combines the measurement of 10 indirect parameters adjusted for age, sex, weight and height: α2-macroglobulin, haptoglobin, apolipoprotein A1, total bilirubin, GGT, ALT, AST, fasting glucose, triglycerides and total cholesterol.

FibroMax serum samples were taken on the day before biopsy from patients in the fasting state. Biochemical parameters were measured using Dade Behring reactants and the Dimension RXL analyzer. Specific proteins, α2-macroglobulin, haptoglobin and apolipoprotein A1, were measured by immunonephelometry using the BN ProSpect Dade Behring system.Using FibroMax, fibrosis was staged on a scale of 0-4 with respect to the METAVIR fibrosis staging. For FibroTest, 0-0.21 fibrosis was staged as F0, 0.22-0.27 as F0-F1, 0.28-0.31 as F1, 0.32-0.48 as F1-F2, 0.49-0.58 as F2, 0.59-0.72 as F3, 0.73-0.74 as F3-F4, and 0.75-1 as F4.

Ultrasound elastography

ARFI imaging is a new tissue strain imaging technology that utilizes sound waves to interrogate the mechanical stiffness properties of tissues. Virtual Touch tissue imaging and Virtual Touch tissue quantification (Siemens AG, Germany) are the first available applications to implement this technology. Unlike conventional B-mode sonography, which provides anatomical details based on differences in acoustic impedance, Virtual Touch imaging describes relative physical tissue stiffness properties. In complement, Virtual Touch™ tissue quantification provides accurate numerical measurements related to tissue stiffness at user-defined anatomical locations.

Conventional elastography, however, requires manual compression of the tissues in order to provide relative displacement between the mass and the surrounding structures and therefore an assessment of strain. This can be difficult to apply to abdominal structures because of the presence of the ribs and abdominal wall. ARFI technology quantifies stiffness without manual compression. Using the Virtual Touch application, the tissue is compressed by acoustic energy. Virtual Touch tissue quantification is a quantitative assessment of tissue stiffness, through measurement of shear wave speed. Shear waves are generated by displacement of tissue and attenuate approximately 10 000 times more rapidly than conventional ultrasound waves.

Prior to liver biopsy, the patients underwent liver ultrasonographic elastography, under fasting conditions, using ARFI imaging [Acuson S2000 (Siemens AG, Germany] with Virtual Touch tissue quantification software). The system uses a standard ultrasonographic probe and offers elastography with a flexible metering box of 1 cm at variable depths. An acoustic push pulse transmitted by the transducer (3.5 MHz) toward the tissue induces an elastic shear wave that propagates through the tissue. The propagation of the shear wave is followed by detection pulses that are used to measure the velocity of shear wave propagation, which is directly related to tissue stiffness: speed increases with stiffness.

Liver stiffness was assessed by the same physician blinded to clinical and biological data. The measurements were performed on the right lobe of the liver through the intercostal spaces, with the patient lying in the decubitus dorsal position, with the right hand under the head and the head turned toward the left. The tip of the probe was covered with coupling gel and placed on the skin between the ribs at the level of the right lobe of the liver. The operator positioned the probe over the area of interest: segment eight of the right lobe, away from motion and portal/hepatic vessels, about 2 cm from the liver capsula, at a depth between 3.8 and 5.5 cm. When the target area had been located after optimizing the B-mode image, the operator pressed the button Virtual Touch tissue quantification, and asked the patient to stop breathing for a moment. Then, we observed the velocity of shear wave on screen. We used this protocol because we saw that, when the right lobe was scanned through the intercostal spaces with normal breathing, the variance of measurement was low. A total of 12 valid measurements per patient were performed. In difficult patients, to obtain better access to the liver without excessive pushing or breath holding, the measurements were performed on patients lying in the left lateral decubitus position, or using a subcostal approach to the left lobe. The results of ARFI ultrasonographic elastography were expressed as liver propagation velocity (m/s). The success rate of liver elasticity measurements was calculated as the ratio between validated and total measurements. Results were expressed as the median value of the total measurements (12 measurements per patient) in m/s, with values ranging from 0.8 to 3.7 m/s. Only procedures with a success rate of at least 60%, and with the interquartile range of all validated measurements < 30% of the median value, were considered reliable.

Histological assessment

Percutaneous liver biopsy was performed by senior operators using the Menghini technique with a 1.4-mm-diameter needle (Hepafix; B Braun Melsungen AG, Germany). After biopsy, the liver samples were fixed in formalin, paraffin embedded, and stained with hematoxylin-eosin and Masson's trichrome. All biopsy specimens were analyzed by an expert pathologist blinded to the biological and clinical data and to the results of ultrasound elastography. The length of each liver biopsy was established in millimeters and the number of portal tracts was counted.

Liver fibrosis and necroinflammatory activity were evaluated by the METAVIR scoring system. Fibrosis was staged on a four-point scale according to this score: F0 represented no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis and a few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis. Necroinflammatory activity was also graded on a four point scale: A0, none; A1, mild; A2, moderate; A3, severe.

Steatosis was assessed according to the number of hepatocytes with degeneration, as follows: 0, none; 1, steatosis in 1%-10% of hepatocytes; 2, steatosis in 11%-33% of hepatocytes; 3, steatosis in 34%-66% and 4, 67%-100% of hepatocytes[3].

Statistical analysis

Results are presented as the mean ± SD, counts and percentages. The results are illustrated as the median and 25th- to 75th-percentile values. The correlation between the noninvasive tests and liver biopsy was tested using the non-parametric Spearman's correlation coefficient. The overall validity was measured using area under the receiver operating characteristic curve (AUROC) with 95% CI. Cutoff values that defined prediction regions for each fibrosis stage were defined by a common optimization step that maximized the sum of the sensitivities in predicting the single stages. Finally, sensitivity, specificity, and positive (PPV) and negative predictive value (NPV) were calculated. The optimal cutoff was chosen at the highest left point on the curve. For all tests, significance was achieved at $P < 0.05$.

Table 1 Patient characteristics

Characteristics	Patients included (n = 74)
Sex (male/female)	32/42
Age (yr)	55.32 ± 9.6 (31-72)
BMI (kg/m^2)	25.59 ± 4.03 (17.23-33.29)
AST (IU/L)	50.06 ± 32.36 (18-188)
ALT (IU/L)	67.06 ± 26.79 (20-147)
INR	1.12 ± 0.15 (0.9-1.74)
Bilirubin (mg/dL)	0.69 ± 0.32 (0.29-2.08)
Albumin (g/dL)	3.9 ± 0.55 (2.6-4.9)
Cholesterol (mg/dL)	192.36 ± 46.58 (120-311)
Triglycerides (mg/dL)	123.24 ± 73.33 (48-467)

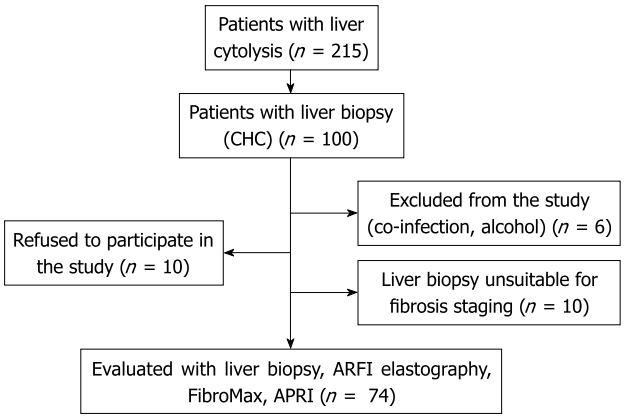

Figure 1 Diagram of patients with liver biopsy for CHC in the University Hospital Bucharest during the 18-mo study period.

Table 2 Patient distribution according to fibrosis stage, activity grade and steatosis

Fibrosis		Activity		Steatosis	
Stage	n (%)	Grade	n (%)	Grade	n (%)
0	1 (1.3)	0	1 (1.3)	0	24 (32.4)
1	9 (12.1)	1	32 (43.2)	1	23 (31.08)
2	25 (33.7)	2	29 (39.1)	2	12 (16.2)
3	19 (25.6)	3	12 (16.2)	3	11 (14.8)
4	20 (27.02)			4	4 (5.40)

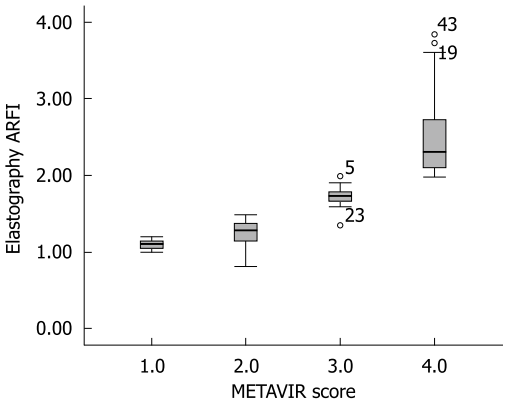

Figure 2 Box plot of METAVIR score and ARFI elastography. Top and bottom of boxes represent first and third quartiles, respectively. Length of box represents interquartile range within which 50% of values are located. Thick line through each box represents the median. Error bars mark minimum and maximum values (range). Small circles represent outliers. Skewed data for fibrosis stage 0/1 can be explained by the small number of patients in this group.

RESULTS

Patient characteristics

A total of 100 patients underwent liver biopsy for CHC. The patients were selected out of 215 patients who attended the University Hospital Bucharest for hepatic cytolisis. Ten patients refused to participate and six were excluded: three had hepatitis B virus coinfection, and three had a current daily alcohol intake of at least 60 g/d. In 10 patients, liver biopsy was unsuitable for fibrosis staging with the length of sample < 15 mm.

Seventy-four patients underwent ARFI elastography, APRI index, FibroMax and successful liver biopsy (Figure 1). Demographic, clinical and biochemical characteristics of the patients included in the study are shown in Table 1.

Histological characteristics

The median biopsy length was 20 mm (range: 15-25 mm); the number of portal tracts was 12 ± 4 (range: 8-16). Patient distribution according to METAVIR fibrosis stage and activity grade and steatosis are presented in Table 2.

Relationship between liver elasticity and histological stages

Figure 2 shows the median value (95% CI) of liver elasticity compared with fibrosis stages according to METAVIR score. A high correlation of increasing liver stiffness with increasing stage of fibrosis was observed. The Spearman's correlation coefficient between the liver stiffness and the histological fibrosis stages was highly significant with a value of 0.919 (P < 0.001). Steatosis is a frequent occurrence in CHC[4]. According to ANOVA test, no influence of the degree of activity or steatosis on elasticity values was observed in this study.

To determine the cutoffs that predicted each degree of fibrosis, we used the ROC curve method (Figure 3). ARFI elastography could predict fibrosis F2 or more, with a validity of 90.2% (95% CI AUROC = 0.831-0.972, P < 0.001). The optimal cutoff point between F1 and F2 was 1.215. At this value, ARFI elastography had a sensitivity of 89.4% and a specificity of 100%.

ARFI sonoelastography predicted even better F3 or F4 fibrosis (AUROC = 99.3%, 95% CI = 0.979-1). The optimal cutoff between F3 and F4 was 1.54, with sensitivity and specificity of 97% and 100%, respectively. The specificity of 100% shows that all study patients who presented with a value of ⩾ 1.54 upon sonoelastography had liver fibrosis F3 or more (confirmed by liver biopsy).

The same high validity was maintained as in predicting cirrhosis: (AUROC = 99.3%, 95% CI = 0.989-1).

The optimal cutoff in predicting cirrhosis was 1.94 with a sensitivity of 100% and a specificity of 98.1%. In other words, every patient in this study that had a sonoelastography result with a value < 1.94 did not have cirrhosis.

The most discriminative cutoff values of ARFI elastography are presented in Figure 4.

Table 3 Most discriminative cutoff values of ARFI elastography

	⩾ F1	⩾ F2	⩾ F3	F4
Cutoff (m/s)	1.185	1.215	1.54	1.94
Sensitivity (95% CI)	89% (79-95)	100% (91-100)	97% (86-100)	100% (83-100)
Specificity (95% CI)	87% (47-99)	71% (53-85)	100% (90-100)	98% (90-99)
PPV (95% CI)	98% (91-99)	79% (65-89)	100% (90-100)	95% (76-100)
NPV (95% CI)	50% (23-76)	100% (86-100)	97% (85-100)	100% (93-100)
Positive likelihood ratio	7.1	3.5	3.5	54

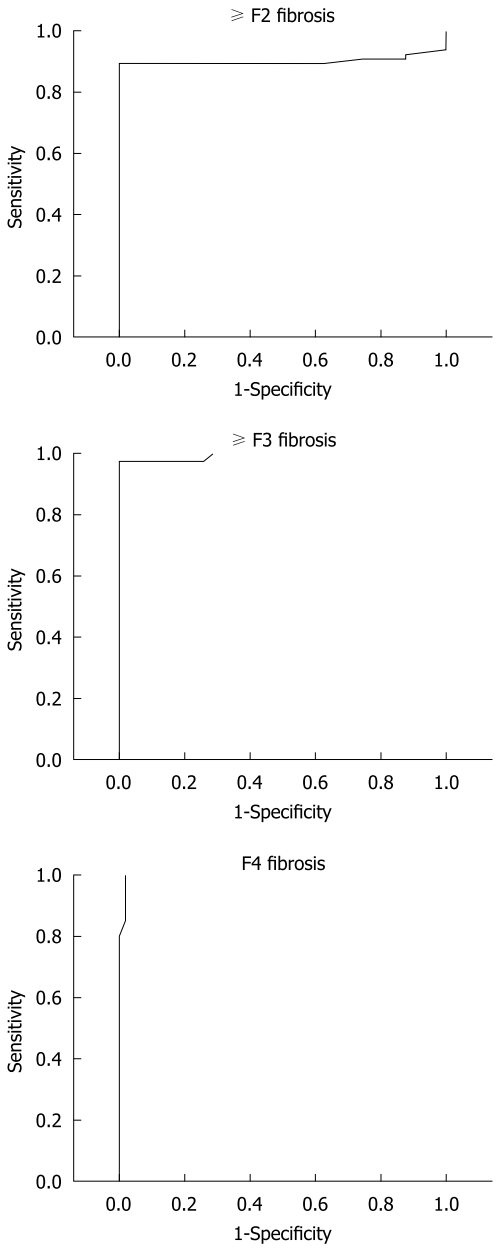

Figure 3 ROC curves: elastography predicting ⩾ F2 fibrosis, ⩾ F3 fibrosis and F4 fibrosis.

The corresponding sensitivity, specificity, PPV and NPV are shown in Table 3.

Relationship between APRI index and FibroMax and histological stages

A high correlation of increasing noninvasive measurements with increasing stage of fibrosis was observed. The Spearman's correlation coefficient between APRI index and FibroMax and the histological fibrosis stages was highly significant: 0.712 for APRI index and 0.674 for FibroMax. Table 4 shows the correlation between noninvasive tests and liver histology.

Table 4 Noninvasive tests and their correlations with liver pathology

	Noninvasive test	Correlation (Spearman coefficient)	95% CI	*P*
Liver biopsy	Elastography ARFI	0.919	0.872-0.949	< 0.001
	APRI index	0.712	0.572-0.811	< 0.001
	FibroMax	0.674	0.521-0.784	< 0.001

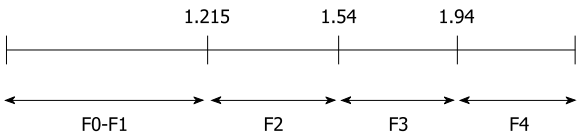

Figure 4 Cutoff points for degrees of fibrosis.

The correlation between two noninvasive tests should be better than with histological assessments for a correct prediction of liver fibrosis stage. The inter-test correlation coefficients, ARFI elastography/APRI index, and ARFI elastography/FibroMax were 0.712 and 0.718, respectively. All noninvasive tests used (Elastography ARFI, APRI index and FibroMax) were highly correlated with each other.

DISCUSSION

In chronic viral hepatitis, the knowledge of the stage of liver fibrosis is important for prognosis and for decisions about antiviral treatment[5]. Significant fibrosis (⩾ F2) in these patients is an indicator for antiviral treatment, hence the great therapeutic value of a highly accurate diagnostic test. At present, liver biopsy is used as the reference standard for the assessment of liver fibrosis[2]. However, liver biopsy is becoming increasingly useless in the management of HCV-related chronic liver disease because of large sampling error, consistent inter-observer disagreement, unavoidable risks and complications, and the fact that it is a snapshot of a process that is everything but a static one. Replacement of liver biopsy in the assessment of chronic liver disease is the goal of any noninvasive technique. Therefore, a test with diagnostic performance for precise estimation

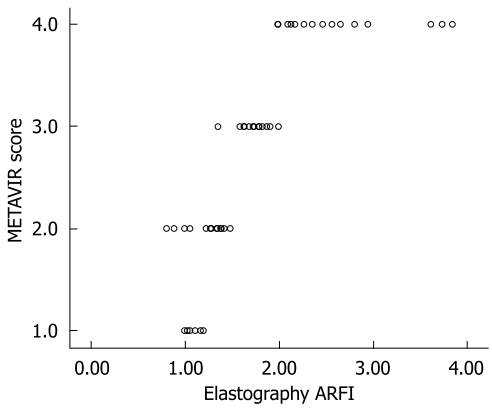

Figure 5 Correlation between METAVIR and ARFI elastography. Correlation between METAVIR and ARFI elastography showed that the overlap between stages F1 and F2 fibrosis limits the discriminatory value of the test in this context.

of the degree of liver fibrosis is of great therapeutic value. Previous studies have reported that transient elastography (FibroScan) can detect liver fibrosis and accurately predict significant fibrosis[5-11]. In the present study, we showed that APRI elastography should be taken into consideration as a noninvasive sonographical method for evaluation of liver fibrosis. A significant positive correlation between liver stiffness and the stages of fibrosis was noticed in patients with CHC. Significant AUROC for fibrosis F2 or more had a validity of 90.2% (95% CI AUROC = 0.831-0.972, $P < 0.001$).

ARFI proved to be an even better predictor of F3 or F4 fibrosis (AUROC = 99.3%, 95% CI = 0.979-1). Early and rapid detection of significant fibrosis using this noninvasive procedure is essential since patients with significant fibrosis are at a high risk of developing complications, such as portal hypertension or hepatocellular carcinoma, and consequently need specific follow-up.

We considered ascites as one of the exclusion criteria from our study since ARFI, similar to Fibro-Scan, is performed in close contact with the liver. Nevertheless, ascites indicates the presence of cirrhosis, which makes noninvasive staging of fibrosis unnecessary[5].

Liver elastometry with FibroScan is unsuccessful in patients with narrow intercostal spaces and in those with morbid obesity[7,8]. These are the major limitations of FibroScan measurements[12]. In the present study, patients with a body mass index (BMI) of up to 30 were enrolled, and ARFI elastography was performed successfully in all included patients. For the majority of the patients, the measurements were performed through a right intercostal space, and patients did not need to hold their breath during the examination. In difficult patients with narrow intercostal spaces, the examination was improved because of the subcostal approach to the left liver lobe. Elastography of the left liver lobe is especially helpful in obese patients. Rifai *et al*[13] have found recently that ARFI results of the right and left liver lobe were comparable ($n = 26$, $r = 0.44$, $P < 0.03$). Furthermore, ARFI elastography is less time consuming than FibroScan. The new software allows one to do 12 measurements in under 1 min, as compared to FibroScan that does 10 measurements in under at least 4 min[9].

The current study confirmed that, in CHC, no influence of steatosis or activity grade on correlation between elasticity values and fibrosis stages was observed. According to ANOVA test, the elasticity values were not affected by steatosis or activity grade. Similar results have been reported from studies analyzing transient elastography with FibroScan[7-9].

Three noninvasive tests were performed. The tests had a good correlation with the liver biopsy results. The most powerful test in predicting fibrosis was ARFI elastography, with a non-parametric correlation coefficient of 0.919 (95% CI = 0.872-0.949, $P < 0.001$). Other correlation coefficients were: 0.712 (95% CI = 0.572-0.811, $P < 0.001$) for APRI index, and 0.674 (95% CI = 0.521-0.784, $P < 0.001$).

In the present study, we found highly significant positive correlations between liver propagation velocity obtained with ARFI elastography and the METAVIR fibrosis stage. The correlation coefficients showed a linear relationship between ARFI elastography and liver biopsy fibrosis, but to determine the cutoffs that predicted each degree of fibrosis, we used the ROC curve method.

An AUC value of 0.902 (0.831-0.972) was obtained using ARFI elastography for the diagnosis of significant fibrosis (⩾ F2), with a cutoff value of 1.215 m/s. In comparison, three recent studies that analyzed the noninvasive assessment of liver fibrosis with FibroScan have revealed AUCs between 0.75 and 0.84 for the diagnosis of significant fibrosis (⩾ F2)[7-9]. The ARFI sonoelastography predicted even better F3 or F4 fibrosis (AUROC = 99.3%, 95% CI = 0.979-1).

Considering the increased values of sensitivity and specificity, ARFI sonoelastography was shown to be a valuable investigative technique that could be of great help in the evaluation and follow-up of liver fibrosis.

At present, it is difficult to determine the real impact of ARFI elastometry in the early diagnosis of fibrosis in patients with CHC. Figure 5 clearly indicates an overlap observed between F0-F1 and F2. The increase in liver propagation velocity was more important between stages F2 (1.21 m/s) and F3 (1.54 m/s) than between stages F1 (1.18 m/s) and F2 (1.21 m/s). This is consistent with the fact that the increase in fibrous tissue is more important between stages F2 and F3 than between stages F1 and F2.

This limit of ARFI elastography (reduced discriminatory value between F1 and F2) was overcome by the fact that significant fibrosis (⩾ F2) is considered a hallmark of progressive liver disease. Studies have shown that antiviral treatment of patients with CHC prolongs life, improves quality of life, and is cost-effective[14,15]. However, treatment may be associated with severe side effects and the decision for treatment needs to be made on an individual basis. Patients with fibrosis stage F2 are at increased risk for developing cirrhosis with its complications (ascites, encephalopathy, or portal hypertension). Therefore, patients with fibrosis stage ⩾ F2 have a stronger indication for treatment as compared with patients with no or mild fibrosis (F1)[16,17].

Compared with fibrosis biomarkers, the disadvantage of liver elastometry is the absence of a large control group to assess the limit of normal values[14-18]. In studies using liver biopsy as a reference method, as in our study, the number of patients without fibrosis (F0) is very small.

Liver biopsy was considered the gold standard in our study because it is the only reference method available at present[1,19]. However, this technique is an invasive procedure that is known to have serious limitations. Various studies have shown that a single-needle liver biopsy can miss the diagnosis of cirrhosis in 20%-50% of patients[19]. To maximize the diagnostic yield of liver biopsy, we selected only the patients with a biopsy length > 15 mm and histological sections with at least eight portal tracts. The major advantage of ARFI liver elastography compared with liver biopsy is that it is painless, rapid, has no risk of complications, is very well tolerated and it is more representative of the entire liver parenchyma than is liver biopsy (1/50000 of the total liver mass)[2].

To assess the performance of ARFI elastography, we have compared the liver propagation velocity with predictive blood tests (FibroMax) and APRI index in the same population. Our results showed that the most powerful test in predicting fibrosis was ARFI elastography. The high correlation between these three noninvasive tests suggests a sequential combination of them in algorithms used for staging liver fibrosis in patients with CHC.

In conclusion, our study showed that ARFI sonoelastography stands out on account of its very good correlation with liver biopsy (gold standard for evaluating liver fibrosis), good sensitivity and excellent specificity. It has proved to be a noninvasive imaging method, which is far superior to other methods investigated (APRI and FibroMax), for staging liver fibrosis. Further investigations on a larger number of patients are necessary to validate ARFI elastography as a noninvasive method of diagnosing liver fibrosis.

COMMENTS

Background

Liver biopsy is an invasive procedure with certain unavoidable risks and complications. Therefore, the development of noninvasive tests to assess hepatic inflammation and fibrosis has been an active area of research. Several noninvasive methods have been proposed to stage liver fibrosis, including biochemical tests and imaging techniques. The biochemical tests consist of sophisticated indices and scores, or a large number of serological markers of liver fibrosis. However, the value of these diagnostic methods remains debatable. Among the imaging methods, transient elastography, based on ultrasound, is a new technique that rapidly and noninvasively measures mean tissue stiffness.

Research frontiers

Transient elastography is a noninvasive method with performance equivalent to that of serum markers for the diagnosis of significant fibrosis in patients with hepatitis C. Combining transient elastography with serum markers as first-line assessment could avoid liver biopsy in the majority of these patients. There is now a general consensus that liver fibrosis is potentially reversible. Follow-up biopsy is too insensitive for real-time monitoring of progression and regression of fibrosis. When considering anti-fibrotic therapy, it is important to recognize that fibrosis is a dynamic process. Clinical proof and monitoring of anti-fibrotic drug effects requires better noninvasive tests for fibrosis. Incorporation of noninvasive tests into large studies and therapeutic trials should be a priority in the next few years.

Innovations and breakthroughs

Acoustic radiation force impulse (ARFI) imaging sonoelastography has been proposed as an alternative method to assess liver elasticity. No comparison of ARFI sonoelastography and biochemical tests (APRI and FibroMax) has been reported for the assessment of liver fibrosis.

Applications

ARFI imaging could be applied to the noninvasive assessment of hepatic fibrosis initially, or after treatment. This study evaluated ARFI imaging in patients with chronic hepatitis C, but probably it could be used for other liver diseases that lead to fibrosis (we need further studies).

Terminology

Elastography is a noninvasive method by which stiffness of soft tissue is evaluated.

Peer review

The present study provides readers with a reliable, relatively inexpensive tool to measure liver fibrosis. It is a well designed, conducted and reported study.

REFERENCES

1 **Goodman ZD**. Fibrosis in chronic hepatits C - assessment by liver biopsy. In: AASLD Postgraduate Course: Liver Diseases: Pathophysiologic Basis for the Therapy of Liver Disease. Boston, USA. 2007: 86-91

2 **Afdhal NH**. Non-biopsy methods to determine hepatic fibrosis. In: AASLD Postgraduate Course: Liver Diseases: Pathophysiologic Basis for the Therapy of Liver Disease. Boston, USA. 2007: 91-95

3 **Friedrich-Rust M**, Ong MF, Herrmann E, Dries V, Samaras P, Zeuzem S, Sarrazin C. Real-time elastography for noninvasive assessment of liver fibrosis in chronic viral hepatitis. *AJR Am J Roentgenol* 2007; **188**: 758-764

4 **Fierbinţeanu-Braticevici C**, Mohora M, Creţoiu D, Creţoiu S, Petrişor A, Usvat R, Ion DA. Role of oxidative stress in the pathogenesis of chronic hepatitis C (CHC). *Rom J Morphol Embryol* 2009; **50**: 407-412

5 **Friedrich-Rust M**, Ong MF, Martens S, Sarrazin C, Bojunga J, Zeuzem S, Herrmann E. Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. *Gastroenterology* 2008; **134**: 960-974

6 **Kettaneh A**, Marcellin P, Douvin C, Poupon R, Ziol M, Beaugrand M, de Lédinghen V. Features associated with success rate and performance of FibroScan measurements for the diagnosis of cirrhosis in HCV patients: a prospective study of 935 patients. *J Hepatol* 2007; **46**: 628-634

7 **Fraquelli M**, Rigamonti C, Casazza G, Conte D, Donato MF, Ronchi G, Colombo M. Reproducibility of transient elastography in the evaluation of liver fibrosis in patients with chronic liver disease. *Gut* 2007; **56**: 968-973

8 **Castéra L**, Vergniol J, Foucher J, Le Bail B, Chanteloup E, Haaser M, Darriet M, Couzigou P, De Lédinghen V. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology* 2005; **128**: 343-350

9 **Chang J**, Tan HH, Yew BS. Transient elastography (FibroScan ®) to assess hepatic fibrosis in Chinese with chronic hepatitis B. 17th Asian Pacific Association for the Study of Liver Conference. 2007

10 **Kim KM**, Choi WB, Park SH, Yu E, Lee SG, Lim YS, Lee HC, Chung YH, Lee YS, Suh DJ. Diagnosis of hepatic steatosis and fibrosis by transient elastography in asymptomatic healthy individuals: a prospective study of living related potential liver donors. *J Gastroenterol* 2007; **42**: 382-388

11 **Ziol M**, Handra-Luca A, Kettaneh A, Christidis C, Mal F, Kazemi F, de Lédinghen V, Marcellin P, Dhumeaux D, Trinchet JC, Beaugrand M. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C. *Hepatology* 2005; **41**: 48-54

12 **Roulot D**, Czernichow S, Le Clésiau H, Costes JL, Vergnaud AC, Beaugrand M. Liver stiffness values in apparently

healthy subjects: influence of gender and metabolic syndrome. *J Hepatol* 2008; **48**: 606-613

13 **Rifai K**, Bahr MJ, Mederacke I, Bantel H, Bayer D, Boozari B, Wedemeyer H, Manns MP, Gebel M. Acoustic Radiation Force Imaging (ARFI) as a new method of Ultrasonographic elastography allows accurate and flexible assessment of liver stiffness. Copenhagen: Poster presentation EASL, 2009

14 **Poynard T**, Halfon P, Castera L, Charlotte F, Le Bail B, Munteanu M, Messous D, Ratziu V, Benhamou Y, Bourlière M, De Ledinghen V. Variability of the area under the receiver operating characteristic curves in the diagnostic evaluation of liver fibrosis markers: impact of biopsy length and fragmentation. *Aliment Pharmacol Ther* 2007; **25**: 733-739

15 **Poynard T**, Halfon P, Castera L, Munteanu M, Imbert-Bismut F, Ratziu V, Benhamou Y, Bourlière M, de Ledinghen V. Standardization of ROC curve areas for diagnostic evaluation of liver fibrosis markers based on prevalences of fibrosis stages. *Clin Chem* 2007; **53**: 1615-1622

16 **Huwart L**, Sempoux C, Vicaut E, Salameh N, Annet L, Danse E, Peeters F, ter Beek LC, Rahier J, Sinkus R, Horsmans Y, Van Beers BE. Magnetic resonance elastography for the noninvasive staging of liver fibrosis. *Gastroenterology* 2008; **135**: 32-40

17 **Poynard T**, Munteanu M, Imbert-Bismut F, Charlotte F, Thabut D, Le Calvez S, Messous D, Thibault V, Benhamou Y, Moussalli J, Ratziu V. Prospective analysis of discordant results between biochemical markers and biopsy in patients with chronic hepatitis C. *Clin Chem* 2004; **50**: 1344-1355

18 **Ngo Y**, Munteanu M, Messous D, Charlotte F, Imbert-Bismut F, Thabut D, Lebray P, Thibault V, Benhamou Y, Moussalli J, Ratziu V, Poynard T. A prospective analysis of the prognostic value of biomarkers (FibroTest) in patients with chronic hepatitis C. *Clin Chem* 2006; **52**: 1887-1896

19 **Crockett SD**, Kaltenbach T, Keeffe EB. Do we still need a liver biopsy? Are the serum fibrosis tests ready for prime time? *Clin Liver Dis* 2006; **10**: 513-534, viii

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ORIGINAL ARTICLE

Immunotherapy for nonalcoholic steatohepatitis using the multiple cytokine production modulator Y-40138

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Abstract

AIM: To investigate the possible use of the multiple cytokine production modulator, Y-40138, as a novel immunotherapy in the rat nonalcoholic steatohepatitis (NASH) model.

METHODS: We allocated 6-wk-old male F344 rats to choline-supplemented, L-amino acid-defined (CSAA) diet (control group), CSAA diet + Y-40138 (control + Y-40138 group), choline-deficient, L-amino acid-defined (CDAA) diet (NASH group), or CDAA diet + Y-40138 (NASH + Y-40138 group). In each group, we measured the plasma alanine aminotransferase (ALT) levels, and the plasma and liver levels of tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), and interleukin-10 (IL-10). Tissue specimens of phosphate buffered saline-perfused liver were subjected to hematoxylin and eosin staining, Azan staining, Sirius red staining, and immunohistochemical staining (for Kupffer cells and TNF-α). We then extracted Kupffer cells from the collagenase-perfused livers using the Percoll gradient centrifugation method, and measured the TNF-α levels in the supernatant (*in vitro* TNF-α production by Kupffer cells) using an enzyme-linked immunosorbent assay kit.

RESULTS: In comparison to the NASH group, serum ALT elevation was mild, production of serum and liver TNF-α and IFN-γ was inhibited, and IL-10 production was increased in the NASH + Y-40138 group. Amelioration of liver histology was also noted in the NASH + Y-40138 group. Kupffer cell immunohistochemical staining revealed no differences between groups, whereas TNF-α immunohistochemical staining showed fewer stained cells in the NASH + Y-40138 group than in the NASH group. The TNF-α levels in the *in-vitro* Kupffer cell culture supernatant were lower in the NASH + Y-40138 group than in the NASH group.

CONCLUSION: Administration of Y-40138 to NASH model rats reduced hepatic inflammation and suppressed fibrosis. These results indicate that the multiple cytokine production modulator, Y-40138, is promising as a novel treatment for NASH.

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Key words: Nonalcoholic steatohepatitis; Y-40138; Tumor necrosis factor α; Interferon γ; Interleukin-10; Kupffer cell; Innate immunity

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INTRODUCTION

In recent years, nonalcoholic steatohepatitis (NASH) associated with obesity and diabetes mellitus has attracted a great deal of attention. Despite no history of alcohol intake, the histological findings of NASH are similar to

those of alcoholic hepatitis, and it is a progressive condition. A 2-component theory has been proposed for the etiology of NASH, the underlying condition being simple steatosis, with a first component of fatty degeneration as a result of insulin resistance, followed by a second component of aggravating factors such as inflammatory cytokines, including tumour necrosis factor α (TNF-α), oxidative stress and endotoxins[1]. Kupffer cells, part of the innate immune system, produce various cytokines and are known to play a role in pathologies of the liver[2,3]. Abnormally functioning Kupffer cells are suspected to be involved in NASH[4]. Although the etiological mechanisms of NASH have not been fully elucidated, similar histopathological findings in NASH and alcoholic hepatitis suggest the existence of a common underlying mechanism. One possible mechanism is that increased endotoxin levels cause activation of Kupffer cells through surface Toll-like receptor-4 (TLR4), leading to increased expression of TNF-α and increased levels of reactive oxygen species (ROS)[5]. These induce inflammation and fibrosis, progressing to NASH.

It is known that the choline-deficient L-amino acid-defined (CDAA) fed rat model shows fatty accumulation and fibrosis, and after 16 wk the liver shows cirrhotic changes, and nodules appear as in hepatocellular carcinoma[6]. Although this model lacks obesity and insulin resistance, the 2 major characteristics of NASH in humans, the possible relation of Kupffer cells with inflammation, steatosis, and fibrosis may resemble human NASH[7].

In this study, we administered Y-40138, which is a reciprocal cytokine production modulator, inhibiting production of TNF-α and interferon γ (IFN-γ), and promoting production of interleukin-10 (IL-10)[8,9], to a CDAA-fed rat NASH model, to investigate its possible use as a novel immunotherapy for NASH.

MATERIALS AND METHODS

Animals and induction of NASH model

Male F344 rats (6-wk-old, weighing 180-200 g) were purchased from Japan SLC Inc. (Hamamatsu, Japan). The animals were housed in stainless steel mesh cages under controlled temperature (23 ± 3℃) and relative humidity (50% ± 20%) conditions with 10 to 15 air changes per hour, and light illumination for 12 h a day. They were allowed access to tap water *ad libitum* throughout the duration of the study. CDAA and choline-supplemented L-amino acid-defined (CSAA) feeds were purchased from CLEA Japan Inc. (Tokyo, Japan). The details of both diets are described elsewhere[10].

The study groups were fed a CDAA diet for 8 wk to produce NASH. After 8 wk, the control group rats (CSAA diet, n = 5), control + Y-40138 group rats (CSAA + Y-40138 diet, n = 5), NASH group rats (CDAA diet, n = 5), and NASH + Y-40138 group rats (CDAA diet + Y-40138, n = 5) were killed and bled from the portal vein. Plasma was obtained by centrifugation at 3000 g for 15 min, and stored at -20℃. Liver tissues were excised after perfusion with phosphate buffered saline *via* the portal vein. All procedures were approved by our Institutional Animal Care Committee, and conducted in accordance with the Nara Medical University Guidelines for the Care and Use of Laboratory Animals.

Compounds

Y-40138 was obtained from Tanabe Mitsubishi Pharma Co. (Osaka, Japan), and dissolved in 0.5% hydroxypropyl methylcellulose solution. Y-40138 was orally administered to rats (10 mg/kg per day) for 8 wk.

Plasma alanine aminotransferase (ALT)

The plasma ALT levels were measured in samples from rats of each group. The blood samples were centrifuged, and the plasma was collected and stored at -30°C until used for ALT determination. The plasma ALT levels were measured using a 7170 Clinical Analyzer (Hitachi High-Technologies, Tokyo, Japan).

Plasma TNF-α, IFN-γ and IL-10

TNF-α, IFN-γ and IL-10 concentrations were measured in plasma samples (50 μL) from rats from each group using an enzyme-linked immunosorbent assay (ELISA) kit (BioSource International, Camarillo, CA, USA).

Liver TNF-α, IFN-γ and IL-10

Liver tissue samples were frozen in liquid nitrogen, powdered, and 30 mg samples were homogenized in 1 mL of lysis buffer. TNF-α, IFN-γ, IL-10 levels in the supernatant (50 μL) were measured using an ELISA kit (BioSource International).

Histological examination

Liver tissue samples were fixed in 10% formalin, embedded in paraffin, and 5 μm thick sections were cut from the paraffin blocks for staining with hematoxylin and eosin, Azan, and Sirius red. Histological grading and staging were performed using a modified scoring system based on the classifications of Matteoni *et al*[11] and Brunt *et al*[12].

Immunohistochemistry

Kupffer cell dynamics were analyzed by comparing cell counts in each group using immunohistochemical staining with anti-rat macrophage/dendritic cell monoclonal antibody (RM-4: Trans Genic Inc., Kobe, Japan), Vectastain ABC Elite Kit (Vector Laboratories, Burlingame, CA, USA), and DAB peroxidase substrate solution (Vector Laboratories), with counterstaining by Hematoxylin Mayer. The stained areas were analyzed and compared using NIH image software (Version 1.61, U. S. National Institute of Health, Bethesda, MD, USA).

TNF-α immunohistochemical staining was performed on the paraffin sections using an anti-rat TNF-α/TNFSF1A antibody (R&D Systems, Minneapolis, MN, USA). We then used Vectastain ABC Elite Kit, and DAB peroxidase substrate solution, with counterstaining by Hematoxylin Mayer. The stained areas were analyzed using NIH image software.

Isolation and culture of Kupffer cells

Kupffer cells were harvested from the liver using isolation buffers according to a previously described procedure[13,14]. The livers were perfused *in situ* with Ca^{2+}-free minimum essential medium (Sigma, St. Louis, MI, USA), followed by 0.3% pronase (Roche Diagnostics Co., Indianapolis, IN, USA) and 0.05% collagenase type Ⅳ (Wako, Osaka, Japan) in Dulbecco's modified Eagle's medium/F-12 (Sigma) at a rate of 10 mL/min through the portal vein. The liver was carefully removed and minced using scissors. The minced livers were further incubated in a shaker water bath with 0.035% pronase and 62.5 U/mL DNase (Sigma) in Dulbecco's modified Eagle's medium/F-12 at 37℃ for 20 min. The samples were then filtered through gauze mesh, and the parenchymal cells were removed using low-speed centrifugation. Percoll (GE Healthcare, Little Chalfont, UK) was used for density gradient centrifugation of the cells. Kupffer cells were collected from the layer between 1.04 and 1.06, and cultured in RPMI 1640 supplemented with 10% fetal bovine serum and SAG (50 μg/mL streptomycin, 50 μg/mL ampicillin, 0.3 g/L L-glutamine). The viability of the cultured Kupffer cells was determined using trypan blue exclusion. Kupffer cells were seeded at a density of 5×10^5 cells/mL on 12-well plastic plates with RPMI-1640 medium. For the lipopolysaccharide (LPS)-stimulated group, Kupffer cells were then exposed to 1 μg/mL of LPS (Sigma-Aldrich Corp.) and incubated for 4.5 h at 37℃ in 50 mL/L CO_2-humidified air. Cells not treated with LPS were used as a control. TNF-α levels in the supernatant were measured using an ELISA kit.

The purity of the isolated Kupffer cells was over 98%, as examined by the uptake of 1 μm latex beads[15], and the viability was over 97% using the trypan blue dye exclusion test.

Statistical analysis

All results are expressed as mean ± SD. Analyses were conducted using Statview software (version 5.0, SAS Institute, Cary, NC, USA). The statistical differences between groups were evaluated using the paired *t*-test. A *P*-value < 0.05 was considered statistically significant.

RESULTS

Plasma ALT levels

The mean plasma ALT level was 35.8 ± 4.7 IU/L in the control group, 38.3 ± 6.7 IU/L in the control + Y-40138 group, 279.0 ± 32.8 IU/L in the NASH group, and 171.3 ± 28.5 IU/L in the NASH + Y-40138 group. The NASH group showed a statistically significant elevation in the plasma ALT level in comparison to the control group ($P < 0.01$). The plasma ALT levels were significantly decreased in the NASH + Y-40138 group in comparison to the NASH group ($P < 0.01$) (Figure 1).

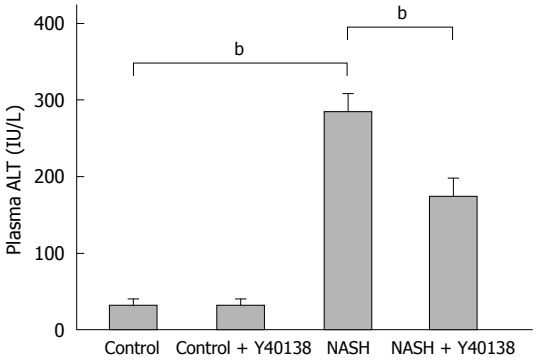

Figure 1 The plasma alanine aminotransferase (ALT) levels in the nonalcoholic steatohepatitis (NASH) group were significantly elevated in comparison to the control group. The plasma ALT levels in the NASH + Y-40138 group were significantly decreased in comparison to the NASH group (each group $n = 5$). $^bP < 0.01$.

Plasma TNF-α, IFN-γ and IL-10 levels

In comparison to the control group, the plasma TNF-α levels were significantly elevated in the NASH group (5.1 ± 1.4 pg/mL *vs* 35.2 ± 7.3 pg/mL, $P < 0.01$). The plasma TNF-α levels were significantly lower in the NASH + Y-40138 group (9.6 ± 3.6 pg/mL) than in the NASH group ($P < 0.01$). The plasma IFN-γ levels in the NASH group (62.4 ± 11.0 pg/mL, $P < 0.01$) were significantly elevated in comparison to the control group (12.3 ± 2.3 pg/mL). The plasma IFN-γ levels in the NASH + Y-40138 group (12.8 ± 6.3 pg/mL) were significantly decreased in comparison to the NASH group ($P < 0.01$). The plasma IL-10 levels in the control + Y-40138 group (2860 ± 350 pg/mL, $P < 0.01$) and the NASH group (1360 ± 380 pg/mL, $P < 0.01$) were significantly elevated in comparison to the control group (520 ± 96 pg/mL). The plasma IL-10 levels in the NASH + Y-40138 group (3820 ± 480 pg/mL) were significantly increased in comparison to the NASH group ($P < 0.01$) (Figure 2A).

Liver TNF-α, IFN-γ and IL-10 levels

The liver TNF-α level in the NASH group was 39.1 ± 6.2 pg/μg protein, significantly higher than that in the control group (15.8 ± 3.6 pg/μg protein, $P < 0.01$). The liver TNF-α level in the NASH + Y-40138 group (12.4 ± 4.1 pg/μg protein) was significantly lower than that in the NASH group ($P < 0.01$). The liver IFN-γ level in the NASH group was 136 ± 39 pg/μg protein, significantly higher than that in the control group (36.3 ± 5.6 pg/μg protein, $P < 0.01$). The liver IFN-γ level in the NASH + Y-40138 group (45.6 ± 18.5 pg/μg protein) was significantly lower than that in the NASH group ($P < 0.01$). The liver IL-10 level in the control + Y-40138 group (24.7 ± 5.3 pg/μg protein) and the NASH group (19.3 ± 4.1 pg/μg protein) were significantly elevated in comparison to the control group (10.4 ± 2.5 pg/μg protein). The liver IL-10 level in the NASH + Y-40138 group (34.5 ± 4.3 pg/μg protein) was significantly higher than that in the NASH group ($P < 0.01$) (Figure 2B).

Histological findings

In contrast to the control group, marked inflammation, fat deposition, and fibrosis were seen in the NASH

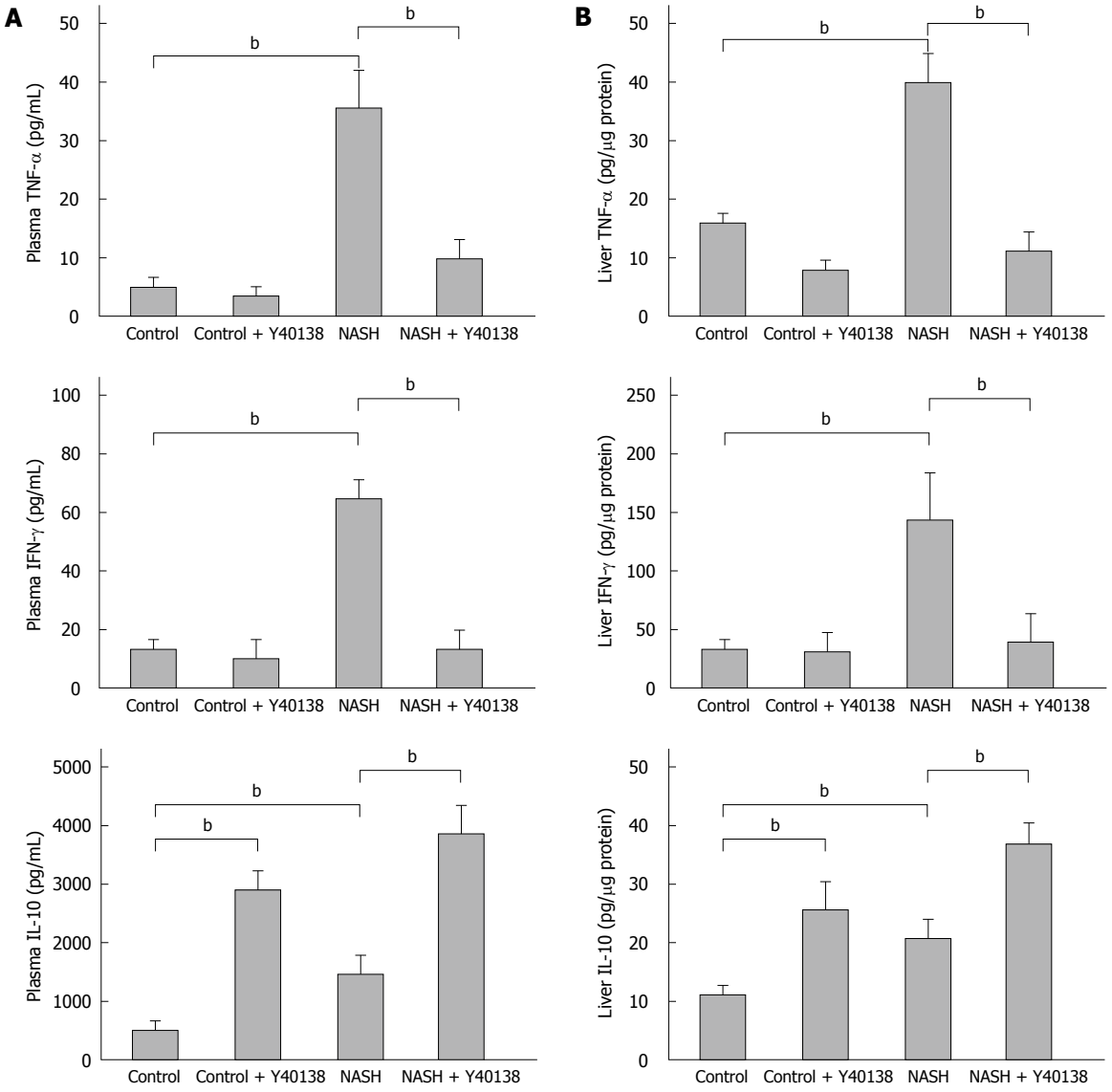

Figure 2 Tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), and interleukin-10 (IL-10) levels in the plasma and liver (ELISA method) in each group (*n* = 5). A: The plasma TNF-α and IFN-γ levels were significantly elevated in the NASH group, and the increase was attenuated in the NASH + Y-40138 group. The IL-10 levels were increased in the NASH group and further increased in the NASH + Y-40138 group rats. $^{b}P < 0.01$; B: The liver TNF-α, IFN-γ and IL-10 levels were significantly elevated in the livers of the NASH group rats. TNF-α and IFN-γ levels were attenuated in the NASH + Y-40138 group. The IL-10 levels further increased in NASH + Y-40138 group rats. $^{b}P < 0.01$.

group, corresponding to Matteoni's type 4 and grade 3/stage 3 of Brunt's NASH classification. The NASH + Y-40138 group findings corresponded to Matteoni's type 3 and Brunt's grade 2/stage 2, and showed amelioration of inflammation and fibrosis in comparison to the NASH group (Figure 3).

Immunohistochemical findings

Kupffer cell immunohistochemical staining showed no significant difference in the number of stained cells per field between groups (Figure 4A and B). Moreover, quantitative analysis using NIH Image analysis software showed no significant difference between groups (Figure 4C).

The TNF-α immunoreactivity in the liver tissue samples from the NASH group rats was increased in comparison to the control group rats. Moreover, the immunopositive areas in those animals were localized either to the periphery of fatty spots or to the non-parenchymal cells (Figure 5A). Semiquantitative analysis of TNF-α staining revealed significantly increased immunoreactivity in the livers of the NASH group rats (3250 ± 390) in comparison to the control group rats (260 ± 80) ($P < 0.01$). Semiquantitative analysis of TNF-α staining revealed significantly decreased immunoreactivity in the livers of the NASH + Y-40138 group rats (820 ± 350) in comparison to the NASH group rats ($P < 0.01$) (Figure 5B).

In-vitro TNF-α production by Kupffer cells

The viability of the cultured Kupffer cells was over 98% as determined by trypan blue exclusion. The basal TNF-α production by Kupffer cells isolated from the NASH group (26.6 ± 3.4 pg/mL) was equal to that

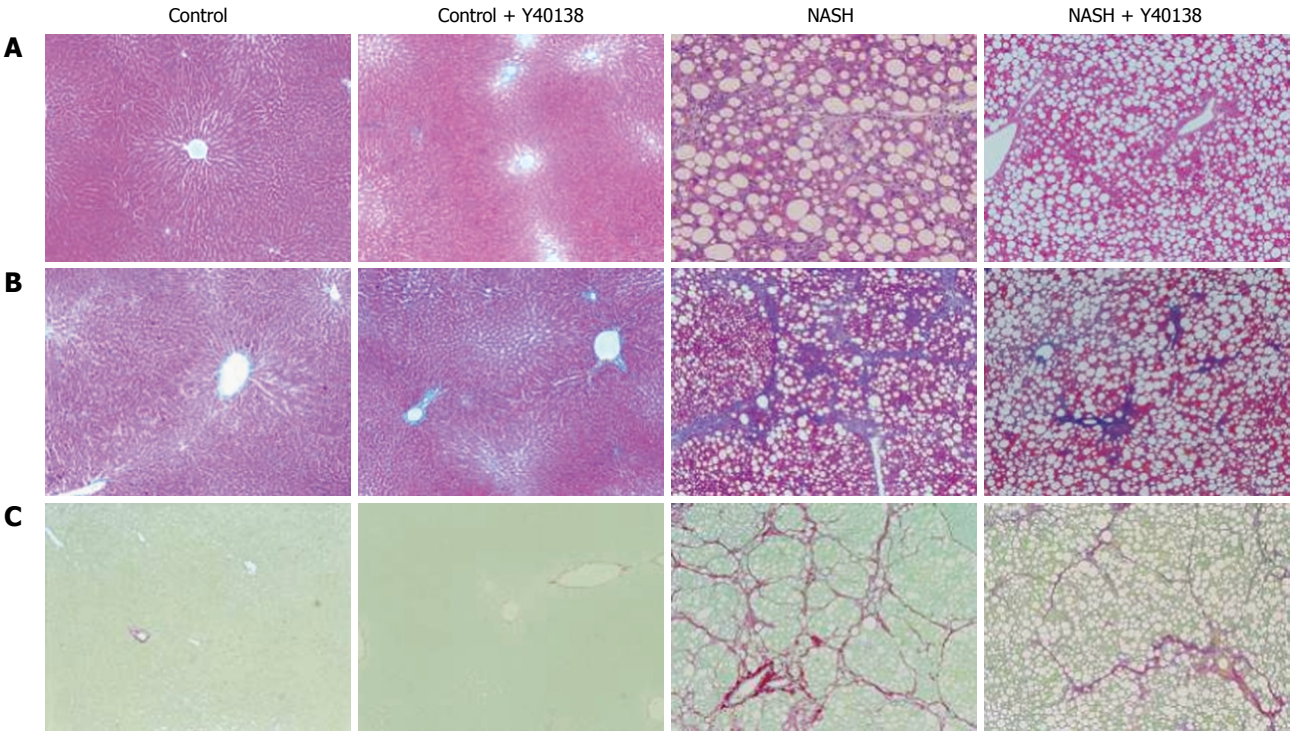

Figure 3 Histological analysis of liver sections. A: Hematoxylin and eosin stain (× 100); B: Azan stain (× 100); C: Sirius red stain (× 100). Histological findings of liver tissue from the NASH group rats corresponded to Matteoni's type 4 and grade 3/stage 3 of Brunt's NASH classification. Sirius red staining revealed abundant collagen. NASH + Y-40138 group findings corresponded to Matteoni's type 3 and Brunt's grade 2/stage 2, and showed amelioration of inflammation and fibrosis in comparison to the NASH group (*n* = 5).

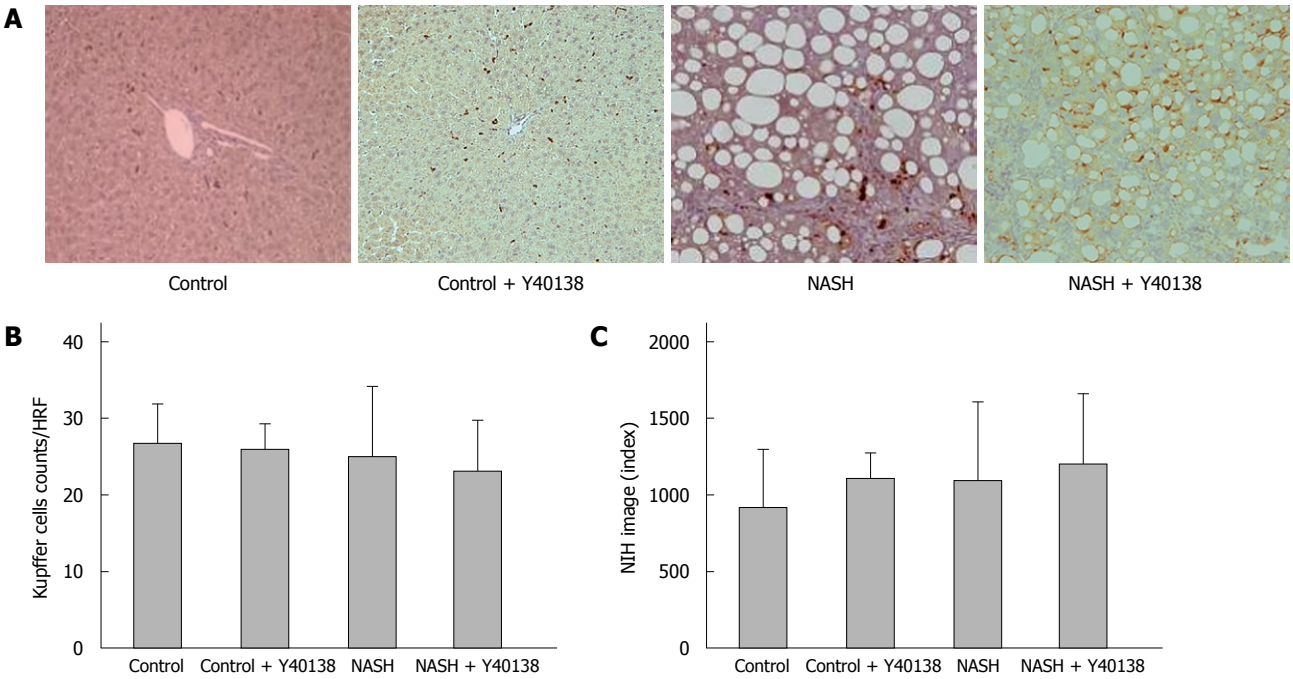

Figure 4 Kupffer cell immunohistochemical staining, Kupffer cell number and semiquantification in the liver. A: Kupffer cell immunohistochemical staining (× 200). Brown-stained cells are positive; B: No significant differences were seen between groups in the stained cell counts per field; C: Quantitative analysis revealed no significant differences between groups (*n* = 5). HRF: High rise filed.

from the control group (23.4 ± 4.8 pg/mL). After LPS stimulation, TNF-α production in the control group (48.9 ± 7.5 pg/mL) was significantly higher than the basal control level, and that in the NASH group (73.8 ± 8.4 pg/mL) was significantly higher than that in the control group ($P < 0.01$). TNF-α production in the NASH + Y-40138 group (57.2 ± 10.3 pg/mL) was significantly lower than that in the NASH group ($P < 0.05$) (Figure 6).

DISCUSSION

Kupffer cells located in the hepatic sinusoids are important

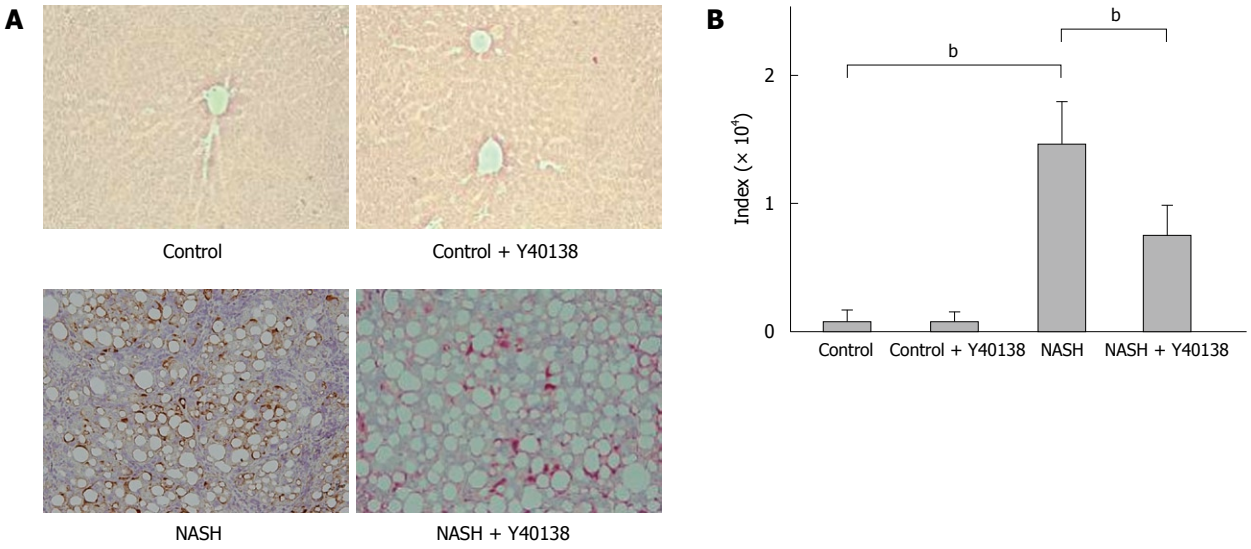

Figure 5 TNF-α immunohistochemical staining and semiquantification. A: TNF-α immunohistochemical staining (× 200). Brown-stained cells are positive; B: TNF-α immunoreactivities in the tissue samples from the NASH group were increased in comparison to the control group. The TNF-α immunoreactivities in the NASH + Y-40138 group were decreased in comparison to the NASH group (n = 5). $^{b}P < 0.01$.

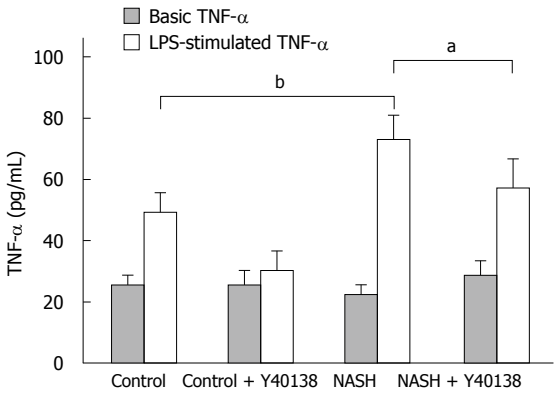

Figure 6 *In-vitro* TNF-α production by Kupffer cells. The basal TNF-α production by Kupffer cells isolated from the NASH group (26.6 ± 3.4 pg/mL) was equal to that in the control group (23.4 ± 4.8 pg/mL). After lipopolysaccharide (LPS) stimulation, TNF-α production in the control group (48.9 ± 7.5 pg/mL) was significantly higher than the basal control level, and was significantly higher in the NASH group (73.8 ± 8.4 pg/mL) than in the control group (n = 5). $^{b}P < 0.01$. TNF-α production in the NASH + Y-40138 group (57.2 ± 10.3 pg/mL) was significantly decreased in comparison to the NASH group (n = 5). $^{a}P < 0.05$.

hepatic cells that possess phagocytic activity, antigen presenting activity, and produce inflammatory cytokines, making them responsible for the hepatic innate immunity. Inflammatory cytokines, in particular TNF-α, are reportedly involved in the etiology and progression of alcoholic hepatitis[16]. The histopathological findings in NASH are similar to those detected in alcoholic hepatitis, suggesting the existence of a common underlying mechanism. A possible mechanism is that increased endotoxin levels cause activation of Kupffer cells through TLR4, leading to increased expression of TNF-α and increased levels of ROS. These induce inflammation and fibrosis, progressing to NASH. The elevation in the hepatic tissue and plasma levels of TNF-α noticed in NASH[2] has been attributed to a combination of secretion by fat cells associated with obesity, and secretion by Kupffer cells activated by endotoxins[17].

Once the trigger of TLR4 signal transmission has been initiated, Kupffer cells produce inflammatory cytokines including TNF-α, IL-1β, and IL-6, as well as the anti-inflammatory cytokine IL-10[18]. Kupffer cells also induce the inflammatory response through production of transforming growth factor-1, matrix metalloproteinase, platelet-derived growth factor, and ROS[19]. NASH is characterized by fatty deposition in the hepatocytes, inflammatory cell infiltration, with consequent liver fibrosis[20].

Methionine/choline deficient (MCD) and CDAA diets are widely used to produce NASH animal models[4]. Although these animals models lack obesity and insulin resistance, 2 of the major characteristics of NASH in humans, the possible relation of Kupffer cells with inflammation, steatosis, and fibrosis may resemble the human NASH[7]. We also demonstrated decreased Kupffer cell phagocytic activity in this model[4]. Increased sensitivity to LPS, the TLR4 ligand, occurs in MCD and CDAA-induced NASH, triggering the production of inflammatory cytokines[3].

TNF-α is a cytokine widely involved in biodefence and immune mechanisms based on inflammation, and continued overproduction is harmful to the organisms, with potentially serious toxicity, resulting in various morbidities[21]. Anti-cytokine antibodies such as infliximab are now widely used clinically as pharmacotherapy for inflammatory diseases[19].

In contrast, IL-10 is produced by macrophages and T-cells and has anti-inflammatory properties, inhibiting the production of inflammatory cytokines such as TNF-α, IL-1β, IL-6, and IL-8[22]. Therefore, IL-10 has shown efficacy in models of sepsis and rheumatoid arthritis[23] and clinically useful therapeutic effects have been reported for IL-10 in the treatment of steroid-resistant Crohn's disease[24].

The chemical name of the new synthetic compound Y-40138 is N-[1-(4-[4-(pyrimidin-2-yl) piperazin-1-yl]

methylphenyl) cyclopropyl] acetamide/hydrochloride. Its molecular formula is $C_{20}H_{25}N_5O/HCl$, its molecular weight is 387.91 (free base: 351.45). Although the site of action of Y-40138 has not yet been elucidated, it is known to have reciprocal actions in inhibiting production of TNF-α and IFN-γ, and increasing production of IL-10, as well as inhibiting production of chemokines and inflammatory cell chemotaxis, mechanisms not seen in any other agents[8,9]. Previous studies on Y-40138 have demonstrated improved survival rates in a D-galactosamine/LPS-induced mouse fulminant hepatic failure model[25,26] and a LPS-stimulated mouse sepsis model[27], and reduced ALT levels in a concanavalin A-induced mouse hepatitis model[28].

In this study using the rat NASH model, we detected increased plasma levels of ALT and TNF-α, and confirmed increased TNF-α levels in the liver using ELISA and immunohistochemical staining. These results indicate that TNF-α is involved in this NASH model, as in alcoholic hepatitis. Administration of Y-40138 to these same model rats reduced the plasma levels of ALT and TNF-α, and also decreased the TNF-α levels in the liver. We detected increased levels of IL-10 in the peripheral blood and liver. The TNF-α production by the isolated Kupffer cells was similar in the control and NASH rats. Kupffer cells from the control rats were sensitive to LPS stimulation. Furthermore, those from NASH were more sensitive to LPS stimulation, suggesting that continuous inflammation leads to up-regulation of the LPS-induced TNF-α production by the activated Kupffer cells. Administration of Y-40138 to the same model rats decreased LPS-induced TNF-α production *in vitro*. We presume that IL-10 restrains the production of TNF-α and IFN-γ *via* inhibition of the activation of Kupffer cells through STAT3 (signal transducer and activator of transcription 3).

In conclusion, the multiple cytokine production modulator Y-40138 ameliorated hepatic inflammation and inhibited fibrotic changes in the liver. These results indicate that Y-40138 is promising as a novel treatment for NASH. Y-40138 may have a role in suppressing the progress of hepatitis and prolonging the lives of patients with liver damage such as NASH in which cytokines and/or chemokines are involved.

ACKNOWLEDGMENTS

We gratefully acknowledge Tanabe Mitsubishi Pharma Co. for generously providing us with Y-40138 for our study.

COMMENTS

Background

Kupffer cells, part of the innate immune system, produce various cytokines and are known to play a role in pathologies of the liver. Abnormally functioning Kupffer cells are thought to be involved in nonalcoholic steatohepatitis (NASH). Although the etiological mechanisms of NASH have not been fully elucidated yet, observing similar histopathological findings in NASH and alcoholic hepatitis suggests the existence of a common underlying mechanism. In this study, the authors administered the multiple cytokine production modulator, Y-40138, to NASH model rats, to investigate its possible use as a novel immunotherapy for NASH.

Research frontiers

Administration of Y-40138 to NASH model rats reduced hepatic inflammation and suppressed fibrosis. These results indicate that the multiple cytokine production modulator, Y-40138, is promising as a novel treatment for NASH. Therefore, Y-40138 may contribute to suppressing the progress of hepatitis and prolonging the life of patients with liver damage such as NASH.

Applications

The present study is an experimental research using the rat NASH model, which was made by feeding a choline-deficient L-amino-acid-defined diet to rats, to examine their innate immunity. The authors believe that Y-40138 is a useful immunotherapy for NASH.

Terminology

The chemical name of the new synthetic compound Y-40138 is N-[1-(4-[4-(pyrimidin-2-yl) piperazin-1-yl] methylphenyl) cyclopropyl] acetamide/hydrochloride. Although the mechanism of action of Y-40138 has not been fully elucidated yet, it is known to have reciprocal actions in inhibiting the production of tumour necrosis factor-α and interferon-γ, and increasing the production of IL-10, as well as inhibiting the production of chemokines and inflammatory cell chemotaxis, and no other agents are known to have such mechanisms.

Peer review

This is an interesting study on the use of a cytokine modulator in the treatment of an experimental NASH model. The paper is well written and the research is well designed and executed.

REFERENCES

1 **Day CP**, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology* 1998; **114**: 842-845

2 **Tsujimoto T**, Kuriyama S, Yamazaki M, Nakatani Y, Okuda H, Yoshiji H, Fukui H. Augmented hepatocellular carcinoma progression and depressed Kupffer cell activity in rat cirrhotic livers. *Int J Oncol* 2001; **18**: 41-47

3 **Kawaratani H**, Tsujimoto T, Kitazawa T, Kitade M, Yoshiji H, Uemura M, Fukui H. Innate immune reactivity of the liver in rats fed a choline-deficient L-amino-acid-defined diet. *World J Gastroenterol* 2008; **14**: 6655-6661

4 **Tsujimoto T**, Kawaratani H, Kitazawa T, Hirai T, Ohishi H, Kitade M, Yoshiji H, Uemura M, Fukui H. Decreased phagocytic activity of Kupffer cells in a rat nonalcoholic steatohepatitis model. *World J Gastroenterol* 2008; **14**: 6036-6043

5 **Tomita K**, Tamiya G, Ando S, Ohsumi K, Chiyo T, Mizutani A, Kitamura N, Toda K, Kaneko T, Horie Y, Han JY, Kato S, Shimoda M, Oike Y, Tomizawa M, Makino S, Ohkura T, Saito H, Kumagai N, Nagata H, Ishii H, Hibi T. Tumour necrosis factor alpha signalling through activation of Kupffer cells plays an essential role in liver fibrosis of non-alcoholic steatohepatitis in mice. *Gut* 2006; **55**: 415-424

6 **Sakaida I**, Kubota M, Kayano K, Takenaka K, Mori K, Okita K. Prevention of fibrosis reduces enzyme-altered lesions in the rat liver. *Carcinogenesis* 1994; **15**: 2201-2206

7 **Koteish A**, Diehl AM. Animal models of steatosis. *Semin Liver Dis* 2001; **21**: 89-104

8 **Haddad JJ**, Safieh-Garabedian B, Saadé NE, Land SC. The biphasic immunoregulation of pyrimidylpiperazine (Y-40138) is IL-10 sensitive and requires NF-kappa B targeting in the alveolar epithelium. *Br J Pharmacol* 2001; **133**: 49-60

9 **Hisadome M**, Fukuda T, Adachi K, Komatsu H. Combination benefit of a pyrimidylpiperazine derivative (Y-40138) and methotrexate in arthritic rats. *Eur J Pharmacol* 2004; **497**: 351-359

10 **Nakae D**, Mizumoto Y, Yoshiji H, Andoh N, Horiguchi K, Shiraiwa K, Kobayashi E, Endoh T, Shimoji N, Tamura K. Different roles of 8-hydroxyguanine formation and 2-thiobarbituric acid-reacting substance generation in the early phase of liver carcinogenesis induced by a choline-deficient, L-amino acid-defined diet in rats. *Jpn J Cancer Res* 1994; **85**: 499-505

11 **Matteoni CA**, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology* 1999; **116**: 1413-1419

12 **Brunt EM**, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999; **94**: 2467-2474

13 **Seglen PO**. Preparation of rat liver cells. 3. Enzymatic requirements for tissue dispersion. *Exp Cell Res* 1973; **82**: 391-398

14 **Olynyk JK**, Clarke SL. Isolation and primary culture of rat Kupffer cells. *J Gastroenterol Hepatol* 1998; **13**: 842-845

15 **Kubo S**, Rodriguez T Jr, Roh MS, Oyedeji C, Romsdahl MM, Nishioka K. Stimulation of phagocytic activity of murine Kupffer cells by tuftsin. *Hepatology* 1994; **19**: 1044-1049

16 **Hansen J**, Cherwitz DL, Allen JI. The role of tumor necrosis factor-alpha in acute endotoxin-induced hepatotoxicity in ethanol-fed rats. *Hepatology* 1994; **20**: 461-474

17 **Schwabe RF**, Seki E, Brenner DA. Toll-like receptor signaling in the liver. *Gastroenterology* 2006; **130**: 1886-1900

18 **Seki E**, Tsutsui H, Nakano H, Tsuji N, Hoshino K, Adachi O, Adachi K, Futatsugi S, Kuida K, Takeuchi O, Okamura H, Fujimoto J, Akira S, Nakanishi K. Lipopolysaccharide-induced IL-18 secretion from murine Kupffer cells independently of myeloid differentiation factor 88 that is critically involved in induction of production of IL-12 and IL-1beta. *J Immunol* 2001; **166**: 2651-2657

19 **Rutgeerts P**, Vermeire S, Van Assche G. Biological therapies for inflammatory bowel diseases. *Gastroenterology* 2009; **136**: 1182-1197

20 **Bugianesi E**, Leone N, Vanni E, Marchesini G, Brunello F, Carucci P, Musso A, De Paolis P, Capussotti L, Salizzoni M, Rizzetto M. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. *Gastroenterology* 2002; **123**: 134-140

21 **Kitazawa T**, Tsujimoto T, Kawaratani H, Fujimoto M, Fukui H. Expression of Toll-like receptor 4 in various organs in rats with D-galactosamine-induced acute hepatic failure. *J Gastroenterol Hepatol* 2008; **23**: e494-e498

22 **Yoshimura A**, Mori H, Ohishi M, Aki D, Hanada T. Negative regulation of cytokine signaling influences inflammation. *Curr Opin Immunol* 2003; **15**: 704-708

23 **Walmsley M**, Katsikis PD, Abney E, Parry S, Williams RO, Maini RN, Feldmann M. Interleukin-10 inhibition of the progression of established collagen-induced arthritis. *Arthritis Rheum* 1996; **39**: 495-503

24 **van Deventer SJ**, Elson CO, Fedorak RN. Multiple doses of intravenous interleukin 10 in steroid-refractory Crohn's disease. Crohn's Disease Study Group. *Gastroenterology* 1997; **113**: 383-389

25 **Fukuda T**, Hisadome M, Komatsu H. Treatment with Y-40138, a multiple cytokine production modulator, inhibits lipopolysaccharide- or tumour necrosis factor-alpha-induced production of pro-inflammatory cytokines and augments interleukin-10. *J Pharm Pharmacol* 2005; **57**: 1461-1466

26 **Fukuda T**, Mogami A, Tanaka H, Yoshikawa T, Hisadome M, Komatsu H. Y-40138, a multiple cytokine production modulator, protects against D-galactosamine and lipopolysaccharide-induced hepatitis. *Life Sci* 2006; **79**: 822-827

27 **Fukuda T**, Sumichika H, Murata M, Hanano T, Adachi K, Hisadome M. A novel dual regulator of tumor necrosis factor-alpha and interleukin-10 protects mice from endotoxin-induced shock. *Eur J Pharmacol* 2000; **391**: 317-320

28 **Fukuda T**, Mogami A, Hisadome M, Komatsu H. Therapeutic administration of Y-40138, a multiple cytokine modulator, inhibits concanavalin A-induced hepatitis in mice. *Eur J Pharmacol* 2005; **523**: 137-142

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ORIGINAL ARTICLE

Macrophage migration inhibitory factor regulates proliferation of gastric cancer cells *via* the PI3K/Akt pathway

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Abstract

AIM: To investigate the effects of macrophage migration inhibitory factor (MIF) on proliferation of human gastric cancer MGC-803 cells and expression of cyclin D1 and $p27^{Kip1}$ in them, and further determine whether the effects are related to the PI3K/Akt signal transduction pathway.

METHODS: Gastric cancer MGC-803 cells were cultured and then treated with 50 μg/L recombinant human MIF (rhMIF) with and without a PI3K inhibitor, LY294002 (25 μmol/L). MTT assay was used to detect the proliferation of MGC-803 cells. Cell cycle was detected by flow cytometry. Expression of cyclin D1 and $p27^{Kip1}$ mRNA was by reverse transcription-polymerase chain reaction. Protein expression of phosphorylated Akt (p-Akt), Akt, cyclin D1 and $p27^{Kip1}$ was examined by immunocytochemistry and Western blotting.

RESULTS: rhMIF significantly stimulated the proliferation of MGC-803 cells and cell cycle progression from G1 phase to S phase in a concentration- and time-dependent manner. After the MGC-803 cells were treated with rhMIF for 24 h, the expression of cyclin D1 was significantly up-regulated compared with the cells not treated with rhMIF at both mRNA and protein levels (0.97 ± 0.02 *vs* 0.74 ± 0.01, P = 0.002; 0.98 ± 0.05 *vs* 0.69 ± 0.04, P = 0.003). The $p27^{Kip1}$ was down-regulated but only statistically significant at the protein level. rhMIF significantly increased the expression of p-Akt, which reached the peak at 30 min, but did not affect the expression of Akt. However, LY294002 inhibited all the effects of rhMIF.

CONCLUSION: Macrophage MIF increases the proliferation of gastric cancer cells, induces the expression of cyclin D1 at the transcriptional level and inhibits the expression of $p27^{Kip1}$ at the post-transcriptional level *via* the PI3K/Akt pathway.

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Key words: Macrophage migration inhibitory factor; Gastric cancer; Proliferation; Cell cycle; Cyclin D1; $p27^{Kip1}$; PI3K/Akt

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INTRODUCTION

Gastric cancer is the most common gastroenterological malignancy worldwide, with its incidence rate being the fourth among all cancers[1,2]. There are approximately 934 000 new cases, and 700 000 patients die of gastric cancer each year. Gastric cancer is the second leading cause of cancer-related death worldwide[3-5], and remains a disease of poor prognosis. The 5-year survival rate of gastric cancer patients is generally below 20%. The current major therapies for gastric cancer include surgery, chemotherapy combined with or without radiotherapy, but their efficacy is not satisfactory. It is, therefore,

necessary to explore and investigate the tumorigenesis of gastric cancer and its novel therapy targets[6].

Macrophage migration inhibitory factor (MIF) is a multi-functional cytokine, which is associated with inflammation and tumorigenesis[7,8]. Recent studies have shown that MIF is related to the initiation and progression of gastric cancer, but its underlying mechanism is not very clear[9-11].

It has been reported that the effect of MIF on cell proliferation is relevant to cell cycle factors[12]. Cyclin Dl and 27^{Kip1} belong to the family of cyclin proteins and cyclin-dependent kinase inhibitors (CDKI), which regulates cell cycle progression from the G1 phase to the S phase[13,14]. Over-expression of cyclin D1 and aberrant expression of $p27^{Kip}$ can lead to the loss of control of cell proliferation and differentiation, thus promoting tumorigenesis[15].

The phosphoinositol-3-kinases (PI3K)/Akt pathway has been considered an important signaling pathway that is involved in the regulation of cell proliferation and differentiation[16]. It has been reported that NF-κB activates the transcription target, *via* PI3K/Akt signaling, to promote gastric tumorigenesis[17]. In a recent study with a gastric cancer cell line, AGS, Kim *et al*[18] reported that inhibition of the PI3K/Akt/PKB pathway could enhance the mitochondrial death pathway. Another recent study indicates that anti-cancer effects of celecoxib on gastric cancer cells are partly mediated by down-regulation of Akt signaling[19].

The aims of the present study were to investigate the effects of recombinant human MIF (rhMIF) on cell proliferation of human gastric cancer MGC-803 cells and cell cycle. Activity of Akt and expression of cyclin D1 and $p27^{kip1}$ were examined. Whether the PI3K/Akt pathway is involved in the effects of rhMIF was further investigated, using a PI3K/Akt inhibitor, LY294002.

MATERIALS AND METHODS

Chemicals

rhMIF and P13K specific inhibitor, LY294002, were purchased from PROSPEC (Rehovot, Israel) and Cell Signaling (Danvers, MA, USA), respectively. Primers for GAPDH, cyclin D1 and p27 were produced by Shanghai Sangon Biological Engineering Technology & Service Company, Ltd. (Shanghai, China). Mouse anti-human β-actin primary antibody was bought from Beyotime Institute of Biotechnology (Shanghai, China). Rabbit anti-human cyclin D1 primary antibody was purchased from Epitomics (Burlingame, CA, USA), and rabbit anti-human p27, rabbit anti-human phosphorylated Akt (p-Akt) and Akt primary antibodies were bought from Santa Cruz Biotechnology (CA, USA). Goat anti-mouse and goat anti-rabbit secondary antibodies were purchased from Beyotime Institute of Biotechnology (Shanghai, China) and BioDev-Tech (BioDev, Beijing, China), respectively. Epics-XL II flow cytometer was purchased from Beckman Coulter (Beckman, Fullerton, CA, USA).

Cell culture

Gastric cancer cell line, MGC-803, was provided by the Institute of Cancer Research, Nanhua University (China), and cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 100 μg/mL streptomycin and 100 μ/mL penicillin, and maintained at 37℃ in a humidified atmosphere containing 5% CO_2. The cultured cells were then used in different experiments as described below.

Cell proliferation assay (MTT assay)

Approximately 2×10^3 cells/well were grown in 96-well microtiter plates and incubated overnight in 100 μL of the culture medium. Cells were starved without FBS for 24 h at 70%-80% confluence and then treated with rhMIF at different concentrations (25, 50 and 100 μg/L) with or without 1-h pretreatment with LY294002 (25 μmol/L), for 12, 24 and 48 h, respectively. Cells without any treatment were used as controls. Twenty microliters of 5 mg/mL MTT (Sigma, St Louis, MO) labeling reagent was added to the designated well, and cells were incubated at 37℃ for 4 h. The supernatant was removed, and then 150 μL dimethyl sulfoxide (DMSO) was added to the designated well. After the plate was incubated at 37℃ for 15 min, the absorbency was measured with a micro ELISA reader (Bio-Tek, Winooski, VT, USA) at a wavelength of 570 nm.

Flow cytometry

Cells were harvested after treated with rhMIF, with or without 1-h pretreatment with LY294002 for 24 h as described earlier, and fixed with 75% cold alcohol at 4℃ overnight. After washed with phosphate buffered saline (PBS), propidium iodide (PI) was added and cells were incubated at 4℃ for 30 min. Cell cycle distribution was detected with an Epics-XL II flow cytometer (Beckman Coulter, Inc., Fullerton, CA, USA).

Reverse transcriptase polymerase chain reaction (RT-PCR)

Cells were harvested after treated with rhMIF, with or without 1-h pretreatment with LY294002 for 24 h as described earlier. Total RNA was extracted from cells with a total RNA kit (BioDev, Beijing, China). One microgram of RNA was reversely transcribed to cDNA with thermoscript RT system reagent (MBI Fermentas, Glen Burnie, USA) according to its manufacturer's instructions. The primer sequences are as follows: cyclin D1, forward: 5'-CGTCCATGCGGAAGATC-3', reverse: 5'-CAGAGGGCAACGAAGGT-3' (406 bp); $p27^{Kip1}$, forward: 5'-CGCAAGTGGAATTTCGATTT-3', reverse: 5'-ATGCGTGTCCTCAGAGTTAGC-3' (215 bp); GAPDH, forward: 5'-CGGAGTCAACGGATTTGGTCGTAT-3', reverse: 5'-AGCCTTCTCCATGGTGGTGAAGAC-3' (306 bp). PCR was performed according to the following conditions: denaturation at 94℃ for 3 min, 40 cycles of denaturation at 94℃ for 30 s, annealing at 57℃ for 30 s and extension at 72℃ for 2 min, followed by a final extension at 72℃ for 10 min. Five microlitres of PCR product was used for agarose gel electrophoresis and analyzed using Alphalmager 2200 (Imaging System, CA, USA).

Western blotting

Whole cell lysates were prepared from cells with RIPA

lysis buffer containing protease inhibitors (Beyotime, Shanghai, China). Protein concentration was measured with a BCA protein assay kit (Beyotime, Shanghai, China). An aliquot of 40 μg protein of each sample was subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis. Then, the protein was transferred to a PVDF membrane (Millipore, Billerica, MA). Non-specific binding was blocked with 5% non-fat milk for 2 h at 37℃. The membrane was immunodetected with anti-cyclin D1 (1:500), anti-p27^{kip1} (1:500), anti-p-Akt (1:500), anti-Akt (1:500), anti-β-actin (1:1000) primary antibodies overnight at 4℃, and then with corresponding HRP-conjugated secondary antibodies (1:2000). Antigen-antibody complexes were visualized with a luminol reagent (Santa Cruz, CA, USA), and the results were analyzed with Alphalmager 2200. β-actin was detected as the loading control.

Immunocytochemistry

Cells were grown on slides at a concentration of 1.0 × 10^6 cells/mL, treated with rhMIF (50 μg/L) with or without 1-h pretreatment with LY294002 (25 μmol/L) for 24 h at 70%-80% confluence. Cells without any treatment were used as controls. The treated and control cells were fixed with 75% cold alcohol at 4℃ overnight and then subjected to immunostaining using the streptavidin-peroxidase technique. In brief, endogenous peroxidase was blocked with 3% H_2O_2 in PBS, and then with a normal goat serum blocking solution for 20 min at room temperature. The slides were incubated with anti-p-Akt, anti-cyclin D1 and anti-p27^{kip1} primary antibodies (1:200 diluted with 0.01 mol/L PBS), respectively, for 60 min at room temperature. Slides that were incubated with PBS instead of primary antibodies were used as negative controls. After washed with PBS, the slides were incubated with corresponding HRP-conjugated secondary antibodies for 30 min. The color of immunostaining was developed with a diaminobenzidine solution and counterstained with hematoxylin.

Statistical analysis

Data were analyzed using SPSS 16.0 software. The results were expressed as mean ± SD. Student *t*-test was used to determine the difference in numeric parameters between different groups. $P < 0.05$ was considered statistically significant.

RESULTS

Effects of rhMIF and LY294002 on the proliferation of MGC-803 cells

The MTT assay showed that treatment with rhMIF significantly increased the proliferation of MGC-803 cells in a dose- and time-dependent manner compared with the controls ($P < 0.05$). The proliferation of MGC-803 cells reached its peak (1.823 ± 0.04/0.647 ± 0.02, 282%) when they were treated with 50 μg/L rhMIF for 48 h (Figure 1A), which was significantly inhibited after 1-h pretreatment with 25 μmol/L LY294002 ($P < 0.05$, Figure 1B). DMSO (the menstruum of LY294002, less than 0.1%) had little effect on the proliferation of MGC-803 cells.

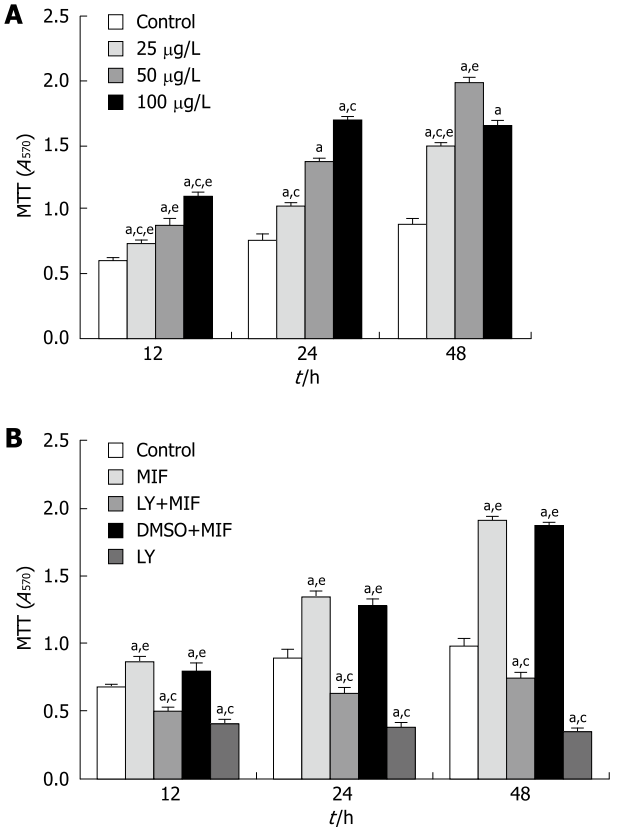

Figure 1 Effects of recombinant human migration inhibitory factor (rhMIF) and LY294002 on the proliferation of MGC-803 cells. A: MGC-803 cells were treated with 25, 50 and 100 μg/L rhMIF for the indicated periods of time and cell proliferation was examined by MTT assay. $^aP < 0.05$ *vs* control group; $^cP < 0.05$ *vs* 50 μg/L MIF group; $^eP < 0.05$ *vs* 24 h group; B: With or without 1-h pretreatment with 25 μmol/L LY294002, MGC-803 cells were exposed to 50 μg/L rhMIF for the indicated periods of time, with a vehicle control of dimethyl sulfoxide (DMSO) (less than 0.1%) included. $^aP < 0.05$ *vs* control group; $^cP < 0.05$ *vs* MIF group; $^eP < 0.05$ *vs* LY+MIF group. Data were represented as mean ± SD of three independent experiments. DMSO (the menstruum of LY294002, less than 0.1%) had little impact on the rhMIF effect.

Effects of rhMIF and LY294002 on the cell cycle of MGC-803 cells

The effect of rhMIF on cell cycle distribution is shown in Figure 2. rhMIF (50 μg/L) significantly induced the progression of MGC-803 cells from G1 phase to S phase ($P < 0.05$), thus increasing the accumulation of cells in S phase. However, the cells treated with LY294002 were accumulated in G1 phase, namely G1 arrest, irrespective of rhMIF treatment ($P < 0.05$). DMSO had little impact on the rhMIF effect (Figure 2).

Effects of rhMIF and LY294002 on the mRNA and protein expression of cyclin D1 and p27^{kip1} in MGC-803 cells

After treatment with 50 μg/L rhMIF for 24 h, the mRNA level of cyclin D1 was increased significantly ($P < 0.05$). However, 1-h pre-treatment with LY294002 reduced the mRNA expression irrespective of subsequent rhMIF treatment ($P < 0.05$, Figure 3A). DMSO had little impact on the rhMIF effect. Treatment with rhMIF or LY294002 had no effect on the mRNA level of p27^{kip1} (Figure 3A).

Western blotting showed that after treatment with 50 μg/L rhMIF for 24 h, the expression of cyclin D1

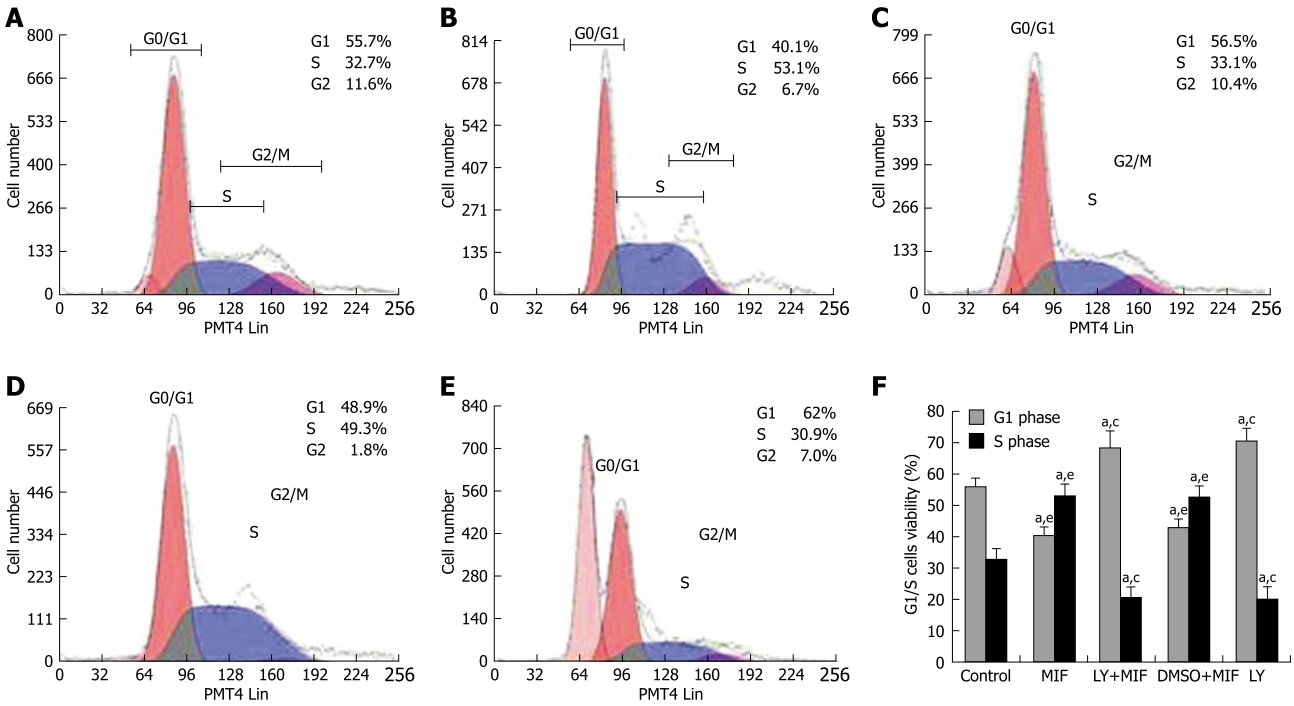

Figure 2 Effects of rhMIF and LY294002 on cell cycle of MGC-803 cells. With or without 1-h pretreatment with 25 μmol/l LY294002, MGC-803 cells were exposed to 50 μg/L rhMIF for 24 h. A vehicle control of DMSO (less than 0.1%) was included. Cell lysates were prepared for the cell cycle detection. The cell cycle distribution was analyzed using an Eics-XL II flow cytometer. A: Control; B: MIF group; C: LY+MIF group; D: DMSO+MIF group; E: LY group; F: Quantification of G1/S viability(%) analysis of MGC-803 cells. Data were represented as mean ± SD of three independent experiments. $^{a}P < 0.05$ *vs* control group; $^{c}P < 0.05$ *vs* MIF group; $^{e}P < 0.05$ *vs* LY+MIF group. DMSO (the menstruum of LY294002, less than 0.1%) had little impact on the rhMIF effect.

protein was significantly up-regulated, but the expression of $p27^{kip1}$ protein was down-regulated ($P < 0.05$, Figure 3B). However, 1-h pretreatment with LY294002 prior to rhMIF treatment abrogated the effects of rhMIF on the expression of cyclin D1 and $p27^{kip1}$ proteins ($P < 0.05$). Again, DMSO had little impact on the rhMIF effect (Figure 3B).

Immunocytochemistry demonstrated that the positive expression of cyclin D1 and $p27^{kip1}$, in yellow or brown, was mostly located in the nuclei of MGC-803 cells, but rarely in the cytoplasm. rhMIF increased the expression of cyclin D1, but decreased the expression of $p27^{kip1}$ (Figure 4). However, 1-h pretreatment with LY294002 down-regulated the expression of cyclin D1 (Figure 4A), but up-regulated the expression of $p27^{kip1}$ (Figure 4B), which was consistent with the results obtained by Western blotting.

Effects of rhMIF on the expression of p-Akt and Akt in MGC-803 cells

Western-blotting showed that after treatment with different concentrations of rhMIF (25, 50, 100 μg/L) for 30 min, the expression of p-Akt in MGC-803 cells was up-regulated in a dose-dependent manner (Figure 5A, $P < 0.05$). In addition, the cells treated with 50 μg/L rhMIF for different periods of time (5, 15, 30 and 60 min) showed that the expression of activated Akt in MGC-803 cells started to increase at 5 min, reached its peak at 30 min, then decreased but remained at a level higher than that in the untreated control cells ($P < 0.05$, Figure 5B). Pretreatment with LY294002 prior to rhMIF treatment, however, inhibited the expression of p-Akt irrespective of rhMIF treatment (Figure 5C). DMSO had little impact on the rhMIF effect. Both rhMIF and LY294002 had no effect on the expression of Akt (Figure 5A and C).

Immunocytochemistry showed that p-Akt was positively expressed in cytoplasm of the cells. rhMIF increased the expression of p-Akt. However, pre-treatment with LY294002 down-regulated the expression of p-Akt (Figure 5D).

DISCUSSION

MIF has multiple biological functions and plays an important role in inflammation as well as in carcinogenesis as a cytokine[20,21]. Increased expression of MIF has been reported in pre-cancerous, cancerous and metastatic tumors[10,22]. It has been shown that MIF-modulated cancer progress is associated with the ERK/MAPK, PI3K/Akt, and Rb-E2F pathways[22-25]. However, its detailed mechanism underlying inflammatory and malignant transformation as well as tumorigenesis needs to be further investigated.

In our study, rhMIF increased the MGC-803 cell proliferation in a dose- and time-dependent manner, and induced progression from G1 phase to S phase, leading to S phase arrest. It has been demonstrated that cyclin, cyclin-dependent kinase (CDK) and CDK inhibitor (CDKI) play a critical role in the orderly progression of cell cycle during the transition from G1 phase to S phase[26,27]. Since cyclin D1 is an important regulator of cell cycle progression, its increased expression contributes significantly to tumor development and malignant transformation[13,28]. $p27^{Kip1}$, an inhibitor of CDK, functions as

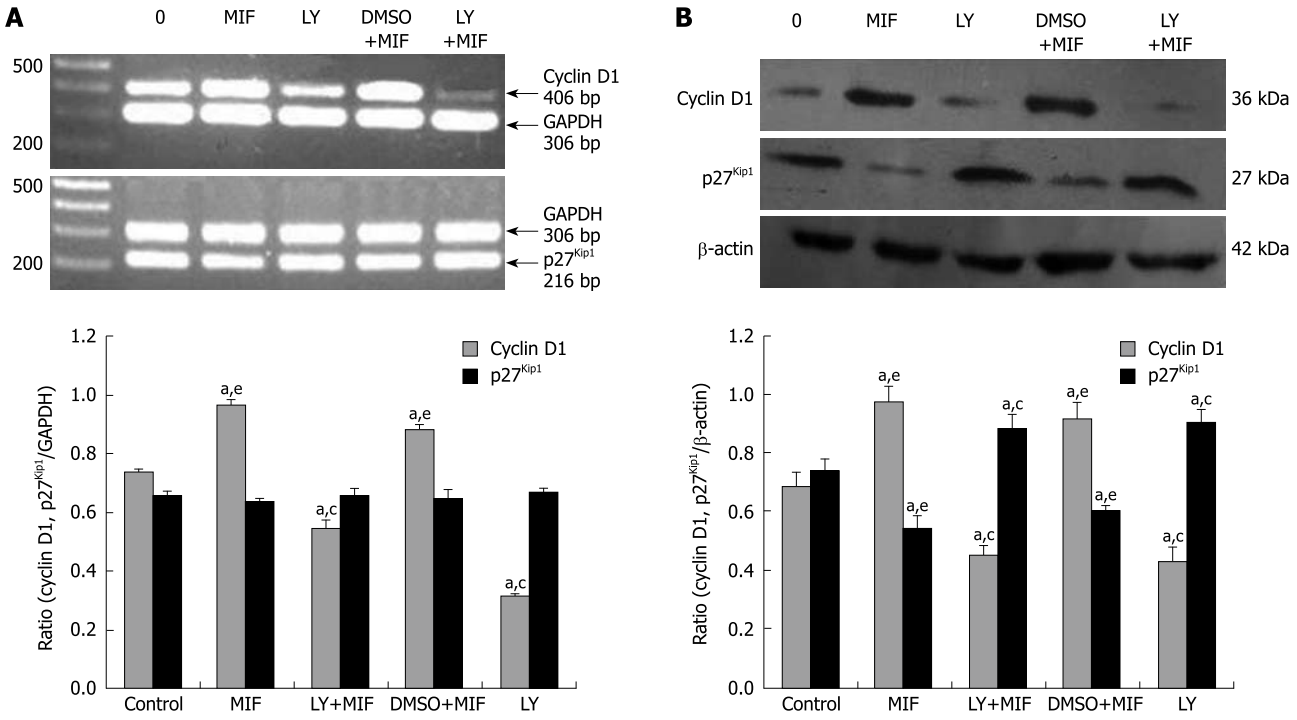

Figure 3 Effects of rhMIF and LY294002 on the expression of cyclin D1 and p27^{kip1} in MGC-803 cells. With or without 1-h pretreatment with 25 μmol/L LY294002, MGC-803 cells were exposed to 50 μg/L rhMIF for 24 h. A vehicle control of DMSO (less than 0.1%) was included. A: RT-PCR; B: Western blotting. The results were represented as mean ± SD of three independent experiments. $^{a}P < 0.05$ *vs* control group; $^{c}P < 0.05$ *vs* MIF group; $^{e}P < 0.05$ *vs* LY+MIF group. DMSO (the menstruum of LY294002, less than 0.1%) had little impact on the rhMIF effect.

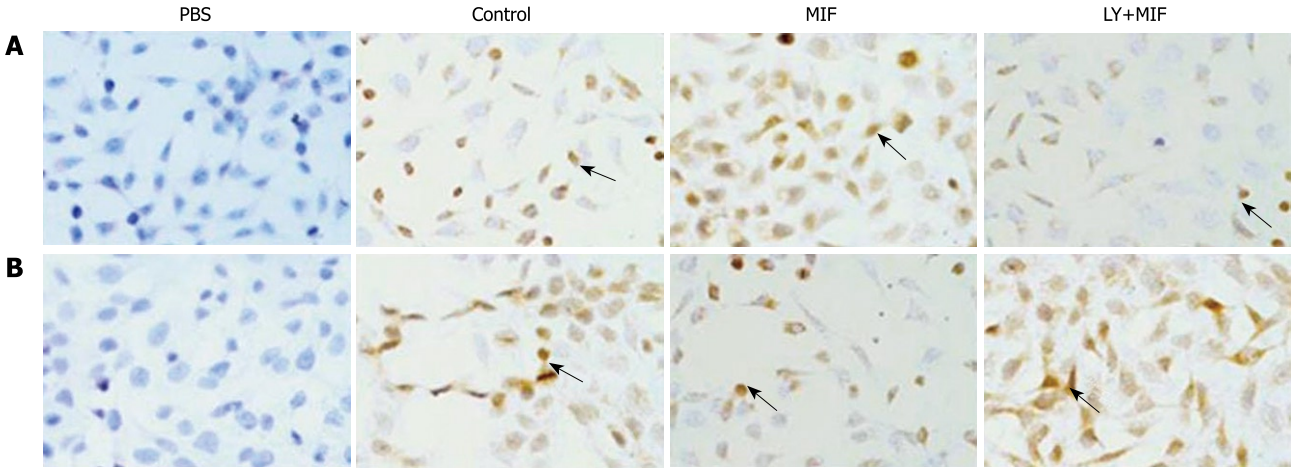

Figure 4 Effects of rhMIF and LY294002 on the translocation of cyclin D1 (A) and p27^{kip1} (B) in MGC-803 cells. With or without 1-h pretreatment with 25 μmol/L LY294002, MGC-803 cells were exposed to 50 μg/L rhMIF for 24 h. The arrow symbols represent immunocytochemical staining for nuclear and cytoplasmic expression of cyclin D1 (Original magnification, × 200) and p27^{kip1} (Original magnification, × 200) in MGC-803. PBS: Phosphate buffered saline.

an integral brake of the cell cycle, thus also playing an important role in tumor suppression[29]. For example, deregulation of p27^{Kip1} has a profound effect on tumor progression in colorectal cancer and is suggested to be an accurate and independent prognostic marker[30]. Moreover, p27^{Kip1} may be a central target for growth regulation of the normal endometrium and for the pathogenesis of endometrial carcinoma[31]. In our study, rhMIF increased cyclin D1 mRNA expression, but had no effect on p27^{Kip1} mRNA expression. However, immunocytochemistry showed that rhMIF increased the expression of cyclin D1 protein and decreased the expression of p27^{Kip1} protein. These results were identified further by Western blotting. Therefore, our results indicate that rhMIF affects the cell cycle by inducing cyclin D1 and inhibiting p27^{Kip1}, thus promoting the proliferation of MGC-803 cells. In addition, rhMIF up-regulates the transcriptional cyclin D1, which is in contrast with the posttranscriptional protein reduction of p27^{Kip1} in cells. This phenomenon may be related to the degradation by its ubiquitin ligase[32,33].

The PI3K/Akt pathway is crucial in tumorigenesis because the p-Akt can regulate the cell proliferation, apoptosis, angiogenesis and cell cycle by activating the

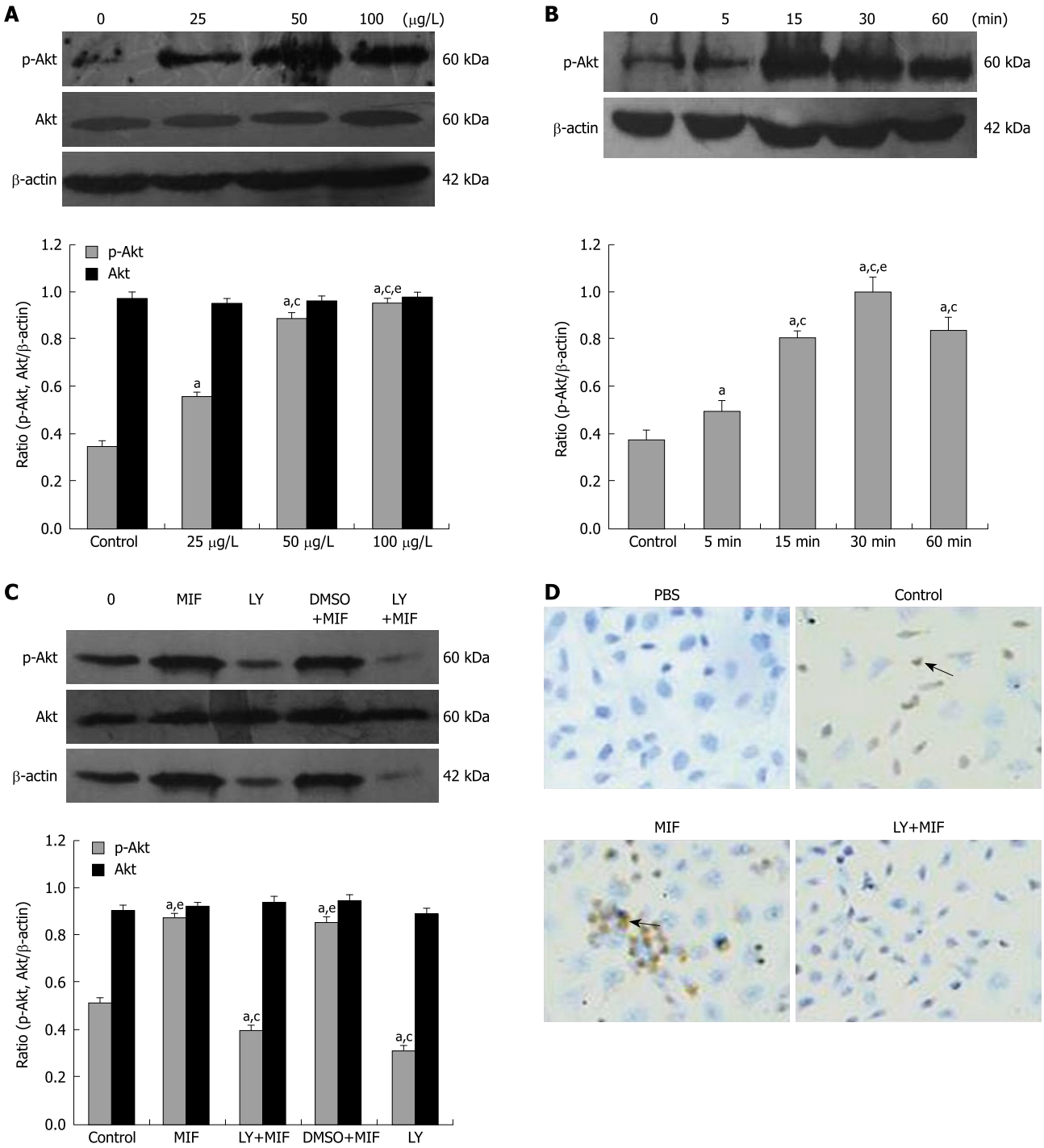

Figure 5 Effects of rhMIF on the expression of p-Akt and Akt in MGC-803 cells. Cell lysates were isolated for Western blotting to detect the expression of p-Akt and Akt (A, B). A: MGC-803 cells were treated with 25, 50 and 100 μg/L rhMIF for 30 min. $^{a}P < 0.05$ *vs* control group; $^{c}P < 0.05$ *vs* 25 μg/L MIF group; $^{e}P < 0.05$ *vs* 50 μg/L MIF group; B: MGC-803 cells were treated with 50 μg/L rhMIF at different time points (5, 15, 30 and 60 min). $^{a}P < 0.05$ *vs* control group; $^{c}P < 0.05$ *vs* 5 min group; $^{e}P < 0.05$ *vs* 15 min and 60 min groups; C: With or without 1-h pretreatment with 25 μmol/L LY294002, MGC-803 cells were exposed to 50 μg/L rhMIF for 30 min with a vehicle control of DMSO (less than 0.1%) included, and then the expression of p-Akt and Akt was determined by Western blotting. $^{a}P < 0.05$ *vs* control group; $^{c}P < 0.05$ *vs* MIF group; $^{e}P < 0.05$ *vs* LY+MIF group; D: The arrow symbols represent immunocytochemical staining for nuclear and cytoplasmic expression of p-Akt (original magnification, × 200) in MGC-803 cells. The results were represent as mean ± SD of three independent experiments. DMSO (the menstruum of LY294002, less than 0.1%) had little impact on the rhMIF effect.

downstream cell receptors or effectors[34-36]. Cinti *et al*[37] studied 50 cases of advanced gastric carcinoma using immunohistochemistry, showing a statistically significant direct correlation between p-Akt expression and depth of infiltration of the tumor, between the number of infiltrated lymph nodes and p34/cdc2 expression, and between prevalently nuclear p-Akt and cyclin D1 and cyclin E, but an inverse correlation between nuclear pAkt and apoptotic index, and between cytoplasmatic and nuclear pAkt and patient survival, suggesting that p-Akt may be considered an indicator of tumor progression and a prognostic factor for the survival of gastric cancer patients. Liang *et al*[38] reported that cellular prion protein up-regulates cyclin D1 at both mRNA and protein levels, thus promoting

cell proliferation and transition from G1 phase to S phase in gastric cancer GC7901 and AGS cells, at least partially by activating the PI3K/Akt pathway. Moreover, a PI3K specific inhibitor, LY294002, can significantly suppress the effects of cellular prion protein. In the present study, immunocytochemistry showed that rhMIF increased the p-Akt expression, activated p-Akt in a dose- and time-dependent manner, and induced the maximum Akt phosphorylation at 30 min. However, the total expression level of Akt remained unchanged, indicating that rhMIF affects specifically the activated Akt, namely the p-Akt. To further validate the involved Akt pathway in the effect of rhMIF on MGC-803 cells, the cells were pretreated with a PI3K specific inhibitor, LY294002, showing that LY294002 reversed or abolished all the effects induced by rhMIF, including the increased proliferation, cell cycle S arrest, up-regulation of cyclin D1 and p-Akt, down-regulation of $p27^{Kip1}$, suggesting that rhMIF can induce the proliferation and cell cycle progression of MGC-803 cells *via* the PI3K/Akt pathway.

In conclusion, MIF increases the proliferation of gastric cancer cells, induces the expression of cyclin D1 at the transcriptional level and inhibits the expression of $p27^{Kip1}$ at the post-transcriptional level *via* the PI3K/Akt pathway. Further investigation is required to reveal whether macrophage MIF is a novel potential therapeutic target for the treatment of gastric cancer.

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COMMENTS

Background

Gastric cancer is one of the most common gastroenterological malignancies worldwide and remains a disease of poor prognosis. It is necessary to investigate the gastric tumorigenesis and potential new therapies. Macrophage migration inhibitory factor (MIF) is a multi-function cytokine involved in inflammation, auto-immune disease and development of several cancers. Recent studies have shown that over-expression of MIF is associated with tumorigenesis of gastric cancer, but the underlying mechanism is unclear.

Research frontiers

MIF induces cell proliferation through many signal pathways, but the mechanism underlying the regulation of immunoreaction and tumorigenesis is complicated. The PI3K/Akt signaling pathway plays an important role in the regulation of cell growth, proliferation and differentiation. Recent studies have shown that the proliferation of cancer cells is suppressed by blocking the PI3K/Akt pathway. In this study, we investigated the effects of rhMIF on the proliferation of gastric cells and cell cycle progression, and whether the expression of cyclin D1 and $p27^{Kip1}$ and the regulation of PI3K/Akt pathway are involved in the effects of rhMIF.

Innovations and breakthroughs

The study found that MIF induced the proliferation of MGC-803 cells and promoted the cell cycle progression from G1 phase to S phase in gastric cancer MGC-803 cells, and that rhMIF executed its effects by up-regulating cyclin D1 and down-regulating $p27^{Kip1}$ through the PI3K/Akt signal pathway.

Applications

The study explored the mechanism by which MIF induces the proliferation of gastric cancer MGC-803 cells and promotes cell cycle, indicating that MIF may be the potential target for gastric cancer therapy.

Terminology

MIF, a T-cell-derived cytokine, was identified and named by Bloom and David in 1966. PI3Ks is involved in the regulation of cell proliferation, differentiation and apoptosis. The signal pathway through PI3K and its down-stream protein PKB or Akt are closely related to the human tumorigenesis.

Peer review

In this study, the authors investigated whether the PI3K/Akt pathway is involved in the effect of rhMIF on the proliferation of MGC-803 cells, using a PI3K/Akt inhibitor, LY294002. Overall, the study is interesting and significant, and the data are presented in a clear and logical manner.

REFERENCES

1 **Crew KD**, Neugut AI. Epidemiology of gastric cancer. *World J Gastroenterol* 2006; **12**: 354-362
2 **Gallo A**, Cha C. Updates on esophageal and gastric cancers. *World J Gastroenterol* 2006; **12**: 3237-3242
3 **Inoue M**, Tsugane S. Epidemiology of gastric cancer in Japan. *Postgrad Med J* 2005; **81**: 419-424
4 **Ferlay J**, Autier P, Boniol M, Heanue M, Colombet M, Boyle P. Estimates of the cancer incidence and mortality in Europe in 2006. *Ann Oncol* 2007; **18**: 581-592
5 **Kamangar F**, Dores GM, Anderson WF. Patterns of cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions of the world. *J Clin Oncol* 2006; **24**: 2137-2150
6 **Sutter AP**, Fechner H. Gene therapy for gastric cancer: is it promising? *World J Gastroenterol* 2006; **12**: 380-387
7 **Leng L**, Bucala R. Macrophage migration inhibitory factor. *Crit Care Med* 2005; **33**: S475-S477
8 **Bach JP**, Rinn B, Meyer B, Dodel R, Bacher M. Role of MIF in inflammation and tumorigenesis. *Oncology* 2008; **75**: 127-133
9 **Shun CT**, Lin JT, Huang SP, Lin MT, Wu MS. Expression of macrophage migration inhibitory factor is associated with enhanced angiogenesis and advanced stage in gastric carcinomas. *World J Gastroenterol* 2005; **11**: 3767-3771
10 **He XX**, Yang J, Ding YW, Liu W, Shen QY, Xia HH. Increased epithelial and serum expression of macrophage migration inhibitory factor (MIF) in gastric cancer: potential role of MIF in gastric carcinogenesis. *Gut* 2006; **55**: 797-802
11 **Camlica H**, Duranyildiz D, Oguz H, Oral EN, Yasasever V. The diagnostic value of macrophage migration inhibitory factor (MIF) in gastric cancer. *Pathol Oncol Res* 2008; **14**: 79-83
12 **Liu L**, Ji C, Chen J, Li Y, Fu X, Xie Y, Gu S, Mao Y. A global genomic view of MIF knockdown-mediated cell cycle arrest. *Cell Cycle* 2008; **7**: 1678-1692
13 **Tashiro E**, Tsuchiya A, Imoto M. Functions of cyclin D1 as an oncogene and regulation of cyclin D1 expression. *Cancer Sci* 2007; **98**: 629-635
14 **Zheng JY**, Wang WZ, Li KZ, Guan WX, Yan W. Effect of p27(KIP1) on cell cycle and apoptosis in gastric cancer cells. *World J Gastroenterol* 2005; **11**: 7072-7077
15 **Ge JR**, Ren GP, Yao HP. [Expression of cyclin D1 and p27kip1 in renal cell carcinoma and its significance] *Zhejiang Daxue Xuebao Yixueban* 2007; **36**: 483-487
16 **Chang F**, Lee JT, Navolanic PM, Steelman LS, Shelton JG, Blalock WL, Franklin RA, McCubrey JA. Involvement of PI3K/Akt pathway in cell cycle progression, apoptosis, and neoplastic transformation: a target for cancer chemotherapy. *Leukemia* 2003; **17**: 590-603
17 **Kang MJ**, Ryu BK, Lee MG, Han J, Lee JH, Ha TK, Byun DS, Chae KS, Lee BH, Chun HS, Lee KY, Kim HJ, Chi SG. NF-kappaB activates transcription of the RNA-binding factor HuR, via PI3K-AKT signaling, to promote gastric tumorigenesis. *Gastroenterology* 2008; **135**: 2030-2042, 2042. e1-e3
18 **Kim JH**, Go HY, Jin DH, Kim HP, Hong MH, Chung WY, Park JH, Jang JB, Jung H, Shin YC, Kim SH, Ko SG. Inhibition of the PI3K-Akt/PKB survival pathway enhanced an ethanol

extract of Rhus verniciflua Stokes-induced apoptosis via a mitochondrial pathway in AGS gastric cancer cell lines. *Cancer Lett* 2008; **265**: 197-205

19 **Kim N**, Kim CH, Ahn DW, Lee KS, Cho SJ, Park JH, Lee MK, Kim JS, Jung HC, Song IS. Anti-gastric cancer effects of celecoxib, a selective COX-2 inhibitor, through inhibition of Akt signaling. *J Gastroenterol Hepatol* 2009; **24**: 480-487

20 **Bucala R**, Donnelly SC. Macrophage migration inhibitory factor: a probable link between inflammation and cancer. *Immunity* 2007; **26**: 281-285

21 **Calandra T**, Roger T. Macrophage migration inhibitory factor: a regulator of innate immunity. *Nat Rev Immunol* 2003; **3**: 791-800

22 **Mitchell RA**. Mechanisms and effectors of MIF-dependent promotion of tumourigenesis. *Cell Signal* 2004; **16**: 13-19

23 **Lue H**, Kapurniotu A, Fingerle-Rowson G, Roger T, Leng L, Thiele M, Calandra T, Bucala R, Bernhagen J. Rapid and transient activation of the ERK MAPK signalling pathway by macrophage migration inhibitory factor (MIF) and dependence on JAB1/CSN5 and Src kinase activity. *Cell Signal* 2006; **18**: 688-703

24 **Lue H**, Thiele M, Franz J, Dahl E, Speckgens S, Leng L, Fingerle-Rowson G, Bucala R, Lüscher B, Bernhagen J. Macrophage migration inhibitory factor (MIF) promotes cell survival by activation of the Akt pathway and role for CSN5/JAB1 in the control of autocrine MIF activity. *Oncogene* 2007; **26**: 5046-5059

25 **Petrenko O**, Moll UM. Macrophage migration inhibitory factor MIF interferes with the Rb-E2F pathway. *Mol Cell* 2005; **17**: 225-236

26 **Santamaria D**, Ortega S. Cyclins and CDKS in development and cancer: lessons from genetically modified mice. *Front Biosci* 2006; **11**: 1164-1188

27 **Johansson M**, Persson JL. Cancer therapy: targeting cell cycle regulators. *Anticancer Agents Med Chem* 2008; **8**: 723-731

28 **Alao JP**. The regulation of cyclin D1 degradation: roles in cancer development and the potential for therapeutic invention. *Mol Cancer* 2007; **6**: 24

29 **Belletti B**, Nicoloso MS, Schiappacassi M, Chimienti E, Berton S, Lovat F, Colombatti A, Baldassarre G. p27(kip1) functional regulation in human cancer: a potential target for therapeutic designs. *Curr Med Chem* 2005; **12**: 1589-1605

30 **Hershko DD**, Shapira M. Prognostic role of p27Kip1 de-regulation in colorectal cancer. *Cancer* 2006; **107**: 668-675

31 **Lecanda J**, Parekh TV, Gama P, Lin K, Liarski V, Uretsky S, Mittal K, Gold LI. Transforming growth factor-beta, estrogen, and progesterone converge on the regulation of p27Kip1 in the normal and malignant endometrium. *Cancer Res* 2007; **67**: 1007-1018

32 **Nakayama K**, Nagahama H, Minamishima YA, Miyake S, Ishida N, Hatakeyama S, Kitagawa M, Iemura S, Natsume T, Nakayama KI. Skp2-mediated degradation of p27 regulates progression into mitosis. *Dev Cell* 2004; **6**: 661-672

33 **Lahav-Baratz S**, Ben-Izhak O, Sabo E, Ben-Eliezer S, Lavie O, Ishai D, Ciechanover A, Dirnfeld M. Decreased level of the cell cycle regulator p27 and increased level of its ubiquitin ligase Skp2 in endometrial carcinoma but not in normal secretory or in hyperstimulated endometrium. *Mol Hum Reprod* 2004; **10**: 567-572

34 **Yu BH**, Zhou XY. [Advances on PI3K/Akt/mTOR signalling pathway in malignancies] *Zhonghua Binglixue Zazhi* 2005; **34**: 674-676

35 **Tokunaga E**, Oki E, Egashira A, Sadanaga N, Morita M, Kakeji Y, Maehara Y. Deregulation of the Akt pathway in human cancer. *Curr Cancer Drug Targets* 2008; **8**: 27-36

36 **Michl P**, Downward J. Mechanisms of disease: PI3K/AKT signaling in gastrointestinal cancers. *Z Gastroenterol* 2005; **43**: 1133-1139

37 **Cinti C**, Vindigni C, Zamparelli A, La Sala D, Epistolato MC, Marrelli D, Cevenini G, Tosi P. Activated Akt as an indicator of prognosis in gastric cancer. *Virchows Arch* 2008; **453**: 449-455

38 **Liang J**, Pan Y, Zhang D, Guo C, Shi Y, Wang J, Chen Y, Wang X, Liu J, Guo X, Chen Z, Qiao T, Fan D. Cellular prion protein promotes proliferation and G1/S transition of human gastric cancer cells SGC7901 and AGS. *FASEB J* 2007; **21**: 2247-2256

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BRIEF ARTICLE

Short-chain fatty acids act as antiinflammatory mediators by regulating prostaglandin E_2 and cytokines

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Abstract

AIM: To investigate the effect of short-chain fatty acids (SCFAs) on production of prostaglandin E_2 (PGE_2), cytokines and chemokines in human monocytes.

METHODS: Human neutrophils and monocytes were isolated from human whole blood by using 1-Step Polymorph and RosetteSep Human Monocyte Enrichment Cocktail, respectively. Human GPR41 and GPR43 mRNA expression was examined by quantitative real-time polymerase chain reaction. The calcium flux assay was used to examine the biological activities of SCFAs in human neutrophils and monocytes. The effect of SCFAs on human monocytes and peripheral blood mononuclear cells (PBMC) was studied by measuring PGE_2, cytokines and chemokines in the supernatant. The effect of SCFAs *in vivo* was examined by intraplantar injection into rat paws.

RESULTS: Human GPR43 is highly expressed in human neutrophils and monocytes. SCFAs induce robust calcium flux in human neutrophils, but not in human monocytes. In this study, we show that SCFAs can induce human monocyte release of PGE_2 and that this effect can be enhanced in the presence of lipopolysaccharide (LPS). In addition, we demonstrate that PGE_2 production induced by SCFA was inhibited by pertussis toxin, suggesting the involvement of a receptor-mediated mechanism. Furthermore, SCFAs can specifically inhibit constitutive monocyte chemotactic protein-1 (MCP-1) production and LPS-induced interleukin-10 (IL-10) production in human monocytes without affecting the secretion of other cytokines and chemokines examined. Similar activities were observed in human PBMC for the release of PGE_2, MCP-1 and IL-10 after SCFA treatment. In addition, SCFAs inhibit LPS-induced production of tumor necrosis factor-α and interferon-γ in human PBMC. Finally, we show that SCFAs and LPS can induce PGE_2 production *in vivo* by intraplantar injection into rat paws ($P < 0.01$).

CONCLUSION: SCFAs can have distinct antiinflammatory activities due to their regulation of PGE_2, cytokine and chemokine release from human immune cells.

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Key words: Short-chain fatty acids; GPR43; GPR41; Human monocytes; Prostaglandin E_2; Chemokines; Cytokines

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INTRODUCTION

The receptors for free fatty acids, including GPR40, GPR41, GPR42 and GPR43, constitute a subfamily of recently deorphanized G-protein-coupled receptors (GPCRs) that are clustered on human chromosome 19q13.1[1]. They exhibit 30%-40% homology to one another and have diverse tissue distributions, yet all are activated by various free fatty acids, which function as intercellular lipid mediators through GPCRs[2,3].

The free fatty acid receptors are functionally involved in both metabolism and the immune system. In contrast to GPR40 which is activated by medium- and long-chain fatty acids and abundantly expressed in the pancreatic islets[4-7], GPR41 and GPR43 are functionally activated by short-chain fatty acids (SCFAs), including acetate, propionate and butyrate[8-10]. Although both receptors are activated by SCFAs, they have distinct tissue distribution profiles. GPR41 is preferentially expressed in adipose tissue, while GPR43 is highly expressed in hematopoietic tissues (such as spleen and bone marrow) and immune cells (particularly monocytes and neutrophils). Interestingly, the murine ortholog of GPR43 was initially identified as leukocyte-specific STAT-induced GPCR (LSSIG). It was reported that expression of both murine LSSIG and human GPR43 is induced during the differentiation of leukocyte progenitor cells to monocytes or neutrophils, suggesting their involvement in leukocyte function and host defense[11].

SCFAs have long been known to modulate the immune response. Acetate, propionate and butyrate represent the most often described SCFAs that are capable of immune activation. SCFAs affect neutrophil function and migration[12-15], and inhibit tumor necrosis factor-α (TNF-α) or interleukin-1β (IL-1β)-induced vascular cell adhesion molecule-1 (and intercellular adhesion molecule-1) surface expression[16]. In addition, SCFAs reduce adherence of monocytes or lymphocytes to cytokine-stimulated human umbilical vein endothelial cells[17], and inhibit interferon-γ (IFN-γ) signaling[18,19], a possible regulator of inflammation in inflammatory bowel disease (IBD). Although the molecular mechanisms of the effects of SCFAs remain to be elucidated, evidence in the literature indicates that SCFA-induced biological effects involve G-protein-mediated signaling[12,20]. GPR43, which is highly expressed in immune cells, could be the potential missing GPCR activated by SCFAs. GPR41 is also expressed in immune cells, but at lower levels compared to GPR43 and thus may play a lesser role[8-10]. As there are no well-validated specific drugs that can be used as tools to investigate the receptor, the emerging biology of these receptors is dependent on expression profiling. It is believed that GPR43 is likely the main receptor contributing to the effect of SCFAs in immune cells. In human neutrophils, it was reported that SCFAs induced calcium flux and chemotaxis possibly through GPR43[9]. Nonetheless, the precise role of GPR43 in the effect of SCFAs in neutrophils needs to be established with the generation of a knockout model and specific small molecules. In addition, GPR43 is also highly expressed in monocytes and peripheral blood mononuclear cells (PBMC)[9], and the effects of SCFAs in these important immune cells are of high interest, but are largely unknown.

SCFAs are produced by microbial anaerobic fermentation in the hindgut in millimolar (mmol/L) concentrations; therefore, the physiological site of GPR43 activation may be in the gut. Within the human colon, concentrations of acetate, propionate and butyrate were reported to be in the range of 20-43 mmol/L, 6-13 mmol/L and 6-15 mmol/L, respectively[21]. GPR43 has been reported to be expressed in human, mouse and rat colons[22-24]. Induction of anti-inflammatory activities by SCFAs in the colon may contribute to the bacterial evasion of the immune system[25]. GPR43-mediated activation may provide a link between the intestinal bacteria and the immune response in physiological and pathophysiological conditions.

We report the identification of new functional roles of SCFAs in production of prostaglandin E_2 (PGE_2), cytokines and chemokines in human monocytes and in PBMC. We speculate that the effect of SCFAs is most likely through activation of GPR43, a potential therapeutic target for the development of an innovative treatment for colitis.

MATERIALS AND METHODS

Isolation of human neutrophils, monocytes and PBMCs

Human neutrophils were isolated from human whole blood by using 1-Step Polymorph (Accurate Chemical & Scientific Corp., Westbury, NY) according to the manufacturer's instructions. Human monocytes were isolated by RosetteSep Human Monocyte Enrichment Cocktail (StemCell Technologies, London, UK). Human PBMC were prepared by the Ficoll-Hypaque centrifugation method.

For the calcium flux assay, both human neutrophils and monocytes were treated with formate, acetate and propionate (Sigma, St. Louis, MO, USA) for 5-10 min. For measurement of PGE_2, cytokines and chemokines, human monocytes and PBMC cells were incubated with formate, acetate, propionate and butyrate (Sigma, St. Louis, MO, USA) overnight.

Quantitative real-time polymerase chain reaction (PCR) analysis

TaqMan primers and probes were designed with Primer Express software and purchased from ABI (Applied Biosystems, Foster City, CA, USA). The sequences of the human primers and probes for GPR41 and GPR43 are shown in Table 1. Quantitative real-time PCR was carried out with an ABI Prism 7900HT Sequence Detection System. The PCR reactions were prepared

Table 1 Real-time polymerase chain reaction primers and probes designed for the detection and quantification of human GPR41 and GPR43

Gene	RefSeq ID	Forward primer	Reverse primer	Probe
GPR41	NM_005304	GCTTTGGGCCCTACAACGT	CCATGCCGGGCTTTCA	TCCCATGTCGTGGGCTATATCTGCG
GPR43	NM_005306	GGCTTTCCCCGTGCAGTAC	ACCAGAGCTGCAATCACTCCAT	AGCTCTCCCGCCGGCCTCTG

using the components from the iScript Custom One-Step RT-PCR Kit with ROX and assembled according to the manufacturer's instructions (Bio-Rad, Hercules, CA, USA). The final concentrations of the primers and probe in the PCR reactions were 200 nmol/L and 100 nmol/L, respectively. The fluorogenic probes were labeled with 6-carboxyfluorescein as the reporter and 6-carboxy-4,7,2,7'-tetramethylrhodamine as a quencher. Each 10 μL PCR reaction contained 10 ng of total RNA. The RT-PCR reactions were performed in triplicate in a 384-well plate according to the following protocol: one cycle for 10 min at 50℃, followed by one 5 min cycle at 95℃, followed by 40 cycles at 95℃ for 15 s and 60℃ for 1 min. A eukaryotic 18S rRNA endogenous control probe/primer set (ABI) was used as an internal control for RNA quality.

The PCR data were quantified, based on a 12-point standard curve generated using 4-fold serial dilutions of a cDNA containing the gene of interest. The 4-fold dilutions began at 20000 fg. This procedure provides an absolute quantification of the amounts of GPR41 and GPR43 mRNA in a given sample.

Calcium flux assay

Intracellular Ca^{2+} assays were carried out as previously described with modifications[26]. Freshly isolated human neutrophils were resuspended at 1×10^6 cells/mL in cell culture medium (RPMI 1640 with 2 mmol/L GlutaMAX and 5% fetal bovine serum) and incubated at 37℃, 50 mL/L CO_2, for 1 h. Cells were spun and resuspended at 1×10^6 cells/mL in a 1:1 mixture of cell culture medium and Calcium-4 no wash dye (Molecular Devices, Sunnyvale, CA, USA) containing a final concentration of 5 mmol/L probenecid (Sigma). The cells were seeded (50000/well) into poly-D-lysine-coated 384-well, black-wall, clear bottom microtiter plates (Becton Dickinson). Cells were sedimented in plates by spinning down briefly at low speed with no brake then incubated at 37℃, 50 mL/L CO_2, for 1 h before assaying on a FLIPR-384 (Molecular Devices). Freshly isolated human monocytes were resuspended at 1×10^9 cells/L in a 1:1 mixture of cell culture medium and Calcium-3 no wash dye containing a final concentration of 2.5 mmol/L probenecid. The cells were seeded (50000/well) into poly-D-lysine coated 384-well plates. Cells were dye loaded for 45 min at 37℃, 50 mL/L CO_2, before assaying on a FLIPR-384.

Formate, acetate, propionate and butyrate (Sigma) were prepared in Hank's balanced salt solution, 25 mmol/L Hepes, and 0.1% bovine serum albumin. The maximum change in fluorescence over baseline was used to determine agonist response. The dose-response curves and EC_{50} values were obtained by nonlinear regression (Prism 4; Graph Pad Software, San Diego, CA, USA).

Measurement of PGE_2, cytokines and chemokines

PGE_2 was measured by ELISA kit from Assay Designs (Ann Arbor, MI, USA). Cytokines and chemokines were measured by MesoScale ELISA (MesoScale Discovery, Gaithersburg, MD, USA) according to the manufacturer's instructions. To generate the conditioned media, 1×10^6 cells per mL were resuspended in RPMI 1640 medium supplemented with 10% fetal bovine serum, 1 mmol/L L-glutamine, 10 mmol/L Hepes, 50000 U/L penicillin and 50 μg/mL streptomycin.; and seeded in 24-well plates for stimulation with SCFAs and/or incubation with selective inhibitors (Cayman Chemical, Ann Arbor, MI, USA), including MLnFP (methyl α-linolenyl fluorophosphonate), a phospholipase inhibitor; 1400W, a selective iNOS inhibitor; SC-560, a selective cyclooxygenase-1 (COX-1) inhibitor; CAY10404, a selective COX-2 inhibitor; PTX, pertussis toxin. The tested concentrations of each inhibitor (except PTX) was chosen to be about 100 times of its IC_{50} as reported in the data sheet provided by the manufacturer.

Intraplantar injection of SCFAs into rat paws

Sprague-Dawley male rats from Charles River Laboratories, 4 per group and approximately 200 g body weight, were anesthetized with isoflurane gas and the subplantar space of the paw injected with 0.1 mL of 100 mmol/L solutions of the SCFAs, either formate, acetate, propionate or butyrate prepared fresh in 0.9% saline. Lipopolysaccharide (LPS), *Escherichia coli* 0127:B8 (Sigma) was also injected at 3 μg in saline either alone or in combination with 0.1 mL of 200 mmol/L sodium butyrate. Rats in the normal group were not injected. At 3 h post-injection, the rats were humanely euthanized and a uniform punch biopsy of the injected site was taken from each rat. The punch biopsies were immediately placed in PMSF (phenylmethanesulphonyl fluoride) buffer containing 10 g/L of indomethacin and frozen at -20℃. The tissues were homogenized in this collection buffer and assayed for PGE_2.

All statistical analysis was performed by Mann-Whitney *U* test using GraphPad Instat version 3.06 for Windows XP (GraphPad Software, San Diego, CA, USA). All studies in animals were performed in accordance with the regulations specified by the National Institutes of Health Principles of Laboratory Animal Care (1985 revised version) and the Schering-Plough Research Institute Animal Care and Use Committee.

RESULTS

GPR43 is highly expressed in human neutrophils and monocytes

Both GPR43 and GPR41 are activated by SCFAs and

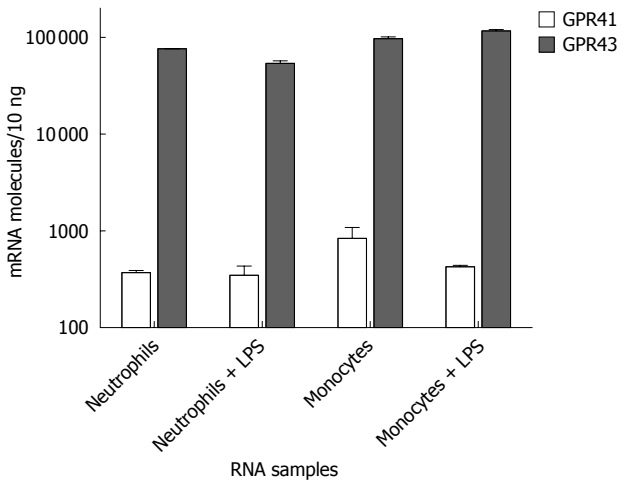
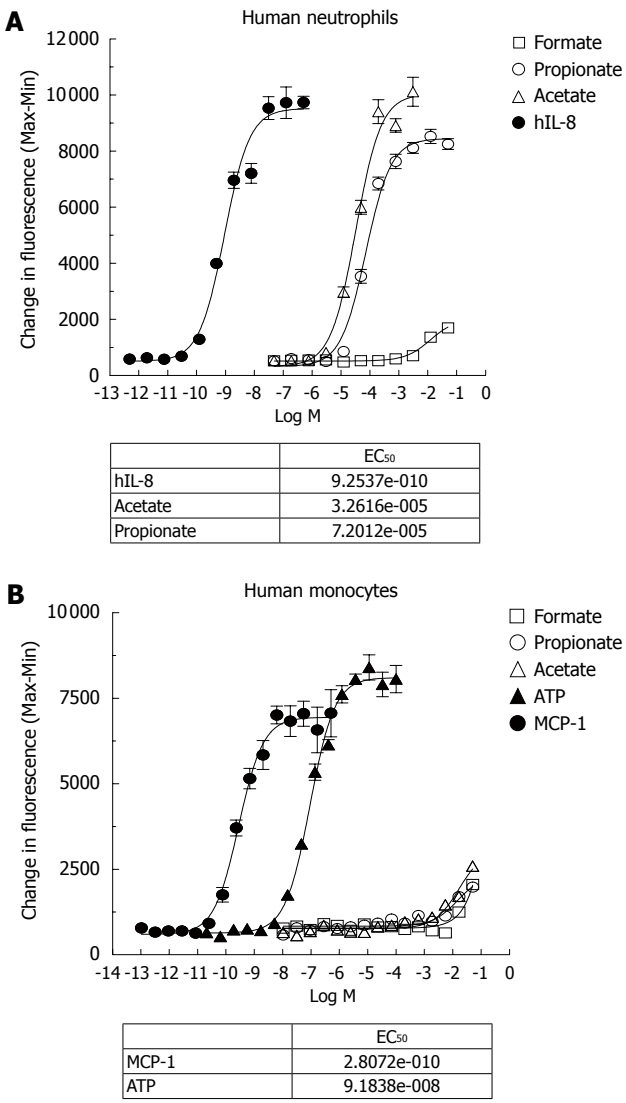


Figure 1 GPR43 is highly expressed in human neutrophils and monocytes. Human neutrophils and monocytes were isolated from human whole blood as described in Materials and Methods. Isolated human neutrophils or monocytes were stimulated with 100 ng/mL of lipopolysaccharide (LPS) for 3 h. RNAs were isolated and evaluated by Taqman analysis for absolute quantities of GPR43 and GPR41 mRNA molecules.

reported to be expressed in immune cells. To examine the role of GPR43 and GPR41 in human immune cells, we initially quantified their expression levels in human neutrophils and monocytes by Taqman analysis. Human neutrophils and monocytes were each isolated from human donors to 95% purity. Some of them were stimulated with LPS. RNAs were isolated and analyzed for GPR43 and GPR41 expression by Taqman. Figure 1 shows that GPR43 is expressed in both human neutrophils and monocytes at a much higher level than GPR41. It also appears that LPS stimulation did not affect their expression levels.

SCFAs induce robust calcium flux in human neutrophils, but not in human monocytes

To investigate the biological activities of SCFAs, both purified human neutrophils and monocytes were exposed to various concentrations of SCFAs (formate, acetate and propionate) in a calcium flux assay. Formate was used as a negative control for the SCFAs. In addition, IL-8 was included as a positive control for neutrophil activation, while monocyte chemotactic protein-1 (MCP-1) and ATP were used as the positive controls for monocyte activation. Since GPR41 couples to $G_{i/o}$ only, SCFAs should not cause a calcium flux through this receptor, which was confirmed in a recombinant cell line expressing GPR41 (data not shown). Indeed, the agonist potency profile of the calcium response in human neutrophils (Figure 2A) was consistent with the GPR43 receptor response that has been described[9]. From 8 human donors, acetate had an average EC_{50} of 58.25 ± 12.44 μmol/L (n = 8), and propionate had an EC_{50} of 200.6 ± 42.42 μmol/L (n = 8). Formate was inactive and IL-8 had an EC_{50} about 1-2 nmol/L in human neutrophils. Acetate and propionate achieved the same maximal activation response as IL-8 in human neutrophils using this calcium flux assay.



	EC_{50}
hIL-8	9.2537e-010
Acetate	3.2616e-005
Propionate	7.2012e-005

	EC_{50}
MCP-1	2.8072e-010
ATP	9.1838e-008

Figure 2 Short-chain fatty acids (SCFAs) induce robust calcium flux in human neutrophils, but not in human monocytes. Human neutrophils (A) and monocytes (B) were isolated and exposed to the indicated concentrations of formate, acetate and propionate for a calcium flux assay as described in Materials and Methods. Interleukin-8 (IL-8) is included as a positive control for human neutrophils, and monocyte chemotactic protein-1 (MCP-1) and ATP for human monocytes. The dose-response curves and EC_{50} values were obtained by nonlinear regression using Graph Pad Prism 4 software.

On the other hand, Figure 2B shows that acetate and propionate, up to 100 mmol/L, did not induce significant calcium flux in human monocytes, while MCP-1 and ATP were able to induce robust calcium flux in the human monocyte preparation. It appears that the response of GPR43 to SCFAs for calcium flux is dependent either on cell type or on the state of differentiation of the cells. Indeed, it has been reported that propionic acid induced calcium mobilization in human neutrophils, but not in monocytes, platelets and lymphocytes[12].

SCFAs strongly induce PGE_2 production in human monocytes and the effect is receptor-mediated

To further explore the biological effects of SCFAs, human monocytes were isolated and incubated overnight with various concentrations of SCFAs in the presence

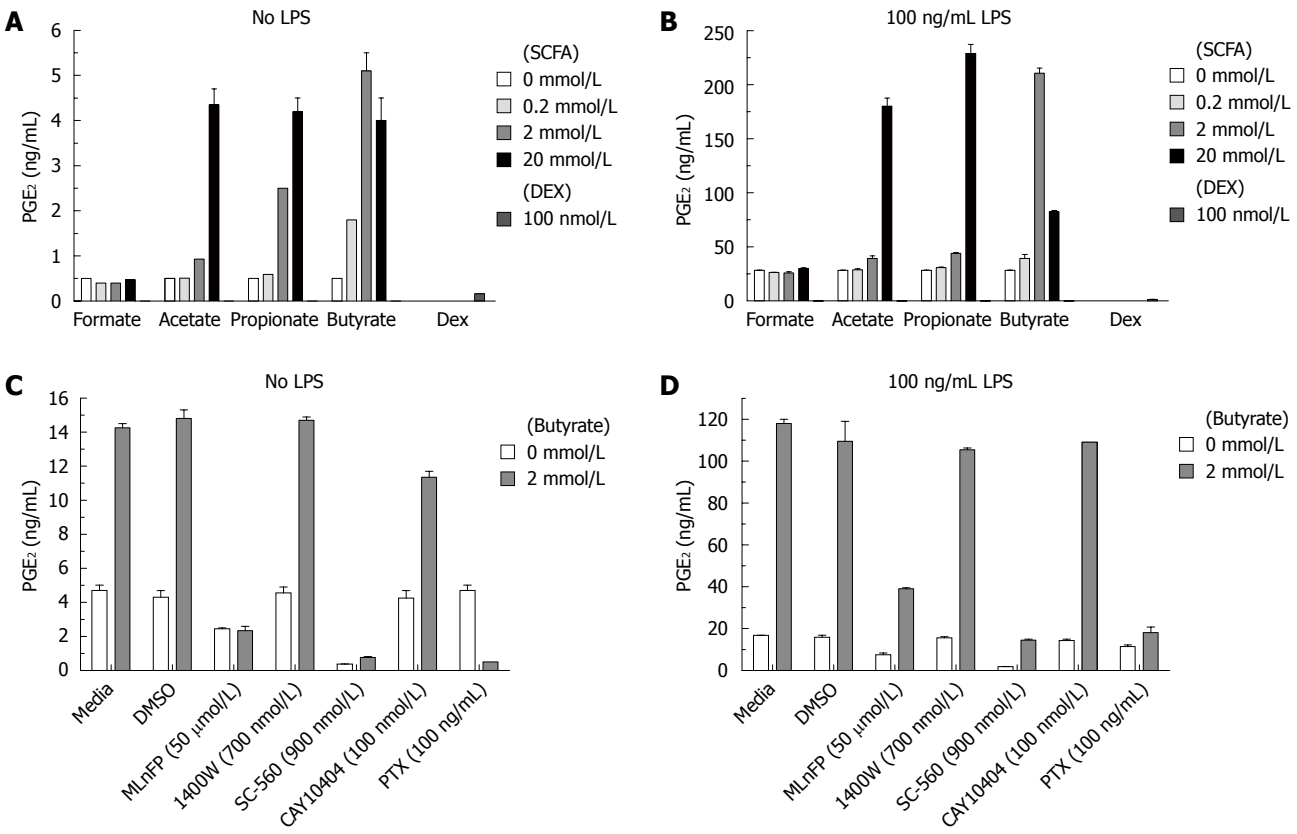


Figure 3 SCFAs strongly induce PGE_2 production in human monocytes and the effect is receptor-mediated. Freshly isolated human monocytes (1×10^9 cells/L) were incubated with the indicated concentrations of SCFAs or 100 nmol/L dexamethasone (Dex), and then cultured for 22 h without (A) or with (B) 100 ng/mL LPS. In a separate experiment (C, D), human monocytes were incubated with the indicated concentrations (in the parentheses) of inhibitors (described below) for 30 min, then with or without 2 mmol/L butyrate for another 30 min, and finally cultured for 22 h without (C) or with (D) 100 ng/mL LPS. The media and DMSO samples were used as controls with no inhibitors. All culture supernatants were assayed for PGE_2 by ELISA. MLnFP (methyl α-linolenyl fluorophosphonate): Phospholipase inhibitor; 1400W: Selective iNOS inhibitor; SC-560: Selective cyclooxygenase-1 (COX-1) inhibitor; CAY10404: Selective COX-2 inhibitor; PTX: Pertussis toxin. (Cayman Chemical, Ann Arbor, MI). The tested concentrations of each inhibitor (except PTX) were chosen to be about 100 times its IC_{50} as reported in the data sheet provided by the manufacturer.

or absence of LPS (100 ng/mL) as a proinflammatory stimulus. The supernatants were assayed for lipid mediators such as PGE_2, proinflammatory cytokines and chemokines by either ELISA or Meso Scale Multi-Spot Discovery Technology. Dexamethasone was included in the experiment as a positive control for the induction of inhibition. Figure 3A shows that SCFAs alone strongly enhanced PGE_2 production in human monocytes across a panel of 10 human donors in a concentration-dependent manner. Furthermore, SCFAs synergistically enhanced the PGE_2 production induced by LPS in human monocytes (10 human donors), as shown in Figure 3B. The rank order of potency was butyrate > propionate > acetate, while formate was inactive. Dexamethasone at 100 nmol/L potently inhibited basal PGE_2 and LPS-induced PGE_2 production (Figure 3A and B). Similar results were obtained from 10 human donors. The culture supernatants were also assayed for PGI_2, leukotriene B_4 (LTB_4) and thromboxane B_2 (TXB_2). SCFAs had no significant effect on these lipid mediators (data not shown), suggesting its effect on PGE_2 production is specific.

To examine possible mechanisms of PGE_2 production induced by SCFAs, human monocytes were preincubated with several inhibitors and then added to culture with butyrate and/or LPS. Figure 3C and D show that the PGE_2 production induced by butyrate is inhibited by pretreatment with PTX, COX-1 and phospholipase inhibitors either in the absence or presence of LPS. In contrast, COX-2 and iNOS inhibitors had no effect on PGE_2 production induced by butyrate. The sensitivity to PTX suggests PGE_2 production induced by butyrate is receptor-mediated.

SCFAs specifically inhibit LPS-induced IL-10 production and constitutive MCP-1 expression in human monocytes

The same culture supernatants described above were also analyzed for 8 proinflammatory cytokines including IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12p70, IFN-γ, TNF-α (Meso Scale Multi-Spot Discovery Technology). In the absence of LPS, SCFAs had no effect on the level of any of the 8 cytokines including IL-10, as shown in Figure 4A. In contrast, SCFAs specifically inhibited IL-10 production stimulated with LPS (Figure 4B), but had no effect on the other 7 cytokines with LPS stimulation (including LPS-induced production of IL-1β and TNF-α. The effect of dexamethasone on the cytokine profile is consistent with what has been previously described; briefly, it inhibited LPS-induced production of IL-1β, IL-10 and TNF-α.

It is consistently observed that isolated human monocytes produce high levels of MCP-1 in the culture

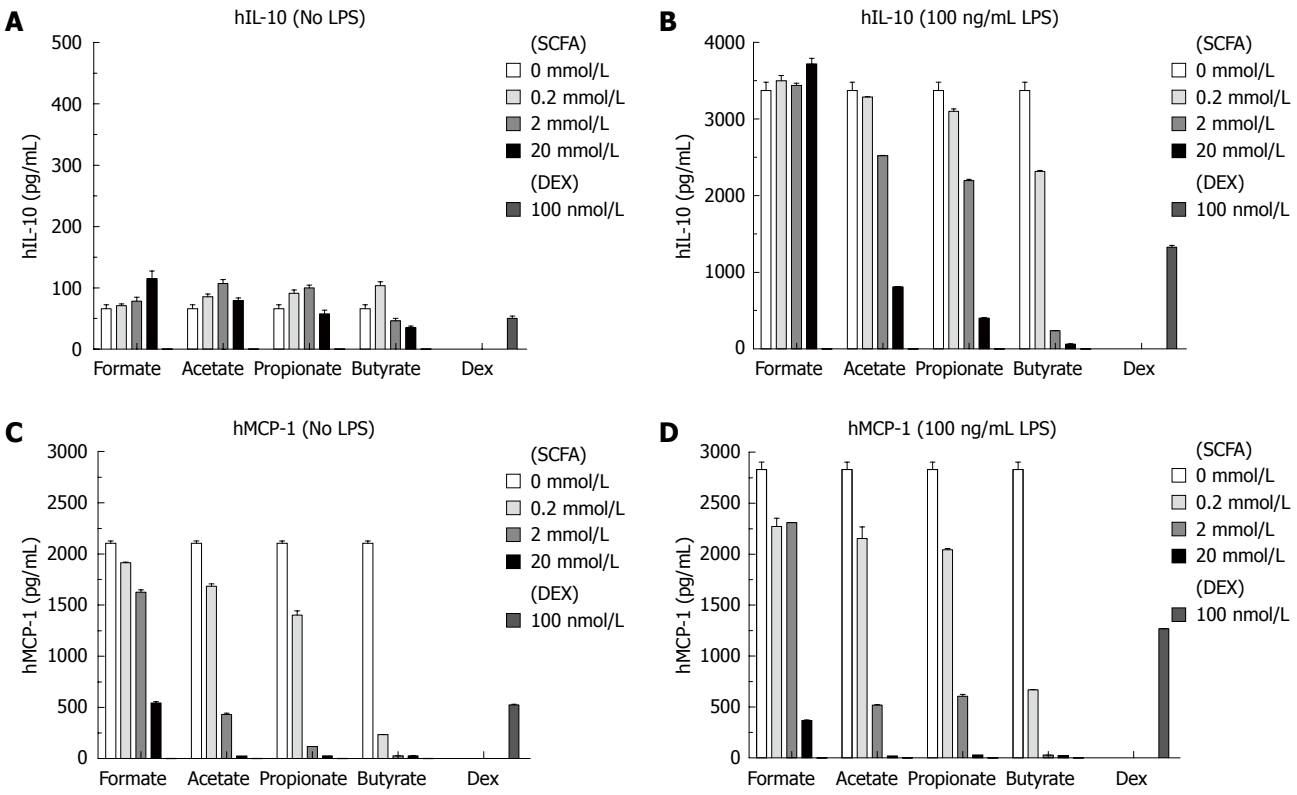


Figure 4 SCFAs specifically inhibit LPS-induced IL-10 production and constitutive MCP-1 expression in human monocytes. The culture supernatants as in Figure 3A and B were analyzed for 8 proinflammatory cytokines (including IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12p70, IFN-γ, TNF-α) or 9 proinflammatory chemokines (including MCP-1, MCP-4, eotaxin, eotaxin-3, IL-8, IFN-γ inducible protein-10, macrophage inflammatory protein-1β, macrophage-derived chemokine, thymus and activation-regulated chemokine) by Meso Scale Multi-Spot Discovery Technology. Only the data for IL-10 (A, B) and MCP-1 (C, D) are shown here. Similar results were obtained from 10 human donors.

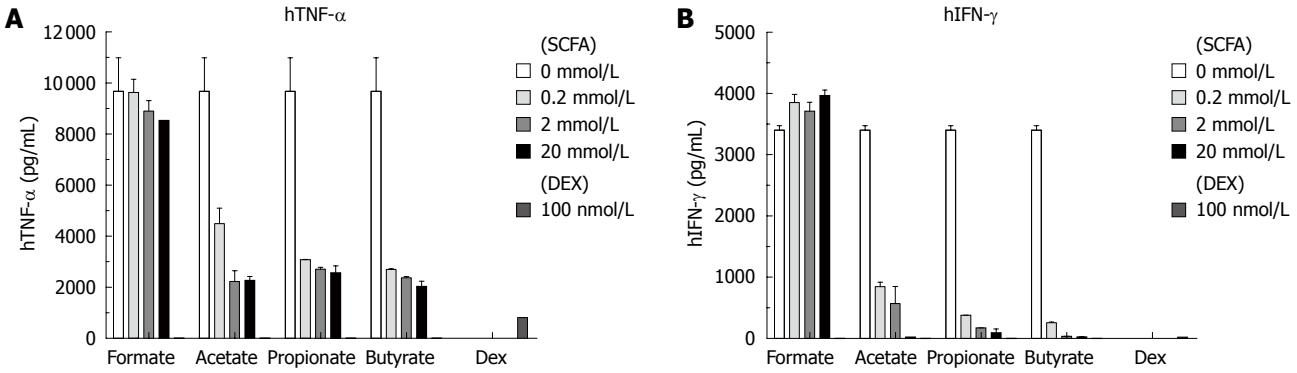


Figure 5 SCFAs inhibit LPS-induced production of TNF-α and IFN-γ in human peripheral blood mononuclear cells (PBMC) in a dose-dependent manner. Human PBMC isolated by Ficoll-Hypaque centrifugation procedure (1×10^6 cells/mL) were incubated with the indicated concentrations of short-chain fatty acids (SCFA) or 100 nmol/L dexamethasone (Dex), and then cultured for 22 h with or without 100 ng/mL LPS. Culture supernatants were analyzed for 8 proinflammatory cytokines including IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12p70, IFN-γ, TNF-α by Meso Scale Multi-Spot Discovery Technology. The data for TNF-α (A) and IFN-γ (B) with LPS stimulation are shown here.

supernatants without stimulation. Figure 4C and D show that SCFAs inhibited this constitutive MCP-1 production in a concentration-dependent manner either in the absence or presence of LPS. SCFAs did not inhibit the constitutive high levels of eotaxin-3, macrophage-derived chemokine and macrophage inflammatory protein-3β in the cultures, suggesting that the effect of SCFAs on MCP-1 production is specific. In this experiment, 20 mmol/L formate showed some inhibition, which is likely the result of a nonspecific effect.

SCFAs inhibit LPS-induced production of TNF-α and IFN-γ in human PBMCs in a concentration-dependent manner

To further confirm the effect of SCFAs, human PBMC were isolated by Ficoll-Hypaque centrifugation procedure and incubated with SCFAs and/or LPS. The culture supernatants were analyzed as before for PGE_2, cytokines and chemokines. The effects of SCFAs on PGE_2, MCP-1 and IL-10 production were similar to that observed in the isolated human monocytes. However, as shown in

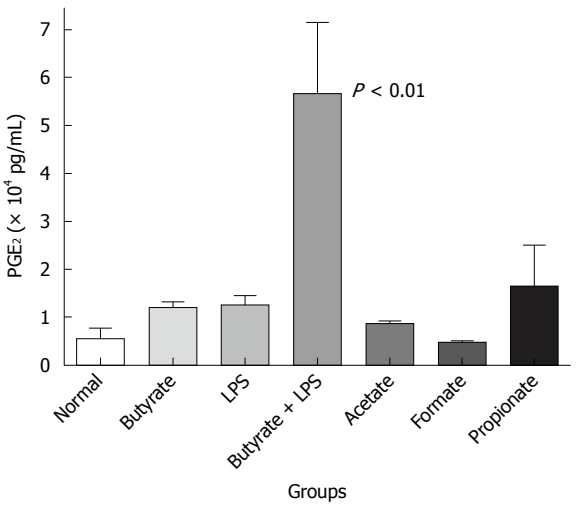


Figure 6 SCFAs induce PGE_2 production by intraplantar injection into rat paws. The paw of male Sprague-Dawley rats was injected intraplantarly with 0.1 mL of 100 mmol/L formate, acetate, propionate or butyrate. Three micrograms of LPS in saline was also injected alone or in combination with 0.1 mL of 200 mmol/L butyrate. There were 4 rats per group. The normal group was not injected. Three hours post injection, rat paws were punched and fluids were collected in phenylmethanesulphonyl fluoride buffer with indomethacin. All punch fluids were assayed for PGE_2 by ELISA. $P < 0.01$ for butyrate + LPS group *vs* normal group, Mann-Whitney *U* test.

Figure 5, SCFAs inhibited LPS-induced production of TNF-α and IFN-γ in human PBMC in a concentration-dependent manner, an effect not observed in isolated human monocytes where IFN-γ production was low and not stimulated by LPS addition. In the human PBMC experiment, dexamethasone inhibited LPS-induced production of IL-1β, IL-10, IFN-γ and TNF-α.

SCFAs induce PGE_2 production by intraplantar injection into rat paws

To demonstrate the effect of SCFAs *in vivo*, SCFAs and LPS alone or in combination were injected by the intraplantar route into a rat paw. This was done to initiate an inflammatory response and determine what effect the butyrate would have on lipid mediator and cytokine induction. In the representative experiment shown in Figure 6, acetate, propionate and butyrate alone could induce PGE_2 production in rat paw. Formate was inactive and the level of PGE_2 was equivalent to that extracted from a normal (not injected) paw. In addition, suboptimal LPS also induced PGE_2 production and the combination of LPS and butyrate strongly enhanced PGE_2 production in a synergistic manner ($P < 0.01$). These *in vivo* data, although preliminary in nature, support the effects of SCFAs on PGE_2 production observed in human monocytes and PBMC. Cytokine analysis was performed on the tissue homogenates but no clear pattern emerged that was similar to that obtained in the human monocytes and PBMC *in vitro* experiments. Sodium butyrate is extremely labile with a very high diffusion rate. Maintenance of a sufficient concentration in the paw subcutaneous space was a significant problem in these experiments.

DISCUSSION

In this study, we have shown that SCFAs can induce either robust PGE_2 production alone or in synergy with LPS in human monocytes and PBMC. Since LPS stimulation did not affect the mRNA level of GPR43 and GPR41, the synergy is likely from 2 independent signaling pathways. This effect on PGE_2 production is specific, since other lipid mediators including PGI_2, LTB_4 and TXB_2, were not affected by SCFAs. Furthermore, this effect is receptor-mediated since it is sensitive to PTX.

Interestingly, we showed that SCFAs induced robust calcium flux in human neutrophils, but not in human monocytes. Our finding is consistent with the previous report that propionic acid induced calcium mobilization in human neutrophils, but not in monocytes, platelets and lymphocytes[12]. It is likely that SCFAs activate neutrophils and monocytes for the immune response through different signaling pathways that would be consistent with the different temporal roles of these cells in an inflammatory response. SCFAs induce calcium flux and chemotaxis in neutrophils which arrive early in an inflammatory response, while they regulate production of PGE_2, cytokines and chemokines in monocytes which are also present in the inflammatory site but mature into macrophages during extravasation and remain at the site of inflammation until resolution.

This *in vitro* effect of SCFAs on human monocytes may have relevance to human diseases that target the intestinal tract. Among various prostanoids, PGE_2, in particular, seems to play a critical role in inflammatory bowel disease (IBD) *via* the EP4 receptor, one of the four PGE_2 receptor subtypes(EP1-4)[27-31]. Among 8 prostanoid receptor-deficient mice, only EP4-deficient mice developed severe colitis with 3% dextran sodium sulphate (DSS) treatment. It is suggested that EP4 maintains intestinal homeostasis by maintaining mucosal integrity and downregulating the immune response[32]. Therefore, it is possible that SCFAs induce production of PGE_2 and play a protective role *via* the GPR43-expressing cells of the colon. Furthermore, we demonstrate that PGE_2 production induced by SCFAs was inhibited by a COX-1 inhibitor, but not a COX-2 inhibitor. This is consistent with the notion that in DSS-induced colitis, the significant reduction in PGE_2 resulted from decreased expression of COX-1, but not COX-2[33].

In human monocytes, SCFAs specifically inhibited constitutive MCP-1 production and LPS-induced IL-10 production out of the total 16 cytokines and chemokines examined. The activities of SCFAs in our human monocyte cultures were all confirmed in human PBMC, which contain both monocytes and lymphocytes. We made the additional observation in these PBMC cultures that SCFAs would inhibit LPS-induced TNF-α and IFN-γ production.

This unique effect of SCFAs on the production of cytokines and chemokines in monocytes and PBMC is relevant to the design of new therapies for colitis. Intestinal inflammation present in inflammatory bowel disease

is driven by the production of cytokines, chemokines and growth factors that draw in immune cells to the mucosa. It has been shown that MCP-1, IL-10, IFN-γ and TNF-α were significantly upregulated in experimental colitis models[34,35]. Furthermore, the increased production of SCFAs from dietary fiber supplementation or probiotics administration inhibited the production of proinflammatory mediators and recovered damaged colonic mucosa in colitic animals[36,37]. These experiments show that SCFAs can inhibit multiple inflammatory mediators which may control intestinal inflammation. Clinical trial evidence for this use of SCFAs is still not widely available or accepted as a mainstream therapy.

We tried to design a model system *in vivo* to duplicate our *in vitro* findings. We present the results of a model system using the intraplantar space in the rat paw, a space from which there was limited diffusion of the injected SCFAs. We had previously tried injecting SCFAs into the pleural cavity and into the peritoneal cavity to induce an inflammatory response with and without suboptimal inflammatory stimuli such as LPS. However, SCFAs are extremely labile with a very high diffusion rate and require high concentrations to achieve their effect *in vivo*. Maintenance of a sufficient concentration of SCFAs at a local site or tissue space (such as the colon) has been a major challenge to obtain clear cytokine profiles and *in vivo* efficacy. Therefore, better pharmacological tools, such as GPR43 specific small molecules[38], are needed to confirm our *in vitro* findings and further elucidate their biology. We were able to utilize the intraplantar space of the paw to illustrate the ability of SCFAs to synergize with LPS and induce mediator production but there was significant variability in this response.

The present findings of antiinflammatory activities of SCFAs in immune cells expand the database on these fatty acids as lipid mediators and may help explain the known beneficial effects of SCFAs in the colon and on colitis. Future studies are needed in knockout mice and with specific small molecules (both agonists and antagonists) to prove the exact molecular target for these effects of SCFAs.

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COMMENTS

Background

Short-chain fatty acids (SCFAs) are produced by bacterial fermentation of dietary fiber in the hindgut in millimolar concentrations. They have long been known to modulate the immune response and have a beneficial effect in the colon and on colitis.

Research frontiers

The molecular mechanism for the effect of SCFAs in immune cells of the gastrointestinal tract has not been characterized. Recently, a free fatty acid receptor GPR43 was found to be highly expressed in both neutrophils and monocytes and identified to be activated by SCFAs. In neutrophils, SCFAs induce calcium flux and chemotaxis. However, the effect of SCFAs in monocytes is of high interest, but is largely unknown.

Innovations and breakthroughs

This study showed that SCFAs can induce specific and robust prostaglandin E_2 (PGE_2) production either alone or in synergy with lipopolysaccharide in human monocytes and peripheral blood mononuclear cells (PBMC). Furthermore, SCFAs can specifically regulate production of monocyte chemotactic protein-1, interleukin-10, tumor necrosis factor-α and interferon-γ in human monocytes and/or PBMC. Finally, we showed that SCFAs can induce PGE_2 production *in vivo* by intraplantar injection into rat paws.

Applications

The present findings of antiinflammatory activities of SCFAs in immune cells expand the database on these fatty acids and may help explain the known beneficial effects of SCFAs in the colon and on colitis. Future studies are needed in knockout mice and with specific small molecules to prove the exact molecular target for these effects of SCFAs. The authors speculate that the action of SCFAs is most likely through activation of the free fatty acid receptor GPR43, a potential therapeutic target for the development of an innovative treatment of colitis.

Peer review

This is a study in an important area of inflammatory activities of SCFAs and its effects on the related cytokines and chemokines. In the present paper, the authors examined the inflammatory activities of SCFAs and the production of the related receptors and analyzed the mechanisms. The experiments were well organized.

REFERENCES

1 **Sawzdargo M**, George SR, Nguyen T, Xu S, Kolakowski LF, O'Dowd BF. A cluster of four novel human G protein-coupled receptor genes occurring in close proximity to CD22 gene on chromosome 19q13.1. *Biochem Biophys Res Commun* 1997; **239**: 543-547

2 **Im DS**. Discovery of new G protein-coupled receptors for lipid mediators. *J Lipid Res* 2004; **45**: 410-418

3 **Brown AJ**, Jupe S, Briscoe CP. A family of fatty acid binding receptors. *DNA Cell Biol* 2005; **24**: 54-61

4 **Briscoe CP**, Tadayyon M, Andrews JL, Benson WG, Chambers JK, Eilert MM, Ellis C, Elshourbagy NA, Goetz AS, Minnick DT, Murdock PR, Sauls HR Jr, Shabon U, Spinage LD, Strum JC, Szekeres PG, Tan KB, Way JM, Ignar DM, Wilson S, Muir AI. The orphan G protein-coupled receptor GPR40 is activated by medium and long chain fatty acids. *J Biol Chem* 2003; **278**: 11303-11311

5 **Itoh Y**, Kawamata Y, Harada M, Kobayashi M, Fujii R, Fukusumi S, Ogi K, Hosoya M, Tanaka Y, Uejima H, Tanaka H, Maruyama M, Satoh R, Okubo S, Kizawa H, Komatsu H, Matsumura F, Noguchi Y, Shinohara T, Hinuma S, Fujisawa Y, Fujino M. Free fatty acids regulate insulin secretion from pancreatic beta cells through GPR40. *Nature* 2003; **422**: 173-176

6 **Kotarsky K**, Nilsson NE, Olde B, Owman C. Progress in methodology. Improved reporter gene assays used to identify ligands acting on orphan seven-transmembrane receptors. *Pharmacol Toxicol* 2003; **93**: 249-258

7 **Steneberg P**, Rubins N, Bartoov-Shifman R, Walker MD, Edlund H. The FFA receptor GPR40 links hyperinsulinemia, hepatic steatosis, and impaired glucose homeostasis in mouse. *Cell Metab* 2005; **1**: 245-258

8 **Brown AJ**, Goldsworthy SM, Barnes AA, Eilert MM, Tcheang L, Daniels D, Muir AI, Wigglesworth MJ, Kinghorn I, Fraser NJ, Pike NB, Strum JC, Steplewski KM, Murdock PR, Holder JC, Marshall FH, Szekeres PG, Wilson S, Ignar DM, Foord SM, Wise A, Dowell SJ. The Orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. *J Biol Chem* 2003; **278**: 11312-11319

9 **Le Poul E**, Loison C, Struyf S, Springael JY, Lannoy V, Decobecq ME, Brezillon S, Dupriez V, Vassart G, Van Damme J, Parmentier M, Detheux M. Functional characterization of human receptors for short chain fatty acids and their role

in polymorphonuclear cell activation. *J Biol Chem* 2003; **278**: 25481-25489
10 **Nilsson NE**, Kotarsky K, Owman C, Olde B. Identification of a free fatty acid receptor, FFA2R, expressed on leukocytes and activated by short-chain fatty acids. *Biochem Biophys Res Commun* 2003; **303**: 1047-1052
11 **Senga T**, Iwamoto S, Yoshida T, Yokota T, Adachi K, Azuma E, Hamaguchi M, Iwamoto T. LSSIG is a novel murine leukocyte-specific GPCR that is induced by the activation of STAT3. *Blood* 2003; **101**: 1185-1187
12 **Naccache PH**, Faucher N, Caon AC, McColl SR. Propionic acid-induced calcium mobilization in human neutrophils. *J Cell Physiol* 1988; **136**: 118-124
13 **Nakao S**, Fujii A, Niederman R. Alteration of cytoplasmic Ca2+ in resting and stimulated human neutrophils by short-chain carboxylic acids at neutral pH. *Infect Immun* 1992; **60**: 5307-5311
14 **Nakao S**, Moriya Y, Furuyama S, Niederman R, Sugiya H. Propionic acid stimulates superoxide generation in human neutrophils. *Cell Biol Int* 1998; **22**: 331-337
15 **Böcker U**, Nebe T, Herweck F, Holt L, Panja A, Jobin C, Rossol S, B Sartor R, Singer MV. Butyrate modulates intestinal epithelial cell-mediated neutrophil migration. *Clin Exp Immunol* 2003; **131**: 53-60
16 **Zapolska-Downar D**, Siennicka A, Kaczmarczyk M, Kołodziej B, Naruszewicz M. Butyrate inhibits cytokine-induced VCAM-1 and ICAM-1 expression in cultured endothelial cells: the role of NF-kappaB and PPARalpha. *J Nutr Biochem* 2004; **15**: 220-228
17 **Menzel T**, Lührs H, Zirlik S, Schauber J, Kudlich T, Gerke T, Gostner A, Neumann M, Melcher R, Scheppach W. Butyrate inhibits leukocyte adhesion to endothelial cells via modulation of VCAM-1. *Inflamm Bowel Dis* 2004; **10**: 122-128
18 **Klampfer L**, Huang J, Sasazuki T, Shirasawa S, Augenlicht L. Inhibition of interferon gamma signaling by the short chain fatty acid butyrate. *Mol Cancer Res* 2003; **1**: 855-862
19 **Park JS**, Lee EJ, Lee JC, Kim WK, Kim HS. Anti-inflammatory effects of short chain fatty acids in IFN-gamma-stimulated RAW 264.7 murine macrophage cells: involvement of NF-kappaB and ERK signaling pathways. *Int Immunopharmacol* 2007; **7**: 70-77
20 **Brunkhorst BA**, Kraus E, Coppi M, Budnick M, Niederman R. Propionate induces polymorphonuclear leukocyte activation and inhibits formylmethionyl-leucyl-phenylalanine-stimulated activation. *Infect Immun* 1992; **60**: 2957-2968
21 **Topping DL**, Clifton PM. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiol Rev* 2001; **81**: 1031-1064
22 **Karaki S**, Tazoe H, Hayashi H, Kashiwabara H, Tooyama K, Suzuki Y, Kuwahara A. Expression of the short-chain fatty acid receptor, GPR43, in the human colon. *J Mol Histol* 2008; **39**: 135-142
23 **Dass NB**, John AK, Bassil AK, Crumbley CW, Shehee WR, Maurio FP, Moore GB, Taylor CM, Sanger GJ. The relationship between the effects of short-chain fatty acids on intestinal motility in vitro and GPR43 receptor activation. *Neurogastroenterol Motil* 2007; **19**: 66-74
24 **Karaki S**, Mitsui R, Hayashi H, Kato I, Sugiya H, Iwanaga T, Furness JB, Kuwahara A. Short-chain fatty acid receptor, GPR43, is expressed by enteroendocrine cells and mucosal mast cells in rat intestine. *Cell Tissue Res* 2006; **324**: 353-360
25 **Säemann MD**, Böhmig GA, Zlabinger GJ. Short-chain fatty acids: bacterial mediators of a balanced host-microbial relationship in the human gut. *Wien Klin Wochenschr* 2002; **114**: 289-300
26 **Cox MA**, Gomes B, Palmer K, Du K, Wiekowski M, Wilburn B, Petro M, Chou CC, Desquitado C, Schwarz M, Lunn C, Lundell D, Narula SK, Zavodny PJ, Jenh CH. The pyrimidinergic P2Y6 receptor mediates a novel release of proinflammatory cytokines and chemokines in monocytic cells stimulated with UDP. *Biochem Biophys Res Commun* 2005; **330**: 467-473
27 **Jiang GL**, Nieves A, Im WB, Old DW, Dinh DT, Wheeler L. The prevention of colitis by E Prostanoid receptor 4 agonist through enhancement of epithelium survival and regeneration. *J Pharmacol Exp Ther* 2007; **320**: 22-28
28 **Nitta M**, Hirata I, Toshina K, Murano M, Maemura K, Hamamoto N, Sasaki S, Yamauchi H, Katsu K. Expression of the EP4 prostaglandin E2 receptor subtype with rat dextran sodium sulphate colitis: colitis suppression by a selective agonist, ONO-AE1-329. *Scand J Immunol* 2002; **56**: 66-75
29 **Narumiya S**. Prostanoids in immunity: roles revealed by mice deficient in their receptors. *Life Sci* 2003; **74**: 391-395
30 **Matsuoka T**, Narumiya S. Prostaglandin receptor signaling in disease. *ScientificWorldJournal* 2007; **7**: 1329-1347
31 **Fujino H**, Regan JW. EP(4) prostanoid receptor coupling to a pertussis toxin-sensitive inhibitory G protein. *Mol Pharmacol* 2006; **69**: 5-10
32 **Kabashima K**, Saji T, Murata T, Nagamachi M, Matsuoka T, Segi E, Tsuboi K, Sugimoto Y, Kobayashi T, Miyachi Y, Ichikawa A, Narumiya S. The prostaglandin receptor EP4 suppresses colitis, mucosal damage and CD4 cell activation in the gut. *J Clin Invest* 2002; **109**: 883-893
33 **Martín AR**, Villegas I, Alarcón de la Lastra C. The COX-2 inhibitor, rofecoxib, ameliorates dextran sulphate sodium induced colitis in mice. *Inflamm Res* 2005; **54**: 145-151
34 **Sun FF**, Lai PS, Yue G, Yin K, Nagele RG, Tong DM, Krzesicki RF, Chin JE, Wong PY. Pattern of cytokine and adhesion molecule mRNA in hapten-induced relapsing colon inflammation in the rat. *Inflammation* 2001; **25**: 33-45
35 **Rodríguez-Cabezas ME**, Gálvez J, Lorente MD, Concha A, Camuesco D, Azzouz S, Osuna A, Redondo L, Zarzuelo A. Dietary fiber down-regulates colonic tumor necrosis factor alpha and nitric oxide production in trinitrobenzenesulfonic acid-induced colitic rats. *J Nutr* 2002; **132**: 3263-3271
36 **Rodríguez-Cabezas ME**, Gálvez J, Camuesco D, Lorente MD, Concha A, Martinez-Augustin O, Redondo L, Zarzuelo A. Intestinal anti-inflammatory activity of dietary fiber (Plantago ovata seeds) in HLA-B27 transgenic rats. *Clin Nutr* 2003; **22**: 463-471
37 **Peran L**, Sierra S, Comalada M, Lara-Villoslada F, Bailón E, Nieto A, Concha A, Olivares M, Zarzuelo A, Xaus J, Gálvez J. A comparative study of the preventative effects exerted by two probiotics, Lactobacillus reuteri and Lactobacillus fermentum, in the trinitrobenzenesulfonic acid model of rat colitis. *Br J Nutr* 2007; **97**: 96-103
38 **Lee T**, Schwandner R, Swaminath G, Weiszmann J, Cardozo M, Greenberg J, Jaeckel P, Ge H, Wang Y, Jiao X, Liu J, Kayser F, Tian H, Li Y. Identification and functional characterization of allosteric agonists for the G protein-coupled receptor FFA2. *Mol Pharmacol* 2008; **74**: 1599-1609

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BRIEF ARTICLE

Analysis of *monocyte chemotactic protein-1* gene polymorphism in patients with spontaneous bacterial peritonitis

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Abstract

AIM: To investigate a genetic polymorphism of the *monocyte chemotactic protein-1* (*MCP-1*) gene in patients with spontaneous bacterial peritonitis (SBP).

METHODS: MCP-1 genotyping was performed in 23 patients with SBP and 83 cirrhotic control patients with non-infected ascites.

RESULTS: The frequency of carriers of the G-allele was lower in SBP patients but this difference did not reach statistical significance. However, in the subgroup of patients with alcoholic cirrhosis (n = 80), carriers of the G-allele were significantly less frequent in SBP-patients (38.1%) than in cirrhotic controls (67.8%, P = 0.021).

CONCLUSION: In patients with alcoholic liver cirrhosis, the -2518 MCP-1 genotype AA is a risk factor for the development of SBP.

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Key words: Monocyte chemotactic protein-1; Chemokines; Spontaneous bacterial peritonitis; Polymorphism; Liver cirrhosis

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INTRODUCTION

Spontaneous bacterial peritonitis (SBP) is a common and potentially life-threatening complication in patients with cirrhosis. It is a prototypical infective disease in cirrhotic patients characterized by peritoneal neutrophil infiltration, which also serves as a diagnostic criterion for SBP (e.g. an ascites neutrophil count greater than 250/mm^3)[1].

Factors influencing the development of SBP in patients with liver cirrhosis are poorly understood. Previous studies have indicated that peritoneal macrophages of cirrhotic patients might contribute to the control of SBP or influence its associated pathology in human cirrhosis by producing high quantities of angiogenic peptides and nitric oxide[2-5]. Accordingly, elevated concentrations of proinflammatory cytokines are found in ascites of patients[6,7].

Therefore, it is of particular interest to identify mechanisms underlying the recruitment and activation of macrophages/monocytes that infiltrate the ascites of cirrhotic patients. Chemotactic cytokines are known to be critical mediators of inflammatory cell trafficking into sites of injury and are crucial for the modulation of tissue injury, inflammation, and repair.

Monocyte chemotactic protein-1 (MCP-1) is one of the most potent chemokines for monocytes/macrophages and activated lymphocytes during infections[8]. In addition, several studies have shown that neutrophil infiltration is affected either directly or indirectly *via* MCP-1[9,10].

Several lines of evidence indicate that MCP-1 might play a role in the recruitment and maintenance of the inflammatory infiltrate during liver injury. MCP-1 secretion is up-regulated during chronic hepatitis and correlates with the severity of hepatic inflammation[11-13].

Furthermore, a previous study showed elevated MCP-1 levels in ascites of cirrhotic patients as compared to controls[14]. Moreover, during SBP, even higher MCP-1 levels were measured in ascites suggesting that this potent chemokine plays a pathophysiological role during the development and course of SBP.

Recently, a mutation in the distal regulatory region of the *MCP-1* gene at position -2518 relatively to the transcription start site (based on the published sequence, Gene Bank accession number D26087) was identified (A-2518G)[15]. *In vitro* stimulated monocytes from individuals carrying a G-allele at -2518 produced more MCP-1 than cells from A/A homozygous subjects[15]. Several further studies have confirmed the functional effect of this single nucleotide polymorphism (SNP)[13,16-18].

The prevalence of high MCP-1 producing genotypes has been shown to be associated with increased susceptibility and severity of several diseases, including bronchial asthma[19], premature kidney graft failure[20], Crohn's disease[21], cutaneous vasculitis[22], and tuberculosis[16]. Furthermore, this genetic polymorphism has been shown to be a risk factor for the progression of chronic viral hepatitis C infection[13].

The aim of this study was to analyze the association of this functional promoter SNP of the *MCP-1* gene with SBP.

MATERIALS AND METHODS

Patients and controls

We studied 106 patients with liver cirrhosis and ascites. Clinical charts of patients diagnosed with chronic liver disease at the medical department of the University of Regensburg during the period 2001-2007 were studied retrospectively. Based on the availability of DNA samples in an established tissue bank, patients were included in the study. Liver carcinoma, other neoplasms or HIV-infection were exclusion criteria. Clinical charts revealed diagnosis of SBP in 23 cases, while no SBP had ever occurred in 83 patients. In the majority of patients, chronic alcohol abuse was the cause of the underlying liver disease (Table 1). In the other patients, chronic hepatitis B or C infection and hemochromatosis were the cause of cirrhosis. Patients were considered to have alcohol related cirrhosis if alcohol intake had been in excess of 100 g/d for more than 10 years and if tests for viral, metabolic, and immune etiologies were negative. Patient characteristics are summarized in Table 1.

As an additional control group, 118 heavy drinkers without evidence of liver damage (90 male, 28 female; mean age: 42.5 ± 9.1 years) were analyzed. These unrelated individuals of German descent were recruited from an in-patient abstention program at the Department of Psychiatry of the University of Regensburg. Each subject met the criteria of alcohol dependence according to ICD-10 alcohol dependence according to DSM-IV (American Psychiatric Association, 1994) and ICD-10 (World Health Organization, 1992).

Table 1 Characteristics of cirrhotic patients with SBP and cirrhotic control patients with ascites (mean ± SD)

	Control cirrhotic patients (n = 83)	Cirrhotic patients with SBP (n = 23)
Age (yr)	55.8 ± 10.5	58.7 ± 8.6
Male gender (%)	75.9	78.3
Albumin (g/dL)	3.0 ± 0.6	3.0 ± 0.6
Prothrombin activity (%)	63.8 ± 22.1	54.9 ± 20.8
Bilirubin (mg/dL)	4.9 ± 6.3	4.9 ± 5.9
Chronic alcohol abuse as etiology of cirrhosis (%)	71.1	91.3

SBP: Spontaneous bacterial peritonitis.

At the day of admission, several serum parameters were analyzed, including transaminases (alanine aminotransferase, aspartate aminotransferase, γ glutamyltransferase), and anti hepatitis B virus (HCV)- and hepatitis B virus (HBV)-antibodies. Previous and current drinking habits, family history of non-alcoholic liver diseases, and potential previous complications of advanced liver damages, such as ascites or esophageal varices, were examined. Furthermore, within 14 d after admission a clinical and ultrasound examination of the abdomen was performed. Only alcohol-dependent subjects with an alcohol consumption of at least 100 g/d for more than 5 years were included. Furthermore, clinical or sonomorphological indications for advanced liver damage and serological evidence of HBV or HCV infection were exclusion criteria.

Patients and controls were Caucasians and their geographical origin was Southern Germany. Informed consent was obtained from all patients and the study was approved by the local ethics committee.

DNA isolation and MCP-1 genotyping

DNA isolation and MCP-1 genotyping were performed as described previously[13,23]. Briefly, genomic DNA specimens were prepared from 200 μL blood using the QIAamp blood kit, following the manufacturer's instructions (Qiagen, Hilden, Germany). The G to A polymorphisms at position -2518 of the *MCP-1* gene was analyzed by performing PCR and restriction fragment length polymorphism analysis. PCR was performed under standard conditions (35 cycles, annealing temperature: 55℃) in a total reaction volume of 50 μL containing 2 μL of diluted genomic DNA, using the following pair of primers: forward*:* 5'-CCGAGATGTTCCCAGCACAG-3' and reverse*:* 5'-CTGCTTTGCTTGTGCCTCTT-3'. PCR products were digested by *Pvu*Ⅱ, and the resulting fragments were separated by electrophoresis in a 2% agarose gel and visualized by ethidium bromide staining. With the -2518 A polymorphic base, the recognition sequence 5'-CAG/CTG-3' is modified to 5'-CAG/CTA-3', which is not cut by *Pvu*Ⅱ.

Table 2 Frequencies of -2518 MCP-1 genotypes *n* (%)

-2518 MCP-1 genotype	Cirrhotic patients with ascites (*n* = 106)	Patients with alcoholic cirrhosis and ascites (*n* = 80)	Heavy drinkers w/o cirrhosis (*n* = 118)
A/A	45 (42.5)	32 (40.0)	64 (54.2)
A/G	51 (48.1)	39 (48.8)	47 (39.8)
G/G	10 (9.4)	9 (11.3)	7 (5.9)

Table 3 Frequencies of carriers of the G-allele of the -2518 MCP-1 promoter polymorphism in patients with SBP and cirrhotic control patients with ascites *n* (%)

-2518 MCP-1 genotype	Whole cohort (*n* = 106)		Alcoholic cirrhosis (*n* = 80)	
	No SBP (*n* = 83)	SBP (*n* = 23)	No SBP (*n* = 59)	SBP (*n* = 21)
A/G or G/G	52 (62.7)	9 (39.1)	40 (67.8)	8 (38.1)
A/A	31 (37.3)	14 (60.9)	19 (32.2)	13 (61.9)

Statistical analysis

Results are expressed as mean ± SD (range) or, when indicated, as absolute number and percent. Genotype frequencies are reported with their group percentages. Contingency table analysis and two-sided Fisher's exact tests were used for comparison of qualitative variables. $P < 0.05$ was considered statistically significant.

RESULTS

Characteristics of the study population

The clinical and epidemiological characteristics of SBP patients and cirrhotic control patients are summarized in Table 1. There was no statistical difference between patients with SBP and non-infected cirrhotic patients with ascites with respect to age, distribution of sexes, and serum laboratory findings, such as albumin, bilirubin, or prothrombin activity. However, the percentage of patients with chronic excessive alcohol abuse as the cause of liver cirrhosis was significantly higher in the SBP group (91.3% *vs* 71.1% in the cirrhosis group, $P = 0.046$), which agreed with previous studies that showed greater susceptibility to infections, especially SBP, in alcohol-induced liver cirrhosis[24].

Frequency of the -2518 MCP-1 polymorphism in patients and controls

The frequencies of the different MCP-1 genotypes in our cohort of patients with liver cirrhosis and ascites are summarized in Table 2. In addition, genotype distribution in the subgroup of patients with chronic excessive alcohol consumption as an underlying cause of liver disease and cirrhosis is depicted. Furthermore, MCP-1 genotypes were analyzed in heavy drinkers without evidence of liver damage.

The frequency of individual genotypes in the group of heavy drinkers without cirrhosis was similar to those previously reported in other Caucasian control populations[19,20,22,25]. Genotype distribution did not differ significantly between the whole group of cirrhotic patients with ascites and the subgroup of patients with alcohol related cirrhosis. However, irrespective of the underlying cause of liver disease, the genotype AA was less frequent in cirrhotic patients with and without ascites (40.0% and 42.5%, respectively) as compared to heavy drinkers without evidence of liver damage (54.2%) although these differences did not reach statistical significance ($P = 0.084$ and $P = 0.060$, respectively).

Most previous *in vitro* and epidemiological studies reported functional and disease-related differences between -2518 MCP-1 genotypes AA and non-AA (i.e. carriers of the G-allele: genotypes AG and GG); therefore, we continued this differentiation throughout the following analysis and focused on the comparison of patients with no G-allele (AA-homozygotes) and carriers of the G-allele (genotypes AG and GG).

Frequency of the -2518 MCP-1 polymorphism in patients with and without SBP

In our cohort of 106 patients with ascites, SBP has been diagnosed in 23 individuals (21.7%), while in 83 cirrhotic patients (78.3%), despite mostly long lasting episodes of ascites, no SBP had occurred. The frequency of carriers of the G-allele was lower in the SBP group but this difference did not reach statistical significance [9/23 (39.1%) *vs* 52/83 (62.7%), $P = 0.057$, Table 3]. Also, in the subgroup of patients with alcoholic cirrhosis ($n = 80$), carriers of the G-allele were less frequent in SBP-patients compared to patients without SBP, and in this case, the difference reached statistical significance [8/21 (38.1%) *vs* 40/59 (67.8%), $P = 0.021$, Table 3].

DISCUSSION

The aim of this study was to analyze the association of the functional MCP-1 promoter polymorphism (A-2518G) with SBP. Interestingly, in patients with alcoholic liver cirrhosis, the genotype AA was significantly more frequent in SBP-patients than in cirrhotic control patients with ascites only. Previously, this MCP-1 genotype has been shown to be associated with reduced MCP-1 release of monocytes *in vitro* and MCP-1 tissue levels *in vivo*, respectively[13,16].

Furthermore, in a previous study[14], MCP-1 levels in ascites were significantly higher when compared with their levels in serum, suggesting a chemotactic gradient towards the peritoneal cavity, even in the absence of infection. This chemotactic gradient could be operative in the chemotaxis of monocytes/macrophages. Antigenic stimuli drive these cells to synthesize further proinflammatory cytokines and chemokines, and thus might also modify the systemic response to the infection. Furthermore, the chemotactic MCP-1 gradient might directly or indirectly favor leukocyte migration towards the peritoneal cavity, even in the absence of infection. We could speculate that in patients with SBP, higher MCP-1 ascites fluid levels are implicated in the infiltration of the peritoneal cavity. Furthermore, in several experimental models of acute peritonitis, high concentrations of MCP-1 have

been shown to have beneficial effects by recruiting macrophages and neutrophils to the site of infection[26-28]. Taken together these data indicate that, in patients with alcoholic liver cirrhosis, the -2518 MCP-1 genotype AA is a risk factor for the development of SBP, possibly *via* reduced MCP-1 ascites levels as compared to patients carrying the G-allele.

Interestingly, only in the subgroup of patients with chronic excessive alcohol abuse as the underlying cause of liver cirrhosis was the association between MCP-1 genotype and SBP found. In a previous study, patients with alcohol induced cirrhosis and patients with other hepatopathies showed similar MCP-1 serum and ascitic fluid levels[14].

Notably, carriers of the G-allele of the MCP-1 polymorphism were more frequent in patients with alcohol induced cirrhosis than in heavy drinkers without evidence of liver damage. This difference did not reach statistical significance, but taken together with our previous finding that carrying the -2518 MCP-1 G-allele is a risk factor for disease progression in patients with chronic hepatitis C infection[13], these data indicate that this functional SNP also affects fibrosis progression in patients with alcoholic liver disease. Thus, the same SNP appears to affect two related pathophysiological mechanisms (namely development of cirrhosis and SBP) but to different extents, depending on the underlying liver disease. Inflammatory and immune responses are altered in cirrhotic patients in general[29,30]; however, it has to be considered that there are differences between the underlying liver diseases leading to liver cirrhosis. This might be one explanation why it is more difficult to identify an association with the functional MCP-1 polymorphism and SBP in patients with liver disease other than alcoholic liver disease. Furthermore, in patients with alcoholic cirrhosis, the mean endotoxin concentration was significantly higher than in patients with non-alcoholic cirrhosis[31], and bacterial translocation and endotoxin concentration have been shown to play an important role in the pathophysiology of SBP development[32]. In addition, it has been shown that LPS downregulates the specific MCP-1 receptor, CCR2, and abolishes macrophage infiltration in an animal model of acute infection[33]. Therefore, we speculate that higher LPS levels in patients with alcoholic liver disease are the reason why an association between the -2518 MCP-1 polymorphism and SBP was found only in the subgroup of patients with this underlying liver disease.

Finally, as in most genetic association studies, it has to be considered that the SNP investigated is in linkage disequilibrium to a different disease-associated genetic variation. Theoretically, this potential genetic variation might particularly affect the pathophysiology of alcoholic but not viral or other liver diseases. However, the high levels of MCP-1 in ascites in patients suffering from SBP and the functional relevance of the -2518 MCP-1 polymorphism demonstrated in several studies, argue against this hypothesis.

In summary, this is one of the first genetic association studies in patients with SBP. Our data indicate that the -2518 MCP-1 genotype AA is a risk factor for the development of SBP in patients with alcoholic cirrhosis. Of course, our results have to be confirmed in a larger cohort of patients with independent and prospective studies. However, it is intriguing to speculate that genotyping might be helpful to identify cirrhotic patients with a higher risk of developing this life threatening complication and to apply prophylactic treatment specifically to this subset of patients.

COMMENTS

Background

Spontaneous bacterial peritonitis (SBP) is a potentially life threatening complication in patients with liver cirrhosis and ascites, caused by peritoneal neutrophil infiltration. However, factors leading to enhanced neutrophil infiltration of the peritoneal cavity with consecutive SBP are poorly characterized.

Research frontiers

Monocyte-chemotactic protein-1 (MCP-1) is an important chemokine for several proinflammatory cells, and there exists a functional single nucleotide polymorphism (SNP) of the MCP-1 promoter (A-2518G) leading to higher MCP-1 expression in carriers of the G-allele. In the present study, the frequency of this MCP-1 SNP was analyzed in patients with SBP and liver cirrhosis.

Innovations and breakthroughs

Accumulating evidence suggests that genotypes leading to increased MCP-1 production contribute to susceptibility and severity of diseases such as asthma, kidney graft failure, Crohn's disease, cutaneous vasculitis, tuberculosis, and chronic hepatitis C infection. This is the first genetic association study in patients with SBP to show that the -2518 MCP-1 genotype AA is a risk factor to development of SBP in patients with alcoholic cirrhosis.

Applications

Genotyping of the -2518 MCP-1 SNP in cirrhotic patients might be beneficial to identify patients with increased risk for the development of SBP. Thus, early antibiotic treatment of such patients could help to decrease the mortality of this severe complication in liver cirrhosis.

Terminology

SBP is an infection of the ascites in patients with liver cirrhosis, diagnosed by a neutrophil count greater the 250/mm^3 in the ascitic fluid. MCP-1 is one of the most potent chemokines for the recruitment of inflammatory cell-types during infection. For example, MCP-1 is upregulated in chronic hepatitis.

Peer review

This retrospective study identified an association of a genetic polymorphism of the chemokine MCP-1 with increased risk to develop SBP in patients with alcohol-related liver cirrhosis. This is one of the first genetic association studies in patients with SBP and the results might be of high clinical relevance.

REFERENCES

1 **Sheer TA**, Runyon BA. Spontaneous bacterial peritonitis. *Dig Dis* 2005; **23**: 39-46

2 **Cejudo-Martín P**, Ros J, Navasa M, Fernández J, Fernández-Varo G, Ruiz-del-Arbol L, Rivera F, Arroyo V, Rodés J, Jiménez W. Increased production of vascular endothelial growth factor in peritoneal macrophages of cirrhotic patients with spontaneous bacterial peritonitis. *Hepatology* 2001; **34**: 487-493

3 **Jiménez W**, Ros J, Morales-Ruiz M, Navasa M, Solé M, Colmenero J, Sort P, Rivera F, Arroyo V, Rodés J. Nitric oxide production and inducible nitric oxide synthase expression in peritoneal macrophages of cirrhotic patients. *Hepatology* 1999; **30**: 670-676

4 **Pérez-Ruiz M**, Ros J, Morales-Ruiz M, Navasa M, Colmenero J, Ruiz-del-Arbol L, Cejudo P, Clária J, Rivera F, Arroyo V, Rodés J, Jiménez W. Vascular endothelial growth factor production in peritoneal macrophages of cirrhotic patients: regulation by cytokines and bacterial lipopolysaccharide. *Hepatology* 1999; **29**: 1057-1063

5 **Bories PN**, Campillo B, Scherman E. Up-regulation of

nitric oxide production by interferon-gamma in cultured peritoneal macrophages from patients with cirrhosis. *Clin Sci* (Lond) 1999; **97**: 399-406
6 **Andus T**, Gross V, Holstege A, Schölmerich J. High interleukin-6 concentrations in hepatic ascites. *Dig Dis Sci* 1994; **39**: 219-220
7 **Andus T**, Gross V, Holstege A, Ott M, Weber M, David M, Gallati H, Gerok W, Schölmerich J. High concentrations of soluble tumor necrosis factor receptors in ascites. *Hepatology* 1992; **16**: 749-755
8 **Luster AD**. Chemokines--chemotactic cytokines that mediate inflammation. *N Engl J Med* 1998; **338**: 436-445
9 **Li P**, Garcia GE, Xia Y, Wu W, Gersch C, Park PW, Truong L, Wilson CB, Johnson R, Feng L. Blocking of monocyte chemoattractant protein-1 during tubulointerstitial nephritis resulted in delayed neutrophil clearance. *Am J Pathol* 2005; **167**: 637-649
10 **Maus U**, Huwe J, Maus R, Seeger W, Lohmeyer J. Alveolar JE/MCP-1 and endotoxin synergize to provoke lung cytokine upregulation, sequential neutrophil and monocyte influx, and vascular leakage in mice. *Am J Respir Crit Care Med* 2001; **164**: 406-411
11 **Marra F**, DeFranco R, Grappone C, Milani S, Pastacaldi S, Pinzani M, Romanelli RG, Laffi G, Gentilini P. Increased expression of monocyte chemotactic protein-1 during active hepatic fibrogenesis: correlation with monocyte infiltration. *Am J Pathol* 1998; **152**: 423-430
12 **Fisher NC**, Neil DA, Williams A, Adams DH. Serum concentrations and peripheral secretion of the beta chemokines monocyte chemoattractant protein 1 and macrophage inflammatory protein 1alpha in alcoholic liver disease. *Gut* 1999; **45**: 416-420
13 **Mühlbauer M**, Bosserhoff AK, Hartmann A, Thasler WE, Weiss TS, Herfarth H, Lock G, Schölmerich J, Hellerbrand C. A novel MCP-1 gene polymorphism is associated with hepatic MCP-1 expression and severity of HCV-related liver disease. *Gastroenterology* 2003; **125**: 1085-1093
14 **Girón-González JA**, Rodríguez-Ramos C, Elvira J, Galán F, Del Alamo CF, Díaz F, Martín-Herrera L. Serial analysis of serum and ascitic fluid levels of soluble adhesion molecules and chemokines in patients with spontaneous bacterial peritonitis. *Clin Exp Immunol* 2001; **123**: 56-61
15 **Rovin BH**, Lu L, Saxena R. A novel polymorphism in the MCP-1 gene regulatory region that influences MCP-1 expression. *Biochem Biophys Res Commun* 1999; **259**: 344-348
16 **Flores-Villanueva PO**, Ruiz-Morales JA, Song CH, Flores LM, Jo EK, Montaño M, Barnes PF, Selman M, Granados J. A functional promoter polymorphism in monocyte chemoattractant protein-1 is associated with increased susceptibility to pulmonary tuberculosis. *J Exp Med* 2005; **202**: 1649-1658
17 **Papachristou GI**, Sass DA, Avula H, Lamb J, Lokshin A, Barmada MM, Slivka A, Whitcomb DC. Is the monocyte chemotactic protein-1 -2518 G allele a risk factor for severe acute pancreatitis? *Clin Gastroenterol Hepatol* 2005; **3**: 475-481
18 **McDermott DH**, Yang Q, Kathiresan S, Cupples LA, Massaro JM, Keaney JF Jr, Larson MG, Vasan RS, Hirschhorn JN, O'Donnell CJ, Murphy PM, Benjamin EJ. CCL2 polymorphisms are associated with serum monocyte chemoattractant protein-1 levels and myocardial infarction in the Framingham Heart Study. *Circulation* 2005; **112**: 1113-1120
19 **Szalai C**, Kozma GT, Nagy A, Bojszkó A, Krikovszky D, Szabó T, Falus A. Polymorphism in the gene regulatory region of MCP-1 is associated with asthma susceptibility and severity. *J Allergy Clin Immunol* 2001; **108**: 375-381
20 **Krüger B**, Schröppel B, Ashkan R, Marder B, Zülke C, Murphy B, Krämer BK, Fischereder M. A Monocyte chemoattractant protein-1 (MCP-1) polymorphism and outcome after renal transplantation. *J Am Soc Nephrol* 2002; **13**: 2585-2589
21 **Herfarth H**, Göke M, Hellerbrand C, Mühlbauer M, Vogl D, Schölmerich J, Rogler G. Polymorphism of monocyte chemoattractant protein 1 in Crohn's disease. *Int J Colorectal Dis* 2003; **18**: 401-405
22 **Aguilar F**, González-Escribano MF, Sánchez-Román J, Núñez-Roldán A. MCP-1 promoter polymorphism in Spanish patients with systemic lupus erythematosus. *Tissue Antigens* 2001; **58**: 335-338
23 **Karrer S**, Bosserhoff AK, Weiderer P, Distler O, Landthaler M, Szeimies RM, Müller-Ladner U, Schölmerich J, Hellerbrand C. The -2518 promotor polymorphism in the MCP-1 gene is associated with systemic sclerosis. *J Invest Dermatol* 2005; **124**: 92-98
24 **Rosa H**, Silvério AO, Perini RF, Arruda CB. Bacterial infection in cirrhotic patients and its relationship with alcohol. *Am J Gastroenterol* 2000; **95**: 1290-1293
25 **Szalai C**, Duba J, Prohászka Z, Kalina A, Szabó T, Nagy B, Horváth L, Császár A. Involvement of polymorphisms in the chemokine system in the susceptibility for coronary artery disease (CAD). Coincidence of elevated Lp(a) and MCP-1 -2518 G/G genotype in CAD patients. *Atherosclerosis* 2001; **158**: 233-239
26 **Matsukawa A**, Hogaboam CM, Lukacs NW, Lincoln PM, Strieter RM, Kunkel SL. Endogenous monocyte chemoattractant protein-1 (MCP-1) protects mice in a model of acute septic peritonitis: cross-talk between MCP-1 and leukotriene B4. *J Immunol* 1999; **163**: 6148-6154
27 **Ajuebor MN**, Das AM, Virág L, Szabó C, Perretti M. Regulation of macrophage inflammatory protein-1 alpha expression and function by endogenous interleukin-10 in a model of acute inflammation. *Biochem Biophys Res Commun* 1999; **255**: 279-282
28 **Ajuebor MN**, Gibbs L, Flower RJ, Das AM, Perretti M. Investigation of the functional role played by the chemokine monocyte chemoattractant protein-1 in interleukin-1-induced murine peritonitis. *Br J Pharmacol* 1998; **125**: 319-326
29 **Navasa M**, Rimola A, Rodés J. Bacterial infections in liver disease. *Semin Liver Dis* 1997; **17**: 323-333
30 **Garcia-Tsao G**. Bacterial infections in cirrhosis. *Can J Gastroenterol* 2004; **18**: 405-406
31 **Fukui H**, Brauner B, Bode JC, Bode C. Plasma endotoxin concentrations in patients with alcoholic and non-alcoholic liver disease: reevaluation with an improved chromogenic assay. *J Hepatol* 1991; **12**: 162-169
32 **Wiest R**, Garcia-Tsao G. Bacterial translocation (BT) in cirrhosis. *Hepatology* 2005; **41**: 422-433
33 **Zhou Y**, Yang Y, Warr G, Bravo R. LPS down-regulates the expression of chemokine receptor CCR2 in mice and abolishes macrophage infiltration in acute inflammation. *J Leukoc Biol* 1999; **65**: 265-269

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BRIEF ARTICLE

Endoscopic ultrasound and magnetic resonance imaging for re-staging rectal cancer after radiotherapy

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Abstract

AIM: To compare the sensitivity and specificity of two imaging techniques, endoscopic ultrasound (EUS) and magnetic resonance imaging (MRI), in patients with rectal cancer after neoadjuvant chemoradiation therapy. And we compared EUS and MRI data with histological findings from surgical specimens.

METHODS: Thirty-nine consecutive patients (51.3% Male; mean age: 68.2 ± 8.9 years) with histologically confirmed distal rectal cancer were examined for staging. All patients underwent EUS and MRI imaging before and after neoadjuvant chemoradiation therapy.

RESULTS: After neoadjuvant chemoradiation, EUS and MRI correctly classified 46% (18/39) and 44% (17/39) of patients, respectively, in line with their histological T stage ($P > 0.05$). These proportions were higher for both techniques when nodal involvement was considered: 69% (27/39) and 62% (24/39). When patients were sorted into T and N subgroups, the diagnostic accuracy of EUS was better than MRI for patients with T0-T2 (44% *vs* 33%, $P > 0.05$) and N0 disease (87% *vs* 52%, $P = 0.013$). However, MRI was more accurate than EUS in T and N staging for patients with more advanced disease after radiotherapy, though these differences did not reach statistical significance.

CONCLUSION: EUS and MRI are accurate imaging techniques for staging rectal cancer. However, after neoadjuvant RT-CT, the role of both methods in the assessment of residual rectal tumors remains uncertain.

Key words: Endoscopic ultrasound; Magnetic resonance imaging; Rectal cancer; Neoadjuvant chemoradiation therapy; Diagnostic accuracy

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INTRODUCTION

Endoscopic ultrasound (EUS) is the most accurate imaging technique for evaluating local invasion of rectal cancer and perirectal lymph nodes. Its overall accuracy for T staging before radiation therapy (RT) ranges from 73% to 94%, and from 70% to 80% for N staging[1]. The findings of rectal EUS serve to decide the type of treatment: surgery or preoperative chemoradiation (RT-CT)[2]. The response to neoadjuvant therapy must also be assessed accurately in patients with rectal cancer.

The benefits of this treatment have been reported in different studies, reporting lower local recurrence rates in patients with locally advanced rectal tumors. Rectal EUS helps select a group with advanced locoregional disease (stage T3 or T4) in whom this preoperative treatment offers most benefit[3-5].

The accuracy of EUS in staging rectal cancer ranges from 80% to 95% compared to 65%-75% for computed

tomography (CT) and 75%-85% for magnetic resonance imaging (MRI)[6-9]. There is still debate about the role of cross-sectional imaging to restage rectal cancer after RT-CT, and data are scanty on accuracy, sensitivity, and specificity rates, suggesting that these modalities are not efficient enough for restaging.

The aim of this prospective study was to evaluate the diagnostic accuracy of two imaging techniques, EUS and MRI, in patients with rectal cancer, before and after neoadjuvant RT-CT. We compared the EUS and MRI data with histological findings on surgical specimens.

MATERIALS AND METHODS

Over a 2-year period (January 2007 to January 2009), 50 patients with histologically confirmed distal rectal cancer were referred to our Unit for rectal endosonography to stage the disease. Out of these, thirty-nine consecutive cases (51.3% Male; mean age: 68.2 ± 8.9 years) were enrolled (drop-out from the study: 11 patients).

Both examinations were done, in random order, by two experienced endosonographers (> 300 rectal examinations/year) and an expert radiologist, before and after RT. The second examiner was blind to the first one's conclusions. Patients were included in the study if they had rectal cancer shown by either EUS or MRI, and were scheduled for neoadjuvant RT.

Usually, 30-40 d after termination of the RT protocol, patients were re-assessed by either EUS or MRI, followed by surgical excision during the same week. All patients gave informed consent.

Subjects with a history of rectal surgery, recurrent rectal cancer, or severe systemic illness were excluded from the study. Ten patients had neoplastic sub-stenosis of the lumen; therefore, it was not possible to evaluate their iliac lymph nodes by EUS.

These two subgroups were formed to see whether the diagnostic accuracy of EUS or MRI was related to the tumor T stage after RT.

EUS protocol: Rectal EUS was performed using an oblique-forward viewing echo-endoscope (Pentax: FG-G36UX, FG-38UX, EG-3630U). All examinations were done with the patient lying on the left side under conscious sedation (midazolam i.v.). Patients were prepared with colonic lavage before EUS. The echo-endoscope was inserted and advanced beyond the lesion, under direct vision, to the rectosigmoid junction, high enough to detect iliac vessels on the EUS picture. Tumors were targeted to determine the depth of infiltration into or through the rectal wall. Frequencies commonly employed ranged from 7.5 to 10 MHz (Figure 1).

MRI protocol: All MRI examinations were performed on a 1.5T MRI scanner (Gyroscan Intera, Philips, The Nederlands). Iron contrast medium rectal distension and IM hypotonizing medication were performed. MRI examinations were performed before and after iv contrast media infusion (0.2 mL/kg). Multiplanar TSE T2 weighted sequences (TR: 4450, TE: 120, matrix: 512 × 344, NSA: 6, FOV: 240) were performed in order to local stage rectal cancer; axial TSE T2 weighted sequences (TR: 3950, TE: 80, matrix: 528 × 372, NSA: 3, FOV: 370) were performed to analyze the presence of pathological lymph nodes (N). Axial FFE T1 weighted sequences (TR: 196, TE: 4.6, matrix: 320 × 216, NSA: 4, FOV: 250) with and without Fat Saturation, before and after contrast medium and sagittal FFE T1 weighted sequences (TR: 196, TE: 4.6, matrix: 320 × 216, NSA: 4, FOV: 250) with Fat Saturation, after contrast medium, were also performed. The MRI protocol used in our hospital is very similar to that commonly reported in the literature. Our Institute can be considered a medium-high flow hospital for rectal cancer staging; in fact, we performed at least 50 rectal contrast enhanced MRI in 1 year (Figure 2).

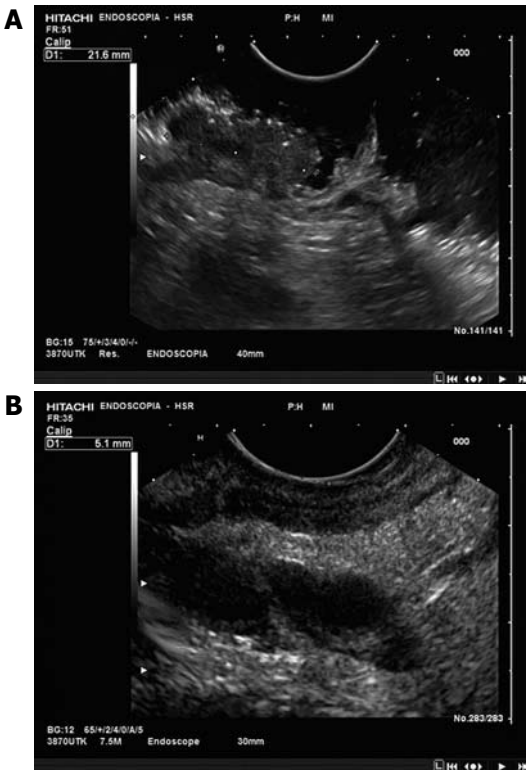

Figure 1 Rectal EUS. A: Before neoadjuvant chemoradiation treatment: evidence of perirectal fat tissue invasion, with fat speculation; B: After neoadjuvant chemoradiation treatment: reduction of tumor size and perirectal fat invasion.

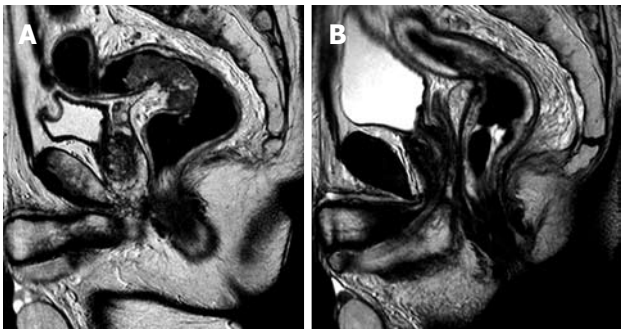

Figure 2 Rectal RMI. A: Before neoadjuvant chemoradiation treatment: evidence of perirectal fat tissue invasion, with fat spiculation, and enlarged mesorectal lymph nodes; B: After neoadjuvant chemoradiation treatment: reduction of tumor size, perirectal fat invasion, enlarged lymph nodes; non-homogeneous mesorectal fat tissue.

Table 1 Post-treatment stage: imaging diagnosis *vs* surgical specimens

Stage	EUS	MRI
T0N0	6	4
T0N1	0	0
T0N2	0	0
T1N0	1	0
T1N1	0	0
T1N2	0	0
T2N0	0	0
T2N1	0	1
T2N2	0	0
T3N0	2	2
T3N1	4	6
T3N2	0	0
T4N0	0	0
T4N1	0	0
T4N2	0	0

Neoadjuvant chemoradiation treatment: Chemoradiotherapy consisted of administration of Oxaliplatin 100 mg/m^2 every 2 wk for three courses plus continuous infusion of 5-FU 200 mg/m^2/die for six consecutive weeks. Concomitant hyperfractionated radiotherapy at a total dose of 45 Gy (1.25 Gy twice daily for 5 d every week with a four-field box technique, with 6-18 MeV photons) was started the same day as the second course of Oxaliplatin. EUS and pelvic MRI were repeated just before surgery to establish the clinical response.

Surgical treatment: Laparoscopic or laparotomic nerve sparing surgical resection (either low anterior or abdomino-perineal resection) with total mesorectal excision (TME) was scheduled 6-8 wk after completion of neoadjuvant treatment. Criteria for sphincter preservation were acceptable sphincter function, assessed with preoperative rectal manometry; and absence of direct invasion of the sphincter apparatus. These criteria remained unchanged throughout the treatment period. Creation of temporary loop ileostomy or colostomy was performed in selected cases as judged necessary by the surgeon.

Rectal cancer staging

Rectal tumors were staged using the tumor-node-metastasis system (TNM)[10].

Statistical analysis

Pre-operative EUS and MRI findings were compared to assess the concordance between the two methods. κ-statistics were used to check how well EUS and MRI classified subjects in the T and N stage groups. The degree of agreement was quantified by weighted κ, which assumes the categories (Tl, T2, *etc.*) are ordered and accounts for how far apart EUS and MRI are in classifying them.

EUS and MRI were repeated after RT, and the findings were compared with the T and N stages established on the basis of histological examination of surgical specimens. The proportions of concordant results between each method and histology were compared using the χ^2 test (2 × 2 table). Patients were considered all together and sorted into two subgroups according to their histological T stage: the first group had T0-T2 stage cancer and the second T3-T4.

Table 2 Diagnostic accuracy (T and N-stage)

	All T	T0-T2	T3-T4	All N	N0	N 1-2
n	39	18	21	39	23	16
MRI	44%	33%	52%	62%	52%	75%
EUS	46%	44%	48%	69%	87%	44%

MRI: Magnetic resonance imaging; EUS: Endoscopic ultrasound.

RESULTS

After neoadjuvant chemoradiation therapy, histological examination of surgical specimens was done for all patients, with the following results: 9 T0; 1 Tl; 8 T2; 21 T3. Nodal involvement was N0 in 23; N1 in 14; N2 in 2. There were 18 patients with T0-T2 disease, 21 T3 (no T4), 23 without nodal metastasis (N0 disease) and 16 with N1-N2 disease. EUS and MRI correctly classified patients in line with their histological T stage in 46% (18/39) and 44% (17/39) (NS) of cases, respectively. These proportions were higher for both techniques when nodal involvement was considered: staging was correct in 69% (27/39) and 62% (24/39). Interestingly, when patients were sorted into T and N subgroups, the diagnostic accuracy of EUS was better than MRI for patients with T0-T2 (44% *vs* 33%, NS) and N0 disease (87% *vs* 52%, $P = 0.013$). However, MRI was more accurate for patients with more advanced disease (both T and N) after RT, though these differences did not reach statistical significance (Tables 1 and 2).

DISCUSSION

The prognosis for rectal cancer strongly correlates with the histopathological stage at diagnosis. Many imaging techniques are available, but each one differs in accuracy and applicability[11-13]. Accurate staging is important for planning surgery and deciding on adjuvant treatment.

ERUS (endorectal ultrasound) and MRI are considered to be the most accurate modalities for determining local tumor stage. Two recent meta-analyses have compared EUS, CT and MRI for rectal cancer staging. Bipat *et al*[14] found that ERUS was the most accurate modality when compared with CT and MRI for the evaluation of the T stage in rectal cancer. For lymph node involvement, the results of ERUS, CT and MRI were comparable. However, the T-staging system does not discriminate between T3 tumors with close or involved circumferential resection clearance. In this meta-analysis, the distance of the tumor from the rectal fascia or the anticipated circumferential resection clearance was not evaluated. Lahaye *et al*[15] conducted another meta-analysis regarding the accuracy of preoperative imaging for predicting the two most important risk factors that they recognized for local recurrence in rectal cancer; the circumferential resection

clearance and the lymph node status. Major progress has been made in the preoperative staging in rectal tumors by MRI and several authors have indicated that a tumor-free circumferential clearance of more than 1 mm can be predicted using this method. For nodal status, ERUS was slightly, but not significantly, better than MRI.

The introduction of trans-rectal EUS has made it easier to see the pattern of the layers of the rectal wall, improving treatment allocation by establishing the depth of tumor invasion more accurately[16-18]. EUS is the most accurate tool for evaluating local invasion of rectal cancer and perirectal lymph nodes. Its overall accuracy before radiation ranges from 73% to 94% in T staging, and from 70% to 80% in N staging. Comparative studies found EUS very accurate in staging rectal cancer from 80% to 95%, compared to 65% to 75% for CT and 75% to 85% for MRI[6-9].

CT and MRI have proved disappointing for detecting small neoplastic lesions. MRI is not significantly superior to CT because of the limited resolution of conventional MRI techniques. It has, however, been reported useful in determining the status of the circumferential resection margin (meso-rectum), which is important for assessing the risk of local recurrence[19].

Recent data suggest that EUS staging of rectal cancer after RT-CT is inaccurate[1], while MRI seems to be cost-effective in selecting appropriate patients for neoadjuvant therapy and its use is justified for local staging. After RT-CT, EUS and MRI might be useful to demonstrate tumor shrinkage and down-staging in responsive tumors, which might occasionally disappear completely[17,18,20-23]. Increasing reflectivity and signal changes indicate fibrosis, but unless significant tumor bulk remains, neither modality seems to be able to exclude the persistence of tumor cells within fibrosis[24-28].

Finally, EUS-FNA (endoscopic ultrasound-guided fine-needle aspiration) was proposed for N staging of rectal cancer following neoadjuvant chemoradiation[29].

This study compared rectal EUS and MRI for staging rectal cancer before and, in particular, after neoadjuvant RT-CT. As reported in other studies, at the first staging examination after the diagnosis of rectal cancer, EUS and MRI offer similar accuracies. However, if we consider the degree of agreement between the two procedures in rectal cancer, EUS (without lymph node biopsy) and MRI give concordant results in T and N staging only in 64% and 54% of patients. Moreover, they only give concordant results for both T and N stages in one third of patients. The strength of agreement between EUS and MRI in staging advanced rectal cancer was very poor, implying that each method has limitations.

After RT, both EUS and MRI offered poor diagnostic performance in the assessment of T and N stages compared to the "gold standard", i.e. histological examination of surgical specimens. In the present study, compared to MRI, EUS offered significantly superior diagnostic accuracy in assessing nodal involvement after RT, giving much better results in patients with N0 disease. A higher proportion of false-positive results of MRI in patients without nodal metastasis are probably due to the effects of RT on perirectal tissues (edema, inflammation, and fibrosis), which renders MRI unreliable for excluding nodal disease. Both methods were weak in restaging the persistence and degree of tumor invasion of the rectal wall, and this might be mainly due to microscopic persistence (under-staging) in the wall or to inflammatory and scar changes in perirectal fat (over-staging). These might explain the lower sensitivity and accuracy of both methods in re-staging after RT.

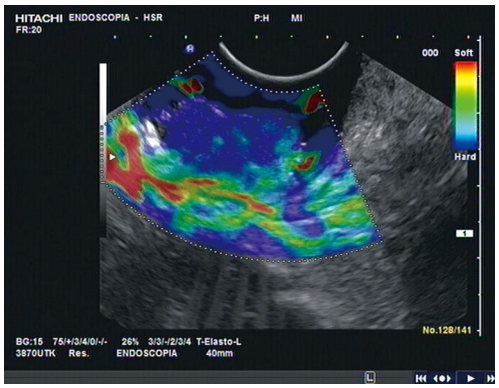

Figure 3 Rectal EUS and elastosonography before neoadjuvant chemoradiation treatment. Elastography showing hard tissue inside the lesion.

The recent development of elastosonography, a new real-time EUS modality that gives a fairly qualitative image of tissue elasticity, might improve the accuracy and sensitivity of EUS in this setting. In a preliminary report, we showed that adding elastosonography to real-time EUS boosted the accuracy in T staging of the disease[30]. The ability of elastosonography to distinguish tissues with different levels of elasticity means it can detect inflammatory (soft) tissues and tumor (hard) separately, particularly when the real-time modality does not exclude the suspicion of perirectal invasion (Figure 3).

Therefore, in the modern era of GI-oncology it seems that patients benefit mostly from interdisciplinary approaches. In considering the value of a diagnostic technique, based on its ability to influence the therapeutic choices by allowing a better selection of patients, we believe that in this situation these two modalities are complementary.

In conclusion, EUS and MRI are both accurate for staging rectal cancer. In particular, in pre-therapy staging, EUS is a good modality for T staging while MRI obtains other information, including the clearance. However, after RT-CT, neither method is reliable for establishing the T stage. EUS seems significantly better than MRI for assessing the N stage, but until significant improvements in both methods are achieved, their use in this setting should be considered only in controlled trials.

COMMENTS

Background

Endoscopic ultrasound (EUS) and magnetic resonance imaging (MRI) are both accurate for staging rectal cancer. However, after radiation therapy-computed tomography (RT-CT), neither method is reliable for establishing the T stage. EUS

seems significantly better than MRI for assessing the N stage but until significant improvements in both methods are achieved, their use in this setting should be considered only in controlled trials.

Innovations and breakthroughs

This study compared rectal EUS and MRI for staging rectal cancer after neoadjuvant RT-CT.

Peer review

This study compared rectal EUS and MRI for staging rectal cancer after neoadjuvant RT-CT and provides a good basis for selecting the best surgical or medical strategy in patients with rectal cancer.

REFERENCES

1 **Vanagunas A**, Lin DE, Stryker SJ. Accuracy of endoscopic ultrasound for restaging rectal cancer following neoadjuvant chemoradiation therapy. *Am J Gastroenterol* 2004; **99**: 109-112

2 **Savides TJ**, Master SS. EUS in rectal cancer. *Gastrointest Endosc* 2002; **56**: S12-S18

3 **Pahlman L**, Glimelius B. Pre- or postoperative radiotherapy in rectal and rectosigmoid carcinoma. Report from a randomized multicenter trial. *Ann Surg* 1990; **211**: 187-195

4 Improved survival with preoperative radiotherapy in resectable rectal cancer. Swedish Rectal Cancer Trial. *N Engl J Med* 1997; **336**: 980-987

5 **Harewood GC**. Assessment of clinical impact of endoscopic ultrasound on rectal cancer. *Am J Gastroenterol* 2004; **99**: 623-627

6 **Kwok H**, Bissett IP, Hill GL. Preoperative staging of rectal cancer. *Int J Colorectal Dis* 2000; **15**: 9-20

7 **Guinet C**, Buy JN, Ghossain MA, Sezeur A, Mallet A, Bigot JM, Vadrot D, Ecoiffier J. Comparison of magnetic resonance imaging and computed tomography in the preoperative staging of rectal cancer. *Arch Surg* 1990; **125**: 385-388

8 **Rifkin MD**, Ehrlich SM, Marks G. Staging of rectal carcinoma: prospective comparison of endorectal US and CT. *Radiology* 1989; **170**: 319-322

9 **Thaler W**, Watzka S, Martin F, La Guardia G, Psenner K, Bonatti G, Fichtel G, Egarter-Vigl E, Marzoli GP. Preoperative staging of rectal cancer by endoluminal ultrasound vs magnetic resonance imaging. Preliminary results of a prospective, comparative study. *Dis Colon Rectum* 1994; **37**: 1189-1193

10 **American Joint Committee on Cancer**. Colon and rectum. Manual for staging of cancer. 4th ed. Philadelphia: JB Lippincott, 1992: 75-79

11 **Heriot AG**, Grundy A, Kumar D. Preoperative staging of rectal carcinoma. *Br J Surg* 1999; **86**: 17-28

12 **Muthusamy VR**, Chang KJ. Optimal methods for staging rectal cancer. *Clin Cancer Res* 2007; **13**: 6877s-6884s

13 **Halefoglu AM**, Yildirim S, Avlanmis O, Sakiz D, Baykan A. Endorectal ultrasonography versus phased-array magnetic resonance imaging for preoperative staging of rectal cancer. *World J Gastroenterol* 2008; **14**: 3504-3510

14 **Bipat S**, Glas AS, Slors FJ, Zwinderman AH, Bossuyt PM, Stoker J. Rectal cancer: local staging and assessment of lymph node involvement with endoluminal US, CT, and MR imaging--a meta-analysis. *Radiology* 2004; **232**: 773-783

15 **Lahaye MJ**, Engelen SM, Nelemans PJ, Beets GL, van de Velde CJ, van Engelshoven JM, Beets-Tan RG. Imaging for predicting the risk factors--the circumferential resection margin and nodal disease--of local recurrence in rectal cancer: a meta-analysis. *Semin Ultrasound CT MR* 2005; **26**: 259-268

16 **Brown G**, Davies S, Williams GT, Bourne MW, Newcombe RG, Radcliffe AG, Blethyn J, Dallimore NS, Rees BI, Phillips CJ, Maughan TS. Effectiveness of preoperative staging in rectal cancer: digital rectal examination, endoluminal ultrasound or magnetic resonance imaging? *Br J Cancer* 2004; **91**: 23-29

17 **Puli SR**, Bechtold ML, Reddy JB, Choudhary A, Antillon MR, Brugge WR. How good is endoscopic ultrasound in differentiating various T stages of rectal cancer? Meta-analysis and systematic review. *Ann Surg Oncol* 2009; **16**: 254-265

18 **Puli SR**, Reddy JB, Bechtold ML, Choudhary A, Antillon MR, Brugge WR. Accuracy of endoscopic ultrasound to diagnose nodal invasion by rectal cancers: a meta-analysis and systematic review. *Ann Surg Oncol* 2009; **16**: 1255-1265

19 **LeBlanc JK**. Imaging and management of rectal cancer. *Nat Clin Pract Gastroenterol Hepatol* 2007; **4**: 665-676

20 **Bartram C**, Brown G. Endorectal ultrasound and magnetic resonance imaging in rectal cancer staging. *Gastroenterol Clin North Am* 2002; **31**: 827-839

21 **Maor Y**, Nadler M, Barshack I, Zmora O, Koller M, Kundel Y, Fidder H, Bar-Meir S, Avidan B. Endoscopic ultrasound staging of rectal cancer: diagnostic value before and following chemoradiation. *J Gastroenterol Hepatol* 2006; **21**: 454-458

22 **Dinter DJ**, Hofheinz RD, Hartel M, Kaehler GF, Neff W, Diehl SJ. Preoperative staging of rectal tumors: comparison of endorectal ultrasound, hydro-CT, and high-resolution endorectal MRI. *Onkologie* 2008; **31**: 230-235

23 **Shihab OC**, Moran BJ, Heald RJ, Quirke P, Brown G. MRI staging of low rectal cancer. *Eur Radiol* 2009; **19**: 643-650

24 **Suzuki C**, Torkzad MR, Tanaka S, Palmer G, Lindholm J, Holm T, Blomqvist L. The importance of rectal cancer MRI protocols on interpretation accuracy. *World J Surg Oncol* 2008; **6**: 89

25 **Siddiqui AA**, Fayiga Y, Huerta S. The role of endoscopic ultrasound in the evaluation of rectal cancer. *Int Semin Surg Oncol* 2006; **3**: 36

26 **Torricelli P**. Rectal cancer staging. *Surg Oncol* 2007; **16** Suppl 1: S49-S50

27 **Rovera F**, Dionigi G, Boni L, Cutaia S, Diurni M, Dionigi R. The role of EUS and MRI in rectal cancer staging. *Surg Oncol* 2007; **16** Suppl 1: S51-S52

28 **Lennon AM**, Penman ID. Endoscopic ultrasound in cancer staging. *Br Med Bull* 2007; **84**: 81-98

29 **Harewood GC**. Utility of endoscopic ultrasound for restaging rectal cancer following neoadjuvant chemoradiation therapy. *Am J Gastroenterol* 2004; **99**: 953; author reply 954

30 **Mezzi G**, Arcidiacono PG, Carrara S, Boemo C, Testoni PA. Elastosonography in malignant rectal disease: preliminary data. *Endoscopy* 2007; **39**: 375; author reply 375

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BRIEF ARTICLE

Routine modified D2 lymphadenectomy performance in pT1-T2N0 gastric cancer

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Abstract

AIM: To evaluate routine modified D2 lymphadenectomy in gastric cancer, based on immunohistochemically detected skip micrometastases in level Ⅱ lymph nodes.

METHODS: Among 95 gastric cancer patients who were routinely submitted to curative modified D2 lymphadenectomy, from January 2004 to December 2008, 32 were classified as pN0. All level Ⅰ lymph nodes of these 32 patients were submitted to immunohistochemistry for micrometastases detection. Patients in whom micrometastases were detected in the level Ⅰ lymph node stations (n = 4) were excluded from further analysis. The level Ⅱ lymph nodes of the remaining 28 patients were studied immunohistochemically for micrometastases detection and constitute the material of the present study.

RESULTS: Skip micrometastases in the level Ⅱ lymph nodes were detected in 14% (4 out of 28) of the patients. The incidence was further increased to 17% (4 out of 24) in the subgroup of T1-2 gastric cancer patients. All micrometastases were detected in the No. 7 lymph node station. Thus, the disease was upstaged from stage ⅠA to ⅠB in one patient and from stage ⅠB to Ⅱ in three patients.

CONCLUSION: In gastric cancer, true R0 resection may not be achieved without modified D2 lymphadenectomy. Until D2+/D3 lymphadenectomy becomes standard, modified D2 lymphadenectomy should be performed routinely.

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Key words: Gastric cancer; D2 gastrectomy; D2 lymphadenectomy; Micrometastases; Skip metastases; Skip micrometastases

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INTRODUCTION

The lymphatic stream from a gastric tumor is wide and complicated, thus the exact pattern of lymphatic drainage remains obscure and poorly understood. However, histologically confirmed metastatic infiltration of perigastric and extragastric lymph nodes has been defined as the strongest independent dismal prognostic factor for both early[1] and large[2] gastric cancer patients.

Micrometastases and/or isolated tumor cells have been reported as immunohistochemically detectable in 10% of early gastric cancer patients[3], in 52.6% of T2N0 patients[4], and in 21%[5] to 49%[6] of all node-negative gastric cancer patients.

Skip metastases are defined as the detection of metastatically infiltrated extragastric lymph nodes (level Ⅱ), in the absence of perigastric lymph node (level Ⅰ) involve-

ment[7]. Particularly in the subgroup of level I lymph node negative patients, the incidence of histologically detected metastases in the level Ⅱ lymph nodes (skip metastases) ranges between 2.8% in cases of early[7] and 5%[8] to 17.4%[9] in all other gastric cancers. Moreover, even in patients with histologically classified level I lymph node negative early gastric cancer, micrometastatically infiltrated level Ⅱ lymph nodes are immunohistochemically detected in 10% of them[10].

Most authors[11-13] agree that, except for early gastric cancer patients[3], patients with immunohistochemically detected micrometastases have significantly worse 5-year survival rates compared to patients with undetectable micrometastases. However, the incidence, clinical implications and clinical significance of skip micrometastases in level Ⅱ extragastric lymph nodes in patients with gastric cancer have not been properly studied.

The aim of the present study was to evaluate retrospectively the necessity for routine modified D2 lymphadenectomy in all gastric cancer patients, (as a prerequisite for R0 resection for locoregional control of the disease), based on the immunohistochemical detection of micrometastases in level Ⅱ lymph node stations in patients who had been classified histologically and immunohistochemically as level I lymph node negative and histologically as level Ⅱ lymph node negative.

MATERIALS AND METHODS

Between January 2004 and December 2008, 207 patients with a preoperative diagnosis of gastric adenocarcinoma were subjected to surgery with curative intent, in our department. None of them had undergone preoperative chemotherapy or radiotherapy. In 95 patients, a modified D2 lymphadenectomy was offered as the standard surgical procedure. Postoperatively, the standard histological examination by hematoxylin and eosin (HE) staining disclosed metastatic infiltration of at least one lymph node in the level I or Ⅱ lymph node stations in 63 of these patients. Thirty-two patients were classified as pN0, since standard histology did not disclose any evidence of metastatic infiltration of level I and level Ⅱ peri- and extragastric lymph node stations. All level I lymph nodes of these 32 patients were submitted to immunohistochemistry for micrometastases detection. Patients in whom micrometastases were detected in the level I lymph node stations (n = 4) were excluded from further analysis.

The level Ⅱ lymph nodes of the remaining 28 patients were studied immunohistochemically for micrometastases detection and constituted the material of the present study.

Surgical technique

The proximal resection margin of the stomach was calculated according to the location of the primary tumor. At least a 6-cm tumor-free (based on the frozen section result) proximal resection margin from the most proximal macroscopic border[14] was achieved in all cases. The dissection of the regional lymph nodes was based on the Japanese Classification of Gastric Carcinoma[15]. Thus, for D1 lymphadenectomy, the appropriate (depending on the location of the primary tumor) nos. 1-6 lymph node stations were included in the gastrectomy specimen, whereas in the modified D2 lymphadenectomy, the nos. 7, 8a, 9, 11p, 11d and 12a lymph node stations, were routinely dissected. The level Ⅱ lymph node stations were recognizable as they had been sent separately to the Pathology Department with special indices demonstrating their exact location. Dissection of the No. 10 lymph node station, splenectomy or distal pancreatectomy was not performed in any of the patients. For staging of the tumors, the TNM classification system according to the AJCC Staging Manual, 6th edition, was used[16].

Histopathology and immunohistochemistry

Primary tumors and lymph nodes were fixed in formalin and embedded in paraffin. The presence or absence of lymph node metastasis was examined routinely by HE staining by using a representative cut section through the largest diameter of the lymph nodes.

One additional section of 4-μm thickness from each node was prepared for immunohistochemical staining with a monoclonal anti-cytokeratin (CK) antibody cocktail (AE1/AE3; Dako, Glostrup, Denmark) that reacts with a broad spectrum of human CKs, to detect micrometastases and/or clusters of isolated tumor cells. Briefly, for AE1/AE3 immunostaining, paraffin-embedded sections were deparaffinized in xylene and rehydrated through graduated ethanol to water. Endogenous peroxidase activity was blocked by incubation for 30 min with a solution of 1% hydrogen peroxide, and antigen retrieval was performed by autoclaving sections in 0.01 mol/L citrate buffer, pH 6.0 for 20 min at 800 W. A monoclonal mouse anti-human CK antibody (clone AE1/AE3) was applied at a dilution of 1:50. The Dako Real Envision kit was then used. Diaminobenzidine was used as a chromogen. Lymphoid tissue was used as an internal negative control, while additional sections from the primary tumors were used as positive controls.

Based on the 6th TNM classification[16], micrometastasis (N1mi) was defined as metastatic focus > 0.2 mm but ⩽ 2 mm, and cluster of tumor cells [N0 (i+)] was defined as cluster < 0.2 mm according to previously accepted conventions.

RESULTS

Pathologic review did not detect patients with previously missed evidence of lymph node metastasis on conventional HE staining.

In four patients, micrometastases were detected in the level I lymph node stations. These patients were excluded from further analysis.

The remaining 28 patients were 16 men with a median age of 72.5 years (IR 69-75) and 12 women with a median age of 66.5 years (IR 58-69.5) (Table 1). Skip micrometastases in the level Ⅱ lymph node stations were immunohistochemically detected in four patients (n = 4). All micrometastases were detected in the No. 7 lymph node station.

Table 1 Characteristics of the study population

Parameter	*n*
Sex	16
Males	12
Females	0
Age (yr) (median + IR)	70.5 (63.5–74)
Tumor location	
Upper third	0
Middle third	6
Lower third	22
Histological type (WHO classification)	
Enteric type	19
Diffuse type	5
Mixed type	4
Differentiation	
High	4
Moderate	19
Low	5
T	
T1	4
T2	20
T3	4

The profiles of these patients are presented in Table 2. There were three female and one male patients, with T1 (n = 1), T2a (n = 2) and T2b (n = 1) tumors, located in the lower third (n = 2) or middle third (n = 2) of the stomach. Thus, following micrometastases detection, the disease was upstaged from stage I A to I B in one patient and from stage I B to Ⅱ in three patients.

Based on the above, the overall incidence of micrometastases detection was 25% (8 out of 32 patients), while skip micrometastases in the level Ⅱ lymph nodes were detected in 14% (4 out of 28) of gastric cancer patients, who had been classified histologically and immunohistochemically as level I lymph node negative. Furthermore, the incidence of skip micrometastases was increased to 17% (4 out of 24) in the subgroup of T1-2 gastric cancer patients.

DISCUSSION

The present study disclosed that skip micrometastases in the level Ⅱ lymph node stations were detected in 14% (4 out of 28) of the patients, who had been classified histologically and immunohistochemically as level I negative. This incidence was further increased to 17% (4 out of 24 patients) in the subgroup of T1-2 gastric cancer tumors.

Despite AJCC/UICC guidelines, which require the pathological examination of at least 15 lymph nodes for accurate gastric cancer staging[16], only 29% of gastric cancer patients had more than 15 lymph nodes retrieved[17]. However, D2 lymphadenectomy clearly offers the mean number of the required lymph nodes for pathological examination, independently to the pathologist[18].

Although D2 lymphadenectomy is recommended by the Japanese Surgical Society as the surgical option for gastric cancer treatment[19], its performance has not gained popularity worldwide, since prospective randomized studies[20-24] and meta-analysis[25] have revealed significantly higher postoperative morbidity and mortality rates and no 5- and 11-year survival benefit compared to D1 lymphadenectomy.

D2 lymphadenectomy increases the long-term survival of gastric cancer patients with lymph node metastases, however, it has been proposed as unnecessary for patients without lymph node metastases[9]. On the other hand, D2 lymphadenectomy improves survival even in node-negative early gastric cancer patients, probably due to the resection of the coexisting micrometastases[26].

Thus, the favorable overall survival rates which were published following the Japanese-type, compared to the Western-type gastric cancer surgery, indicate that, with more extended lymph node dissections, more R0 resections are achieved[27]. This probably leads to locoregional control of the disease, better outcome and increased survival[28].

Three methods have been used for the identification of micrometastasis, serial sectioning, immunohistochemical staining, and reverse-transcriptase polymerase chain reaction (RT-PCR). Serial sectioning constitutes a histological method, which can detect lymph node metastasis previously missed by the conventional technique, but it may still fail to identify isolated tumor deposits[13]. RT-PCR has been reported as highly sensitive[29], but it is compromised by false-positive results caused by biological contamination[30]. Positive RT-PCR results indicate the presence of tumor DNA, however, they may not indicate the presence of viable tumor cells[31]. Thus, immunohistochemistry with human anti-CK antibodies represents the most accurate method for micrometastasis detection[32] and the most frequently applied technique in research[3]. One limitation of the method is CK expression by some dendritic cells in the lymph nodes[33].

Based on the results of studies in colorectal[34] and non-small cell lung cancer[35], it has been proposed that N1(-)/N2(+) patients represent a subgroup of pN2 disease with more favorable prognosis[35]. However, the clinical significance of skip metastases in gastric cancer patients remains controversial. The controversies are related mainly to the small number of patients enrolled in skip metastasis studies[26], the probably different prognosis of patients with histologically *vs* micrometastatically detected skip metastases[32], and the concern that patients with histologically detected skip metastases may represent cases of overlooked histological metastasis or micrometastasis in level I lymph nodes, thus being misclassified as patients with skip metastasis[36].

Saito *et al*[36] have reported 5-year survival rates of 70.2%, 62.0% and 31.2% in patients with skip metastases, metastases in level I lymph nodes and metastases in level Ⅱ lymph nodes, respectively. The prognosis of patients with metastases in the level Ⅱ lymph nodes was significantly worse than that of the patients with either skip metastases or metastases in the level I lymph nodes. The authors have indicated that the clinicopathological characteristics and the prognosis of patients with skip metastases were similar to patients with level I lymph node metastases, but not to the patients with level Ⅱ lymph node metastases. On the other hand, Li *et al*[9] have concluded that the cumulative survival rate is not statistically different between gastric cancer patients with solitary skip lymph node metastases, compared to

Table 2 Profiles of the patients with skip micrometastases

Pt	Sex	Age (yr)	No. of LN retrieved	(+) LN station	Location of the tumor	T size (mm)	T stage	Histological type	Lymphatic invasion	Vessel invasion
1	F	62	51	7	L, post	4	T2b	Mixed	+	-
2	F	56	16	7	M, less	30	T1	Diffuse	-	-
3	M	54	38	7	M, less	17	T2a	Enteric	-	-
4	F	71	29	7	L, post	26	T2a	Diffuse	-	-

Pt: Patient; LN: Lymph node; T: Tumor; L: Lower third; M: Middle third.

patients with solitary level I lymph node metastases. Moreover, Park *et al*[26] have reported that, in patients with positive nodes extending into the level Ⅱ lymph nodes, the survival curves did not show significant differences between skip(+) and skip(-) groups of patients, which further supports the theory that the number but not the level of lymph node metastases has prognostic significance.

The result of the present study, that all skip micrometastases were detected in the left gastric artery lymph node station, probably indicates that the clinical application of the sentinel node biopsy technique in selected cases might be useful, and lead to selective lymphadenectomy. Although the method has been reported as highly accurate (< 10% false-negative results) in breast cancer surgery[37], similar findings have not been confirmed in gastric cancer surgery, since 20%-36% of positive lymph nodes were located outside of the sentinel lymph node basin[38]. Thus, the method is recommended currently to be used in conjunction with D2 lymphadenectomy[39].

It has been suggested that the most likely route for para-aortic lymph node metastases is from the left gastric artery nodes, passing by the celiac artery[40]. Other common sites of skip metastasis are the 8a and 9 lymph nodes (around the celiac artery). Thus, these lymph nodes should always be evaluated, regardless of the mode of operation, even in the case of minimally invasive surgery. Moreover, Yanagita *et al*[41] have investigated the clinical significance of morphological distribution of metastatic foci (metastasis, micrometastasis or isolated tumor cells) in sentinel lymph nodes with gastric cancer, and have concluded that, in patients with non-marginal sinus type sentinel node metastasis, attention should be paid to the possibility of non-sentinel node or even pN2 metastases. Thus, if the sentinel node cannot be identified in the perigastric lymph nodes, those around the celiac artery lymph nodes should be explored to reduce the likelihood of false-negative results in sentinel node mapping[7].

In conclusion, the present study addressed the fact that, in up to 17% of T1-2 gastric cancer patients, true R0 resection may not be achieved without modified D2 lymphadenectomy. Thus, until D2+ or even D3 lymphadenectomy becomes the standard surgical option, modified D2 lymphadenectomy should be considered as the surgical option of choice in gastric cancer patients.

COMMENTS

Background

R0 resection constitutes the prerequisite for the locoregional control of gastric cancer. However, the extent of the oncologically required lymphadenectomy (D1 or D2) remains a matter of debate and contradictory results have been published.

Research frontiers

True R0 resection is characterized by complete resection of all viable tumor cells. As histology detects metastases > 2 mm, immunohistochemistry can detect micrometastases between 0.2 and 2 mm or even isolated tumor cells.

Innovations and breakthroughs

Immunohistochemistry with human anti-cytokeratin antibodies showed clearly that, in level Ⅱ extragastric lymph nodes, viable tumor deposits (skip micrometastases) were present in 17% of the gastric cancer patients who had been classified previously as node-negative by conventional histology.

Applications

By understanding that, without modified D2 lymphadenectomy, true R0 resection may not be achieved, the present study indicates the usefulness of routine modified D2 lymphadenectomy in all gastric cancer patients.

Terminology

Modified D2 lymphadenectomy offering the mean number of the required lymph nodes for pathological examination, contributes to accurate staging of the disease, probably defying the subgroups of patients who may benefit from adjuvant therapy.

Peer review

The authors suggest that D2 lymphadenectomy might be necessary for complete resection of T1-2 N0 gastric cancer, which highlights clearly that skip metastases may be an overlooked problem with standard resection performed routinely in many countries.

REFERENCES

1 **Folli S**, Morgagni P, Roviello F, De Manzoni G, Marrelli D, Saragoni L, Di Leo A, Gaudio M, Nanni O, Carli A, Cordiano C, Dell'Amore D, Vio A. Risk factors for lymph node metastases and their prognostic significance in early gastric cancer (EGC) for the Italian Research Group for Gastric Cancer (IRGGC). *Jpn J Clin Oncol* 2001; **31**: 495-499

2 **Shiraishi N**, Sato K, Yasuda K, Inomata M, Kitano S. Multivariate prognostic study on large gastric cancer. *J Surg Oncol* 2007; **96**: 14-18

3 **Morgagni P**, Saragoni L, Scarpi E, Zattini PS, Zaccaroni A, Morgagni D, Bazzocchi F. Lymph node micrometastases in early gastric cancer and their impact on prognosis. *World J Surg* 2003; **27**: 558-561

4 **Fukagawa T**, Sasako M, Shimoda T, Sano T, Katai H, Saka M, Mann GB, Karpeh M, Coit DG, Brennan MF. The prognostic impact of isolated tumor cells in lymph nodes of T2N0 gastric cancer: comparison of American and Japanese gastric cancer patients. *Ann Surg Oncol* 2009; **16**: 609-613

5 **Lee HS**, Kim MA, Yang HK, Lee BL, Kim WH. Prognostic implication of isolated tumor cells and micrometastases in regional lymph nodes of gastric cancer. *World J Gastroenterol* 2005; **11**: 5920-5925

6 **Doekhie FS**, Mesker WE, van Krieken JH, Kok NF, Hartgrink HH, Kranenbarg EK, Putter H, Kuppen PJ, Tanke HJ, Tollenaar RA, van de Velde CJ. Clinical relevance of occult tumor cells in lymph nodes from gastric cancer patients. *Am J Surg Pathol* 2005; **29**: 1135-1144

7 **Lee SE**, Lee JH, Ryu KW, Cho SJ, Lee JY, Kim CG, Choi IJ, Kook MC, Nam BH, Park SR, Lee JS, Kim YW. Sentinel node mapping and skip metastases in patients with early gastric

cancer. *Ann Surg Oncol* 2009; **16**: 603-608
8 **Kikuchi S**, Kurita A, Natsuya K, Sakuramoto S, Kobayashi N, Shimao H, Kakita A. First drainage lymph node(s) in gastric cancer: analysis of the topographical pattern of lymph node metastasis in patients with pN-1 stage tumors. *Anticancer Res* 2003; **23**: 601-604
9 **Li C**, Kim S, Lai JF, Oh SJ, Hyung WJ, Choi WH, Choi SH, Noh SH. Solitary lymph node metastasis in gastric cancer. *J Gastrointest Surg* 2008; **12**: 550-554
10 **Miyake K**, Seshimo A, Kameoka S. Assessment of lymph node micrometastasis in early gastric cancer in relation to sentinel nodes. *Gastric Cancer* 2006; **9**: 197-202
11 **Cai J**, Ikeguchi M, Maeta M, Kaibara N, Sakatani T. Clinicopathological value of immunohistochemical detection of occult involvement in pT3N0 gastric cancer. *Gastric Cancer* 1999; **2**: 95-100
12 **Kim JH**, Park JM, Jung CW, Park SS, Kim SJ, Mok YJ, Kim CS, Chae YS, Bae JW. The significances of lymph node micrometastasis and its correlation with E-cadherin expression in pT1-T3N0 gastric adenocarcinoma. *J Surg Oncol* 2008; **97**: 125-130
13 **Lee E**, Chae Y, Kim I, Choi J, Yeom B, Leong AS. Prognostic relevance of immunohistochemically detected lymph node micrometastasis in patients with gastric carcinoma. *Cancer* 2002; **94**: 2867-2873
14 **Bozzetti F**. Principles of surgical radicality in the treatment of gastric cancer. *Surg Oncol Clin N Am* 2001; **10**: 833-854, ix
15 Japanese Gastric Cancer Association. Japanese Classification of Gastric Carcinoma, 2nd English Edition. *Gastric Cancer* 1998; **1**: 10-24
16 **Greene F**L, Page DL, Fleming ID, Fritz A, Balch CM. AJCC cancer staging handbook. 6th ed. New York: Springer, 2002: 111-118
17 **Coburn NG**, Swallow CJ, Kiss A, Law C. Significant regional variation in adequacy of lymph node assessment and survival in gastric cancer. *Cancer* 2006; **107**: 2143-2151
18 **Bunt AM**, Hermans J, van de Velde CJ, Sasako M, Hoefsloot FA, Fleuren G, Bruijn JA. Lymph node retrieval in a randomized trial on western-type versus Japanese-type surgery in gastric cancer. *J Clin Oncol* 1996; **14**: 2289-2294
19 **Nakajima T**. Gastric cancer treatment guidelines in Japan. *Gastric Cancer* 2002; **5**: 1-5
20 **Bonenkamp JJ**, Songun I, Hermans J, Sasako M, Welvaart K, Plukker JT, van Elk P, Obertop H, Gouma DJ, Taat CW. Randomised comparison of morbidity after D1 and D2 dissection for gastric cancer in 996 Dutch patients. *Lancet* 1995; **345**: 745-748
21 **Cuschieri A**, Fayers P, Fielding J, Craven J, Bancewicz J, Joypaul V, Cook P. Postoperative morbidity and mortality after D1 and D2 resections for gastric cancer: preliminary results of the MRC randomised controlled surgical trial. The Surgical Cooperative Group. *Lancet* 1996; **347**: 995-999
22 **Bonenkamp JJ**, Hermans J, Sasako M, van de Velde CJ, Welvaart K, Songun I, Meyer S, Plukker JT, Van Elk P, Obertop H, Gouma DJ, van Lanschot JJ, Taat CW, de Graaf PW, von Meyenfeldt MF, Tilanus H. Extended lymph-node dissection for gastric cancer. *N Engl J Med* 1999; **340**: 908-914
23 **Hartgrink HH**, van de Velde CJ, Putter H, Bonenkamp JJ, Klein Kranenbarg E, Songun I, Welvaart K, van Krieken JH, Meijer S, Plukker JT, van Elk PJ, Obertop H, Gouma DJ, van Lanschot JJ, Taat CW, de Graaf PW, von Meyenfeldt MF, Tilanus H, Sasako M. Extended lymph node dissection for gastric cancer: who may benefit? Final results of the randomized Dutch gastric cancer group trial. *J Clin Oncol* 2004; **22**: 2069-2077
24 **Degiuli M**, Sasako M, Calgaro M, Garino M, Rebecchi F, Mineccia M, Scaglione D, Andreone D, Ponti A, Calvo F. Morbidity and mortality after D1 and D2 gastrectomy for cancer: interim analysis of the Italian Gastric Cancer Study Group (IGCSG) randomised surgical trial. *Eur J Surg Oncol* 2004; **30**: 303-308
25 **McCulloch P**, Nita ME, Kazi H, Gama-Rodrigues J. Extended versus limited lymph nodes dissection technique for adenocarcinoma of the stomach. *Cochrane Database Syst Rev* 2004; CD001964
26 **Park SS**, Ryu JS, Min BW, Kim WB, Kim SJ, Kim CS, Mok YJ. Impact of skip metastasis in gastric cancer. *ANZ J Surg* 2005; **75**: 645-649
27 **Brennan MF**. Current status of surgery for gastric cancer: a review. *Gastric Cancer* 2005; **8**: 64-70
28 **Hartgrink HH**, van de Velde CJ. Status of extended lymph node dissection: locoregional control is the only way to survive gastric cancer. *J Surg Oncol* 2005; **90**: 153-165
29 **Arigami T**, Natsugoe S, Uenosono Y, Mataki Y, Ehi K, Higashi H, Arima H, Yanagida S, Ishigami S, Hokita S, Aikou T. Evaluation of sentinel node concept in gastric cancer based on lymph node micrometastasis determined by reverse transcription-polymerase chain reaction. *Ann Surg* 2006; **243**: 341-347
30 **Davids V**, Kidson SH, Hanekom GS. Melanoma patient staging: histopathological versus molecular evaluation of the sentinel node. *Melanoma Res* 2003; **13**: 313-324
31 **Yamamoto N**, Kato Y, Yanagisawa A, Ohta H, Takahashi T, Kitagawa T. Predictive value of genetic diagnosis for cancer micrometastasis: histologic and experimental appraisal. *Cancer* 1997; **80**: 1393-1398
32 **Yanagita S**, Natsugoe S, Uenosono Y, Arigami T, Arima H, Kozono T, Funasako Y, Ehi K, Nakajo A, Ishigami S, Aikou T. Detection of micrometastases in sentinel node navigation surgery for gastric cancer. *Surg Oncol* 2008; **17**: 203-210
33 **Domagala W**, Bedner E, Chosia M, Weber K, Osborn M. Keratin-positive reticulum cells in fine needle aspirates and touch imprints of hyperplastic lymph nodes. A possible pitfall in the immunocytochemical diagnosis of metastatic carcinoma. *Acta Cytol* 1992; **36**: 241-245
34 **Shiozawa M**, Akaike M, Yamada R, Godai T, Yamamoto N, Saito H, Sugimasa Y, Takemiya S, Rino Y, Imada T. Clinicopathological features of skip metastasis in colorectal cancer. *Hepatogastroenterology* 2007; **54**: 81-84
35 **Riquet M**, Assouad J, Bagan P, Foucault C, Le Pimpec Barthes F, Dujon A, Danel C. Skip mediastinal lymph node metastasis and lung cancer: a particular N2 subgroup with a better prognosis. *Ann Thorac Surg* 2005; **79**: 225-233
36 **Saito H**, Tsujitani S, Ikeguchi M. Clinical significance of skip metastasis in patients with gastric cancer. *Gastric Cancer* 2007; **10**: 87-91
37 **van Deurzen CH**, de Boer M, Monninkhof EM, Bult P, van der Wall E, Tjan-Heijnen VC, van Diest PJ. Non-sentinel lymph node metastases associated with isolated breast cancer cells in the sentinel node. *J Natl Cancer Inst* 2008; **100**: 1574-1580
38 **Gretschel S**, Bembenek A, Hunerbein M, Dresel S, Schneider W, Schlag PM. Efficacy of different technical procedures for sentinel lymph node biopsy in gastric cancer staging. *Ann Surg Oncol* 2007; **14**: 2028-2035
39 **Iddings DM**, Bilchik A. Gastric carcinoma: applying the sentinel node paradigm to improve the understanding of metastatic patterns and the possible role of selective lymphadenectomy. *Ann Surg Oncol* 2007; **14**: 2432-2434
40 **Nomura E**, Sasako M, Yamamoto S, Sano T, Tsujinaka T, Kinoshita T, Furukawa H, Shimizu T, Hiratsuka M, Kobayashi O, Kurokawa Y, Tanigawa N. Risk factors for para-aortic lymph node metastasis of gastric cancer from a randomized controlled trial of JCOG9501. *Jpn J Clin Oncol* 2007; **37**: 429-433
41 **Yanagita S**, Natsugoe S, Uenosono Y, Arima H, Kozono T, Ehi K, Arigami T, Higashi H, Aikou T. Morphological distribution of metastatic foci in sentinel lymph nodes with gastric cancer. *Ann Surg Oncol* 2008; **15**: 770-776

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BRIEF ARTICLE

Comparison of a modified anoscope and the purse-string anoscope in stapled haemorrhoidopexy

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Abstract

AIM: To compare the results of the anoscope of the PPH kit and a modified anoscope during stapled haemorrhoidopexy.

METHODS: The hospital records of 37 patients who underwent stapled haemorrhoidopexy between 2001 and 2006 were reviewed. The purse-string suture anoscope in the PPH kit was used on 15 patients (Group 1), and the modified anoscope was used on 22 patients (Group 2). Demographic characteristics of the patients, operation time, surgeon's performance, analgesic requirement, and complications were compared.

RESULTS: Operation time was significantly longer in Group 1 (42.0 ± 8.4 min *vs* 27.7 ± 8.0 min, $P = 0.039$). The surgeons reported their operative performance as significantly better in Group 2 (the results of the assessments were poor in ten, medium in four and good in one in Group 1, while good in all patients in Group 2, $P < 0.001$). The need for haemostatic sutures was significantly higher in Group 1 (six cases) and was needed in two cases in Group 2 ($P = 0.034$).

CONCLUSION: Operation time decreased and the surgeon's satisfaction increased with use of the modified anoscope, and fewer haemostatic sutures were required if the surgeon waited longer before and after firing the stapler.

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Key words: Haemorrhoidal disease; Modified anoscope; Purse-string suture; Stapled haemorrhoidopexy; Stapled anopexy

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INTRODUCTION

Stapled haemorrhoidopexy is not only a safe method that can be compared with conventional haemorrhoidectomy procedures, but also has advantages such as reduced postoperative pain and early return to work[1,2]. Moreover, some studies suggest the applicability of stapled haemorrhoidopexy as a day case procedure[3]. However, some complications were noted after stapled haemorrhoidopexy, such as rectal perforation, intra-abdominal haemorrhage, recto-vaginal fistula, and peri-rectal haematoma. These complications are not observed after conventional haemorrhoidectomy. In addition, although stapled haemorrhoidopexy is the most recent surgical method for the treatment of haemorrhoidal disease, the number of patients that developed pelvic sepsis after this surgical technique has reached the same number as after conventional haemorrhoidectomy[4-8]. It was suggested that these serious complications could be due to technical errors and could be avoided by eliminating the bite of the suture in deep layers of the rectum[9].

The ideal purse-string suture must enable removal of a ring of the desired thickness from the most appropriate part of the rectum. One major obstacle to the surgeon during the operation is blockage of vision due

to internal haemorrhoids. If these internal haemorrhoids can be eliminated, then optimal purse-string suture can be performed[10]. Some studies suggest that after modification of the anoscope of the PPH stapler kit (Ethicon Endo-Surgery, Cincinnati, OH, USA), purse-string suture applications have been performed with relative ease[10-12].

We first performed the stapled haemorrhoidopexy procedure in our clinic in 2001. We noticed the need for a modification of the anoscope of the PPH kit due to difficulties during the placement of the purse-string suture[10,11]. Subsequently, we tried several modifications of the anoscope and using the modified anoscope, purse-string sutures were performed with ease, and stapled haemorrhoidopexy was completed effectively.

In this study, the surgical findings and early postoperative results of patients in whom the purse-string suture anoscope of the PPH kit was used were compared with those of patients in whom the modified anoscope was used during stapled haemorrhoidopexy.

MATERIALS AND METHODS

Hospital and follow-up records of patients with grade Ⅲ and Ⅳ haemorrhoidal disease who underwent stapled haemorrhoidopexy in our clinic between 2001 and 2006 were reviewed retrospectively. Gender, degree of haemorrhoidal disease, preoperative treatment, operation time, performance of the surgeon, analgesic requirement, haemostatic sutures, histopathological examination of the excised ring, postoperative complications, and hospital stay were evaluated. Patients with associated proctological diseases such as anal abscess, anal fissure and fistula were excluded from the study. The patients were informed about the details of the procedure and written informed consent was obtained. The patients were grouped as: (1) the patients on whom the purse-string suture anoscope in the PPH kit was used (PA group), and (2) the patients on whom the modified anoscope was used (MA group). The modified anoscope was sterilized by ethylene oxide before use in each patient. The purse-string suture technique which has been described previously, was used in the MA group[10,11]. Stapled haemorrhoidopexy was completed using the stapler in the PPH (Ethicon Endo-Surgery, Cincinnati, OH, USA) kit in both groups. In the PA group, the surgeon waited for 30 s with the stapler closed after firing the stapler to ensure haemostasis. In the MA group, the surgeon waited for a total of 3 min, 90 s before and 90 s after firing the stapler. In the MA group, some of the operations were performed by inexperienced surgeons under the supervision of an experienced surgeon, whereas in the PA group an experienced surgeon performed the operations. The procedures were performed by six general surgeons, three of which were experienced. Diclofenac sodium 75 mg, IM was administered on demand after surgery, and pethidine HCl 50 mg, IM was given if diclofenac was insufficient. Patient age, gender, degree of haemorrhoidal disease, previous medical or surgical treatments, previous blood transfusions, histopathological evaluation of the excised ring, the performance of the surgeons, postoperative analgesic requirement, early (during the first 30 d) or late (after the first 30 d) complications in both groups were investigated, and the differences between the groups were analyzed statistically.

In the first few postoperative hours, the surgeons were asked two questions in order to evaluate the procedure: Was the anoscope simple to use during the manipulation, and how would you evaluate your performance during the procedure? The surgeons who performed the operations were asked to evaluate their performances as good, moderate, or poor. The surgeons who performed operations in both groups evaluated their performances by making comparisons with their earlier experiences, however, the surgeons who performed operations only in Group 2 made their decisions without comparisons.

Statistical analysis

SPSS 10.0 was used for statistical analysis. Considering numerical values, Student's *t* test was used for parametric measurements, and Mann Whitney *U* test for non-parametric measurements. Considering categoric values, Pearson's Chi-Square test was applied for parametric measurements and Fisher's exact correct test for non-parametric measurements. A *P* value less than 0.05 was considered statistically significant.

RESULTS

The records of 37 patients, who underwent stapled haemorrhoidopexy for the treatment of haemorrhoidal disease were investigated. Two types of anoscopes were used to aid purse-string suture application: the purse-string anoscope in the PPH kit was used on 15 patients (40.5) between September 2001 and March 2004, and the modified anoscope was used on 22 patients (59.5) between December 2003 and May 2006.

Mean age of the patients was 47.9 ± 15.2 years (range, 27-78 years), and 49.0 ± 11.9 years (range, 30-70 years) for the PA group and the MA group, respectively. There were 11 male and four female patients in the PA group and 17 male and five female patients in the MA group. There were no statistically significant differences between the two groups with respect to age ($P = 0.802$) and gender ($P = 0.541$). Mean duration of complaints was 94.8 ± 83.5 mo (range, 6 mo-20 years) for the PA group, and 112.1 ± 98.5 mo (range, 6 mo-30 years) for the MA group. Mean postoperative follow up time was 46.1 ± 8.7 mo (range, 36-66 mo) in the PA group and 21.9 ± 9.9 mo (range, 10-39 mo) in the MA group.

When the disease stage was considered, nine patients had third degree, and six patients had fourth degree haemorrhoidal disease in the PA group, whereas; 17 patients had third degree, and five patients had fourth degree haemorrhoidal disease in the MA group. There were no statistically significant differences between the two groups with respect to stage of the disease ($P = 0.222$).

Table 1 General characteristics of the patients, operative findings, and the differences between the two groups *n* (%)

	Group 1 (PA)	Group 2 (MA)	*P* value
Number of patients	15 (40.5)	22 (59.5)	
Gender			
Male	11 (73.3)	17 (77.3)	0.541
The degree of haemorrhoidal disease			0.222
Ⅲ	9 (60.0)	17 (77.3)	
Ⅳ	6 (40.0)	5 (22.7)	
Preoperative treatment			0.564
Medical	14 (93.3)	17 (77.3)	
Blood transfusion	-	1 (4.5)	
Surgery	-	1 (4.5)	
Not required	1 (6.7)	3 (13.6)	
Haemostatic suture			0.034
Required	6 (40)	2 (9.1)	
Early complication			0.683
Bleeding	5 (33.3)	7 (31.8)	
Urinary retention	1 (6.7)	1 (4.5)	
Anal prolapse	-	1 (4.5)	
Late complication			0.418
Anal fissure	1 (6.7)	-	
External pile	-	1 (4.5)	
Thrombosis of haemorrhoids	-	1 (4.5)	
Histopathology of the donut			0.933
Normal	13 (86.7)	19 (86.4)	
Smooth muscle	1 (6.7)	2 (9.1)	
Adenoma	1 (6.7)	1 (4.5)	
Evaluation of the performance of the surgeon			< 0.001
Poor	10 (66.7)	-	
Medium	4 (26.7)	-	
Good	1 (6.7)	22 (100)	

When previous treatment modalities (medical, blood transfusion, surgery) were considered, there were no statistically significant differences between the two groups (P = 0.564). General characteristics of the patients, intraoperative findings, and the differences between the two groups are shown in Table 1.

Mean duration of surgery was 42.0 ± 8.4 min (range, 30-60 min) for the PA group, and 27.7 ± 8.0 min (range, 18-45 min) for the MA group, and the difference was statistically significant (P = 0.039) (Table 2). Additional haemostatic suture application after removal of the stapler was required in six cases (40.0) in the PA group and in two cases (9.1) in the MA group; and the difference was statistically significant (P = 0.034). When the need for analgesics was considered, mean diclofenac sodium usage was 1.9 ± 1.5 times (range, 0-6 times) in the PA group, and 1.5 ± 1.1 times (range, 0-4 times) in the MA group. Mean pethidine HCL administration was 0.8 ± 1.1 times (range, 0-3 times) in the PA group, and 0.3 ± 0.6 times (range, 0-2 times) in the MA group. These differences between the groups were not statistically significant (P = 0.284, and P = 0.070, for diclofenac and pethidine, respectively) (Table 2). Urinary retention developed as an early complication in one patient in each group, and was relieved by urinary catheterization. Anal prolapse developed in one patient due to straining 8 h after the operation. Digital rectal examination revealed that the staple line was intact. Prolapsed mucosa was reduced, and gauze sponges were applied to avoid recurrence. Gauze sponges were removed 12 h later and the patient was warned about straining. The patient was free of complaints 24 h later. Anal prolapse did not recur and the patient had normal bowel movements. Bleeding occurred in five patients (33.3) in the PA group and in seven patients (31.8) in the MA group. Bleeding was minor in all cases and no additional treatment was required. Bleeding ceased spontaneously within 24 h in five patients in each group, and after 48 h in two patients in the MA group. No delayed haemorrhage occurred. When early complications were considered, there were no statistically significant differences between the two groups (P = 0.685). The mean hospitalization period was 1.7 ± 1.1 d (range, 1-3 d) in the PA group, and 1.5 ± 0.7 d (range, 1-3 d) in the MA group, and the difference was not statistically significant (P = 0.449) (Table 2). The surgeons ranked their performance as poor (n = 10), moderate (n = 4), and good (n = 1) in the PA group, and as good in all 22 operations in the MA group. The difference was statistically significant (P < 0.001). An anal fissure developed 6 mo after surgery in one patient in the PA group, and treatment with lateral internal sphincterotomy was unsuccessful. Thrombosed external haemorrhoids developed 1 mo after surgery in one patient in the MA group, and excision of the piles was performed under local anesthesia. A skin tag excision was performed in one patient in the MA group 7 mo postoperatively. When late complications in the two groups were considered, the difference was not statistically significant (P = 0.418). Histopathological evaluation of the donuts showed that the specimens from 13 patients in the PA group included only mucosa and submucosa. Additionally, a mucosal adenoma was reported in one patient, and muscularis propria in another patient in this group. In the MA group, 19 donuts included only mucosa and submucosa, two donuts also included muscularis propria, and one included a mucosal adenoma. There were no statistically significant differences between the two groups (P = 0.933).

Table 2 Operation time, need for analgesics, and length of hospital stay of the patients, and the differences between the two groups (mean ± SD)

	Group 1 (PA)	Group 2 (MA)	*P* value
Operation time (min)	42.0 ± 8.4	27.7 ± 8.0	0.039
Needs for analgesics (average dose)			
Diclofenac sodium	1.9 ± 1.5	1.5 ± 1.1	0.284
Pethidine HCl	0.8 ± 1.1	0.3 ± 0.6	0.070
Hospital stay (d)	1.7 ± 1.1	1.5 ± 0.7	0.449

DISCUSSION

Achieving a perfect purse-string may be difficult in stapled haemorrhoidopexy, even after meticulous application of the technique[1]. The surgeon starts by placing the purse-string suture at a distance of 3-4 cm above the dentate line

and just deep enough to take the mucosa and submucosa, however, if internal haemorrhoids obscure the surgical field and if the tip of the needle causes bleeding while passing through the mucosa, further bites of the suture may not pass at the same distance and at the same depth[10,13,14]. This may result in an uneven purse-string suture which is closer to the dentate line, goes through deeper tissues, or which skips the mucosa in some parts. Consequently, some parts of the stapler line may be closer to the dentate line, or some parts of the donut may be thicker, or it may not be intact[4,5,14]. A stapler line close to the dentate line may cause acute or persistent pain, or bring about a risk of incontinence[15,16]. It has been reported that patients with a stapler line uniformly 22 mm above the dentate line needed fewer postoperative narcotic analgesics and returned to work earlier[1]. In the case of an incomplete donut or a donut with thin parts, this may increase the risk of bleeding during the early postoperative period or recurrence in the late period[17].

Various modifications of the purse-string anoscope have been used for easier application of the suture and a new anoscope has been designed[10-12,18,19]. Yamamoto *et al*[12] indicated that they have been using their modified anoscope over the last 2 years. We have been using our modified anoscope since 2003.

This is the first study to compare the conventional anoscope, and a modified anoscope used during stapled haemorrhoidopexy. Although this study is limited by its retrospective nature, the two groups of patients were statistically comparable in terms of age, gender, stage of haemorrhoidal disease and previous treatment modalities.

Median operation time for stapled haemorrhoidopexy has been reported to be between 15 and 38 min (range, 5-150 min)[13,20-23]. Median operation time for the PA group in our study was higher than that reported in the literature; however, the range in operation time was similar. When the MA group is considered, both median operation time and median range were in accordance with the literature. There may be a number of explanations for the difference in operation time between the two groups in the present study. First of all, the PA group covered a time period where the surgeons who applied this method were less experienced. In addition, the purse-string suture had to be repeated several times in some patients in this group, and the number of patients who required haemostatic sutures was higher in this group. We propose that all of these factors prolonged the operation time. One explanation for the shorter operation time in the MA group may be that the application of the purse-string suture with the modified anoscope was much easier. It can also be said that the surgeons performed the operation in a shorter time, as they had gained more experience with the procedure. However, it should be emphasized that inexperienced surgeons performed some of the operations under the supervision of an experienced surgeon in this group. Moreover, the purse-string suture was possible on the first attempt in most patients, and haemostatic sutures were required in a smaller percentage of patients in this group (9.1% in the MA group, and 40% in the PA group). The necessity for haemostatic sutures was reported to be 84% in a study in which the time elapsed before and after firing the stapler was not noted[21]. Nahas *et al*[22] reported that they waited 30 s after firing the stapler and the necessity for haemostatic sutures was 20%. Racalbuto *et al*[20], in their study in which one min elapsed before and after firing the stapler, did not refer to the rate of haemostatic sutures, but reported the rate of bleeding as 6%. The PA group consisted of the initial patients who underwent haemorrhoidopexy surgery. Initially we paused for 30 s after firing the stapler for haemostasis. After the team gained some experience we increased the time before and after firing to 180 s considering that more efficient haemostasis could be achieved by longer compression on the vessels at the surgical site. When the two groups in the present study were compared with respect to the application of haemostatic sutures, it was observed that the number of patients who required additional sutures was significantly less in the MA group than in the PA group. This was an extra finding, and was not due to the features of the videoanoscope. A 3 min pause after closing the stapler may be tedious, but it is better than trying to apply additional haemostatic sutures if bleeding occurs after the stapler is removed. The haemostatic efficacy of keeping the stapler closed needs to be investigated by prospective controlled studies.

The rate of bleeding reportedly ranges between 5% and 85%[9,13,17,22]. The rates of bleeding in the PA group and in the MA group in the present study were 33.3% and 31.8%, respectively. Bleeding was minor in all cases and stopped in most patients within 48 h without any need for additional interventions. Urine retention rates observed in the PA and MA groups were in accordance with the literature[21]. We could not find a similar complication in the literature to the anal prolapse that we observed in one patient in the MA group. It did not seem to be due to anastomotic dehiscence because the stapler line was intact. The reasons for anal prolapse as an early postoperative complication may be due to excessive venous congestion and edema of internal haemorrhoids. Improvement of the clinical picture after reduction of the haemorrhoids confirmed this hypothesis.

The need for analgesics in each group was consistent with that reported in the literature[1,23]. None of the patients suffered from persistent pain. Hospital stay, reported in the literature as 0-2 d was similar to that in the present study[20]. When the two groups were compared in terms of late complications, there were no significant differences, also consistent with the literature[17,20,21,24]. Results of the histopathological examination of the specimens obtained in this study were similar to those reported in the literature, and no significant differences between the two groups in this study were noted[15].

Satisfaction of the surgeons regarding technical performance during surgery was significantly higher in the MA group than in the PA group. As this was a retrospective study, it was difficult for the surgeons to remember their initial operations and commented so.

The decisions made by the surgeons were based on a comparison of the two anoscopes as they performed surgery in both groups, and they described their operative performances as better with the modified anoscope. In the MA group, the surgeons who performed the procedure for the first time did not have the chance to compare the anoscopes, but they too described their operative performance as good or sufficient. As all the surgeons noted that they would prefer to use the modified anoscope in future operations, we have been using only the modified anoscope for 4 years. These results need to be supported by prospective randomized studies.

In conclusion, with the help of our modified anoscope, the purse-string suture is easy to perform, the operation is completed in a shorter time, and the surgeons were more satisfied with their operative performances. Additionally, by waiting a total of 3 min, 90 s before and after firing the stapler, fewer haemostatic sutures were required. Using the modified anoscope, optimum application of the purse-string suture is achieved during stapled haemorrhoidopexy.

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COMMENTS

Background

Modification of the purse-string anoscope was required to achieve an easier purse-string suture during stapled haemorrhoidopexy, because the purse-string anoscope could not prevent protrusion of large haemorrhoids.

Research frontiers

Stapled haemorrhoidopexy is not only a safe method that can be compared with conventional haemorrhoidectomy procedures, but also has advantages such as reduced postoperative pain and early return to work. However, some complications were noted after stapled haemorrhoidopexy. It was suggested that these serious complications could be due to technical errors and could be avoided by eliminating the bite of the suture in deep layers of the rectum. This study compared the surgical findings and early postoperative results of patients in whom the purse-string suture anoscope of the PPH kit was used, with patients in whom the modified anoscope was used during stapled haemorrhoidopexy.

Innovations and breakthroughs

Recent reports have highlighted the importance of purse-string suture during stapled haemorrhoidopexy. Various modifications of the purse-string anoscope have been used for easier application of the suture. We have been using our modified anoscope since 2003. This is the first study to compare the conventional anoscope with a modified anoscope during stapled haemorrhoidopexy.

Applications

Using the modified anoscope, optimum application of the purse string can be performed during stapled haemorrhoidopexy, and it may prevent serious complications of the procedure.

Terminology

Stapled haemorrhoidopexy is an alternative operative procedure for haemorrhoidal disease. It depends on the removal and the anastomosis of the mucosa above the haemorrhoidal tissue using the PPH kit or a circular stapler. Purse-string suture placement is an important part of the procedure. Anoscopes help to perform the purse string suture placement.

Peer review

The authors have compared modified anoscope for the application of the purse string to the standard purse string suture anoscope in the PPH kit. Although the technique using the modified anoscope is well described, this is the first paper comparing the 2 techniques. The manuscript is well written and flows well.

REFERENCES

1 **Plocek MD**, Kondylis LA, Duhan-Floyd N, Reilly JC, Geisler DP, Kondylis PD. Hemorrhoidopexy staple line height predicts return to work. *Dis Colon Rectum* 2006; **49**: 1905-1909

2 **Slawik S**, Kenefick N, Greenslade GL, Dixon AR. A prospective evaluation of stapled haemorrhoidopexy/rectal mucosectomy in the management of 3rd and 4th degree haemorrhoids. *Colorectal Dis* 2007; **9**: 352-356

3 **Beattie GC**, McAdam TK, McIntosh SA, Loudon MA. Day case stapled haemorrhoidopexy for prolapsing haemorrhoids. *Colorectal Dis* 2006; **8**: 56-61

4 **Wong LY**, Jiang JK, Chang SC, Lin JK. Rectal perforation: a life-threatening complication of stapled hemorrhoidectomy: report of a case. *Dis Colon Rectum* 2003; **46**: 116-117

5 **Aumann G**, Petersen S, Pollack T, Hellmich G, Ludwig K. Severe intra-abdominal bleeding following stapled mucosectomy due to enterocele: report of a case. *Tech Coloproctol* 2004; **8**: 41-43

6 **McDonald PJ**, Bona R, Cohen CR. Rectovaginal fistula after stapled haemorrhoidopexy. *Colorectal Dis* 2004; **6**: 64-65

7 **Grau LA**, Budó AH, Fantova MJ, Sala XS. Perirectal haematoma and hypovolaemic shock after rectal stapled mucosectomy for haemorrhoids. *Int J Colorectal Dis* 2005; **20**: 471-472

8 **McCloud JM**, Jameson JS, Scott AN. Life-threatening sepsis following treatment for haemorrhoids: a systematic review. *Colorectal Dis* 2006; **8**: 748-755

9 **Ravo B**, Amato A, Bianco V, Boccasanta P, Bottini C, Carriero A, Milito G, Dodi G, Mascagni D, Orsini S, Pietroletti R, Ripetti V, Tagariello GB. Complications after stapled hemorrhoidectomy: can they be prevented? *Tech Coloproctol* 2002; **6**: 83-88

10 **Bozdağ AD**. A modified anoscope to facilitate the purse-string suture for stapled hemorrhoidopexy. *Tech Coloproctol* 2005; **9**: 239-242

11 **Bozdag AD**, Nazli O, Tansug T, Derici H, Deniz V. Special anoscope for easy purse-string suture application in stapled hemorrhoidopexy. *World J Surg* 2007; **31**: 538-541

12 **Yamamoto J**, Nagai M, Smith TB, Tamaki S, Kubota T, Sasaki K, Ohmori T, Maeda K. Adaptation of the pursestring suture anoscope with a small hole in a case of stapled hemorrhoidectomy. *Dis Colon Rectum* 2006; **49**: 925-926

13 **Koh DC**, Cheong DM, Wong KS. Stapled haemorrhoidectomy: bothersome staple line bleeding. *Asian J Surg* 2005; **28**: 193-197

14 **Jayne DG**, Seow-Choen F. Modified stapled haemorrhoidopexy for the treatment of massive circumferentially prolapsing piles. *Tech Coloproctol* 2002; **6**: 191-193

15 **Shanmugam V**, Watson AJ, Chapman AD, Binnie NR, Loudon MA. Pathological audit of stapled haemorhoidopexy. *Colorectal Dis* 2005; **7**: 172-175

16 **Bulut A**, Evcimen S, Kaya O, Hoca O. Comparison of the Milligan Morgan hemorrhoidectomy and stapled hemorrhoidopexy for the treatment of the hemorrhoidal disease (in Turkish). *Turk J Surg* 2006; **22**: 67-71

17 **Au-Yong I**, Rowsell M, Hemingway DM. Randomised controlled clinical trial of stapled haemorrhoidectomy vs conventional haemorrhoidectomy; a three and a half year follow up. *Colorectal Dis* 2004; **6**: 37-38

18 **Carriero A**, Dal Borgo P, Pucciani F. Stapled mucosal prolapsectomy for haemorrhoidal prolapse with Lone Star Retractor System. *Tech Coloproctol* 2001; **5**: 41-46

19 **Regadas FS**, Regadas SM, Rodrigues LV, Misici R, Tramujas

I, Barreto JB, Lins MA, Silva FR, Filho FS. New devices for stapled rectal mucosectomy: a multicenter experience. *Tech Coloproctol* 2005; **9**: 243-246

20 **Racalbuto A**, Aliotta I, Corsaro G, Lanteri R, Di Cataldo A, Licata A. Hemorrhoidal stapler prolapsectomy vs. Milligan-Morgan hemorrhoidectomy: a long-term randomized trial. *Int J Colorectal Dis* 2004; **19**: 239-244

21 **Senagore AJ**, Singer M, Abcarian H, Fleshman J, Corman M, Wexner S, Nivatvongs S. A prospective, randomized, controlled multicenter trial comparing stapled hemorrhoidopexy and Ferguson hemorrhoidectomy: perioperative and one-year results. *Dis Colon Rectum* 2004; **47**: 1824-1836

22 **Nahas SC**, Borba MR, Brochado MC, Marques CF, Nahas CS, Miotto-Neto B. Stapled hemorrhoidectomy for the treatment of hemorrhoids. *Arq Gastroenterol* 2003; **40**: 35-39

23 **Habr-Gama A**, e Sous AH Jr, Roveló JM, Souza JV, Benício F, Regadas FS, Wainstein C, da Cunha TM, Marques CF, Bonardi R, Ramos JR, Pandini LC, Kiss D. Stapled hemorrhoidectomy: initial experience of a Latin American group. *J Gastrointest Surg* 2003; **7**: 809-813

24 **Fueglistaler P**, Guenin MO, Montali I, Kern B, Peterli R, von Flüe M, Ackermann C. Long-term results after stapled hemorrhoidopexy: high patient satisfaction despite frequent postoperative symptoms. *Dis Colon Rectum* 2007; **50**: 204-212

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BRIEF ARTICLE

Genetic and epigenetic characteristics of gastric cancers with JC virus T-antigen

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Abstract

AIM: To clarify the significance of JC virus (JCV) T-antigen (T-Ag) expression in human gastric cancer.

METHODS: We investigated the relationship between T-Ag detected by immunohistochemistry and Epstein-Barr virus (EBV) infection, microsatellite instability (MSI), and genetic and epigenetic alterations in gastric cancers. Mutations in the *p53*, *β-catenin*, *KRAS*, *BRAF*, *PIK3CA* genes were analyzed by polymerase chain reaction (PCR)-single strand conformation polymorphism and DNA sequencing. Allelic losses were determined by PCR at 7 microsatellite loci. Aberrant DNA methylation was analyzed by MethyLight assay.

RESULTS: JCV T-Ag protein expression was found in 49% of 90 gastric cancer tissues. T-Ag positivity was not correlated with clinicopathological characteristics. T-Ag expression was detected in a similar percentage of EBV positive cancers (4 of 9, 44%) and EBV negative cancers (35 of 73, 48%). T-Ag expression was detected in a significantly lower percentage of MSI-H cancers (14%) than in non MSI-H cancers (55%, P = 0.005). T-Ag expression was detected in a significantly higher percentage of cancers with nuclear/cytoplasmic localization of β-catenin (15 of 21, 71%) than in cancers without (42%, P = 0.018). *p53* mutations were detected in a significantly lower percentage of T-Ag positive cancers (32%) than in T-Ag negative cancers (57%, P = 0.018). T-Ag positive gastric cancers showed a significant increase in the allelic losses and aberrant methylation compared with T-Ag negative gastric cancers (P = 0.008 and P = 0.003).

CONCLUSION: The results suggest that JCV T-Ag is involved in gastric carcinogenesis through multiple mechanisms of genetic and epigenetic alterations.

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Key words: JC virus; T-antigen; Epstein-Barr virus; Microsatellite instability; Gastric cancer

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INTRODUCTION

Viruses have been proposed to play an etiologic role in human cancers. JC virus (JCV) is a polyomavirus that ubiquitously infects humans worldwide, and more than 80% of the adult population carries antibodies against the virus[1]. JCV has been implicated in various types of human cancers[2-9]. It has been reported that JCV

sequences are frequently present throughout the normal human gastrointestinal tract and in colorectal and gastric cancers[2-9].

JCV encodes a transforming gene, T-antigen (T-Ag), which is believed to mediate the oncogenic potential of the virus. T-Ag protein expression was specifically present in the nuclei of colon cancer cells, but not in any adjacent normal colonic epithelium[8]. Previous studies have identified multiple pathways including p53, pRb, IRS, NF1/NF2 and β-catenin, which may be dysregulated by T-Ag[4,10,11]. T-Ag can bind and inactivate p53, pRb, and the spindle assembly checkpoint protein Bub1, resulting in disruption of chromosomal integrity and cell cycle checkpoints[12,13]. T-Ag can also bind and stabilize β-catenin[4,11]. T-Ag protein expression, rather than the simple presence of JCV DNA sequences, has been significantly associated with chromosomal instability (CIN) and the methylator phenotype in colorectal cancers[8]. The possible involvement in aberrant methylation is thought to provide a secondary link to microsatellite instability (MSI) in colorectal cancers[8]. Therefore, it has been suggested that JCV is involved in colorectal cancers through multiple mechanisms of genetic and epigenetic alterations[8]. Bhattacharyya *et al*[14] recently reported the interplay between β-catenin and Rac1 that is initiated by T-Ag and results in stabilization of β-catenin and its presence in cell membrane ruffles. T-Ag and β-catenin synergistically activate Rac1, an event that can trigger several oncogenic factors[14].

The association between T-Ag expression and aberrant promoter methylation in colorectal cancers suggests that this viral oncogene induces the methylator phenotype[8]. Genomic methylation is a host defense mechanism that silences the transcription of transposons and retroviruses that have accumulated in the mammalian genome[8,15,16]. Methylation of host cell genes is not unique to JCV and occurs with other oncogenic viruses. It has been suggested that the key protein triggering methylation events for the polyomavirus SV40 is T-Ag, which mediates cellular transformation of cultured epithelial cells by regulating the activities of key *de novo* DNA methyltransferases, such as DNMT3b[8,17].

A significant correlation has been found between Epstein-Barr virus (EBV) and methylation of multiple genes in gastric cancers[18,19]. A mutually negative association between EBV and MSI has been reported in gastric cancers[20]. T-Ag protein expression was found in 9 (39%) of 23 gastric cancers, whereas no expression was observed in any of the non-cancer tissues[9]. In contrast to colorectal cancers[8], however, little is known about the significance of T-Ag expression in gastric cancers. In the current study, we investigated the relationship between T-Ag protein expression and EBV infection, MSI, and genetic and epigenetic alterations in gastric cancers.

MATERIALS AND METHODS

Tissue samples

A total of 90 paired specimens of gastric adenocarcinoma and adjacent noncarcinoma tissue were obtained from Japanese patients who had undergone surgical treatment. Informed consent was obtained from each patient. The tumor-node-metastasis (TNM) system of the American Joint Committee on Cancer and the International Union against Cancer was used for the pathologic diagnosis and classification of variables. Clinicopathological characteristics were as follows: age (69 ± 10 years), gender (59 male and 31 female), Lauren histology (44 intestinal and 46 diffuse), and pTNM stages (stage Ⅰ, 23; stage Ⅱ, 16; stage Ⅲ, 30; stage Ⅳ, 21).

Immunohistochemistry and in situ hybridization

Immunohistochemistry using a mouse monoclonal antibody against SV40 large T-Ag (clone PAb416, 1:100 dilution; Oncogene Research Products, San Diego, CA, USA), which cross-reacts with T-Ag of JCV, was performed as described previously[8]. Immunohistochemistry with an anti-human β-catenin monoclonal antibody (BD Transduction Laboratories, San Jose, CA, USA) was done as described previously[21]. Cancer cases were categorized into the following 4 groups corresponding to immunostaining patterns of β-catenin as described previously[21]: membranous, membranous staining pattern similar to that in normal epithelium; weak, no staining or weaker staining than normal epithelium; cytoplasmic, diffuse staining in the cytoplasm as well as at the cell membrane; accumulated, strong staining in the nucleus and cytoplasm. EBV infection was analyzed by *in situ* hybridization for *EBER-1*[20].

MSI analysis

MSI was analyzed by polymerase chain reaction (PCR) using the mononucleotide (BAT26 and BAT25) and dinucleotide markers (D2S123, D5S346, and D17S250) proposed by the NCI workshop[22]. Based on the number of markers showing instability per cancer, cancers were divided into 3 groups; those with 2 or more of the 5 markers displaying instability (MSI-H), those with 1 of 5 markers displaying instability (MSI-low; MSI-L), and those with no instability (microsatellite stable; MSS).

Mutation analysis

Mutations in exons 2-9 of *p53*, exon 3 of *β-catenin*, codons 12 and 13 of *KRAS*, codon 600 of *BRAF*, exons 9 and 20 of *PIK3CA* genes were analyzed by PCR-single strand conformation polymorphism and DNA sequencing as described previously[23-25]. Interstitial deletions spanning exon 3 of *β-catenin* was analyzed as described previously[24,25].

LOH analysis

LOH was analyzed as described previously[26]. Seven sets of microsatellite loci that are linked to tumor suppressor genes were used to identify significant allelic losses in gastric cancers. DNA was amplified by PCR at microsatellite loci linked to the *APC* locus on 5q21 (D5S505), possible tumor suppressor/senescence gene locus on 10p15 (D10S501 and D10S602), *p53* locus on 17p13 (TP53), *BRCA1* locus on 17q21 (D17S855), and *DCC* locus on 18q21 (D18S58 and D18S61)[26]. Assessment of LOH was assigned when a tumor allele showed at least a 50% reduction in the relative intensity

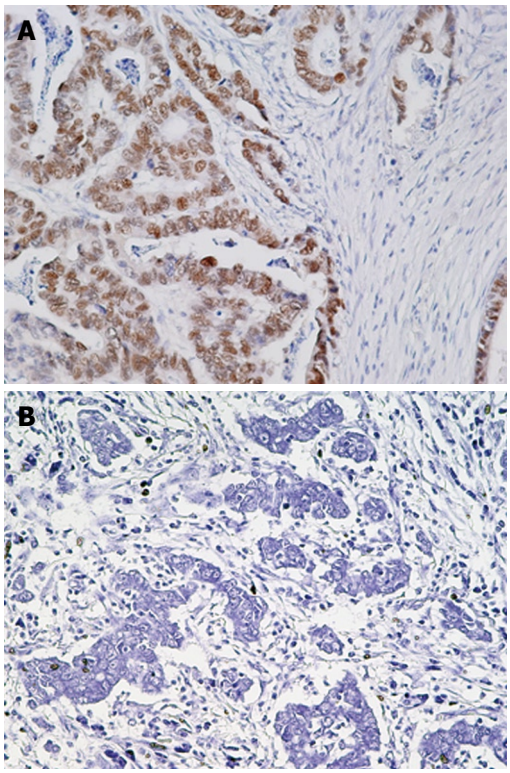

Figure 1 Immunohistochemistry for JCV T-Ag in gastric cancer tissues. A: Gastric adenocarcinoma positive for JCV T-Ag; B: Gastric adenocarcinoma negative for JCV T-Ag. Original magnification, × 200.

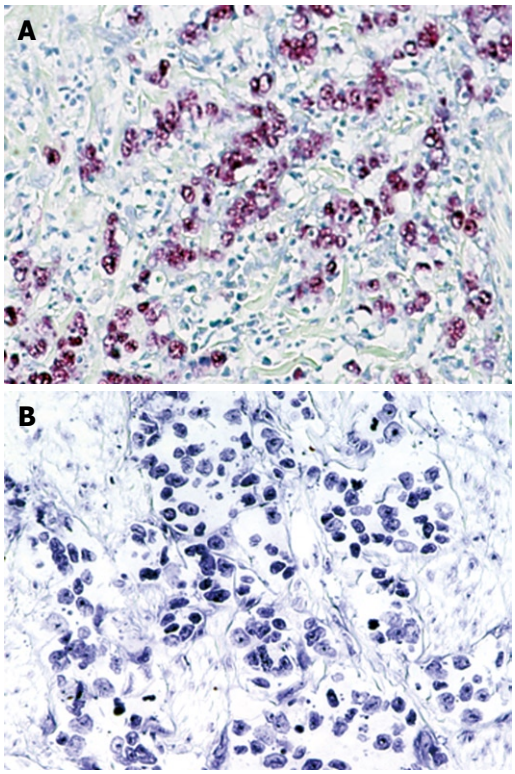

Figure 2 *In situ* hybridization for *EBER-1* in gastric cancer tissues. A: Gastric adenocarcinoma positive for *EBER-1*; B: Gastric adenocarcinoma negative for *EBER-1*. Original magnification, × 200.

of 1 allele in cancer tissue compared with the matched non-cancer DNA as described previously[26].

Quantitative DNA methylation analysis by real-time PCR (MethyLight assay)

Sodium bisulfite treatment of genomic DNA and MethyLight assay were performed as described previously[25,27-30]. We analyzed six promoters: calcium channel, voltage dependent, T type a-1G subunit (CACNA1G), cellular retinoic acid binding protein 1 (CRABP1), neurogenin 1 (NEUROG1), CDKN2A (p16), RUNX3, and SOCS1. Primers, probes, and percentage of methylated reference (PMR, i.e. degree of methylation) were described previously[29,30]. The cutoff value of 4 (except for 6 in *CRABP1*) was based on previously validated data[28].

Statistical analysis

The results were assessed for associations with clinicopathological parameters, using the following statistical tests: Student's *t* test for age, the Mann-Whitney test for depth of invasion, lymph node metastasis, and pTNM stage, and the chi-square test or Fisher's exact test for the remaining parameters. $P < 0.05$ was considered significant. A P value between 0.05 and 0.10 was considered as a trend toward an association.

RESULTS

T-Ag protein expression in gastric cancers

T-Ag protein expression was found in 44 (48.9%) of 90 gastric cancers, whereas no expression was observed in adjacent normal gastric epithelial cells or stromal cells (Figure 1 and data not shown). T-Ag positivity was not correlated with clinicopathological characteristics (data not shown).

EBV infection

EBER-1 expression was found in 9 (11.0%) of 82 gastric cancers, whereas no expression was observed in any of the non-cancer tissues (Figure 2). EBV infection was detected more frequently in diffuse type (7 of 44, 16%) than intestinal type (2 of 38, 5%), although this did not reach statistical significance. T-Ag expression was detected in a similar percentage of EBV positive cancers (4 of 9, 44%) and EBV negative cancers (35 of 73, 48%). The results for T-Ag protein expression and EBV infection were summarized in a Figure 3.

MSI status

Following the NCI criteria, a total of 90 gastric cancers was classified as follows: 14 (16%) MSI-H, 10 (11%) MSI-L, and 66 (73%) MSS (Figure 4). T-Ag expression was detected in a significantly lower percentage of MSI-H cancers (14%) than in non MSI-H cancers (55%, $P = 0.005$). None of the MSI-H tumors were EBV positive.

Alterations of β-catenin

Figure 5 shows representative results of immunohistochemistry for β-catenin in gastric cancer tissues. In normal gastric epithelium cells, β-catenin was moderately stained in a membranous distribution (Figure 5A), whereas fibroblasts, endothelial cells, and smooth muscle

Table 1 The exact mutations and their frequencies in gastric cancer tissues

p53 44% (40/90)	*n*		*n*		*n*	KRAS 6% (5/90)	*n*	BRAF 0% (0/90)	PIK3CA 4% (4/90)	*n*
G524A (R175H)	3	G469T (V157F)	2	G733A (G245S)	2	G35A (G12D)	3		A3140G (H1047R)	3
C742T (R248W)	2	G743A (R248Q)	2	C817T (R273C)	2	G35T (G12V)	2		G1624A (E542K)	1
G818A (R273H)	2	C844T (R282W)	2	C200G (P67R)	1					
G313T (G105C)	1	G422A (C141Y)	1	G461T (G154V)	1					
G473A (R158H)	1	C489G (Y163X)	1	G524T (R175L)	1					
C574T (Q192X)	1	A578G (H193R)	1	G586T (R196X)	1					
G587C (R196P)	1	A614G (Y205C)	1	C637T (R213X)	1					
G638A (R213Q)	1	G646A (V216M)	1	A659G (Y220C)	1					
G725T (C242F)	1	G731T (G244V)	1	G733T (G245V)	1					
C742G (R248G)	1	C817A (R273S)	1	T823G (C275G)	1					
C847T (R283C)	1									

Nucleotide substitution (amino acid change) is shown.

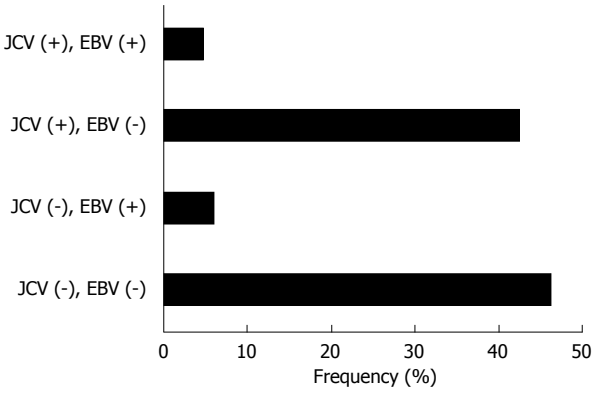

Figure 3 Classification of 90 gastric cancer tissues based on T-Ag protein expression and EBV infection.

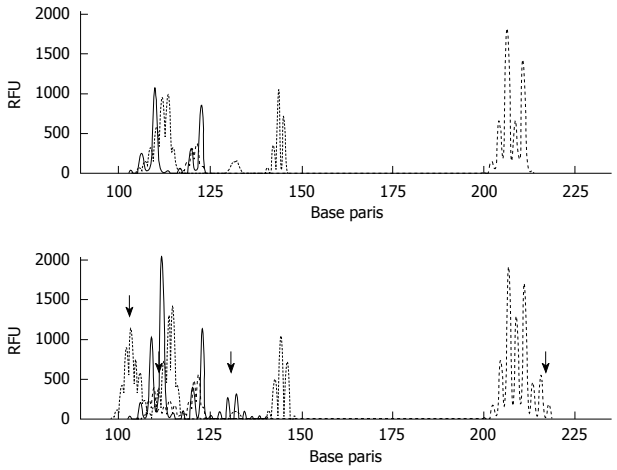

Figure 4 MSI analysis in gastric cancer tissues. MSI was analyzed by PCR using the mononucleotide (BAT26 and BAT25) and dinucleotide markers (D2S123, D5S346, and D17S250). Results of matched normal (upper panel) and tumor (bottom panel) samples (MSI-H) are shown. The arrows mean instability positive marker. RFU: Relative fluorescent units.

cells were negative. Membranous, weak, cytoplasmic, and accumulated pattern was observed in 36 (40%), 33 (37%), 11 (12%), and 10 (11%), respectively. Neither point mutations nor interstitial deletions in exon 3 were detected (data not shown). T-Ag expression was detected in a significantly higher percentage of cancers with nuclear/cytoplasmic localization of β-catenin (15 of 21, 71%) than in cancers without (42%, $P = 0.018$).

Mutations of the p53, KRAS, BRAF, PIK3CA genes in gastric cancer tissues

p53 mutations were detected in 40 (44%) of 90 gastric cancer tissues. *p53* mutations were detected in a significantly lower percentage of T-Ag positive cancers (32%) than in T-Ag negative cancers (57%, $P = 0.018$). *p53* mutations were detected in a significantly lower percentage of MSI-H cancers (14%) than in MSI-L cancers (60%, $P = 0.028$) or in MSS cancers (48%, $P = 0.021$). *KRAS* mutations were detected in only 5 (6%) of 90 gastric cancers. *BRAF* mutations were not detected. *PIK3CA* mutations were detected in 4 (4%) of 90 cancers. The exact mutations and their frequencies were described in a Table 1.

LOH analysis

Overall, 62 (69%) of 90 cancers had at least 1 LOH, with most frequent chromosomal losses observed on 17p (43%), followed by 18q (33%), 10p (27%), 5q (24%), and 17q (12%) (Figure 6A and B). Of 62 cancers with LOH, 4 (6%) were also MSI-H. The data were expressed in terms of an allelic loss index (ALI), which reflects the number of chromosomal losses per the number of chromosomal loci showing heterozygosity but not MSI in each cancer specimen. T-Ag positive gastric cancers showed a significant increase in the ALI compared with T-Ag negative gastric cancers (0.41 ± 0.12 *vs* 0.21 ± 0.09, $P = 0.008$).

Methylation analysis

The results were summarized based on each individual marker (Figure 7A and B). The data were expressed in terms of a methylation index (MI), which reflects the number of abnormally methylated promoters per cancer specimen within the subsets of cancers. T-Ag positive gastric cancers showed a significant increase in the MI compared with T-Ag negative gastric cancers (3.01 ± 0.92 *vs* 1.51 ± 0.71, $P = 0.003$).

DISCUSSION

In the current study, T-Ag protein expression was positive in 44 (49%) of 90 gastric cancer tissues and it was observed specifically in gastric cancer cells but not in

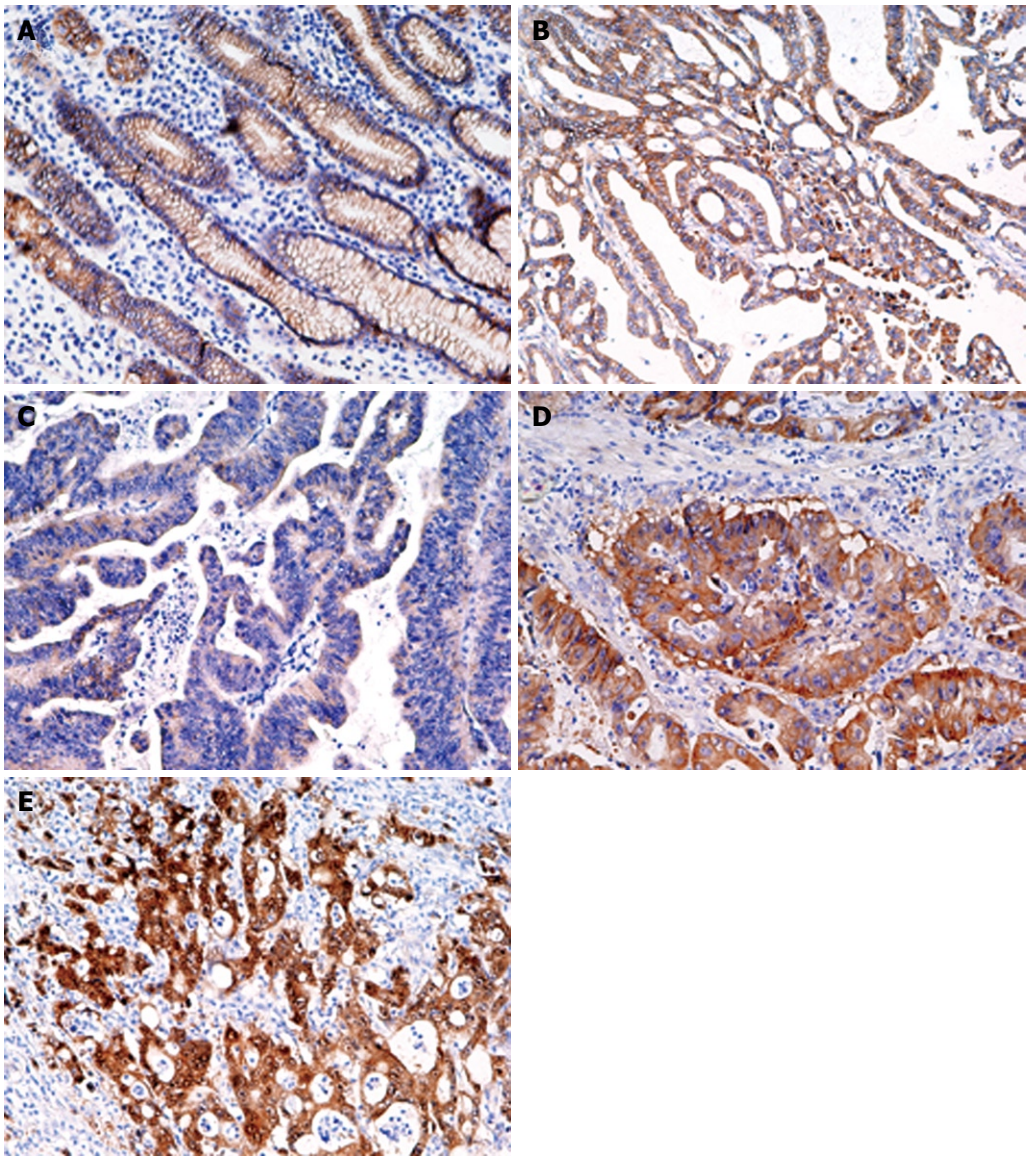

Figure 5 Immunohistochemistry for β-catenin in gastric normal (A) and cancer (B-E) tissues. A: Moderate membrane staining; B: Membrane staining pattern similar to that seen in normal epithelium; C: Weak staining; D: Diffuse staining in the cytoplasm and membrane; E: Strong staining of the nucleus and cytoplasm (Original magnification, × 200).

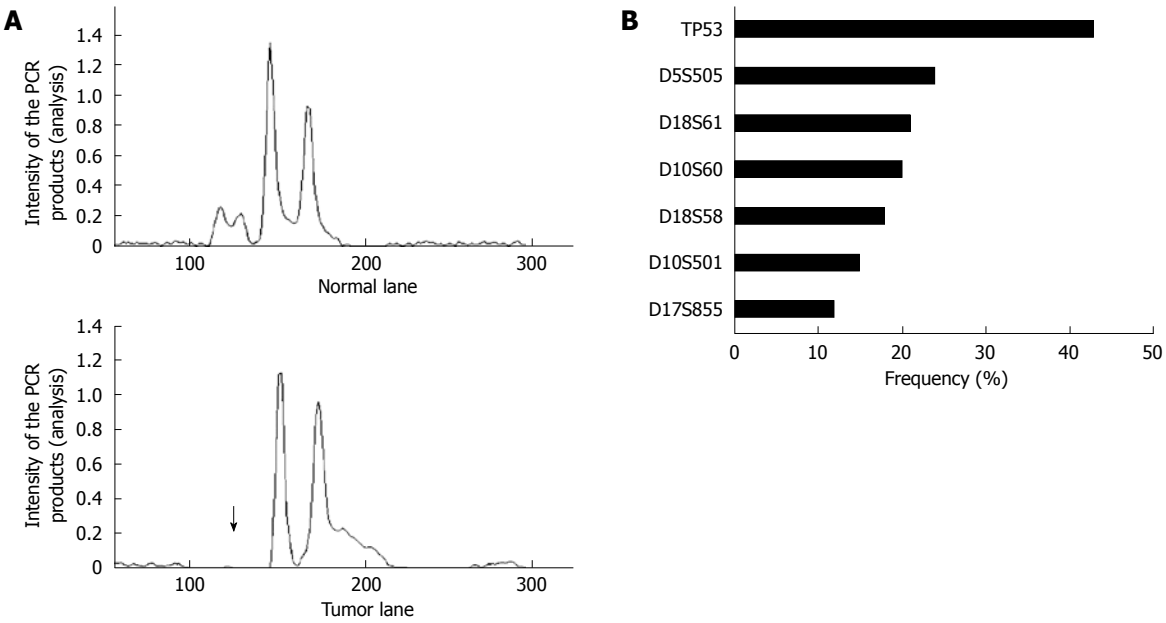

Figure 6 LOH analysis in gastric cancer tissues. A: A representative example of a tumor with allelic loss in 5q21 (D5S505) is shown. The upper and bottom panels show the intensity plots of both the normal and tumor lane, respectively, demonstrating reduced relative intensity of allele one in the tumor sample (arrow) compared to the corresponding normal sample; B: LOH frequencies based on each individual marker.

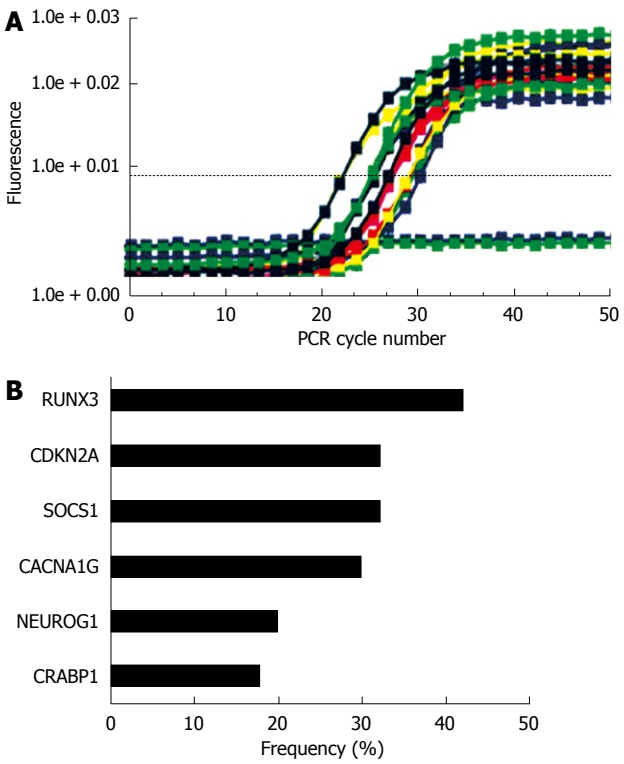

Figure 7 MethyLight analysis in gastric cancer tissues. A: Results of the *CRABP1* gene are shown. Bisulfite-converted DNA was used for quantitative methylation-specific PCR. The amount of hypermethylated DNA was determined by reading the midpoint of the linear portion of the S-shaped real-time curves, called the Ct point or threshold cycle. The Ct refers to the number of cycles it takes a sample to reach a specific fluorescence threshold; B: DNA methylation frequencies based on each individual marker.

the adjacent normal gastric epithelial cells or stromal cells. Previous study has also shown that T-Ag protein expression was found in 9 (39%) of 23 gastric cancers, whereas no expression was observed in any of the non-cancer tissues[9]. These results suggest an active role for this oncogenic protein in gastric carcinogenesis[9].

The frequencies of EBV infection (11%) and MSI-H (16%) in this study were almost consistent with those reported in previous studies[20,31,32]. None of the MSI-H cancers were EBV positive. A mutually negative association between EBV and MSI has been reported in gastric cancers[20]. T-Ag was detected in a significantly lower percentage of MSI-H cancers (14%) than in non MSI-H cancers (55%). In contrast, T-Ag expression was present in similar frequencies in MSI-H and (8/15, 53%) and MSS/MSI-L (35/85, 41%) colorectal cancers[8]. From the view point of MSI, these results suggest a differential role of T-Ag in gastric and colorectal carcinogenesis.

T-Ag expression was detected in a significantly higher percentage of cancers with nuclear/cytoplasmic localization of β-catenin than in cancers without. T-Ag interacts with β-catenin, leading to its stabilization and resultant dysregulation of the WNT-signaling pathway in gastrointestinal cancers[4]. Neither point mutations nor interstitial deletions in exon 3 were detected. Although not analyzed in this study, the frequency of *APC* mutations is relatively low in gastric cancers. Therefore, T-Ag may play an important role in the stabilization of β-catenin in gastric cancers.

p53 mutation was detected in a significantly lower percentage of T-Ag positive cancers (32%) than in T-Ag negative cancers (57%). T-Ag can bind and inactivate p53[12], resulting in disruption of chromosomal integrity and cell cycle checkpoints. Therefore, T-Ag expression may eliminate the selective pressure for *p53* mutations in a subset of gastric cancers.

A significant association was observed between T-Ag expression and allelic losses, being consistent with that in colorectal cancers[8]. It has been previously reported that the introduction of JCV into a diploid cell line leads to the rapid induction of CIN[33]. JCV can also induce CIN in a diploid colon cancer cell line RKO, which has wild-type *APC*, *p53*, and *β-catenin* genes[33]. These results suggest that T-Ag play a role in genomic damage during gastric carcinogenesis.

Significant association was also observed between T-Ag expression and aberrant methylation, being consistent with that in colorectal cancers[8]. A significant correlation has been found between EBV and methylation of multiple genes in gastric cancers[8,18,19]. These results suggest that JCV T-Ag and EBV play a similar role, at least in part, during gastric carcinogenesis.

Taken together, our results suggest that JCV T-Ag is involved in gastric cancers through multiple mechanisms of genetic and epigenetic alterations[34]. Further analysis is required to determine whether there is a plausible molecular mechanism by which JCV can induce genetic and epigenetic alterations in gastric carcinogenesis.

COMMENTS

Background

Gastric cancer is one of the most common cancer and leading cause of cancer-related death in the world. Understanding the molecular biological features of gastric cancer is necessary for early diagnosis and better prognosis. The potential role of viral infection in human cancer is receiving increasing attention.

Research frontiers

A significant correlation has been found between Epstein-Barr virus (EBV) and methylation of multiple genes in gastric cancer. However, little is known about the significance of JC *virus* (JCV) T-antigen (T-Ag) expression in gastric cancers. In this study, the authors demonstrate that JCV T-Ag plays a key role in gastric carcinogenesis.

Innovations and breakthroughs

This is the first study to report that JCV T-Ag plays a key role in gastric carcinogenesis. Furthermore, T-Ag expression was associated with nuclear/cytoplasmic localization of β-catenin, allelic losses and aberrant DNA methylation, suggesting that JCV T-Ag is involved in gastric carcinogenesis through multiple mechanisms of genetic and epigenetic alterations.

Applications

JCV T-Ag expression could be future diagnostic and/or therapeutic targets in clinical settings. Understanding of a plausible molecular mechanism by which JCV can induce genetic and epigenetic alterations may represent a future strategy for therapeutic intervention in the treatment of patients with gastric cancer.

Terminology

JCV: JCV is a polyomavirus that ubiquitously infects humans worldwide, and more than 80% of the adult population carries antibodies against the virus. EBV: EBV is a double-stranded DNA virus and takes a linear form in the viral particles. EBV was the first virus discovered from human neoplastic cells, a Burkitt's lymphoma cell lines. Microsatellite instability (MSI): MSI is a type of genetic instability characterized by length alterations within simple repeated microsatellite sequences.

Peer review

This paper reports the characteristics of gastric cancer with JCV T-Ag expression. The authors showed that JCV T-Ag plays a key role in gastric carcinogenesis. The study sounds interesting and the information could be useful for other gastric cancer researchers.

REFERENCES

1 **Major EO**, Amemiya K, Tornatore CS, Houff SA, Berger JR. Pathogenesis and molecular biology of progressive multifocal leukoencephalopathy, the JC virus-induced demyelinating disease of the human brain. *Clin Microbiol Rev* 1992; **5**: 49-73

2 **Laghi L**, Randolph AE, Chauhan DP, Marra G, Major EO, Neel JV, Boland CR. JC virus DNA is present in the mucosa of the human colon and in colorectal cancers. *Proc Natl Acad Sci USA* 1999; **96**: 7484-7489

3 **Ricciardiello L**, Laghi L, Ramamirtham P, Chang CL, Chang DK, Randolph AE, Boland CR. JC virus DNA sequences are frequently present in the human upper and lower gastrointestinal tract. *Gastroenterology* 2000; **119**: 1228-1235

4 **Enam S**, Del Valle L, Lara C, Gan DD, Ortiz-Hidalgo C, Palazzo JP, Khalili K. Association of human polyomavirus JCV with colon cancer: evidence for interaction of viral T-antigen and beta-catenin. *Cancer Res* 2002; **62**: 7093-7101

5 **Khalili K**, Del Valle L, Otte J, Weaver M, Gordon J. Human neurotropic polyomavirus, JCV, and its role in carcinogenesis. *Oncogene* 2003; **22**: 5181-5191

6 **Niv Y**, Goel A, Boland CR. JC virus and colorectal cancer: a possible trigger in the chromosomal instability pathways. *Curr Opin Gastroenterol* 2005; **21**: 85-89

7 **Casini B**, Borgese L, Del Nonno F, Galati G, Izzo L, Caputo M, Perrone Donnorso R, Castelli M, Risuleo G, Visca P. Presence and incidence of DNA sequences of human polyomaviruses BKV and JCV in colorectal tumor tissues. *Anticancer Res* 2005; **25**: 1079-1085

8 **Goel A**, Li MS, Nagasaka T, Shin SK, Fuerst F, Ricciardiello L, Wasserman L, Boland CR. Association of JC virus T-antigen expression with the methylator phenotype in sporadic colorectal cancers. *Gastroenterology* 2006; **130**: 1950-1961

9 **Shin SK**, Li MS, Fuerst F, Hotchkiss E, Meyer R, Kim IT, Goel A, Boland CR. Oncogenic T-antigen of JC virus is present frequently in human gastric cancers. *Cancer* 2006; **107**: 481-488

10 **Shollar D**, Del Valle L, Khalili K, Otte J, Gordon J. JCV T-antigen interacts with the neurofibromatosis type 2 gene product in a transgenic mouse model of malignant peripheral nerve sheath tumors. *Oncogene* 2004; **23**: 5459-5467

11 **Gan DD**, Khalili K. Interaction between JCV large T-antigen and beta-catenin. *Oncogene* 2004; **23**: 483-490

12 **Ludlow JW**. Interactions between SV40 large-tumor antigen and the growth suppressor proteins pRB and p53. *FASEB J* 1993; **7**: 866-871

13 **Cotsiki M**, Lock RL, Cheng Y, Williams GL, Zhao J, Perera D, Freire R, Entwistle A, Golemis EA, Roberts TM, Jat PS, Gjoerup OV. Simian virus 40 large T antigen targets the spindle assembly checkpoint protein Bub1. *Proc Natl Acad Sci USA* 2004; **101**: 947-952

14 **Bhattacharyya R**, Noch EK, Khalili K. A novel role of Rac1 GTPase in JCV T-antigen-mediated beta-catenin stabilization. *Oncogene* 2007; **26**: 7628-7636

15 **Bestor TH**. The host defence function of genomic methylation patterns. *Novartis Found Symp* 1998; **214**: 187-195; discussion 195-199, 228-232

16 **Doerfler W**. DNA methylation: eukaryotic defense against the transcription of foreign genes? *Microb Pathog* 1992; **12**: 1-8

17 **Soejima K**, Fang W, Rollins BJ. DNA methyltransferase 3b contributes to oncogenic transformation induced by SV40T antigen and activated Ras. *Oncogene* 2003; **22**: 4723-4733

18 **Kang GH**, Lee S, Kim WH, Lee HW, Kim JC, Rhyu MG, Ro JY. Epstein-barr virus-positive gastric carcinoma demonstrates frequent aberrant methylation of multiple genes and constitutes CpG island methylator phenotype-positive gastric carcinoma. *Am J Pathol* 2002; **160**: 787-794

19 **Kusano M**, Toyota M, Suzuki H, Akino K, Aoki F, Fujita M, Hosokawa M, Shinomura Y, Imai K, Tokino T. Genetic, epigenetic, and clinicopathologic features of gastric carcinomas with the CpG island methylator phenotype and an association with Epstein-Barr virus. *Cancer* 2006; **106**: 1467-1479

20 **Chang MS**, Lee HS, Kim HS, Kim SH, Choi SI, Lee BL, Kim CW, Kim YI, Yang M, Kim WH. Epstein-Barr virus and microsatellite instability in gastric carcinogenesis. *J Pathol* 2003; **199**: 447-452

21 **Sasaki Y**, Morimoto I, Kusano M, Hosokawa M, Itoh F, Yanagihara K, Imai K, Tokino T. Mutational analysis of the beta-catenin gene in gastric carcinomas. *Tumour Biol* 2001; **22**: 123-130

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

23 **Kahlenberg MS**, Stoler DL, Basik M, Petrelli NJ, Rodriguez-Bigas M, Anderson GR. p53 tumor suppressor gene status and the degree of genomic instability in sporadic colorectal cancers. *J Natl Cancer Inst* 1996; **88**: 1665-1670

24 **Mikami M**, Nosho K, Yamamoto H, Takahashi T, Maehata T, Taniguchi H, Adachi Y, Imamura A, Fujita M, Hosokawa M, Itoh F, Imai K, Shinomura Y. Mutational analysis of beta-catenin and the RAS-RAF signalling pathway in early flat-type colorectal tumours. *Eur J Cancer* 2006; **42**: 3065-3072

25 **Nosho K**, Yamamoto H, Takahashi T, Mikami M, Taniguchi H, Miyamoto N, Adachi Y, Arimura Y, Itoh F, Imai K, Shinomura Y. Genetic and epigenetic profiling in early colorectal tumors and prediction of invasive potential in pT1 (early invasive) colorectal cancers. *Carcinogenesis* 2007; **28**: 1364-1370

26 **Hiyama T**, Tanaka S, Yoshihara M, Sasao S, Kose K, Shima H, Tuncel H, Ueno Y, Ito M, Kitadai Y, Yasui W, Haruma K, Chayama K. Chromosomal and microsatellite instability in sporadic gastric cancer. *J Gastroenterol Hepatol* 2004; **19**: 756-760

27 **Takahashi T**, Nosho K, Yamamoto H, Mikami M, Taniguchi H, Miyamoto N, Adachi Y, Itoh F, Imai K, Shinomura Y. Flat-type colorectal advanced adenomas (laterally spreading tumors) have different genetic and epigenetic alterations from protruded-type advanced adenomas. *Mod Pathol* 2007; **20**: 139-147

28 **Ogino S**, Kawasaki T, Brahmandam M, Cantor M, Kirkner GJ, Spiegelman D, Makrigiorgos GM, Weisenberger DJ, Laird PW, Loda M, Fuchs CS. Precision and performance characteristics of bisulfite conversion and real-time PCR (MethyLight) for quantitative DNA methylation analysis. *J Mol Diagn* 2006; **8**: 209-217

29 **Weisenberger DJ**, Siegmund KD, Campan M, Young J, Long TI, Faasse MA, Kang GH, Widschwendter M, Weener D, Buchanan D, Koh H, Simms L, Barker M, Leggett B, Levine J, Kim M, French AJ, Thibodeau SN, Jass J, Haile R, Laird PW. CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. *Nat Genet* 2006; **38**: 787-793

30 **Kawasaki T**, Ohnishi M, Suemoto Y, Kirkner GJ, Liu Z, Yamamoto H, Loda M, Fuchs CS, Ogino S. WRN promoter methylation possibly connects mucinous differentiation, microsatellite instability and CpG island methylator phenotype in colorectal cancer. *Mod Pathol* 2008; **21**: 150-158

31 **Yamamoto H**, Imai K, Perucho M. Gastrointestinal cancer of the microsatellite mutator phenotype pathway. *J Gastroenterol* 2002; **37**: 153-163

32 **Imai K**, Yamamoto H. Carcinogenesis and microsatellite instability: the interrelationship between genetics and epigenetics. *Carcinogenesis* 2008; **29**: 673-680

33 **Ricciardiello L**, Baglioni M, Giovannini C, Pariali M, Cenacchi G, Ripalti A, Landini MP, Sawa H, Nagashima K, Frisque RJ, Goel A, Boland CR, Tognon M, Roda E, Bazzoli F. Induction of chromosomal instability in colonic cells by the human polyomavirus JC virus. *Cancer Res* 2003; **63**: 7256-7262

34 **Ottini L**, Falchetti M, Lupi R, Rizzolo P, Agnese V, Colucci G, Bazan V, Russo A. Patterns of genomic instability in gastric cancer: clinical implications and perspectives. *Ann Oncol* 2006; **17** Suppl 7: vii97-vii102

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BRIEF ARTICLE

Unsedated transnasal small-caliber esophagogastroduodenoscopy in elderly and bedridden patients

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Abstract

AIM: To evaluate the safety of unsedated transnasal small-caliber esophagogastroduodenoscopy (EGD) for elderly and critically ill bedridden patients.

METHODS: One prospective randomized comparative study and one crossover comparative study between transnasal small-caliber EGD and transoral conventional EGD was done (Study 1). For the comparative study, we enrolled 240 elderly patients aged > 65 years old. For the crossover analysis, we enrolled 30 bedridden patients with percutaneous endoscopic gastrostomy (PEG) (Study 2). We evaluated cardiopulmonary effects by measuring arterial oxygen saturation (SpO_2) and calculating the rate-pressure product (RPP) (pulse rate × systolic blood pressure/100) at baseline, 2 and 5 min after endoscopic intubation in Study 1. To assess the risk for endoscopy-related aspiration pneumonia during EGD, we also measured blood leukocyte counts and serum C-reactive protein (CRP) levels before and 3 d after EGD in Study 2.

RESULTS: In Study 1, we observed significant decreases in SpO_2 during conventional transoral EGD, but not during transnasal small-caliber EGD (0.24% *vs* -0.24% after 2 min, and 0.18% *vs* -0.29% after 5 min, P = 0.034, P = 0.044). Significant differences of the RPP were not found between conventional transoral and transnasal small-caliber EGD. In Study 2, crossover analysis showed statistically significant increases of the RPP at 2 min after intubation and the end of endoscopy (26.8 and 34.6 *vs* 3.1 and 15.2, P = 0.044, P = 0.046), and decreases of SpO_2 (-0.8% *vs* -0.1%, P = 0.042) during EGD with transoral conventional in comparison with transnasal small-caliber endoscopy. Thus, for bedridden patients with PEG feeding, who were examined in the supine position, transoral conventional EGD more severely suppressed cardiopulmonary function than transnasal small-caliber EGD. There were also significant increases in the markers of inflammation, blood leukocyte counts and serum CRP values, in bedridden patients after transoral conventional EGD, but not after transnasal small-caliber EGD performed with the patient in the supine position. Leukocyte count increased from 6053 ± 1975/L to 6900 ± 3392/L (P = 0.0008) and CRP values increased from 0.93 ± 0.24 to 2.49 ± 0.91 mg/dL (P = 0.0005) at 3 d after transoral conventional EGD. Aspiration pneumonia, possibly caused by the endoscopic examination, was found subsequently in two of 30 patients after transoral conventional EGD.

CONCLUSION: Transnasal small-caliber EGD is a safer method than transoral conventional EGD in critically ill, bedridden patients who are undergoing PEG feeding.

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Key words: Aged; Aspiration pneumonia; Gastrointestinal endoscopy; Critical illness

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INTRODUCTION

Small-caliber endoscopy of the upper gastrointestinal tract was developed and for transnasal esophagogastro duodenoscopy (EGD) and has been used frequently in the past decade[1-4]. Transnasal small-caliber EGD has improved the safety of the endoscopic examination, and has fewer adverse effects on cardiopulmonary function[5-10] and autonomic nerve function[11], compared with transoral conventional EGD. Transnasal small-caliber EGD provides good operability compared to that of transoral conventional EGD[12], and requires no special training[13]. For these reasons, unsedated transnasal small-caliber EGD is used routinely in endoscopic examinations of the upper gastrointestinal tract, often in preference to transoral conventional EGD.

Although we perform endoscopy on elderly and critically ill patients with increasing frequency, we have little information concerning the relative safety of transnasal small-caliber and transoral conventional EGD in these patient groups. Elderly patients do not frequently gag or choke during transoral conventional EGD. If transoral conventional EGD is safe and comfortable for the elderly, the value of transnasal small-caliber EGD is limited, since the smaller charge-coupled device in the endoscope may potentially reduce diagnostic accuracy for minute gastric lesions. The comparative safety of the two techniques should therefore be considered carefully for the treatment of elderly patients with higher risks for gastric cancer and cardiopulmonary diseases.

We compared the safety and tolerability of transnasal and transoral EGD in patients aged > 65 years old and in chronically bedridden elderly patients who required percutaneous endoscopic gastrostomy (PEG) feeding. We used a prospective comparative study and a crossover analysis to evaluate changes in hemodynamic and pulmonary function, and the risk of endoscopy-related aspiration pneumonia during EGD by each method.

MATERIALS AND METHODS

These following prospective and crossover studies were conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of Izumo City General Medical Center. In Study 1, we obtained written informed consent from all the participants. In Study 2, written informed consent was obtained from the key family members of the enrolled patients.

Study 1

We compared changes in cardiopulmonary function during unsedated transnasal small-caliber and transoral conventional EGD in patients aged > 65 years old.

Patients: Between July 2006 and April 2007, we enrolled 240 elderly patients (> 65 years old), who received EGD for their abdominal symptoms and for their annual medical check in the Izumo City General Medical Center, into the prospective comparison study. The first consecutive 120 patients were assigned to the transnasal small-caliber EGD (TN) group and the second consecutive 120 patients to the transoral conventional EGD (TO) group.

Endoscopic procedure: A single experienced endoscopist (M.Y.), certified by the Japan Gastroenterological Endoscopy Society, performed all of the examinations. Patients in the TN group were examined with a small-caliber videoendoscope (EG-530N; Fujinon Toshiba ES Systems Co. Ltd., Tokyo, Japan), 5.9 mm in diameter. Patients in the TO group were examined with a conventional endoscope (GIF-H260; Olympus Medical Systems, Tokyo, Japan), 9.8 mm in diameter. Patients who needed endoscopic biopsy and/or received a time-consuming chromoendoscopic examination were excluded from this study.

Patients in the TO group received throat anesthesia by application of 5 mL 2% lidocaine viscous (Xylocaine viscous; Astra Zeneca, Osaka, Japan) for 5 min. Nasal anesthesia was started in patients in the TN group by spraying a solution of 0.4% lidocaine and 0.5% naphazoline into the nostril. The patients were then instructed to inhale 2% lidocaine jelly (Xylocaine jelly; Astra Zeneca), and a 20 Fr catheter covered with lidocaine jelly was inserted into the deeper nasal cavity for 5 min. All the EGDs were performed with the patient in the left lateral recumbent position, and without administration of scopolamine butylbromide.

Cardiopulmonary monitoring: Systolic and diastolic blood pressure (BP), pulse rate (PR), and oxygen saturation by pulse oximetry (SpO_2) were measured at the following four points during the endoscopic examination, to measure these parameters at the stabilized condition of patients: (1) baseline; (2) at 2 min and (3) at 5 min after endoscopic intubation; and (4) at the end of the examination. The values of BP, PR and SpO_2 were measured simultaneously with high sensitivity by the newly developed measuring system, Bedside Monitor PetiTelemo DS-7001 (Fukuda Denshi Co. Ltd., Tokyo, Japan). The rate-pressure product (PR × systolic BP/100) was also calculated as shown in previous reports[14,15]. Changes in each value were compared between TN and TO groups.

Study 2

In the patients treated with PEG tube feeding, we performed periodic PEG tube exchanges during endoscopic observation every 6 mo. For these observations, an endoscopic method, transoral conventional or transnasal small-caliber was selected randomly for each patient by an envelop method, and switched at the next exchange (a

crossover design). Changes in cardiopulmonary parameters and markers of inflammation were measured during EGD, and blood leukocyte counts and serum C-reactive protein (CRP) concentrations were measured before and after EGD.

Patients: Between March 2007 and April 2008, 30 patients who were undergoing PEG feeding were enrolled. Reasons for PEG feeding included dysphagia caused by cerebral infarction, cerebral hemorrhage, subarachnoid hemorrhage, and other conditions. Their PEG tube exchanges were carried out routinely under endoscopic observation every 6 mo, using an instrument (transnasal small-caliber or transoral conventional endoscope) that was assigned randomly to each patient at enrollment. At the next tube exchange 6 mo later, the alternate method was used for each patient. The 30 patients were thereby divided into one initially transnasal, secondarily transoral (TN-TO, n = 15) group, and one initially transoral, secondarily transnasal (TO-TN, n = 15) group. In all of the enrolled patients, a bumper type kit (Ponsky Non-Balloon Replacement Gastrostomy Tube; Bard Limited, Salt Lake City, UT, USA) was used as a PEG tube. Each procedure for the PEG tube exchanges was not done when the patients had infectious diseases such as pneumonia, or urinary or hepato-biliary tract infection. Before PEG tube exchange, chest X-ray examination, urinalysis, and laboratory blood tests including peripheral blood count, inflammatory markers and hepato-biliary enzymes were done to confirm the absence of infectious diseases. In this study, PEG tube exchanges were not performed in patients who showed increases of leukocyte count or CRP and fever elevation before the procedure.

Endoscopic procedure: A single experienced endoscopist (M.Y., as in Study 1) performed all of the examinations, using the same endoscopes and pre-medication regimens as in Study 1. The procedure for PEG tube exchange by transnasal endoscope was similar to that by transoral endoscope. All the EGDs were performed with the patient in the supine position, and without administration of scopolamine butylbromide.

Cardiopulmonary monitoring: During each endoscopy procedure, systolic and diastolic BP, PR, SpO_2 and RPP were determined at the following three points: (1) baseline; (2) at 2 min after endoscopic intubation; and (3) at the end of the examination. Changes in each value were compared between TN and TO endoscopic studies.

Evaluation of inflammatory response: As markers of inflammation, body temperature, leukocyte counts and serum concentrations of CRP were measured. Body temperature was measured before and every 6 h after the endoscopy-guided exchange of PEG tube. Leukocyte counts and CRP were performed routinely before and 3 d after the procedure. If suspicious pneumonia was found, these parameters were examined more frequently. Further examination of pneumonia such as chest X-ray examination and/or computed tomography (CT) was done when respiratory symptoms, fever elevation, and increased leukocyte count and/or CRP were found.

Table 1 Characteristics of patients enrolled in Study 1 (mean ± SD)

	TN group	TO group	*P* value
Age (range, yr)	77.0 ± 7.7 (65-97)	76.7 ± 7.2 (65-95)	NS
Gender (M:F)	30:66	53:49	NS
Total procedure time (min)	8.46 ± 4.32	6.03 ± 2.50	0.001
Baseline cardiopulmonary parameters			
Systolic BP	131.4 ± 19.3	135.4 ± 21.8	NS
Diastolic BP	67.6 ± 12.3	70.5 ± 12.8	NS
RPP	100.2 ± 27.4	98.82 ± 25.7	NS
SpO_2	98.1 ± 1.4	98.2 ± 1.4	NS

NS: Not significant; M: Male; F: Female.

Increased occurrence of aspiration pneumonia: The incidence of aspiration pneumonia was also compared between the TN and TO groups. Pulmonary aspiration was defined by witnessed aspiration or tracheal suctioning of secretions including saliva as shown by previous studies on PEG procedures[16,17]. In patients with post-procedure elevation of inflammation markers and/or fever, aspiration pneumonia was evaluated by chest X-ray examination and/or CT. Pneumonia was diagnosed by the presence of new infiltrates of the lung associated with fever.

Statistical analysis

Data for each comparison between groups were analyzed using the χ^2 and Wilcoxon signed rank tests. The latter test was done only when the Friedman test showed significant differences. Categorical data were compared using Student's t test or, where unequal variances occurred, Welch's test. $P < 0.05$ was considered to be significant. All statistical analyses were performed using SPSS version 12.0 (SPSS Japan, Tokyo, Japan).

RESULTS

Study 1

Ninety-six and 102 elderly patients were analyzed finally as the TN and TO groups, respectively. Twenty-four and 18 patients were excluded since complete data could not be obtained, mainly because of the delay in measurement in the TN and TO groups, respectively. Characteristics of the enrolled patients in each group are shown in Table 1. Mean ages of these patients were 77.0 and 76.7 years, respectively. Total procedure time for EGD was 8.46 ± 4.32 min in the TN group, and 6.03 ± 2.50 min in the TO group. This difference in procedure time was statistically significant ($P < 0.001$). No serious complications in cardiopulmonary function occurred in this study, although two patients experienced mild epistaxis during pre-medication for transnasal EGD. The baseline cardiopulmonary parameters, RPP and SpO_2, did not show any significant differences (Table 1).

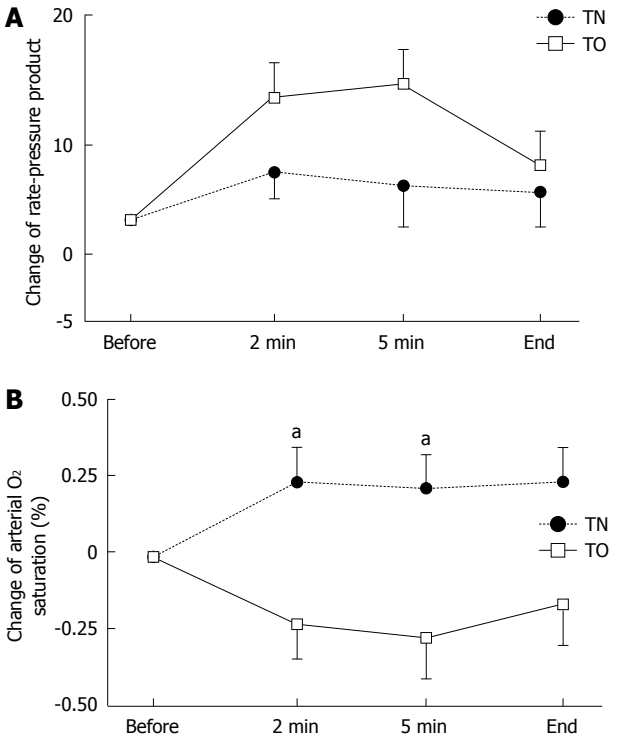

Figure 1 Time course of changes in RPP and SpO_2 (Study 1). A: RPP tended to increase more in the TO group than in the TN group, although this difference did not reach statistical significance; B: SpO_2 fell significantly at 2 and 5 min after the start of transoral endoscopic examination. Bars show SE. $^aP < 0.05$.

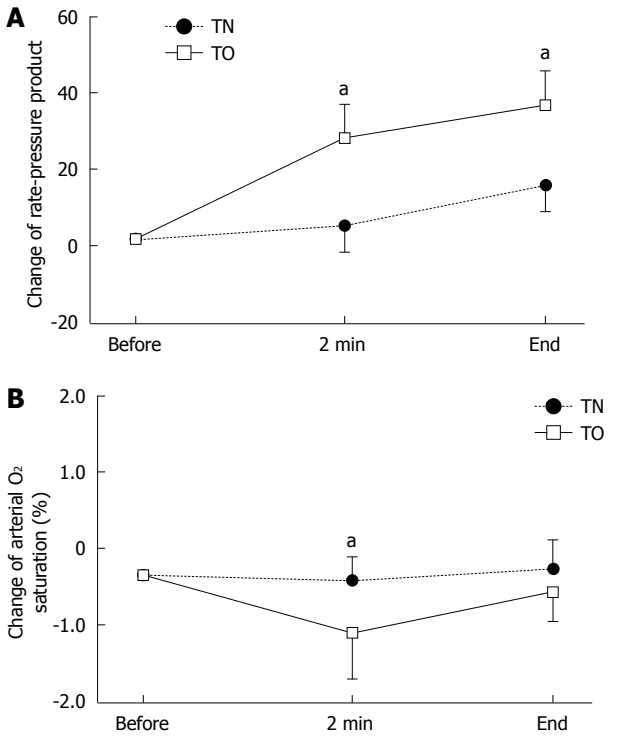

Figure 2 Time course of changes in RPP and SpO_2 (Study 2). A: RPP increased significantly more in the TO group than in the TN group. B: SpO_2 fell significantly at 2 min after the start of examination in the TO group. Bars show SE. $^aP < 0.05$.

Table 2 Characteristics of patients enrolled in Study 2 (mean ± SD)

	TN-TO group	TO-TN group	*P* value
Age (yr)	84.3 ± 7.9	80.7 ± 15.8	NS
Gender (M:F)	3:12	4:11	NS
Background pathological condition for PEG feeding			
Cerebral infarction	10	11	
Cerebral hemorrhage	2	3	
Subarachnoid hemorrhage	1	0	
Others	2	1	

Table 3 The results in total 30 endoscopic procedures (mean ± SD)

	TN-EGD	TO-EGD	*P* value
Total procedure time (min)	4.36 ± 3.46	4.42 ± 3.18	NS
Baseline cardiopulmonary parameter			
Systolic BP	125.5 ± 25.3	122.6 ± 28.4	NS
Diastolic BP	70.1 ± 17.8	64.9 ± 20.1	NS
RPP	107.7 ± 44.6	101.3 ± 37.9	NS
SpO_2	98.7 ± 1.7	95.4 ± 17.5	NS

Changes in BP, PR and RPP during endoscopy did not differ significantly between the TN and TO groups, although these parameters tended to increase more in the TO group (Figure 1A). Values for SpO_2 in the TO group decreased significantly during EGD (-0.24% and -0.29% after 2 and 5 min) compared with those in the TN group (+0.24% and +0.18% after 2 and 5 min, $P < 0.05$). These findings suggested that transoral EGD affects pulmonary function more severely than transnasal EGD does in this group of patients (Figure 1B).

Study 2

We enrolled 30 patients who required PEG feeding: 15 in the TN-TO group and 15 in the TO-TN group. Characteristics of these patients are shown in Tables 2 and 3. Categorical data did not differ significantly between the TN-TO and TO-TN groups. Baseline cardiopulmonary parameters, RPP and SpO_2, did not differ significantly.

Higher values of systolic and diastolic BP were found in the TO group than in the TN group after intubation for endoscopy, but these differences did not reach statistical significance. Values for RPP during and at the end of endoscopy were significantly higher in the TO group (26.8 and 34.6) than those in the TN group (3.1 and 15.2, $P < 0.05$), as shown in Figure 2A. The SpO_2 also significantly decreased at 2 min after the start of transoral EGD (-0.8%) in comparison with transnasal EGD (-0.1%, $P < 0.05$) (Figure 2B). Thus, in the bedridden patients with PEG feeding, examined in the supine position, transoral EGD disturbed cardiopulmonary function more strongly than did transnasal EGD.

Markers of inflammation also changed significantly in conjunction with endoscopy. Peripheral leukocyte count increased from 6053 ± 1975/L to 6900 ± 3392/L at 3 d after transoral EGD, as shown in Figure 3A ($P < 0.001$); and CRP values increased from 0.93 ± 0.24 mg/dL to 2.49 ± 0.91 mg/dL ($P < 0.001$) (Figure 3B). These data

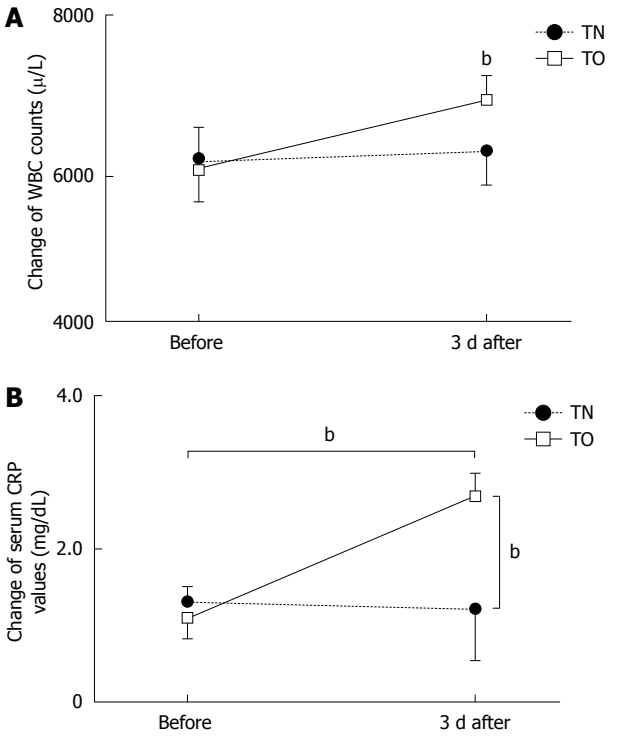

Figure 3 Changes in leukocyte counts (A) and CRP values (B) (Study 2). Counts and CRP increased significantly by the third day after transoral endoscopy (TO), but did not change after transnasal endoscopy (TN). Bars show SE. $^{b}P < 0.001$.

also showed statistically significant differences at 3 d after PEG tube exchange between transnasal and transoral EGD ($P < 0.001$).

Aspiration pneumonia, possibly caused by the endoscopic examination, was subsequently found in two of 30 patients after transoral EGD. In these two cases, a significant drop in SpO_2 values was found during transoral EGD, and the elevation of inflammation makers (CRP values and leukocyte counts) was also found after the procedure with transoral EGD but not with transnasal EGD. Additionally, the witnessed aspiration or tracheal suctioning of secretions including saliva was observed in these two cases of transoral EGD. Both of these patients recovered after the administration of antibiotics.

DISCUSSION

Transnasal small-caliber endoscopy, recently accepted for screening of the upper gastrointestinal tract, presents advantages apart from image quality, a noteworthy feature of transoral conventional endoscopy. Unsedated transoral EGD is well known to increase BP and PR, with an increased cardiopulmonary work load[2,4-6]. Recently, small-caliber endoscopes have been developed and used for EGD with the transnasal route. Several investigators have demonstrated that transnasal EGD with small-caliber endoscopes is feasible and tolerable[7-11], since transnasal small-caliber EDG is less stimulative to the uvula, palatine arches and posterior part of the tongue, and does not induce gag reflex[6]. Therefore, the feasibility and tolerability of unsedated transnasal small-caliber EGD strongly support its value as a standard screening procedure. However, the studies on transnasal small-caliber EGD were done mainly on relatively young patients aged < 60 years old. Elderly patients are believed to tolerate easily transoral EGD without excessive gagging or choking. Therefore, the clinical advantage of transnasal small-caliber EGD for this population may not outweigh the advantage of higher image quality obtained with conventional EGD. To determine the value of the transnasal small-caliber endoscope for screening older patients, we must first establish its tolerability and safety for this group. We have found no studies that specifically have addressed this issue.

In the first part of our study, we found no significant difference between transnasal and transoral endoscopy with respect to hemodynamic parameters. On the other hand, SpO_2, a measure of pulmonary function, significantly declined during transoral endoscopy, although the decrease was small. The small decrease in SpO_2 is not clinically important for elderly patients in good physical condition. However, for elderly patients with cardiopulmonary diseases and resulting decreased basal SpO_2 value, further small decreases in SpO_2 are prone to have serious consequences. Patients long confined to bed (including most who require PEG feeding) may become susceptible to infectious pulmonary diseases as a result of swallowing disturbance or micro-aspiration that accompanies decreased SpO_2. For safety in the periodic tube exchange, endoscopy-guided re-intubation of the PEG kit is recommended for these patients, because wrong replacement of the feeding tube can cause serious complications, such as peritonitis. This procedure requires that the patient remains supine, which increases the risk for aspiration of saliva and refluxed gastric contents. Transoral endoscopy may stimulate salivary secretion and thereby increase the risk of aspiration pneumonia. In addition, the supine position may influence specifically hemodynamic and/or pulmonary parameters during endoscopy. Previous studies have assessed only the hemodynamic changes that occur when PEG tube insertion is monitored with transnasal small-caliber endoscopy[18,19]. We have therefore measured pulmonary function and two markers of inflammation, as well as hemodynamic parameters, in this endoscopic examination (i.e. with the patient in the supine position). We consistently found advantages in the use of the transnasal small-caliber endoscope, with respect to both hemodynamic and pulmonary parameters. Significant increases in leukocyte counts and CRP values, which indicate systemic inflammatory disease, occurred only after transoral conventional endoscopy. Aspiration pneumonia was found in two cases after 30 transoral endoscopic procedures for the replacement of the PEG tube. PEG tube exchange is done in the supine position, therefore, the patients may have a higher risk of aspiration. However, no pneumonia was found after the procedure using the transnasal small-caliber endoscope. The transoral conventional endoscope may stimulate salivary secretion and further increase the risk of aspiration. Additionally, the oral cavity and saliva of bedridden patients with PEG feeding is prone to be infected by bacteria that may cause pneumonia. Use of the transnasal small-

caliber endoscope may therefore reduce the risk of this potentially serious complication of transoral conventional EGD in bedridden patients.

In conclusion, SpO_2 decreased significantly in elderly patients during transoral conventional EGD, but not during transnasal small-caliber EGD. Compared to transoral conventional EGD, transnasal small-caliber endoscopy may reduce the risk of aspiration pneumonia, when the patient must be examined in the supine position. Therefore, transnasal small-caliber EGD is a safer method than transoral conventional EGD in critically ill patients, such as those who are bedridden and undergoing PEG feeding.

COMMENTS

Background

Unsedated transnasal small-caliber esophagogastroduodenoscopy (EGD) is often used to examine the upper gastrointestinal tract. Its efficacy for elderly and critically ill patients, however, has not been fully evaluated. To evaluate the tolerability of transnasal EGD for elderly and critically ill bedridden patients, a prospective randomized comparative and a crossover study were undertaken.

Research frontiers

Elderly patients, including those who are bedridden, do not frequently gag or choke during transoral conventional EGD. If transoral conventional EGD is safe and comfortable for them, the value of transnasal small-caliber EGD could be limited, because the smaller charge-coupled device in the endoscope may potentially reduce diagnostic accuracy for minute gastric lesions. This is believed to be the first published study concerned with the tolerability and safety of transnasal small-caliber endoscopy for critically ill patients.

Innovations and breakthroughs

Other studies evaluating the tolerability and safety of transnasal endoscopy have focused mainly on younger patients, with a mean age < 50 years old. The data and viewpoints originated from elderly and critically ill patients and have not been published elsewhere.

Applications

Significant decreases were observed in SpO_2 saturation during conventional transoral EGD, but not during transnasal small-caliber EGD. Significant increases were also found in the markers of inflammation in bedridden patients after transoral conventional EGD, but not after transnasal small-caliber EGD performed with the patient in the supine position.

Peer review

This is a prospective study that compared the consequences of using two endoscopic devices (conventional and small caliber) in critically ill patients. The importance of this study is related to the fact that the use of small caliber endoscopes in diagnosis is becoming more common because of its tolerability and lack of sedation.

REFERENCES

1 **Dumortier J**, Ponchon T, Scoazec JY, Moulinier B, Zarka F, Paliard P, Lambert R. Prospective evaluation of transnasal esophagogastroduodenoscopy: feasibility and study on performance and tolerance. *Gastrointest Endosc* 1999; **49**: 285-291

2 **Garcia RT**, Cello JP, Nguyen MH, Rogers SJ, Rodas A, Trinh HN, Stollman NH, Schlueck G, McQuaid KR. Unsedated ultrathin EGD is well accepted when compared with conventional sedated EGD: a multicenter randomized trial. *Gastroenterology* 2003; **125**: 1606-1612

3 **Dumortier J**, Napoleon B, Hedelius F, Pellissier PE, Leprince E, Pujol B, Ponchon T. Unsedated transnasal EGD in daily practice: results with 1100 consecutive patients. *Gastrointest Endosc* 2003; **57**: 198-204

4 **Murata A**, Akahoshi K, Sumida Y, Yamamoto H, Nakamura K, Nawata H. Prospective randomized trial of transnasal versus peroral endoscopy using an ultrathin videoendoscope in unsedated patients. *J Gastroenterol Hepatol* 2007; **22**: 482-485

5 **Preiss C**, Charton JP, Schumacher B, Neuhaus H. A randomized trial of unsedated transnasal small-caliber esoph agogastroduodenoscopy (EGD) versus peroral small-caliber EGD versus conventional EGD. *Endoscopy* 2003; **35**: 641-646

6 **Yagi J**, Adachi K, Arima N, Tanaka S, Ose T, Azumi T, Sasaki H, Sato M, Kinoshita Y. A prospective randomized comparative study on the safety and tolerability of transnasal esophagogastroduodenoscopy. *Endoscopy* 2005; **37**: 1226-1231

7 **Trevisani L**, Cifalà V, Sartori S, Gilli G, Matarese G, Abbasciano V. Unsedated ultrathin upper endoscopy is better than conventional endoscopy in routine outpatient gastroenterology practice: a randomized trial. *World J Gastroenterol* 2007; **13**: 906-911

8 **Kawai T**, Miyazaki I, Yagi K, Kataoka M, Kawakami K, Yamagishi T, Sofuni A, Itoi T, Moriyasu F, Osaka Y, Takagi Y, Aoki T. Comparison of the effects on cardiopulmonary function of ultrathin transnasal versus normal diameter transoral esoph agogastroduodenoscopy in Japan. *Hepatogastroenterology* 2007; **54**: 770-774

9 **Thota PN**, Zuccaro G Jr, Vargo JJ 2nd, Conwell DL, Dumot JA, Xu M. A randomized prospective trial comparing unsedated esophagoscopy via transnasal and transoral routes using a 4-mm video endoscope with conventional endoscopy with sedation. *Endoscopy* 2005; **37**: 559-565

10 **Alami RS**, Schuster R, Friedland S, Curet MJ, Wren SM, Soetikno R, Morton JM, Safadi BY. Transnasal small-caliber esophagogastroduodenoscopy for preoperative evaluation of the high-risk morbidly obese patient. *Surg Endosc* 2007; **21**: 758-760

11 **Mori A**, Ohashi N, Tatebe H, Maruyama T, Inoue H, Takegoshi S, Kato T, Okuno M. Autonomic nervous function in upper gastrointestinal endoscopy: a prospective randomized comparison between transnasal and oral procedures. *J Gastroenterol* 2008; **43**: 38-44

12 **Dumortier J**, Josso C, Roman S, Fumex F, Lepilliez V, Prost B, Lot M, Guillaud O, Petit-Laurent F, Lapalus MG, Ponchon T. Prospective evaluation of a new ultrathin one-plane bending videoendoscope for transnasal EGD: a comparative study on performance and tolerance. *Gastrointest Endosc* 2007; **66**: 13-19

13 **Maffei M**, Dumortier J, Dumonceau JM. Self-training in unsedated transnasal EGD by endoscopists competent in standard peroral EGD: prospective assessment of the learning curve. *Gastrointest Endosc* 2008; **67**: 410-418

14 **Robinson BF**. Relation of heart rate and systolic blood pressure to the onset of pain in angina pectoris. *Circulation* 1967; **35**: 1073-1083

15 **Gobel FL**, Norstrom LA, Nelson RR, Jorgensen CR, Wang Y. The rate-pressure product as an index of myocardial oxygen consumption during exercise in patients with angina pectoris. *Circulation* 1978; **57**: 549-556

16 **Carnes ML**, Sabol DA, DeLegge M. Does the presence of esophagitis prior to PEG placement increase the risk for aspiration pneumonia? *Dig Dis Sci* 2004; **49**: 1798-1802

17 **Kitamura T**, Nakase H, Iizuka H. Risk factors for aspiration pneumonia after percutaneous endoscopic gastrostomy. *Gerontology* 2007; **53**: 224-227

18 **Lustberg AM**, Darwin PE. A pilot study of transnasal percutaneous endoscopic gastrostomy. *Am J Gastroenterol* 2002; **97**: 1273-1274

19 **Dumortier J**, Lapalus MG, Pereira A, Lagarrigue JP, Chavaillon A, Ponchon T. Unsedated transnasal PEG placement. *Gastrointest Endosc* 2004; **59**: 54-57

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BRIEF ARTICLE

Association between MDM2-SNP309 and hepatocellular carcinoma in Taiwanese population

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Abstract

AIM: To investigate the risk association and compare the onset age of hepatocellular carcinoma (HCC) patients in Taiwan with different genotypes of MDM2-SNP309.

METHODS: We analyzed MDM2-SNP309 genotypes from 58 patients with HCC and 138 cancer-free healthy controls consecutively. Genotyping of MDM2-SNP309 was conducted by restriction fragment length polymorphism assay.

RESULTS: The proportion of homozygous MDM2-SNP309 genotype (G/G) in cases and cancer-free healthy controls was similar (17.2% *vs* 16.7%). Multivariate analysis showed that the risk of G/G genotype of MDM2-SNP309 *vs* wild-type T/T genotype in patients with HCC was not significant (OR = 1.265, 95% CI = 0.074-21.77) after adjustment for sex, hepatitis B or C virus infection, age, and cardiovascular disease/diabetes. Nevertheless, there was a trend that GG genotype of MDM2-SNP309 might increase the risk in HCC patients infected with hepatitis virus (OR = 2.568, 95% CI = 0.054-121.69). Besides, the homozygous MDM2-SNP309 genotype did not exhibit a significantly earlier age of onset for HCC.

CONCLUSION: Current data suggest that the association between MDM2-SNP309 GG genotype and HCC is not significant, while the risk may be enhanced in patients infected by hepatitis virus in Taiwan.

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Key words: MDM2 protein; Hepatocellular carcinoma; Taiwan; Tumor suppressor protein p53

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INTRODUCTION

Hepatocellular carcinoma (HCC) is a prevalent type of cancer. It represents the fifth most prevalent cancer worldwide, and accounts for the top three causes of death in the Asia-Pacific region[1,2]. The risk factors associated with HCC include age, sex, alcohol, diet, and infection with hepatitis B virus (HBV) and/or hepatitis C virus (HCV)[2]. Most newly diagnosed HCC is reported in Asia (> 70%), in which chronic HBV infection accounts for 75% of cases worldwide[3]. Significantly, 55% of HCC cases are reported from the Chinese population[4]. Although the incidence rate of HCC is plausibly linked to geographic area and geo-economic conditions, it is possible that gene polymorphism may be associated with the risk of HCC[5,6].

Single nucleotide polymorphism (SNPs) occur frequently in human genomes. SNPs can be detected once per 1000 bp in DNA sequences, on average, and may affect gene transcription and amino acid composition if they are located in gene regions. Several lines of evidence have shown that SNPs in genes such as small inducible cytokine B14 precursor (*SCYB14*), glial cell-derived neurotrophic factor family receptor α 1 (*GFRA1*), corticotropin-releasing hormone receptor 2 (*CRHR2*), glucose-regulated protein 78 (*GRP78*), heat shock protein A1B (*HSPA1B*), DNA-methyltransferase-3B (*DNMT3B*), α-fetoprotein (*AFP*) and *p53 R72P* are associated significantly with HCC[6-12]. These SNPs localize in promoter regions, coding sequences, or even introns of individual genes, which suggests that the expression level and functions of affected genes can influence the incidence rate of HCC.

MDM2 oncoprotein is a direct negative regulator for the p53 tumor suppressor protein, which accounts for 50% of human cancers if deleted or with loss-of-function[13,14]. Overexpression of MDM2 by up to fourfold in transgenic mice that harbor wild-type p53 leads to complete tumorigenesis[15]. MDM2 overexpression also is associated with poor survival and is a useful predictive factor for poor prognosis in humans with liver cancer[16,17]. A genetic polymorphism located in intron 1 of the MDM2 gene, so called MDM2-SNP309 (a change from T to G, rs2279744) can enhance the binding of Sp1 general transcription factor to this promoter region and increase *MDM2* gene transcription[18]. It has been suggested that this SNP is associated with the risk and early onset age of various human cancers[19]. Some studies have shown that MDM2-SNP309 is associated with the risk of HCC in Japanese and Moroccan patients with chronic hepatitis C, and Korean patients with chronic hepatitis B[20-22]. Although > 50% of HCC cases are reported from Asia, it remains largely unknown whether MDM2-SNP309 influences the risk and onset age of HCC in other countries in this region, except for Japan and Korea.

In this study, we initiated a hospital-based case-control study to investigate the risk association between MDM2-SNP309 and HCC in Taiwanese patients. We also examined whether HCC onset was earlier in patients with homozygous MDM2-SNP309 (G/G) compared to wild-type MDM2-SNP309 (T/T).

MATERIALS AND METHODS

Patients and cancer-free healthy controls

We studied 58 patients with HCC diagnosed by cancer specialists, and 138 cancer-free healthy adults enrolled from Taipei City Hospital Ren Ai Branch, Taiwan during 2007. All volunteers signed the consent form and filled out the structured questionnaire before providing their blood samples. All patients and 50 cancer-free healthy controls were tested for hepatitis B and C by anti-HBsAg, HBsAg, Anti-HBc IgG, and anti-HCV. Several risk factors associated with HCC were included in the questionnaire, including age, sex, alcohol intake, frequency of exercise, and cardiovascular diseases/diabetes. There was no other cancer type diagnosed in each patient. The subjects were born in the Taiwan Island except five patients and four cancer-free healthy controls who had emigrated from mainland China. This study was approved by institutional review committee board of National Yang-Ming University and Taipei City Hospital.

MDM2-SNP309 genotyping

Analysis of MDM2-SNP309 genotyping was conducted by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), as described previously[23]. Genomic DNA was extracted from 200 μL whole blood sample using the Qiagen Mini Blood DNA Extraction kit (Valencia, CA, USA). The DNA fragment that contained the *MDM2* SNP309 was amplified by PCR using the forward (5'-CGGGAGTTCAGGGTAAAGGT-3') and reverse primer (5'-AGCAAGTCGGTGCTTACCTG-3') (Protech Inc., Taipei, Taiwan). Each PCR reaction was conducted using 100 ng genomic DNA, 0.2 μmol/L primer, 200 μmol/L dNTP, 1.5 mmol/L $MgCl_2$, 20 mmol/L Tris-HCl (pH 8.4), 50 mmol/L KCl and 1 U Platinum *Taq* DNA polymerase (Invitrogen, Carlsbad, CA, USA). The thermal cycler conditions were 94℃ for 1-5 min; 40 cycles with denaturing at 94℃, annealing at 59℃, and elongation at 72℃ for 30 s each; one cycle at 72℃ for 10 min. Subsequently, 10 μL of the PCR product was digested with 1 U *Msp*A1I restriction enzyme (New England Biolabs, Ipswich, MA, USA) at 37℃ for 30-60 min. The T/T, T/G and G/G genotypes were identified as 233 bp/88 bp, 233 bp/187 bp/88 bp, and 187 bp/88 bp running on the 3% NuSieve agarose gel, respectively. The genotypes were confirmed by direct sequencing of the PCR products by the Sequencing Core Facility of Genomic Research Center in National Yang-Ming University.

Statistical analysis

Whether the frequency of MDM2-SNP309 genotype obeyed the Hardy-Weinberg equilibrium was determined by an on-line public statistical tool (http://www.genes.org.uk/software/hardy-weinberg.shtml). Two-sample *t* test was used to evaluate the difference in age and body mass index (BMI) between cases and controls. Differences in sex, hepatitis frequency, HBV and HCV infection, alcohol intake, exercise, and incidence of cardiovascular diseases and diabetes between cases and controls were determined by Fisher's exact test. Multivariate logistic regression analysis was used to calculate the OR and 95% CI to determine the association between HCC risk and MDM2-SNP309 genotypes. The Kaplan-Meier survival analysis was used to describe the onset age of HCC, and the log-rank test was used to compare the median onset age between patients with GG and those with TT genotypes in MDM2 SNP309. $P < 0.05$ was considered statistically significant in all tests. All statistical analyses were performed with Statistical Analysis System ver. 9.1 (SAS Institute, Cary, NC, USA).

RESULTS

We investigated 58 HCC patients and 138 cancer-free healthy controls to evaluate the risk association between

Table 1 The demography of HCC patients and cancer-free healthy controls from Taiwan *n* (%)

Characteristics[1]	Cases (58)	Controls (138)	*P* value
Age (yr)			
mean	65.90	40.20	< 0.0001
SD	10.14	15.24	
Gender			
Male	45 (77.6)	42 (30.2)	< 0.0001
Female	13 (22.4)	96 (69.8)	
Hepatitis			
Yes	49 (84.5)	10 (7.2)	< 0.0001
No	9 (15.5)	79 (57.25)	
Unknown	0	49 (35.5)	
HBV[2]			
+	29	10	0.0002
-	28	40	
HCV[3]			
+	20	0	< 0.0001
-	37	50	
Alcohol intake			
Yes	26 (44.8)	81 (58.7)	0.0851
No	32 (55.2)	57 (41.3)	
Cardiovascular diseases and/or disorders			
Yes	23 (39.7)	26 (18.7)	0.0035
No	35 (59.6)	116 (81.3)	
BMI	22.7 ± 3.13	22.59 ± 3.44	0.8341

[1]Age and BMI (Body Mass Index) were determined by *t* test. Other parameters were analyzed by Fisher's exact test; [2,3]Two HCC cases were infected with both HBV and HCV. HCC: Hepatocellular carcinoma.

Table 2 The risk evaluation of MDM2 SNP309 genotypic frequencies on the development of HCC *n* (%)

SNP309	Cases	Controls	OR (95% CI)[1]	*P* value
TT	11 (19)	35 (25.3)	1	
TG	37 (63.8)	80 (58)	1.016 (0.152-6.8)	0.99
GG	10 (17.2)	23 (16.7)	1.265 (0.074-21.767)	0.87
TG+GG	47 (81)	103 (57.5)	1.037 (0.757-6.862)	0.97
TT+TG	48 (82.8)	115 (83.3)	1	
GG	10 (17.2)	23 (16.7)	1.25 (0.123-12.66)	0.85

[1]The multivariate logistic regression model was used to calculate odds ratio adjusted for gender, infection of hepatitis virus (B or C type), age, cardiovascular disease and/or diabetes.

Table 3 Estimation of odds ratio for different genotypes of MDM2-SNP309 in hepatitis viral infected patients with HCC *n* (%)

Hepatitis[1]	SNP309	Cases	Controls	OR[2]	95% CI	*P* value
Positive	TT	7 (14.3)	1 (10)	1.000		
	TG	32 (65.3)	7 (70)	2.340	0.11-49.655	0.59
	GG	10 (20.4)	2 (20)	2.568	0.054-121.687	0.63
	TG+GG	42 (85.7)	9 (90)	2.376	0.115-48.896	0.57
Negative	TT	4 (44.4)	19 (24)	1.000		
	TG	5 (55.6)	45 (57)	0.512	0.046-5.712	0.59
	GG[3]	0 (0)	15 (19)	-	-	-
	TG+GG	5 (55.6)	60 (76)	0.470	0.041-5.387	0.54

[1]Include HBV or HCV infected patients with HCC; [2]OR was adjusted for age, gender and cardiovascular diseases and/or diabetes; [3]OR was not estimated because the number in case was zero.

MDM2-SNP309 and HCC. The characteristics of these blood donors are summarized in Table 1. The mean ages were significantly different between cases and controls (65.9 ± 10.14 years *vs* 40.2 ± 15.24 years) at the time that they joined this study. These participants were enrolled consecutively without pre-selection for age, therefore, this difference may confirm that HCC usually is found in elderly individuals. In HCC patients, > 80% had been infected with HBV or HCV, and two cases were infected with both HBV and HCV. In addition, the frequency of HCC patients infected with HBV was slightly higher than that of those infected with HCV. Other factors that may affect the incidence of HCC are also summarized in Table 1. Except for BMI and alcohol intake, other confounding factors exhibited a significant difference between HCC cases and cancer-free healthy controls. They were adjusted for the multivariate logistic regression model thereafter.

The frequency of MDM2-SNP309 distributed in wild-type (TT), heterozygous (TG) and homozygous (GG) genotypes are shown in Table 2. The genotype distributions in HCC cases and cancer-free healthy controls slightly departed from Hardy-Weinberg equilibrium (HCC, $\chi^2 = 4.426$, $P = 0.035$; controls, $\chi^2 = 3.907$, $P = 0.048$). It is impossible to predict the genotype of each blood donor, therefore, it is expected that this inconsistency may have been due to the small sample size. Although the frequency of wild-type MDM2-SNP309 genotype was lower in HCC cases than in cancer-free healthy controls, there was no significant difference found in the frequency of homozygosity between these two groups (Table 2). To determine the risk association between HCC and MDM2-SNP309 genotypes, multivariate logistic regression analysis showed that the OR of heterozygous (TG) and homozygous (GG) SNP309 genotypes was 1.016 (95% CI = 0.152-6.8, P = 0.987) and 1.265 (95% CI = 0.074-21.767, P = 0.87) compared to wild-type SNP309 genotype, respectively (Table 2). These OR values were adjusted for age, sex, infection with HBV or HCV, cardiovascular disease and/or diabetes. Comparison of homozygous MDM2-SNP309 (GG) genotype with common (TT) or heterozygous (TG) MDM2-SNP309 carriers showed that the adjusted OR was 1.247 (95% CI = 0.123-12.66, P = 0.852). The TG or GG genotype of MDM2-SNP309 verses TT variant form exhibited an adjusted OR of 1.037 (95% CI = 0.757-6.682, P = 0.97) (Table 2). These OR values are lower than those reported previously in Japanese, Korean and Moroccan populations[20-22]. Thus, current statistical results showed that risk association between MDM2-SNP309 genotype and HCC was not significant in the Taiwanese population.

We next investigated whether HBV or HCV infection influenced the effects of MDM2-SNP309 genotypes on patients with HCC. As shown in Table 1, 49 cases infected with HBV or HCV were analyzed by the logistic regression model. It revealed that the homozygous (GG) MDM2-SNP309 genotypes exhibited increased risk over the common (TT) genotype of MDM2-SNP309 (adjusted OR = 2.568, 95% CI = 0.054-121.687, P = 0.632) (Table 3). The adjusted OR of TG or GG of MDM2-SNP309 genotype was 2.376 compared to TT genotype (95% CI =

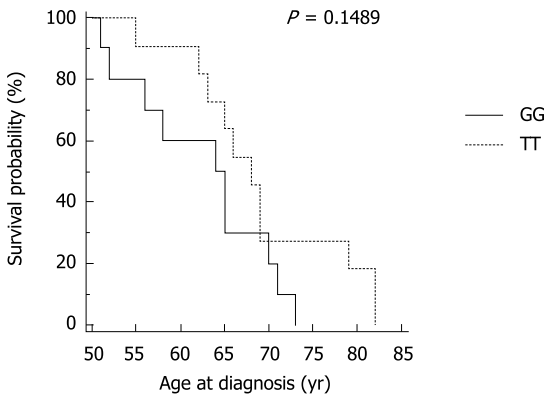

Figure 1 Comparison of age at diagnosis in HCC patients with wild-type (T/T) and homozygous (G/G) MDM2-SNP309 by the Kaplan-Meier method and the log-rank test. The survival fraction was the cumulative case-free survival rate against age at diagnosis of patients with HCC with different MDM2-SNP309 genotypes.

0.115-48.896, $P = 0.575$). The broad range of 95% CI was due to the small sample size, while it seems plausible that the risk association between MDM2-SNP309 and patients with HCC may have been enhanced by viral hepatitis.

We compared the age at diagnosis for HCC patients who had different MDM2-SNP309 genotypes, and the median ages for TT, TG and GG genotypes were 68 (range 55-82), 66 (range 38-87) and 64.5 (range 51-73) years old, respectively. The arithmetic mean ages and SD for TT, TG and GG were 69.1 ± 8.23, 64.3 ± 11.1, and 62.5 ± 7.88 years old, respectively. Although the mean age of patients with homozygous MDM2-SNP309 genotype (GG) was 6.6 years lower than that of patients with wild-type MDM2-SNP309 genotype (TT), there was no significant difference between them by *t* test ($P = 0.0844$, 95% CI = -0.9835-14.1654). Besides, the Kaplan-Meier survival analysis showed that comparison of the age at diagnosis for HCC patients with GG and TT genotypes was not significantly different by log-rank test ($P = 0.1489$, Figure 1). Therefore, the current results suggest that MDM2-SNP309 is not associated with onset age of HCC in the Taiwanese population. However, a larger sample size may be necessary to confirm this observation.

DISCUSSION

HCC commonly occurs in the Asia-Pacific region, and it also accounts for high morbidity and mortality in this area[2]. Although the incidence rate of male HCC patients is high in the Asia-Pacific region (14-36 per 100 000 men), it exhibits significant variance in different countries. The highest incidence rate of male HCC occurs in China and Taiwan, which is 58 and 53 per 100 000 men, respectively[2]. The main etiological agents that result in high incidence of HCC in the Asia-Pacific region include high infection rate with HBV and HCV, cirrhosis, family history, environmental contamination, diet, and α-fetoprotein[4,24-26]. MDM2-SNP309 is a novel predictor for Japanese and Korean patients with HCC infected by HCV and HBV, respectively[20,21]. In this study, we examined the risk association between Taiwanese HCC patients and MDM2-SNP309. Although the multivariate analysis showed that the association between MDM2-SNP309 and HCC in the Taiwanese population was not significant, there was a trend that homozygous (GG) or heterozygous (TG) MDM2-SNP309 genotype exhibited a higher risk in the subset of HCC patients infected with HBV or HCV. This is consistent with previous reports in Japanese and Korean patients with HCC, at least in part. Besides, our data failed to prove that MDM2-SNP309 could accelerate the development of HCC, although the median age at diagnosis of patients with homozygous MDM2-SNP309 genotype (GG) was 3.5 years lower than that of patients with the common genotype (TT). This result also agrees with the studies in the Japanese and Moroccan but not in the Korean population[20-22]. Therefore, the risk association and the effects of age onset between MDM2-SNP309 genotypes and HCC are likely to be dependent on the selected patient subgroups.

MDM2 negatively regulates p53 tumor suppressor protein. It is reasonable to expect that over-produced MDM2 will repress p53 function on cancer prevention. This hypothesis has been demonstrated in an MDM2 overexpression transgenic mouse model that develops systemic tumors. The putative effect of MDM2-SNP309 is to enhance the transcription of the MDM2 gene, and to affect the p53 regulatory pathway for tumor development. Several lines of evidence have demonstrated an association between MDM2-SNP309 and various sporadic or hereditary human cancers, including risk and earlier age onset. Nevertheless, non-supportive data are also reported to disagree that there is a risk association between MDM2-SNP309 and human cancers, even when the same cancer types are studied[27-36]. One of the reasons is likely to be the different subgroups of patients and races. For instance, a recent meta-analysis has demonstrated that the G allele of MDM2-SNP309 may affect breast cancer in the Chinese population rather than non-Chinese population[37]. Although HCC is a common cancer type in the Asia-Pacific region, its association with MDM2-SNP309 has only been reported in Japanese patients with chronic hepatitis C and in Korean patients chronically infected with hepatitis B virus[20,21]. HCC has the second highest cancer mortality in Taiwanese subjects who are vulnerable to be infection with HBV or HCV (http://crs.cph.ntu.edu.tw/). To the best of our knowledge, this is the first study to investigate the Taiwanese population to analyze the effects of MDM2-SNP309 genotypes on HCC development.

The sample size is the primary limitation of our study. The participants were from a single hospital, and most patients who suffered from HCC had a lack of enthusiasm to provide blood samples for analysis. Besides, the age distribution of cases and cancer-free healthy controls was significantly different. The patients were enrolled consecutively, therefore, this bias was not due to selection and was adjusted for in multivariate analysis. Another limitation is that sex distribution and incidence of hepatitis between cases and controls were not comparable. In HCC patients, the ratio of men to women was 3.46, and 84.5% of patients also had hepatitis. The mean ages at diagnosis for HBV- and HCV-infected HCC patients were 59.53 ±

10.72 years and 70.63 ± 6.39 years, respectively ($P = 0.0002$). Male HCC patients were infected mainly by HBV (64.9%), while female HCC patients were infected by HCV (66.7%). Furthermore, the male/female ratio was 6.0 for HCC patients with HBV infection, while it was 1.375 for those with HCV infection. All of these results are similar to a previous study of 18 423 Taiwanese HCC patients enrolled from 1981 to 2001[2,38]. Therefore, the characteristics of HCC patients did not exhibit a significant discrepancy compared to a large cohort study in Taiwan, even though a small sample size was used in this study.

Several potential risk factors were also considered in this study. Frequent alcohol intake is considered to be an etiological agent for the Chinese population (OR = 1.88)[4]. In our study, there was no significant difference between cases and controls regarding alcohol intake. However, identification of alcohol intake is dependent on self-interpretation by participants, and it may lead to apparent bias. It has been reported that metabolic syndrome, such as obesity and diabetes may affect the incidence of HCC[39-41]. We adjusted for these factors in the multivariate logistic regression model to evaluate the risk association between MDM2-SNP309 and HCC in the Taiwanese population. Nevertheless, it remains important to collect more samples to evaluate the potential risk factors for HCC in the presence of various MDM2-SNP309 genotypes in the future.

In summary, this hospital-based case-control study showed that there was no significant association between MDM2-SNP309 and HCC in the Taiwanese population. In the subset with hepatitis B or C, the homozygous or heterozygous MDM2-SNP309 genotype tended to influence the incidence rate of HCC. Homozygous MDM2-SNP309 genotype did not significantly accelerate the development of HCC, even though the median age at diagnosis of patients with homozygous SNP309 was 3.5 years lower than that of patients with wild-type SNP309. In the future, we expect to use a larger sample size to further confirm the effect of MDM2 SNP309 on HCC in the Taiwanese population.

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COMMENTS

Background

The incidence and mortality rate of hepatocellular carcinoma (HCC) are among the highest in Taiwanese cancer patients according to the data from the Taiwan Cancer Registry (http://crs.cph.ntu.edu.tw/main.php?Page=N1). Recently, it has been reported that a single nucleotide polymorphism (SNP) in the promoter region of MDM2 oncogene, MDM2-SNP309, is associated with the risk of HCC in Japanese and Korean patients infected with hepatitis C and hepatitis B virus, respectively. However, it remains unclear whether this observation commonly occurs in other neighboring Asian populations.

Research frontiers

To the best of our knowledge, this is the first study to investigate the association between MDM2-SNP309 and HCC in Taiwanese, a population close to the Han Chinese, which also has a high incidence and mortality of HCC. The data showed that there was no significant association between HCC risk and MDM2-SNP309 in the Taiwanese population, while the association tended to increase in patients with hepatitis B or C virus infection.

Innovations and breakthroughs

The incidence and mortality rate of HCC in Taiwan are comparable to those in Japan and Korea. Previous studies have shown that MDM2-SNP309 is associated with the risk of HCC in Japanese patients with chronic hepatitis C, and Korean patients with chronic hepatitis B. In this study, the authors demonstrated that MDM2-SNP309 genotype may not affect the risk of HCC in the Taiwanese population, except in those with a history of viral hepatitis, regardless of whether hepatitis B or C. This conclusion may be important for previous research groups to reevaluate whether hepatitis can increase the risk effect of MDM2-SNP309 on HCC. It is also an important parameter for other research groups that are dedicated to investigate the association between HCC and genetic polymorphism in the MDM2 gene and its related signaling pathways.

Applications

The results are expected to provide information about MDM2-SNP309 genotyping for routine health and newborn screening for HCC in subjects with or without viral hepatitis.

Terminology

MDM2-SNP309 is a genetic polymorphism that corresponds to nucleotide 309, starting from first nucleotide of intron 1 of the MDM2 gene.

Peer review

This was a well-designed and well-conducted study on the association between polymorphisms in MDM2 gene promoter and HCC. The results are clearly presented and discussed. These results may help dissect the molecular alterations involved in HCC development in Taiwan.

REFERENCES

1 **Bosch FX**, Ribes J, Diaz M, Cleries R. Primary liver cancer: worldwide incidence and trends. *Gastroenterology* 2004; **127**: S5-S16

2 **Yuen MF**, Hou JL, Chutaputti A. Hepatocellular carcinoma in the Asia pacific region. *J Gastroenterol Hepatol* 2009; **24**: 346-353

3 **Parkin DM**, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; **55**: 74-108

4 **Luo RH**, Zhao ZX, Zhou XY, Gao ZL, Yao JL. Risk factors for primary liver carcinoma in Chinese population. *World J Gastroenterol* 2005; **11**: 4431-4434

5 **Laurent-Puig P**, Legoix P, Bluteau O, Belghiti J, Franco D, Binot F, Monges G, Thomas G, Bioulac-Sage P, Zucman-Rossi J. Genetic alterations associated with hepatocellular carcinomas define distinct pathways of hepatocarcinogenesis. *Gastroenterology* 2001; **120**: 1763-1773

6 **Kato N**, Ji G, Wang Y, Baba M, Hoshida Y, Otsuka M, Taniguchi H, Moriyama M, Dharel N, Goto T, Shao RX, Matsuura T, Ishii K, Shiina S, Kawabe T, Muramatsu M, Omata M. Large-scale search of single nucleotide polymorphisms for hepatocellular carcinoma susceptibility genes in patients with hepatitis C. *Hepatology* 2005; **42**: 846-853

7 **Chen GG**, Ho RL, Wong J, Lee KF, Lai PB. Single nucleotide polymorphism in the promoter region of human alpha-fetoprotein (AFP) gene and its significance in hepatocellular carcinoma (HCC). *Eur J Surg Oncol* 2007; **33**: 882-886

8 **Ezzikouri S**, El Feydi AE, Benazzouz M, Afifi R, El Kihal L, Hassar M, Akil A, Pineau P, Benjelloun S. Single nucleotide polymorphism in DNMT3B promoter and its association with hepatocellular carcinoma in a Moroccan population. *Infect Genet Evol* 2009; **9**: 877-881

9 **Wu Y**, Lin JS. DNA methyltransferase 3B promoter polymorphism and its susceptibility to primary hepatocellular carcinoma in the Chinese Han nationality population: a case-control study. *World J Gastroenterol* 2007; **13**: 6082-6086

10 **Zhu X**, Chen MS, Tian LW, Li DP, Xu PL, Lin MC, Xie D, Kung HF. Single nucleotide polymorphism of rs430397 in the fifth intron of GRP78 gene and clinical relevance of

primary hepatocellular carcinoma in Han Chinese: risk and prognosis. *Int J Cancer* 2009; **125**: 1352-1357
11 **Jeng JE**, Tsai JF, Chuang LY, Ho MS, Lin ZY, Hsieh MY, Chen SC, Chuang WL, Wang LY, Yu ML, Dai CY, Chang JG. Heat shock protein A1B 1267 polymorphism is highly associated with risk and prognosis of hepatocellular carcinoma: a case-control study. *Medicine* (Baltimore) 2008; **87**: 87-98
12 **Zhu ZZ**, Cong WM, Liu SF, Dong H, Zhu GS, Wu MC. Homozygosity for Pro of p53 Arg72Pro as a potential risk factor for hepatocellular carcinoma in Chinese population. *World J Gastroenterol* 2005; **11**: 289-292
13 **Jin S**, Levine AJ. The p53 functional circuit. *J Cell Sci* 2001; **114**: 4139-4140
14 **Michael D**, Oren M. The p53-Mdm2 module and the ubiquitin system. *Semin Cancer Biol* 2003; **13**: 49-58
15 **Jones SN**, Hancock AR, Vogel H, Donehower LA, Bradley A. Overexpression of Mdm2 in mice reveals a p53-independent role for Mdm2 in tumorigenesis. *Proc Natl Acad Sci USA* 1998; **95**: 15608-15612
16 **Endo K**, Ueda T, Ohta T, Terada T. Protein expression of MDM2 and its clinicopathological relationships in human hepatocellular carcinoma. *Liver* 2000; **20**: 209-215
17 **Schoniger-Hekele M**, Hanel S, Wrba F, Muller C. Hepatocellular carcinoma--survival and clinical characteristics in relation to various histologic molecular markers in Western patients. *Liver Int* 2005; **25**: 62-69
18 **Bond GL**, Hu W, Bond EE, Robins H, Lutzker SG, Arva NC, Bargonetti J, Bartel F, Taubert H, Wuerl P, Onel K, Yip L, Hwang SJ, Strong LC, Lozano G, Levine AJ. A single nucleotide polymorphism in the MDM2 promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. *Cell* 2004; **119**: 591-602
19 **Bond GL**, Levine AJ. A single nucleotide polymorphism in the p53 pathway interacts with gender, environmental stresses and tumor genetics to influence cancer in humans. *Oncogene* 2007; **26**: 1317-1323
20 **Dharel N**, Kato N, Muroyama R, Moriyama M, Shao RX, Kawabe T, Omata M. MDM2 promoter SNP309 is associated with the risk of hepatocellular carcinoma in patients with chronic hepatitis C. *Clin Cancer Res* 2006; **12**: 4867-4871
21 **Yoon YJ**, Chang HY, Ahn SH, Kim JK, Park YK, Kang DR, Park JY, Myoung SM, Kim do Y, Chon CY, Han KH. MDM2 and p53 polymorphisms are associated with the development of hepatocellular carcinoma in patients with chronic hepatitis B virus infection. *Carcinogenesis* 2008; **29**: 1192-1196
22 **Ezzikouri S**, El Feydi AE, Afifi R, El Kihal L, Benazzouz M, Hassar M, Marchio A, Pineau P, Benjelloun S. MDM2 SNP309T>G polymorphism and risk of hepatocellular carcinoma: a case-control analysis in a Moroccan population. *Cancer Detect Prev* 2009; **32**: 380-385
23 **Walsh CS**, Miller CW, Karlan BY, Koeffler HP. Association between a functional single nucleotide polymorphism in the MDM2 gene and sporadic endometrial cancer risk. *Gynecol Oncol* 2007; **104**: 660-664
24 **Collier J**, Sherman M. Screening for hepatocellular carcinoma. *Hepatology* 1998; **27**: 273-278
25 **Chan HL**, Sung JJ. Hepatocellular carcinoma and hepatitis B virus. *Semin Liver Dis* 2006; **26**: 153-161
26 **Nissen NN**, Martin P. Hepatocellular carcinoma: the high-risk patient. *J Clin Gastroenterol* 2002; **35**: S79-S85
27 **Hu Z**, Ma H, Lu D, Qian J, Zhou J, Chen Y, Xu L, Wang X, Wei Q, Shen H. Genetic variants in the MDM2 promoter and lung cancer risk in a Chinese population. *Int J Cancer* 2006; **118**: 1275-1278
28 **Li G**, Zhai X, Zhang Z, Chamberlain RM, Spitz MR, Wei Q. MDM2 gene promoter polymorphisms and risk of lung cancer: a case-control analysis. *Carcinogenesis* 2006; **27**: 2028-2033
29 **Lind H**, Zienolddiny S, Ekstrom PO, Skaug V, Haugen A. Association of a functional polymorphism in the promoter of the MDM2 gene with risk of nonsmall cell lung cancer. *Int J Cancer* 2006; **119**: 718-721
30 **Ma H**, Hu Z, Zhai X, Wang S, Wang X, Qin J, Jin G, Liu J, Wang X, Wei Q, Shen H. Polymorphisms in the MDM2 promoter and risk of breast cancer: a case-control analysis in a Chinese population. *Cancer Lett* 2006; **240**: 261-267
31 **Millikan RC**, Heard K, Winkel S, Hill EJ, Heard K, Massa B, Mayes L, Williams P, Holston R, Conway K, Edmiston S, de Cotret AR. No association between the MDM2 -309 T/G promoter polymorphism and breast cancer in African-Americans or Whites. *Cancer Epidemiol Biomarkers Prev* 2006; **15**: 175-177
32 **Petenkaya A**, Bozkurt B, Akilli-Ozturk O, Kaya HS, Gur-Dedeoglu B, Yulug IG. Lack of association between the MDM2-SNP309 polymorphism and breast cancer risk. *Anticancer Res* 2006; **26**: 4975-4977
33 **Pine SR**, Mechanic LE, Bowman ED, Welsh JA, Chanock SC, Shields PG, Harris CC. MDM2 SNP309 and SNP354 are not associated with lung cancer risk. *Cancer Epidemiol Biomarkers Prev* 2006; **15**: 1559-1561
34 **Sun YF**, Leu JD, Chen SM, Lin IF, Lee YJ. Results based on 124 cases of breast cancer and 97 controls from Taiwan suggest that the single nucleotide polymorphism (SNP309) in the MDM2 gene promoter is associated with earlier onset and increased risk of breast cancer. *BMC Cancer* 2009; **9**: 13
35 **Talseth BA**, Meldrum C, Suchy J, Kurzawski G, Lubinski J, Scott RJ. MDM2 SNP309 T>G alone or in combination with the TP53 R72P polymorphism does not appear to influence disease expression and age of diagnosis of colorectal cancer in HNPCC patients. *Int J Cancer* 2007; **120**: 563-565
36 **Wilkening S**, Hemminki K, Rudnai P, Gurzau E, Koppova K, Forsti A, Kumar R. No association between MDM2 SNP309 promoter polymorphism and basal cell carcinoma of the skin. *Br J Dermatol* 2007; **157**: 375-377
37 **Economopoulos KP**, Sergentanis TN. Differential effects of MDM2 SNP309 polymorphism on breast cancer risk along with race: a meta-analysis. *Breast Cancer Res Treat* 2009; Epub ahead of print
38 **Lu SN**, Su WW, Yang SS, Chang TT, Cheng KS, Wu JC, Lin HH, Wu SS, Lee CM, Changchien CS, Chen CJ, Sheu JC, Chen DS, Chen CH. Secular trends and geographic variations of hepatitis B virus and hepatitis C virus-associated hepatocellular carcinoma in Taiwan. *Int J Cancer* 2006; **119**: 1946-1952
39 **Amarapurkar DN**, Patel ND, Kamani PM. Impact of diabetes mellitus on outcome of HCC. *Ann Hepatol* 2008; **7**: 148-151
40 **El-Serag HB**, Hampel H, Javadi F. The association between diabetes and hepatocellular carcinoma: a systematic review of epidemiologic evidence. *Clin Gastroenterol Hepatol* 2006; **4**: 369-380
41 **Chen CL**, Yang HI, Yang WS, Liu CJ, Chen PJ, You SL, Wang LY, Sun CA, Lu SN, Chen DS, Chen CJ. Metabolic factors and risk of hepatocellular carcinoma by chronic hepatitis B/C infection: a follow-up study in Taiwan. *Gastroenterology* 2008; **135**: 111-121

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BRIEF ARTICLE

A better parameter in predicting insulin resistance: Obesity plus elevated alanine aminotransferase

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Abstract

AIM: To investigate the association of obesity and elevated alanine aminotransferase with insulin resistance and compare these factors with metabolic syndrome.

METHODS: We enrolled a total of 1308 male workers aged from 22 to 63 years. Data was extracted from the workers' periodic health check-ups in hospitals. All cases were from the community of northern Taiwan. This was a cross-sectional observational study from July to September in 2004. We grouped all cases into four groups, based on the quartile of homeostasis model assessment. The top fourth quartile group was defined as the group with insulin resistance. We performed multivariate logistic regression analysis for the odds ratio of the risk factors for insulin resistance.

RESULTS: Compared with metabolic syndrome, the coexistence of both factors had a 4.3-fold (95% CI: 2.7-6.8) increased risk, which was more than metabolic syndrome with a 3.6-fold (95% CI: 2.6-5.0) increased risk. The two factors had a synergistic effect. The synergistic index of obesity and elevated alanine aminotransferase (ALT) was 2.1 (95% CI: 1.01-4.3).

CONCLUSION: Obesity and elevated ALT are associated with insulin resistance. The effects are synergistic. Coexistence of them is better than metabolic syndrome in predicting insulin resistance.

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Key words: Alanine aminotransferase; Transaminase; Overweight; Obesity; Insulin resistance

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INTRODUCTION

Insulin resistance is a key feature of type 2 diabetes mellitus and it plays an important role in cardiovascular diseases[1]. Predicting insulin resistance is useful in preventing diabetes or cardiovascular disease. Insulin resistance can be directly measured with a hyperinsulinemic euglycemic clamp[2]. In addition, several parameters from less invasive methods have been demonstrated to be related to, or to predict, insulin resistance, such as obesity[3-5], alanine aminotransferase (ALT) level[6], metabolic syndrome, chronic inflammation status[5], nonalcoholic fatty liver disease (NAFLD)[7,8], and adiponectin level[9]. Some of these factors are easily measured (e.g. body weight and ALT) and the others are more complex (e.g. metabolic syndrome), not available in most laboratories (e.g. adiponectin), or are technique-dependent (e.g. sonographic NAFLD).

In Taiwan, the national health program provides a periodic examination for every adult older than forty. The program includes measurements of body height/weight, and ALT level. Measurement of insulin resistance, however, is not included in the program because of its inconvenience, although it is important for preventing cardiovascular disease.

Obesity is a ongoing problem in Taiwan and world-

wide[10,11]. It is associated with several conditions, of which insulin resistance might be the most important[12].

Alanine aminotransferase (ALT) is a widely used laboratory test and abnormal ALT levels are common[13,14]. Elevated ALT has also been noted to be related to insulin resistance.

Besides being non-invasive, both obesity and ALT measurements are easily obtained and are inexpensive. Our purpose was to confirm the belief that that obesity and elevated ALT are important parameters of insulin resistance. We reported that the coexistence of both factors has a synergistic effect and the coexistence might be better than metabolic syndrome for predicting insulin resistance. The possible mechanism will be explored in the article. This result is helpful for preventing cardiovascular disease in public health.

MATERIALS AND METHODS

Data from a total of 1308 male workers' health check-up records were analyzed. Data were collected *via* a questionnaire, including age (years), smoking status (packs per day), and alcohol consumption status (times per week). Anthropometrical data collected included systolic and diastolic blood pressure (mmHg), body weight (kg), height (cm), calculated body mass index, BMI (kg/m^2), and waist circumference (cm). The laboratory measurements were conducted by Beckman Coulter auto-analyzer (model DxC 800 Synchron), and included triglyceride (mg/dL), high-density lipoprotein (HDL, mg/dL), aspartate aminotransferase (AST, U/L), alanine aminotransferase (ALT, U/L), and fasting plasma glucose (mg/dL). The insulin level (U/mL) was measured by the Abbott autoanalyzer (model AxSym). Insulin resistance was defined as the top quartile of $HOMA_{IR}$ (homeostasis model assessment)[2]. Obesity was defined as BMI ⩾ 27 according to the modified criteria in Taiwan[11]. Abdominal obesity (or central obesity) was defined as a waist circumference ⩾ 90 cm in men, according to the modified criteria from ATPⅢ for Asia subjects[15]. Elevated blood pressure, elevated triglyceride, low high-density lipoprotein, and elevated fasting glucose were defined based on previous ATPⅢ criteria for metabolic syndrome as systolic blood pressure ⩾ 130 mmHg or diastolic blood pressure ⩾ 85 mmHg, triglyceride ⩾ 150 mg/dL, high density lipoprotein < 40 mg/dL, and fasting glucose ⩾ 100 mg/dL. Elevated ALT was defined as ALT > 40 U/L (according to our laboratory and any cause, such viral hepatitis or alcoholism, was not ruled out). Smoking was defined as smoking more than one pack per day. Alcohol consumption was defined as drinking more than once per week. The Student's *t* test and chi-square test were used for analyzing continuous variables and categorical variables, respectively. Multivariate logistic regression was used to evaluate the relationship between insulin resistance and the risk factors. We introduced three models for multivariate logistic regression analysis for the odds ratio of the risk factors for insulin resistance. Model 1 showed the odds ratio of the risk factors among which metabolic syndrome is regarded as five separate components. Metabolic syndrome is regarded as a single factor in model 2. Elevated ALT and obesity are regarded as two separate risk factors in model 1 and model 2. The coexistence of the two factors is regarded as a single factor and is compared with metabolic syndrome in model 3. To understand the synergistic effect of the risk factors, we introduced the synergistic index to see if the synergistic effect of obesity and elevated ALT was statistically significant[16]. This analytical study, limited to health check-up data, followed all the ethical criteria for human research[17]. The study protocol (TYGH09702108) was reviewed and approved by the Ethics Committee of the Taoyuan General Hospital.

RESULTS

A total of 1308 male examinees were enrolled in the present study. The baseline characteristics of the study population stratified by $HOMA_{IR}$ are presented in Table 1. The mean age of the total population was 38.7 years (ranging from 22-63 years); increasing age with the higher $HOMA_{IR}$ was noted. A similar increasing trend was also presented in the other parameters except that a decreasing trend was noted in the HDL parameter. We also examined the significance of the trend of the increasing ALT level with insulin resistance through ANOVA analysis.

The abnormality prevalence of the four stratified categories according to the HOMA quartile, and the whole samples, are shown in Table 2. Similarly, the abnormality prevalence rates of all parameters (except smoking and drinking) increase with the higher $HOMA_{IR}$.

In Table 3, after controlling for age, metabolic syndrome, hypercholesterolemia, elevated low-density lipoprotein, drinking, and smoking status, obesity has a 2.5-fold (95% CI: 1.7-3.6) and elevated ALT has a 2.1-fold (1.4-3.0) increased risk of insulin resistance in model 1. Each of these is more than at least three components of metabolic syndrome (abdominal obesity, elevated blood pressure, and hypo-HDL cholesterolemia). In model 2, obesity has a 3.0-fold (2.1-4.2) and elevated ALT has a 2.1-fold (1.5-2.9) increased risk of insulin resistance. Metabolic syndrome has a 2.6-fold (1.8-3.7) increased risk. On their own, both risk factors are similar to, or even more significant than metabolic syndrome. In model 3, we compared the coexistence of obesity and elevated ALT with metabolic syndrome. The coexistence of the two factors has a 4.3-fold (2.7-6.8) increased risk, far more than metabolic syndrome that has a 3.6-fold (2.6-5.0) increased risk. To examine the relationship between obesity and elevated ALT, we presented the synergistic effect of both factors in Table 4. It showed the synergistic index is 2.1 and it is statistically significant (95% CI: 1.0-4.3). Different odds ratios of obesity and elevated ALT in Tables 3 and 4 were noted due to different controlling factors.

Table 1 Basic characteristics of subjects, stratified by $HOMA_{IR}$ (mean ± SD)

	The four sub-groups stratified by $HOMA_{IR}$[1]				Total
	Q1 (327, 25%)	Q2 (326, 25%)	Q3 (328, 25%)	Q4 (327, 25%)	(1308, 100%)
Age (yr)	38.1 ± 10.0	38.5 ± 9.7	38.7 ± 10.0	39.7 ± 10.3	38.7 ± 10.0
Systolic blood pressure (mmHg)	124.4 ± 13.9	125.8 ± 14.7[a]	128.0 ± 15.1[a]	132.5 ± 16.0[a]	127.7 ± 15.2
Diastolic blood pressure (mmHg)	77.3 ± 10.0	77.6 ± 10.4[a]	79.6 ± 11.2[a]	82.1 ± 11.7[a]	79.1 ± 11.0
Body mass index (kg/m^2)	22.8 ± 2.5	23.7 ± 2.5[a]	25.1 ± 2.7[a]	27.0 ± 3.4[a]	24.7 ± 3.3
Waist circumference (cm)	83.6 ± 6.6	85.8 ± 6.1[a]	88.9 ± 6.7[a]	92.6 ± 7.5[a]	87.7 ± 7.5
ALT (U/L)	22.4 ± 16.1	25.5 ± 18.1[a]	36.1 ± 48.4[a]	40.2 ± 26.4[a]	31.0 ± 31.0
AST (U/L)	23.9 ± 11.1	24.2 ± 16.0[a]	27.6 ± 20.2[a]	28.0 ± 11.5[a]	25.9 ± 15.3
Fasting glucose (mg/dL)	95.1 ± 7.6	97.9 ± 7.9[a]	99.4 ± 7.9[a]	102.5 ± 8.5[a]	98.7 ± 8.4
Fasting insulin (U/mL)	3.6 ± 1.0	6.1 ± 0.9[a]	8.9 ± 1.1[a]	15.8 ± 6.5[a]	8.6 ± 5.7
$HOMA_{IR}$	0.8 ± 0.2	1.5 ± 0.2[a]	2.1 ± 0.2[a]	3.9 ± 1.7[a]	2.1 ± 1.5
Triglyceride (mg/dL)	113.9 ± 86.8	127.6 ± 86.2[a]	157.9 ± 105.7[a]	202.8 ± 156.9[a]	150.6 ± 117.6
HDL (mg/dL)	52.8 ± 12.5	50.2 ± 9.8[a]	47.4 ± 10.7[a]	44.4 ± 8.9[a]	48.7 ± 11.0

[1]The subjects were stratified into four groups according to $HOMA_{IR}$; Q1 means the 1st quartile, Q2 means the 2nd, *etc.* ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; HDL: High-density lipoprotein; $HOMA_{IR}$: Insulin resistance assessed by homeostasis model assessment = fasting insulin (U/mL) × glucose (mmol/L)/22.5. [a]$P < 0.05$ *vs* Q1.

Table 2 Abnormality prevalence distribution, stratified by $HOMA_{IR}$ *n* (%)

	The four sub-groups stratified by $HOMA_{IR}$				Total
Abnormality[1]	Q1 327 (25)	Q2 326 (25)	Q3 328 (25)	Q4 327 (25)	1308 (100)
Obesity	20 (6.1)	29 (8.9)	81 (24.7)[a]	153 (46.8)[a]	283 (21.6)
Elevated ALT	25 (7.6)	36 (11.0)	74 (22.6)[a]	118 (36.1)[a]	253 (19.3)
Metabolic syndrome components					
Abdominal obesity	45 (13.8)	74 (22.7)[a]	126 (38.4)[a]	188 (57.5)[a]	433 (33.1)
Elevated blood pressure	110 (33.6)	118 (36.2)	136 (41.5)[a]	187 (57.2)[a]	551 (42.1)
Elevated fasting glucose	16 (4.9)	22 (6.7)	37 (11.3)[a]	62 (19.0)[a]	137 (10.5)
Elevated triglyceride	58 (17.7)	83 (25.5)[a]	132 (40.2)[a]	195 (59.6)[a]	468 (35.8)
Hypo-HDL cholesterolemia	39 (11.9)	47 (14.4)	71 (21.6)[a]	108 (33.0)[a]	265 (20.3)
Alcohol consumption	83 (25.4)	56 (17.2)	74 (22.6)	73 (22.3)	286 (21.9)
Cigarette smoking	122 (37.3)	118 (36.2)[a]	108 (32.9)	109 (33.3)	457 (34.9)

[1]Obesity: Body mass index ⩾ 27 kg/m^2; Elevated ALT: ALT > 40 U/L; Abdominal obesity: waist circumference ⩾ 90 cm in male adults; Elevated blood pressure: blood pressure ⩾ 130/85 mmHg; Elevated fasting glucose: fasting glucose ⩾ 100 mg/dL; Elevated triglyceride: triglyceride ⩾ 150 mg/dL; Hypo-HDL cholesterolemia: HDL cholestero1 < 40 mg/dL; Hypercholesterolemia: total cholesterol ⩾ 200 mg/dL; Elevated LDL: LDL cholesterol ⩾ 130 mg/dL; Alcohol consumption: more than once per week; Cigarette smoking: more than one pack per day. [a]$P < 0.05$ *vs* Q1 (χ^2 test).

Table 3 OR of risk factors, presented as OR (95% CI)

Model[1]	Model 1	Model 2	Model 3
Both obesity and elevated ALT	-	-	4.3 (2.7-6.8)
Obesity	2.5[2] (1.7-3.6)	3.0[2] (2.1-4.2)	-
Elevated ALT	2.1[2] (1.4-3.0)	2.1[2] (1.5-2.9)	-
Metabolic syndrome	-	2.6[2] (1.8-3.7)	3.6 (2.6-5.0)
Abdominal obesity	1.6[2] (1.2-2.2)	-	-
Elevated blood pressure	1.7[2] (1.2-2.4)	-	-
Elevated fasting glucose	2.8[2] (1.8-4.4)	-	-
Elevated triglyceride	2.2[2] (1.6-3.1)	-	-
Hypo-HDL cholesterolemia	1.6[2] (1.1-2.2)	-	-
Alcohol consumption	1.2 (0.8-1.7)	1.2 (0.8-1.6)	1.2 (0.9-1.7)
Cigarette smoking	0.9 (0.7-1.2)	0.9 (0.6-1.2)	0.9 (0.6-1.2)

[1]Risk factors of metabolic syndrome were regarded as five individual factor in model 1 and as a single factor in model 2; both obesity and elevated ALT were regarded as a single factor in model 3; [2]Significant odds ratio (OR), adjusted for age.

Table 4 Synergistic effect of obesity and elevated ALT on insulin resistance[1]

	OR	(95% CI)	SI	95% CI
Neither obesity nor elevated ALT	1.0	Reference	No risk	-
Obesity only	2.3	(1.5-3.5)	One risk only	-
Elevated ALT only	1.8	(1.1-2.9)	One risk only	-
Obesity plus elevated ALT	5.5	(3.2-9.3)	2.1	(1.01-4.3)

[1]Insulin resistance is defined as the 4th quartile of the $HOMA_{IR}$. SI: Synergistic index.

DISCUSSION

Obesity is a worsening problem in Taiwan and worldwide[10]. Due to the variety of definition criteria, the prevalence rate varies. In Taiwan, the prevalence rate of obesity (defined as BMI ⩾ 27 in this article), surveyed in 2000-2001 were 15.9% and 10.7% in men and women separately, and the rate has progressively increased[11]. The prevalence rate of obesity in our study, which was conducted after 2001, is higher and is compatible with the trend of increasing prevalence. Obesity is associated with several comorbidities[10], including coronary heart disease, stroke, NAFLD, and type 2 diabetes mellitus. Insulin resistance is present and important in the above clinical problems.

In our study, obesity alone is an important risk factor of insulin resistance. Similar results have also been published in other studies[3-5]. In contrast, weight reduction improves insulin resistance[18,19], even if it is achieved by surgery[20]. However, not all obese subjects have insulin resistance[12]. Although the exact mechanism is still not fully understood, many factors have been found to induce insulin resistance through different pathways, such as increased non-esterified fatty acids (NEFAs), adipocyte dysfunction, leptin, adiponectin, chronic inflammatory status, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), and fat distribution[10,12,21]. Among these factors, NEFAs, released from dysfunctional adipocytes, might be the single most critical factor in modulating insulin sensitivity[10,12,21].

ALT level is a widely used laboratory test in Taiwan. Elevated ALT is a common laboratory abnormality in a healthy population. The statistical database from the healthy population in the United States in 2003 showed the prevalence rate of abnormal transaminase was 20.8%, similar to previous findings (19.3%)[13]. Another study showed the prevalence rate in the male adolescent population was 12.4%, which was lower, but the population was younger[14].

Our results show elevated ALT is a risk factor of insulin resistance, independent of metabolic syndrome. This conclusion has been also proposed in previous articles[6,22,23]. In Pima, in Indians with normal glucose tolerance, elevated ALT was associated with a decline in clamp-derived whole body insulin sensitivity, according to longitudinal analysis, and it predicted the development of type 2 diabetes in a prospective analysis[6]. This was extended to those with impaired glucose tolerance in another study[22], in which a population of African-Americans and white subjects were analyzed. Burgert *et al*[23] also noted a relationship between elevated ALT and reduced insulin sensitivity in obese children. Our study showed a similar result in Taiwanese adult males using community-based data. A semi-quantified result was also noted in a previous article[22]. Our study also showed the trend of a significantly increasing level of ALT in the more severe insulin-resistant group.

The causes of elevated ALT may be NAFLD, viral hepatitis, alcohol, or medications[24]. NAFLD is also a common clinical problem, and it has been proven to cause elevated ALT[25]. NAFLD was regarded as an explanation of the association between elevated ALT and insulin resistance in previous studies[6,26]. It has also been reported that hepatitis C induced insulin resistance[27]. This might be a partial explanation of the relationship between elevated ALT and insulin resistance. In contrast, alcohol intake in sufficient amounts can improve insulin resistance[8,28]. Many kinds of medication can induce elevated ALT, but there is a lack of evidence that medication leads to insulin resistance. One study in 1983 reported aspirin induced insulin insensitivity, but there were only eight subjects in the study[29]. The C-reactive protein (CRP) level might provide another explanation. A previous study indicated that the CRP level was higher in subjects with elevated ALT than those with normal ALT[30]. Another study showed a high CRP level was related to insulin resistance[31]. From these studies, we might conclude that subjects with elevated ALT have a higher CRP level and further, are more insulin-resistant. The exact mechanism is, however, not well-understood. Further evidence is required.

Interestingly, there is a dramatically higher risk of insulin resistance in subjects with both elevated ALT and obesity. This result has seldom been discussed in the literature.

There are several studies concerning the association between obesity and elevated ALT, but the role of insulin resistance had not been identified in these studies[32,33]. In studies on the association between ALT level and insulin resistance, there was no analysis identifying the effect of obesity, but a significant relationship between body weight and ALT level were shown in the data[6,22]. Elevated ALT was associated with insulin resistance in obese subjects in previous studies, but it lacked comparative analysis between obese and non-obese subjects[23,30].

What mechanism leads to the synergistic effect of obesity and elevated ALT? As discussed earlier, adipocyte dysfunction is regarded as the link between obesity and insulin resistance[21], and its associated release of NEFAs (or free fatty acids) was regarded as the single most critical factor modulating insulin sensitivity[1,10,12,21]. In the model, adipocyte dysfunction results in increased circulating NEFAs, which further mediates insulin resistance. The excessive build-up of NEFAs in the liver leads to NAFLD, of which elevated ALT level is the best marker even within the reference interval[34]. Obesity coexisting with elevated ALT points to excessive NEFAs release and adipocyte dysfunction. This shows the existence of the link "from obesity to insulin resistance". Besides, in the cell module, TNF-α, and its effects on down-regulation of the nuclear hormone receptor peroxisome proliferator activated receptor-γ (PPARγ) strongly contributed to the effects of inflammatory cytokines on adipocytes[21]. Thus the inflammatory status results in adipocyte dysfunction. As elevated ALT is a reflection of systemic inflammation[6], we concluded that coexistence of elevated ALT and obesity means the existence of inflammatory status, the excessive release of NEFAs, and their result: adipocyte dysfunction connecting obesity to insulin resistance.

Based on the models, most subjects with both obesity and elevated ALT should have insulin resistance. In our study, the actual prevalence rate of insulin resistance in the sub-group with both obesity and elevated ALT was almost 70% (not shown in table). This is compatible with our hypothesis.

The limits of this study deserve mention. First, insulin resistance was defined as the top quartile of $HOMA_{IR}$ (homeostasis model assessment), instead of a direct measurement. This might be less representative of insulin resistance, but more cases are available because of the more simple classification. Secondly, although our results showed a trend of increasing

ALT level with more severe insulin resistance, it still lacks an exact quantified relationship. More evidence is needed to confirm if there is a better cutoff value of ALT level or quantified relationship between ALT level and insulin resistance. Thirdly, cigarette smoking and alcohol consumption were recorded as more than one pack per day/once per week, instead of exact quantified data; therefore, it cannot be concluded that there was no relationship between smoking/drinking and insulin resistance based on our results.

In conclusion, obesity and elevated ALT have been shown to be associated with insulin resistance. The coexistence of both factors points to the existence of a linking mechanism between obesity and insulin resistance. The results suggest a synergistic effect on insulin resistance. The coexistence of these parameters might be better than metabolic syndrome for evaluating insulin resistance in clinical practice.

COMMENTS

Background

Insulin resistance is a key feature of type 2 diabetes mellitus and it plays an important role in cardiovascular diseases. Direct measurement of insulin resistance with hyperinsulinemic euglycemic clamp is invasive and inconvenient. For public health, less invasive methods are required. Many parameters have been proposed. Metabolic syndrome is one of the most popularly-used parameter.

Research frontiers

This study proposed that obesity plus elevated alanine aminotransferase (ALT) might be better than metabolic syndrome for predicting insulin resistance due to its being non-invasive and convenience. This article also explored the mechanism linking obesity, elevated ALT, and insulin resistance.

Applications

This study highlights the applications on public health since the measurements of obesity and ALT are easily obtained.

Peer review

The content of the article will be interesting not only for the gastroenterologists, but also for other specialists. For understanding the quantified relationship between body weight or ALT level and insulin resistance, further investigations are required.

REFERENCES

1 **Eckel RH**, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet* 2005; **365**: 1415-1428

2 **Liu CM**, Tung TH, Tsai ST, Liu JH, Tsai YK, Chen VT, Tam TN, Lu HF, Wang KK, Hsu CT, Shih HC, Chan DC, Chou P. Serum insulin, insulin resistance, beta-cell dysfunction, and gallstone disease among type 2 diabetics in Chinese population: a community-based study in Kinmen, Taiwan. *World J Gastroenterol* 2005; **11**: 7159-7164

3 **Viner RM**, Segal TY, Lichtarowicz-Krynska E, Hindmarsh P. Prevalence of the insulin resistance syndrome in obesity. *Arch Dis Child* 2005; **90**: 10-14

4 **Ludvik B**, Nolan JJ, Baloga J, Sacks D, Olefsky J. Effect of obesity on insulin resistance in normal subjects and patients with NIDDM. *Diabetes* 1995; **44**: 1121-1125

5 **Shoelson SE**, Herrero L, Naaz A. Obesity, inflammation, and insulin resistance. *Gastroenterology* 2007; **132**: 2169-2180

6 **Vozarova B**, Stefan N, Lindsay RS, Saremi A, Pratley RE, Bogardus C, Tataranni PA. High alanine aminotransferase is associated with decreased hepatic insulin sensitivity and predicts the development of type 2 diabetes. *Diabetes* 2002; **51**: 1889-1895

7 **Utzschneider KM**, Kahn SE. Review: The role of insulin resistance in nonalcoholic fatty liver disease. *J Clin Endocrinol Metab* 2006; **91**: 4753-4761

8 **Greenfield JR**, Samaras K, Hayward CS, Chisholm DJ, Campbell LV. Beneficial postprandial effect of a small amount of alcohol on diabetes and cardiovascular risk factors: modification by insulin resistance. *J Clin Endocrinol Metab* 2005; **90**: 661-672

9 **Kadowaki T**, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. *J Clin Invest* 2006; **116**: 1784-1792

10 **Eckel RH**, York DA, Rössner S, Hubbard V, Caterson I, St Jeor ST, Hayman LL, Mullis RM, Blair SN. Prevention Conference VII: Obesity, a worldwide epidemic related to heart disease and stroke: executive summary. *Circulation* 2004; **110**: 2968-2975

11 **Chu NF**. Prevalence of obesity in Taiwan. *Obes Rev* 2005; **6**: 271-274

12 **Kahn SE**, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature* 2006; **444**: 840-846

13 **Patt CH**, Yoo HY, Dibadj K, Flynn J, Thuluvath PJ. Prevalence of transaminase abnormalities in asymptomatic, healthy subjects participating in an executive health-screening program. *Dig Dis Sci* 2003; **48**: 797-801

14 **Fraser A**, Longnecker MP, Lawlor DA. Prevalence of elevated alanine aminotransferase among US adolescents and associated factors: NHANES 1999-2004. *Gastroenterology* 2007; **133**: 1814-1820

15 **Tan CE**, Ma S, Wai D, Chew SK, Tai ES. Can we apply the National Cholesterol Education Program Adult Treatment Panel definition of the metabolic syndrome to Asians? *Diabetes Care* 2004; **27**: 1182-1186

16 **Lundberg M**, Fredlund P, Hallqvist J, Diderichsen F. A SAS program calculating three measures of interaction with confidence intervals. *Epidemiology* 1996; **7**: 655-656

17 **Portaluppi F**, Touitou Y, Smolensky MH. Ethical and methodological standards for laboratory and medical biological rhythm research. *Chronobiol Int* 2008; **25**: 999-1016

18 **Goodpaster BH**, Kelley DE, Wing RR, Meier A, Thaete FL. Effects of weight loss on regional fat distribution and insulin sensitivity in obesity. *Diabetes* 1999; **48**: 839-847

19 **Petersen KF**, Dufour S, Befroy D, Lehrke M, Hendler RE, Shulman GI. Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycemia by moderate weight reduction in patients with type 2 diabetes. *Diabetes* 2005; **54**: 603-608

20 **Halverson JD**, Kramer J, Cave A, Permutt A, Santiago J. Altered glucose tolerance, insulin response, and insulin sensitivity after massive weight reduction subsequent to gastric bypass. *Surgery* 1982; **92**: 235-240

21 **Guilherme A**, Virbasius JV, Puri V, Czech MP. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. *Nat Rev Mol Cell Biol* 2008; **9**: 367-377

22 **Hanley AJ**, Wagenknecht LE, Festa A, D'Agostino RB Jr, Haffner SM. Alanine aminotransferase and directly measured insulin sensitivity in a multiethnic cohort: the Insulin Resistance Atherosclerosis Study. *Diabetes Care* 2007; **30**: 1819-1827

23 **Burgert TS**, Taksali SE, Dziura J, Goodman TR, Yeckel CW, Papademetris X, Constable RT, Weiss R, Tamborlane WV, Savoye M, Seyal AA, Caprio S. Alanine aminotransferase levels and fatty liver in childhood obesity: associations with insulin resistance, adiponectin, and visceral fat. *J Clin Endocrinol Metab* 2006; **91**: 4287-4294

24 **Pratt DS**, Kaplan MM. Evaluation of abnormal liver-enzyme results in asymptomatic patients. *N Engl J Med* 2000; **342**: 1266-1271

25 **Angulo P**. Nonalcoholic fatty liver disease. *N Engl J Med* 2002; **346**: 1221-1231

26 **Bloomgarden ZT**. Nonalcoholic fatty liver disease and insulin resistance in youth. *Diabetes Care* 2007; **30**: 1663-1669

27 **Hui JM**, Sud A, Farrell GC, Bandara P, Byth K, Kench JG, McCaughan GW, George J. Insulin resistance is associated with

chronic hepatitis C virus infection and fibrosis progression [corrected]. *Gastroenterology* 2003; **125**: 1695-1704
28 **Kiechl S**, Willeit J, Poewe W, Egger G, Oberhollenzer F, Muggeo M, Bonora E. Insulin sensitivity and regular alcohol consumption: large, prospective, cross sectional population study (Bruneck study). *BMJ* 1996; **313**: 1040-1044
29 **Newman WP**, Brodows RG. Aspirin causes tissue insensitivity to insulin in normal man. *J Clin Endocrinol Metab* 1983; **57**: 1102-1106
30 **Quirós-Tejeira RE**, Rivera CA, Ziba TT, Mehta N, Smith CW, Butte NF. Risk for nonalcoholic fatty liver disease in Hispanic youth with BMI > or =95th percentile. *J Pediatr Gastroenterol Nutr* 2007; **44**: 228-236
31 **McLaughlin T**, Abbasi F, Lamendola C, Liang L, Reaven G, Schaaf P, Reaven P. Differentiation between obesity and insulin resistance in the association with C-reactive protein. *Circulation* 2002; **106**: 2908-2912
32 **Ioannou GN**, Weiss NS, Boyko EJ, Kahn SE, Lee SP. Contribution of metabolic factors to alanine aminotransferase activity in persons with other causes of liver disease. *Gastroenterology* 2005; **128**: 627-635
33 **Park HS**, Han JH, Choi KM, Kim SM. Relation between elevated serum alanine aminotransferase and metabolic syndrome in Korean adolescents. *Am J Clin Nutr* 2005; **82**: 1046-1051
34 **Chang Y**, Ryu S, Sung E, Jang Y. Higher concentrations of alanine aminotransferase within the reference interval predict nonalcoholic fatty liver disease. *Clin Chem* 2007; **53**: 686-692

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BRIEF ARTICLE

Does testosterone prevent early postoperative complications after gastrointestinal surgery?

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Abstract

AIM: To investigate the role of sex hormones in the early postoperative complications of gastrointestinal diseases.

METHODS: A total of 65 patients who underwent operations for gastric and colorectal diseases (mainly malignant diseases) were included in the study. Peripheral venous blood samples were collected at different times for analysis of estradiol, testosterone and progesterone. The only study endpoint was analysis of postoperative complications.

RESULTS: Patients of both sexes were uniform but postoperative complication rate was significantly higher in female patients (P = 0.027). There was no significant association of estradiol and progesterone with postoperative complications. Testosterone levels in complicated patients were significantly lower than in uncomplicated patients (P < 0.05). Area under the receiver operating characteristic curve showed that a lower value of testosterone was a predictor for higher complication rate (P < 0.05), and a lower value of testosterone at later times after surgery was a better predictor of complications.

CONCLUSION: Patients with low testosterone level were prone to higher postoperative complications, which was evident in both sexes. However, further studies are necessary to support this result.

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Key words: Stomach neoplasms; Testosterone; Sex differences; Postoperative complications

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INTRODUCTION

There have been numerous reports of disparity in pathophysiology of different types of disease between male and female subjects, however, the reports have been quite diverse. Female sex is an independent predictor of mortality in patients with enterococcal bloodstream infections[1]. Women, in addition, might have a higher mortality among patients with necrotizing soft tissue infection[2]. Although most studies have suggested increased susceptibility to infectious complications among men, generally they have demonstrated a higher mortality rate for women from infections and sepsis[3-6], but again, this is not universal[7-9]. On the other hand, male sex is an independent risk factor for the development of nosocomial bloodstream infection[10], and is associated with in-hospital mortality in septic surgical patients[11]. Male sex is associated with anastomotic leakage in colorectal surgery[12], as well as in rectal surgery[13-15].

Female sex is an independent risk factor for early

postoperative complications of gastric cancer surgery[16,17]. Early postoperative complications of gastric cancer surgery are significantly more common in female patients, especially mortality and infection rate. Similarly, the duration of postoperative stay is significantly longer in female patients and they have more severe complications than male patients[17].

We observed a significantly higher complication rate in female than male patients, therefore, we assumed that sex hormones may have influenced this disparity. Therefore, we conducted a prospective study to investigate the possible role of sex hormones in early postoperative complications.

MATERIALS AND METHODS

Patient characteristics

A total of 65 patients who underwent operations for gastric and colorectal diseases (mainly malignant diseases) from 2008 to 2009 were included in the study. We applied TNM classification and Duke's classification for gastric cancer and colorectal cancer, respectively. In gastric cancer patients, the majority was diagnosed with loco-regional advanced stage, and few patients were diagnosed as late stage (Table 1). There was one case of gastric polyp disease, one of malignant gastrointestinal stromal tumor, and one of mesenteric metastasis. The median age of the patients was 61 years (range: 22-82 years). All the operations were performed by senior consultants of a single department at Rui Jin Hospital, School of Medicine, Shanghai Jiao Tong University.

All the patients with early and resectable advanced gastric cancer (without significant distant metastases) underwent gastrectomy with D2 lymphadenectomy. Thirty partial and 17 total gastrectomies were performed for gastric cancer. One patient underwent total gastrectomy for gastric polyp disease and partial gastrectomy was performed for one malignant gastrointestinal stromal tumor. Anterior resection was performed on three patients, and one patient underwent abdominoperineal resection for rectal cancer. Three patients had partial colectomy and one underwent subtotal colectomy for colon cancer. One patient case of mesenteric metastasis was resected along with partial enterectomy.

The only study endpoint was analysis of postoperative complications. Complications were recorded in accordance with the definitions reported by Copeland *et al*[18]. Other complications were recorded separately.

Blood sampling

This study was approved by the Hospital Institutional Review Boards for human subject research. All samples were obtained with informed consent. Patients on hormonal therapy or chemotherapy were excluded. Similarly, the patients with concomitant immunological disease were excluded. Peripheral venous blood samples were collected from 65 patients before operation (baseline measurements) and on postoperative day (POD)1, POD3, POD5, and POD7 for extensive analysis of their variation at different times. Samples were collected into Venoject tubes containing EDTA.

Table 1 Demographic data of the patients

Details	Frequency
Age group (yr)	
≤ 60	32
61–70	19
≥ 71	14
Sex	
Male	42
Female	23
No. of procedures	
Gastrectomy	49
Colorectal resection[1]	9
Gastro-jejunal anastomosis	2
Exploratory laparotomy	5
TNM classification (Gastric cancer)	
ⅠA	8
ⅠB	6
Ⅱ	7
ⅢA	10
ⅢB	4
Ⅳ	12
Dukes classification (Colorectal)	
A	7
B	0
C	1

[1]Including one case of small bowel resection combined with mesenteric tumor.

Laboratory tests

Assays for all parameters were performed in the laboratory of the Rui Jin Hospital. Venous blood was centrifuged at a speed 2500 r/min for 10 min, and serum was then preserved at -80℃, until further investigation. Estradiol, total testosterone and progesterone were measured using a Dxl 800 analyzer (Beckman-Coulter, Fullerton, CA, USA).

Statistical analysis

The statistical analysis was performed with SPSS for Windows version 13.0 (SPSS, Inc, Chicago, IL, USA). Test of normality for all related variables were checked by the Shapiro-Wilk method, and a nonparametric statistical method (Mann-Whitney *U* test) was applied to the variables without a normal distribution. The means were compared by Student's *t* test in normally distributed cases. Differences in proportion were compared by Pearson's χ^2 test. $P < 0.05$ was considered statistically significant.

RESULTS

Fifteen patients with gastric disease and with non-gastric disease were compromised by different complications. There were several patients who had pyrexia of unknown origin with a high white blood cell count, but who did not have any diagnosis, mainly because of a lack of further investigation. The definition of postoperative complication introduced by Copeland *et al*[18] does not cover many complications that are common after gastrointestinal surgery, therefore, complications which

Table 2 Overall complications in both sexes

Complications	Frequency	
	Male	Female
Overall	9	11
Intra-abdominal hemorrhage	1	0
Anastomotic leak	1	1
Abdominal infection	2	1
Pyrexia of unknown origin	7	9
Renal failure	1	0
Respiratory failure	0	1
Cardiac failure	0	1
Hypotension	0	1
MODS	0	1
Gastroplegia or enteroplegia	0	2
Anastomosis site bleeding	1	1
Pleural effusion	0	2
Seroperitoneum	2	1
Death	0	1

MODS: Multiple organ dysfunction syndrome.

Table 3 Normal range of different hormones

	Male	Female (postmenopause)
Estradiol (pg/mL)	20-75	20-88
Progesterone (ng/mL)	0.1-0.84	0.08-0.78
Testosterone (ng/mL)	2.62-15.9	0.1-0.8

Table 4 Demographics and clinical characteristics according to sex

	Male (n = 42)	Female (n = 23)	P value
Age (yr)	60.9	58.1	0.356
Physiological score	14	17	0.203
Operative severity score	16	18	0.365
Complications (%)	21.4	47.8	0.027
Baseline estradiol (pg/mL)	23	12	0.320
Baseline progesterone (ng/mL)	0.46	0.37	0.450
Baseline testosterone (ng/mL)	3.26	0.23	0.000

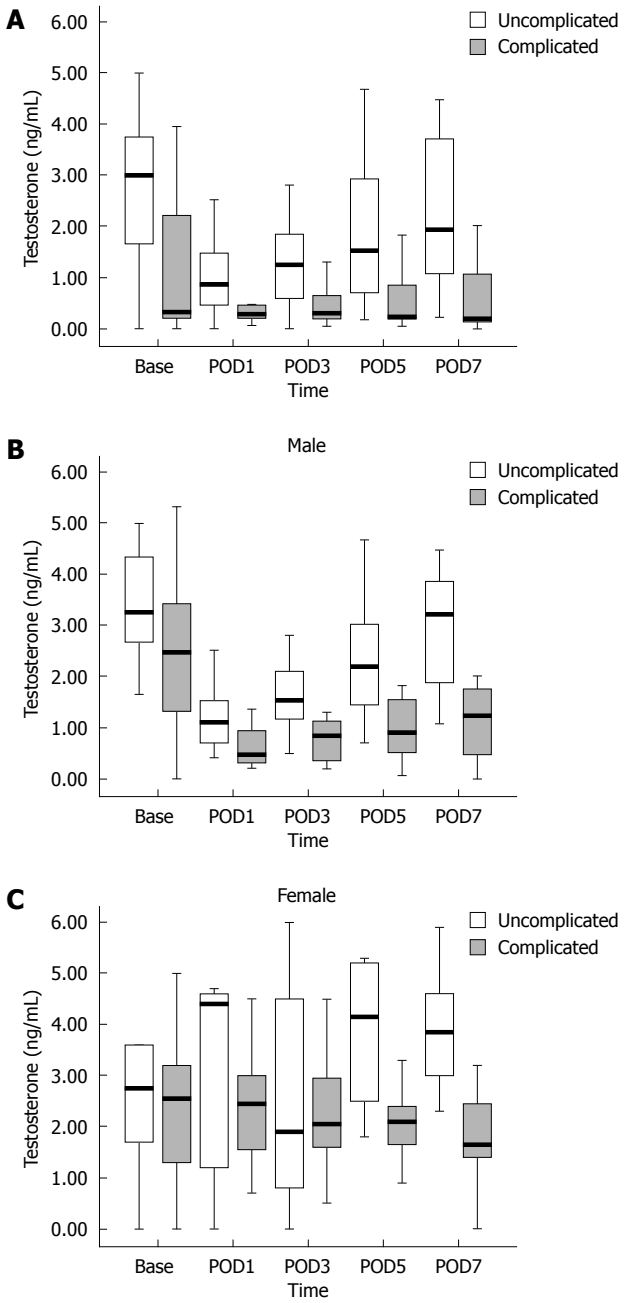

Figure 1 Difference in testosterone level. A: Difference in mean testosterone level in complicated and uncomplicated patients; B: Male patients; C: Female patients.

were confirmed clinically were also included. Details of all complications are summarized in Table 2. The sum of the individual complications was not equal to the total number of complications, and multiple complications were possible in a single patient. Patients of both sexes were uniform and there was no significant difference in age (P = 0.356), physiological score (P = 0.616) and operative severity score (P = 0.304). However, there was a significant difference in complication rate between male and female patients (P = 0.027). The complication rate for male and female patients was 21.4% and 47.8%, respectively. We analyzed the role of sex hormones in postoperative complications. Normal ranges of different sex hormones and demographics of patients according to sex are summarized in Tables 3 and 4, respectively.

There was no significant association of estradiol and progesterone with postoperative complications. Besides, the normal range of these hormones varies greatly at different times in a month in female patients, and there were not sufficient patients to stratify according to age or time period in women. Given the significant association between testosterone and postoperative complications, we focused additional analysis on this relationship. Testosterone decreased significantly after surgery in both sexes. Testosterone level in complicated patients was significantly lower ($P < 0.05$) than in uncomplicated patients (Figure 1A).

Perioperative testosterone level was associated significantly with postoperative complication rate, and the result was similar for either overall complication rate or complications of gastric disease surgery alone (Table 5). Similarly, the difference was also significant after stratification of all complications according to sex. However, after stratification, we found there was no significant

Table 5 Significance of difference in testosterone level at different times

Complication	Baseline	POD1	POD3	POD5	POD7
Overall	0.008	0.002	0.007	0.001	0.000
Gastric disease	0.025	0.008	0.026	0.005	0.005

Table 6 Level of testosterone in different sexes according to outcome

Time	Complications (male)			Complications (female)		
	Absent	Present	*P* value	Absent	Present	*P* value
Base	3.66	2.74	0.104	0.23	0.29	0.689
POD1	0.91	0.87	0.141	0.40	0.23	0.268
POD3	1.54	0.85	0.046	0.19	0.20	0.948
POD5	2.20	0.91	0.040	0.41	0.21	0.038
POD7	3.22	1.24	0.030	0.38	0.16	0.007

Table 7 Area under the ROC for testosterone

Time	Area	*P* value	95% CI	
			Lower bound	Upper bound
BASE	0.729	0.016	0.562	0.897
POD1	0.801	0.002	0.658	0.945
POD3	0.756	0.007	0.593	0.919
POD5	0.813	0.001	0.664	0.963
POD7	0.835	0.000	0.692	0.977

difference of testosterone level between complicated and uncomplicated patients during the first 3 d after surgery, in both sexes (Table 6). The median value of testosterone in complicated patients was approximately half of that in uncomplicated patients. The significance of the difference increased in later days after surgery, especially on POD5 and POD7. The result was similar in both sexes (Figure 1B and C).

Area under the receiver operating characteristic curve showed that a lower value of testosterone was a predictor for higher complication rate (Table 7, Figure 2). A lower value of testosterone at a later time after surgery was a better predictor of complications (Table 8).

DISCUSSION

Sex has been hypothesized to be a determinant of immunological variability after severe traumatic and surgical stress and, at least in animal models, accounts for differences in outcomes[3,7,19-22]. Some studies have demonstrated that altered levels of sex hormones rather than sex itself are related to postoperative complications. In particular, progesterone in male and testosterone in female subjects has an impact on survival. In both sexes, higher 17β-estradiol levels are associated clearly with shorter survival times[23]. However, we did not find a significant relationship between estradiol and postoperative complications. Unlikely, we observed a difference in progesterone level between complicated and uncomplicated patients. However, these observations were limited by the low number of patients, and variation of normal values

Table 8 Sensitivity and specificity for different levels of testosterone

	Testosterone level	Sensitivity	Specificity
Base	≤ 1.95	70.0	68.9
POD1	≤ 0.43	60.0	84.4
POD3	≤ 0.45	73.3	80.0
POD5	≤ 0.24	60.0	90.0
POD7	≤ 0.32	66.7	92.0

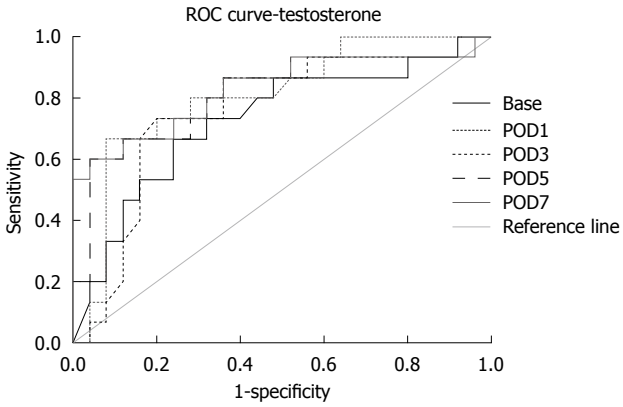

Figure 2 ROC curve for level of hormones at different times.

of this hormone at different times in female subjects made it difficult to obtain any conclusive result.

The uncertainty regarding the role of sex in influencing outcomes in humans may simply result from the clinical, phenotypic, and genetic diversity among populations, and the difficulty in detecting a difference due to these confounders. However, whether men and women respond differently to trauma or surgical insult, either in terms of the host response or the eventual outcome, is important for clinical care and the design of future research on anti-infective treatments or immunological regulators.

As mentioned above, our study was affected by the limitation of statistical calculation due to the low number of postoperative complications. Therefore, we could not analyze the differences in testosterone level in complicated and uncomplicated cases in different age groups, or its effect on different types of complications separately, because the number of patients and their relevant complications were low in those groups. It was still unknown whether the lower level of testosterone caused postoperative complications, or whether it was just a phenomenon observed in complicated patients. However, it was evident that even the preoperative level of testosterone was lower in patients who had complications after surgery. The testosterone level decreased significantly after surgery in all patients and it started to regain its preoperative level, or even higher, on POD3. However, the testosterone level in complicated patients did not regain its preoperative value, and moreover, it kept on decreasing. Even after controlling for sex by stratifying the group and looking at testosterone levels, the testosterone level decreased significantly in male and female patients with postoperative complications. Therefore, this observation

at least confirmed that the testosterone level had some kind of association with postoperative complications. However, without detailed investigation of the effects of other hormones, it was very hard to conclude that only testosterone was responsible for such disparity.

Although there were many limitations and unanswered questions in the present study, at least, our observations reflected that some kind of difference does exist between the sexes, which may be responsible for the disparity in postoperative complication rate. If a difference does indeed exist, it is possible that future studies will have to stratify for sex, infection source, and immunological phenotype. Similarly, future treatment strategy must be tailored for male and female patients separately, and different modes or treatment management will be necessary for the different sexes.

If there is an association between low testosterone level and postoperative complication rate, then serial tests of testosterone levels can easily identify the susceptible patients, and supplementary hormonal replacement therapy might be a novel treatment to prevent postoperative complications in such patients.

In conclusion, there was a significant association between testosterone and overall complications, and also with complications of gastric surgery. Association with testosterone was seen even after controlling for sex by stratifying the group and looking at testosterone levels. Postoperative complications were higher in patients who did not recover normal testosterone levels at POD5 or POD7. However, the results of this small-scale study need confirmation by a large cohort of patients, and prospective studies with hormone supplementation to reevaluate the role of testosterone in postoperative complications.

COMMENTS

Background

There have been numerous reports of disparity in pathophysiology of different types of disease between male and female patients, however, the reports have been quite diverse. Female sex is an independent risk factor for early postoperative complications of gastric cancer surgery. Early postoperative complications of gastric cancer surgery are significantly more common in female patients, especially mortality and infection rate. Similarly, the duration of postoperative stay is significantly longer in female patients, and they have more severe complications than male patients do.

Innovations and breakthroughs

The authors found that there was a significant association between testosterone and overall complications, and also with complications of gastric surgery. Association with testosterone was seen even after controlling for sex by stratifying the group and looking at testosterone levels. Postoperative complications were higher in patients who did not recover normal testosterone levels at POD5 or POD7.

Applications

By understanding the effect of sex hormones on postoperative complications, the present study might represent a future strategy for therapeutic intervention in the treatment of patients with gastrointestinal disease. If there is an association of low testosterone level with postoperative complication rate, then serial testing of testosterone level can easily identify the susceptible patients, and supplementary hormonal replacement therapy could be a novel treatment to prevent postoperative complications in these patients

Terminology

Estradiol, testosterone and progesterone are sex hormones that are measured to understand dysfunction in gonadal activity.

Peer review

This study is unique and interesting. The authors studied the impact of sex hormones, especially testosterone, on the prevalence of complications after gastrointestinal surgery. This study gives us some valuable clues to explain why postoperative complications occur more frequently in female patients, as previously noted. It would also be useful in giving us a basis for clinical research to predict the occurrence of surgical complications.

REFERENCES

1 **Stroud L**, Edwards J, Danzing L, Culver D, Gaynes R. Risk factors for mortality associated with enterococcal bloodstream infections. *Infect Control Hosp Epidemiol* 1996; **17**: 576-580

2 **Elliott DC**, Kufera JA, Myers RA. Necrotizing soft tissue infections. Risk factors for mortality and strategies for management. *Ann Surg* 1996; **224**: 672-683

3 **Wichmann MW**, Zellweger R, DeMaso CM, Ayala A, Chaudry IH. Mechanism of immunosuppression in males following trauma-hemorrhage. Critical role of testosterone. *Arch Surg* 1996; **131**: 1186-1191; discussion 1191-1192

4 **Schneider CP**, Nickel EA, Samy TS, Schwacha MG, Cioffi WG, Bland KI, Chaudry IH. The aromatase inhibitor, 4-hyd roxyandrostenedione, restores immune responses following trauma-hemorrhage in males and decreases mortality from subsequent sepsis. *Shock* 2000; **14**: 347-353

5 **Lephart ED**, Baxter CR, Parker CR Jr. Effect of burn trauma on adrenal and testicular steroid hormone production. *J Clin Endocrinol Metab* 1987; **64**: 842-848

6 **Majetschak M**, Christensen B, Obertacke U, Waydhas C, Schindler AE, Nast-Kolb D, Schade FU. Sex differences in posttraumatic cytokine release of endotoxin-stimulated whole blood: relationship to the development of severe sepsis. *J Trauma* 2000; **48**: 832-839; discussion 839-840

7 **Angele MK**, Wichmann MW, Ayala A, Cioffi WG, Chaudry IH. Testosterone receptor blockade after hemorrhage in males. Restoration of the depressed immune functions and improved survival following subsequent sepsis. *Arch Surg* 1997; **132**: 1207-1214

8 **Martin GS**, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. *N Engl J Med* 2003; **348**: 1546-1554

9 **Schröder J**, Kahlke V, Book M, Stüber F. Gender differences in sepsis: genetically determined? *Shock* 2000; **14**: 307-310; discussion 310-313

10 **Pittet D**, Davis CS, Li N, Wenzel RP. Identifying the hospitalized patient at risk for nosocomial bloodstream infection: a population-based study. *Proc Assoc Am Physicians* 1997; **109**: 58-67

11 **Schröder J**, Kahlke V, Staubach KH, Zabel P, Stüber F. Gender differences in human sepsis. *Arch Surg* 1998; **133**: 1200-1205

12 **Branagan G**, Finnis D. Prognosis after anastomotic leakage in colorectal surgery. *Dis Colon Rectum* 2005; **48**: 1021-1026

13 **Law WI**, Chu KW, Ho JW, Chan CW. Risk factors for anastomotic leakage after low anterior resection with total mesorectal excision. *Am J Surg* 2000; **179**: 92-96

14 **Yeh CY**, Changchien CR, Wang JY, Chen JS, Chen HH, Chiang JM, Tang R. Pelvic drainage and other risk factors for leakage after elective anterior resection in rectal cancer patients: a prospective study of 978 patients. *Ann Surg* 2005; **241**: 9-13

15 **Rullier E**, Laurent C, Garrelon JL, Michel P, Saric J, Parneix M. Risk factors for anastomotic leakage after resection of rectal cancer. *Br J Surg* 1998; **85**: 355-358

16 **Sah BK**, Zhu ZG, Chen MM, Xiang M, Chen J, Yan M, Lin YZ. Effect of surgical work volume on postoperative complication: superiority of specialized center in gastric cancer treatment. *Langenbecks Arch Surg* 2009; **394**: 41-47

17 **Sah BK**, Zhu ZG, Wang XY, Yang QM, Chen MM, Xiang M, Chen J, Yan M. Post-operative complications of gastric cancer surgery: female gender at high risk. *Eur J Cancer Care* (Engl) 2009; **18**: 202-208
18 **Copeland GP**, Jones D, Walters M. POSSUM: a scoring system for surgical audit. *Br J Surg* 1991; **78**: 355-360
19 **Knöferl MW**, Angele MK, Diodato MD, Schwacha MG, Ayala A, Cioffi WG, Bland KI, Chaudry IH. Female sex hormones regulate macrophage function after trauma-hemorrhage and prevent increased death rate from subsequent sepsis. *Ann Surg* 2002; **235**: 105-112
20 **Zellweger R**, Wichmann MW, Ayala A, Stein S, DeMaso CM, Chaudry IH. Females in proestrus state maintain splenic immune functions and tolerate sepsis better than males. *Crit Care Med* 1997; **25**: 106-110
21 **Homo-Delarche F**, Fitzpatrick F, Christeff N, Nunez EA, Bach JF, Dardenne M. Sex steroids, glucocorticoids, stress and autoimmunity. *J Steroid Biochem Mol Biol* 1991; **40**: 619-637
22 **Yokoyama Y**, Kuebler JF, Matsutani T, Schwacha MG, Bland KI, Chaudry IH. Mechanism of the salutary effects of 17beta-estradiol following trauma-hemorrhage: direct downregulation of Kupffer cell proinflammatory cytokine production. *Cytokine* 2003; **21**: 91-97
23 **Angstwurm MW**, Gaertner R, Schopohl J. Outcome in elderly patients with severe infection is influenced by sex hormones but not gender. *Crit Care Med* 2005; **33**: 2786-2793

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BRIEF ARTICLE

Polymorphisms of some cytokines and chronic hepatitis B and C virus infection

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Abstract

AIM: To study the relationship between the polymorphisms in some cytokines and the outcome of hepatitis B virus (HBV) and hepatitis C virus (HCV) infection.

METHODS: Samples were obtained from 203 patients infected with HBV and/or HCV while donating plasma in 1987, and 74 controls were obtained from a rural area of North China. Antibodies to HBV or HCV antigens were detected by enzyme-linked immunoassay. The presence of viral particles in the serum was determined by nested reverse-transcriptase polymerase chain reaction (PCR). Hepatocellular injury, as revealed by alanine aminotransferase (ALT) and aspartate aminotransferase level, was detected by a Beckman LX-20 analyzer. DNA was extracted from blood cells. Then, the single nucleotide polymorphisms of IL-2-330, IFN-γ+874, IL-10-1082/-592 and IL-4-589 were investigated by restriction fragment length polymorphism-PCR or sequence specific primer-PCR.

RESULTS: Persistent infection with HBV, HCV, and HBV/HCV coinfection was associated with IL-2-330 TT genotype and T allele, IFN-γ+874 AA genotype, and IL-10-1082 AA genotype. The clinical outcome of HBV and/or HCV infection was associated with IL-2-330 TT genotype and T allele, IFN-γ+874 AA genotype, and IL-10-1082 AA genotype. IL-2-330 GG genotype frequency showed a negative correlation with clinical progression, IL-10-1082 AA genotype frequency showed a positive correlation and IL-10-1082 AG genotype frequency showed a negative correlation with clinical progression. HCV RNA positive expression was associated with IL-10-1082 AA genotype and the A allele frequency. Abnormal serum ALT level was associated with IL-10-592 AC genotype frequency and IL-4-589 CC genotype, CT genotype, and the C allele.

CONCLUSION: These results suggest that polymorphisms in some cytokine genes influence persistent HBV and HCV infection, clinical outcome, HCV replication, and liver damage.

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Key words: Hepatitis B; Hepatitis C; Single nucleotide polymorphism; Disease susceptibility; Outcome studies; Cytokines

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INTRODUCTION

The natural outcome in hepatitis B virus (HBV) and hepatitis C virus (HCV) infection varies dramatically among individuals. Infection with HCV is self-limited in a fortunate minority, while the majority of subjects develop persistent (chronic) infection[1,2]. Although infection with

HBV in adults progresses to the chronic phase in about 5%[3], among those individuals with persistent HBV or HCV infection, the majority develop chronic hepatitis (CH), progressive fibrosis and even liver cancer[4,5]. However, there are some cases that never evolve into any significant liver disease within the patient's natural lifespan[6,7]. It remains unknown why patients infected with HBV and/or HCV frequently turn to be chronic and the outcome of HBV and/or HCV infection dramatically varies. Besides the pathogenesis of viral factors, the most important factor is the different immune response to HBV or HCV infection between individuals. For example, in patients infected with HBV/HCV, some show a strong reduction in $CD4^+$ T-helper (Th) and $CD8^+$ cytotoxic-T-lymphocyte (CTL) responses. This is likely to be crucial in clearance of acute viremia[8-10]. Another factor is thought to be immune tolerance to HBV/HCV infection. This contributes to explaining the different susceptibility to HBV/HCV infection. Many manifestations show that the immunity level of host correlates with relevant gene polymorphisms, especially with single nucleotide polymorphisms (SNPs) in the promoter region that regulates gene expression. Furthermore, the gene polymorphisms probably determine the outcome of the infection. This study investigated the screened Th1 cytokines, interleukin (IL)-2 (-330), interferon (IFN)-γ (+874), and Th2 cytokines, IL-10 (-1082, -592) and IL-4 (-589) as candidate genes, to investigate the outcome of HBV/HCV infection.

MATERIALS AND METHODS

Subjects

We recruited 277 Han Chinese individuals from a rural area of Northern China (137 male and 140 female, aged 30-70 years, mean age 50.20 ± 10.43 years), including 203 that were infected with HBV and/or HCV when they donated plasma in 1987, and 74 controls who had cleared HBV and HCV spontaneously. Plasma samples were evaluated by nested reverse-transcriptase polymerase chain reaction (nRT-PCR) and PCR. We diagnosed and excluded 28 patients with fatty liver. The infections had been diagnosed in 1993. Written informed consent for enrolling in the study was obtained from all the subjects. Patients were classified into the following groups. (1) Controls: 74 individuals (34 male and 40 female, mean age 48.61 ± 9.39 years) who were negative for HBV and HCV antibodies. (2) Persistent HCV infection: 55 individuals (28 male and 27 female, mean age 49.42 ± 10.01 years) who were positive for HCV antibodies but negative for HBV antibodies. (3) Persistent HBV infection: 69 individuals (39 male and 30 female, mean age 52.74 ± 13.17 years) who had hepatitis B surface antigen and/or anti-hepatitis B core and/or anti-hepatitis B e antibodies, without HCV antibodies. (4) Persistent HBV and HCV coinfection: 79 individuals (36 male and 43 female, mean age 50.01 ± 8.56 years) who had antibodies to HBV and HCV.

Experimental and clinical diagnosis

Antibodies to HBV or HCV antigens were detected with an enzyme-linked immunoassay according to the manufacturer's instructions (Shanghai Kehua Bio-engineering Co. Ltd). The presence of hepatitis C viral particles in serum was determined by nRT-PCR. Hepatocellular injury, as revealed by alanine aminotransferase and aspartate aminotransferase level, was detected by a Beckman LX-20 analyzer. Clinical outcomes were diagnosed by evidence of viremia, liver transaminases and abnormal ultrasonography. Patients were diagnosed into four progressive hepatitis groups. (1) Healthy group: individuals negative for HBV and HCV antibodies, with normal liver transaminase levels and ultrasonography. (2) Mild CH group: patients with antibodies to HBV and/or HCV with mild abnormal liver transaminase and ultrasonography, but no evidence of viremia. (3) Moderate/severe CH: patients with antibodies to HBV and/or HCV with evidence of viremia, abnormal liver transaminase and/or abnormal ultrasonography. (4) Cirrhosis group: patients with antibodies to HBV and/or HCV and significantly abnormal ultrasonography. The diagnosis was performed using established methods for confirming cirrhosis[11], with or without evidence of viremia, which had abnormal liver transaminase levels.

DNA extraction

Blood specimens were collected in sodium citrate sterile tubes. Genomic DNA was extracted from 2-mL samples of whole blood and subsequently stored at -20℃ for genotype analysis. Genomic DNA extraction was performed using the guanidine-HCl method[12].

Gene polymorphism analyses of IL-2-330, Il-4-589 and IL-10-592

Gene amplification: IL-2-T330G, IL-4-C589T and IL-10-A592C gene polymorphisms were typed by restriction fragment length polymorphism-PCR. The primer sequences used are listed in Table 1. The 25-μL final PCR volume was as follows: 20 ng DNA, 2.5 μL 10 × PCR Buffer (100 mmol/L Tris-HCl, pH 8.3, 500 mmol/L KCl), 200 μmol/L dNTPs (400 μmol/L dNTPs for IL-4-C589T), 1.5 mmol/L $MgCl_2$ (2.0 mmol/L $MgCl_2$ for IL-2-T330G), 10 pmol each primer, 1 U *Taq* polymerase (TaKaRa Biotechnology Co. Ltd., Dalian, China). PCR was carried out in an AmpGene DNA thermal cycler 4800 (America PE Corporation). After the initial denaturation step (95℃ for 2 min), the 35 cycles specified in Table 1 were employed. After the final cycle, the samples were kept at 72℃ for 7 min. Successful amplification was confirmed by using 2% agarose gel stained with ethidium bromide. The product size of each cytokine was described as in Table 2.

Restriction enzyme digestion: Restriction enzyme digestion was performed in 10-μL volumes as follows: 2 U restriction enzyme, 1 μL 10 × Buffer, and the appropriate PCR product (4 μL IL-2-T330G, 3 μL IL-

Table 1 The position, polymorphism and primer sequence in IL-2, IL-4 and IL-10 genes

SNP position	Polymorphism	Primer sequence 5′-3′	PCR conditions
IL-2 -330	T→G	F: 5'-TATTCACATGTTCAGTGTAGTTCT-3' R: 5'-ACATTAGCCCACACTTAGGT-3'	94℃, 48℃, 72℃ for 1 min
IL-4 -589	C→T	F: 5'-ACTAGGCCTCACCTGATACG-3' R: 5'-GTTGTAATGCAGTCCTCCTG-3'	94℃, 57℃, 72℃ for 30 s
IL-10 -592	A→C	F: 5'-CCTAGGTCACAGTGACGTGG-3' R: 5'-GGTGAGCACTACCTGACTAGC-3'	94℃/30 s, 60℃/45 s, 72℃/60 s

Table 2 Polymorphism position, product size, restriction enzyme, digestion fragments in IL-2-330, IL-4-589 and IL-10-592

SNP position	Product size (bp)	Restriction enzyme	Digestion fragments (bp)
IL-2-330 T/G	150	MaeI (Bio Basic Inc.)	150 26 + 124
IL-4-589 T/C	252	BsmFI (New England BioLabs)	252 60 + 192
IL-10-592 C/A	412	RsaI (Bio Basic Inc.)	412 236 + 176

4-C589T and 1 μL 10 × BSA, 5 μL IL-10-A592C). The digestion reaction of IL-2-T330G and IL-10-A592C was incubated at 37℃ for 10 h, and the IL-4-C589T reaction at 65℃ for 15 h. The digestive products were visualized on 3% agarose gel stained with ethidium bromide. The wild or mutant genotype was characterized by enzyme digestion fragments, as in Table 2.

Gene polymorphism analysis of IL-10-1082 and IFN-γ+874

IL-10-G1082A and IFN-γ+T874A gene polymorphisms were detected by sequence specific primer-PCR technique. For each sample, two parallel reactions were performed. For IL-10-1082, the forward allele-specific primers were either 5'-ACTACTAAGGCTTCTTTGGGAA-3' (for IL-10 allele A) or 5'-CTACTAAGGCTTCTTTGGGAG-3' (for IL-10 allele G), and the antisense primer was 5'-CAGTGCCAACTGAGAATTTGG-3'. The product size was 258 bp. A human growth hormone sequence was used as an internal control. The control primers were 5'-GCCTTCCCAACCATTCCCTTA-3' and 5'-TCACGGATTTCTGTTGTGTTTC-3', and the product size was 429 bp. The 20-μL final reaction volume contained: 2.0 μL 10 × PCR Buffer (100 mmol/L Tris-HCl, pH 8.3, 500 mmol/L KCl), 200 μmol/L dNTPs, 2.0 mmol/L $MgCl_2$, 3.75 μmol/L specific primer, 0.09 μmol/L control primer, 20 ng DNA, and 0.5 U *Taq* polymerase. PCR was carried out in an AmpGene DNA thermal cycler 4800. The cycling conditions were 95℃ for 2 min, and 10 cycles for 1 min at 95℃, 50 s at 65℃ and 50 s at 72℃, followed by 20 cycles for 1 min at 95℃, 50 s at 59℃, and 50 s at 72℃, followed by a final extension for 7 min at 72℃. The PCR products were visualized on 1.5% agarose gel stained with ethidium bromide.

For IFN-γ+874, the forward allele-specific primers were 5'-TTCTTACAACACAAAATCAAATCT-3' (for allele T) or 5'-TTCTTACAACACAAAATCAAATCA-3' (for allele A), and the anti-sense primer was 5'-TCAACAAAGCTGATACTCCA-3'. The product size was 262 bp. A human growth hormone sequence was used as an internal control. The control forward primer was 5'-TATGATTCTGGCTAAGGAATG-3' and the antisense primer was 5'-TCAACAAAGCTGATACTCCA-3. The product size was 440 bp. The 20-μL final reaction volume contained 2.0 μL 10 × PCR buffer (100 mmol/L Tris-HCl, pH 8.3, 500 mmol/L KCl), 200 μmol/L dNTPs, 2.0 mmol/L $MgCl_2$, 0.5 μmol/L specific primer, 0.1 μmol/L former control primer, 20 ng DNA, and 0.5 U *Taq* polymerase. PCR was carried out in an AmpGene DNA thermal cycler 4800. The cycling conditions were 94℃ for 2 min, and 10 cycles for 30 s at 94℃, 40 s at 60℃ and 40 s at 72℃, followed by 25 cycles for 30 s at 94℃, 40 s at 56℃, and 50 s at 72℃, followed by a final extension for 7 min at 72℃. The PCR products were visualized on 1.5% agarose gel stained with ethidium bromide.

Statistical analysis

The data were tested by the χ^2 test for the goodness of fit to the Hardy-Weinberg equilibrium (HWE) between the observed and expected genotype values. The frequencies of the alleles and genotypes among the groups were compared by the χ^2 test. $P < 0.05$ was considered statistically significant. We calculated the OR and 95% CI.

RESULTS

Hardy-Weinberg equilibrium (HWE) and characteristic distribution of age and sex

An HWE test was performed for all cytokine polymorphisms investigated in this study (Table 3). The distribution of these observed genotypes was not significantly different from the expected distribution according to HWE, except the IL-10-1082 polymorphisms, which revealed a deviation from HWE because of the lower frequency of wild-type homozygosity (the observed *vs* the expected frequency was 1.08% *vs* 10.91%). In IL-2-330, IL-4-589, IL-10-1082, IL-10-592 and IFN-γ+874 gene polymorphisms, there were no significant differences in age and sex ($P > 0.05$).

Genotype and allele distribution of IL-2-330, IFN-γ+874, IL-4-589 and IL-10-1082 and -592 in persistent HBV and/or HCV infection

IL-2-330 TT, -330 GG and -330 TG are shown in Figure 1. The IL-2-330 polymorphisms showed obvious association with persistent HBV and/or HCV infection. IL-2-330 TT was associated with an increased risk, but IL-2-330 GG with a reduced risk of persistent HBV and/or HCV

Table 3 HWE test for cytokines

Locus	Observed homozygosity (%)		Expected homozygosity (%)		*P* value
	Wild-type genotype	Mutant genotype	Wild-type genotype	Mutant genotype	
IL-2-330	40.79	14.08	40.14	13.43	> 0.05
IFN-γ+874	11.55	35.02	14.65	38.10	> 0.05
IL-4-589	3.25	50.54	6.94	54.24	> 0.05
IL-10-592	42.60	10.83	43.40	11.64	> 0.05
IL-10-1082	1.08	35.02	10.91	44.85	< 0.05

Table 4 Polymorphisms of IL-2-330, IFN-γ+874, IL-10-1082 and -592 and IL-4-589 and HBV and HCV infection *n* (%)

Genotype allele	Controls (*n* = 74)	HCV infection (*n* = 55)	HBV infection (*n* = 69)	HBV/HCV coinfection (*n* = 79)	χ^2	*P*
IL-2-330						
TT	22 (29.7)	24 (43.6)	33 (47.8)	34 (43.0)		
GG	19 (25.7)	6 (10.9)	4 (5.8)	10 (12.7)	14.24	0.03
TG	33 (44.6)	25 (45.5)	32 (46.4)	35 (44.3)		
T	77 (52.0)	73 (66.4)	98 (71.0)	103 (65.2)	12.33	0.01
G	71 (48.0)	37 (33.6)	40 (29.0)	55 (34.8)		
IFN-γ+874						
TT	7 (9.5)	5 (9.1)	9 (13.0)	11 (13.9)		
AA	14 (18.9)	23 (41.8)	25 (36.2)	35 (44.3)	16.15	0.01
TA	53 (71.6)	27 (49.1)	35 (50.7)	33 (41.8)		
T	67 (45.3)	37 (33.6)	53 (38.4)	55 (34.8)	4.87	0.18
A	81 (54.7)	73 (66.4)	85 (61.6)	103 (65.2)		
IL-10-1082						
GG	1 (1.4)	2 (3.6)	0 (0.0)	0 (0.0)		
AA	16 (21.6)	21 (38.2)	27 (39.1)	33 (41.8)	13.05	0.04
GA	57 (77.0)	32 (58.2)	42 (60.9)	46 (58.2)		
G	59 (39.9)	36 (32.7)	42 (30.4)	46 (29.1)	4.65	0.20
A	89 (60.1)	74 (67.3)	96 (69.6)	112 (70.9)		
IL-10-592						
AA	34 (45.9)	20 (36.4)	29 (42.0)	35 (44.3)		
CC	9 (12.2)	6 (10.9)	9 (13.0)	6 (7.6)	2.83	0.83
AC	31 (41.9)	29 (52.7)	31 (44.9)	38 (48.1)		
A	99 (66.9)	69 (62.7)	89 (64.5)	108 (68.4)	1.10	0.78
C	49 (33.1)	41 (37.3)	49 (35.5)	50 (31.6)		
IL-4-589						
CC	5 (6.8)	1 (1.8)	1 (1.4)	2 (2.5)		
TT	38 (51.4)	28 (50.9)	35 (50.7)	39 (49.4)	4.41	0.62
CT	31 (41.9)	26 (47.3)	33 (47.8)	38 (48.1)		
C	41 (27.7)	28 (25.5)	35 (25.4)	42 (26.6)	0.26	0.97
T	107 (72.3)	82 (74.5)	103 (74.6)	116 (73.4)		

infection. The OR and 95% CI for IL-2-330 TT compared with -330 GG in HCV infection, HBV infection and HBV/HCV coinfection were 3.46 (1.17-10.20), 7.14 (2.13-23.81) and 2.93 (1.15-7.46), respectively. However, IL-2-330 TT/GG did not differ significantly between patients with HBV and/or HCV infection. The IL-2-330 T allele was associated with an increased risk, but the IL-2-330 G allele was associated with a reduced risk of chronic HCV and/or HBV infection. The OR and 95% CI for the IL-2-330 T allele compared with -330 G allele in HCV infection, HBV infection, and HBV/HCV coinfection were 1.82 (1.09-3.03), 2.26 (1.39-3.69) and 1.73 (1.10-2.73), respectively. However, the IL-2-330 T/G allele did not differ significantly between HBV and/or HCV infection (Table 4).

IFN-γ+874 TT, +874 AA and +874 TA are shown in Figure 2. The IFN-γ+874 polymorphisms showed a significant association with persistent HBV and/or HCV infection. IFN-γ+874 AA was associated with an increased risk, but IFN-γ+874 TA with a reduced risk of persistent HBV and/or HCV infection. The OR and 95% CI for IFN-γ+874 AA compared with +874 TA in HCV infection, HBV infection, and HBV/HCV coinfection were 3.23 (1.43-7.25), 2.70 (1.24-5.92) and 4.02 (1.88-8.55), respectively. However, IFN-γ+874 AA/TA did not differ significantly between HBV and/or HCV infection. The IFN-γ+874 T/A alleles did not differ significantly between all groups (Table 4).

IL-10-1082 GG, -1082 AA and -1082 GA are shown in Figure 3. The IL-10-1082 polymorphisms showed a significant association with persistent HBV and/or HCV infection. IL-10-1082 AA was associated with an increased risk, but -1082 AG with a reduced risk of persistent HBV and/or HCV infection. The OR and 95%

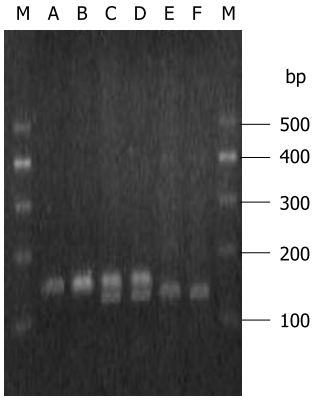

Figure 1 IL-2-330 T/G SNP. M: Marker; A and B: TT wild-type genotype; C and D: TG genotype; E and F: GG mutant genotype.

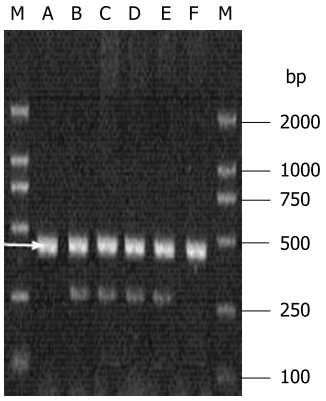

Figure 2 IFN-γ+874 T/A SNP. M: Marker; A and B: AA mutant genotype; C and D: TA genotype; E and F: TT wild-type genotype; →: Internal control gene.

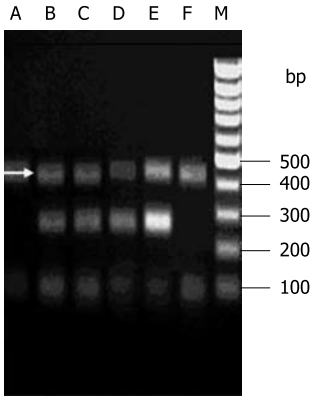

Figure 3 IL-10-1082 A/G SNP. M: Marker; A and B: AA mutant genotype; C and D: AG genotype; E and F: GG wild-type genotype; →: Internal control gene.

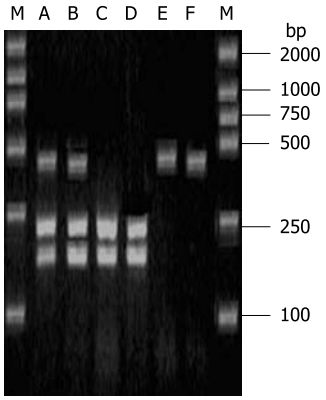

Figure 4 IL-10-592 A/C SNP. M: Marker; A and B: AC genotype; C and D: AA wild-type genotype; E and F: CC mutant genotype.

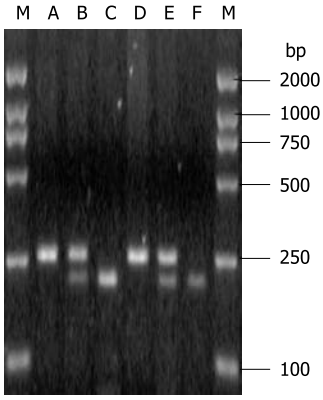

Figure 5 IL-4-589 C/T SNP. M: Marker; A and B: TT mutant genotype; C and D: CT genotype; E and F: CC wild-type genotype.

CI for IL-10-1082 AA compared with -1082 AG in HCV infection, HBV infection, and HBV/HCV coinfection were 2.34 (1.07-5.10), 2.29 (1.10-4.78) and 2.56 (1.25-5.21), respectively. However, IL-10-1082 AA/AG did not differ significantly between the subjects with HBV and/or HCV infection. The IL-10-1082 A/G alleles did not differ obviously between any of the subjects (Table 4).

The IL-10-592 polymorphisms are shown in Figure 4. The IL-10-592 A/C polymorphisms did not differ significantly between any of the groups (Table 4).

IL-10-589 TT, -589 CC and -589 TC are shown in Figure 5. IL-4-589 T/C polymorphisms did not differ significantly between any of the groups (Table 4).

Association of IL-2-330, IFN-γ+874, IL-4-589 and IL-10-1082 and -592 with clinical outcome of HBV and/or HCV infection

The IL-2-330 polymorphisms showed a significant association with the outcome of persistent HBV and/or HCV infection. IL-2-330 TT was associated with an increased risk, but -330 GG with a reduced risk of mild CH, moderate/severe CH, and cirrhosis. The OR and 95% CI for IL-2-330 TT compared with -330 GG genotype in patients with mild CH, moderate/severe CH, and cirrhosis were 3.42 (1.45-8.13), 3.29 (1.10-9.80), and 11.11 (1.32-90.91), respectively. However, IL-2-330 TT/GG did not differ significantly between patients with mild CH, moderate and severe CH, and cirrhosis. The IL-2-330 T allele was associated with an increased risk, but the -330 G allele was associated with a reduced risk of mild CH, moderate and severe CH, or cirrhosis. The OR and 95% CI for the -330 T allele compared with -330 G allele for patients with mild CH, moderate/severe CH and cirrhosis were 1.83 (1.20-2.80), 1.70 (1.02-2.82) and 2.75 (1.32-5.63), respectively. However, IL-2-330 polymorphisms did not differ significantly between patients with mild CH, moderate/severe CH, and cirrhosis (Table 5).

The IFN-γ+874 polymorphisms showed a significant association with the clinical outcome of persistent HBV and/or HCV infection. IFN-γ+874 AA was associated with an increased risk, but +874 TA with a reduced risk of mild CH, moderate/severe CH, and cirrhosis. There was significantly decreased IFN-γ+874 AA genotype frequency in cirrhosis compared with moderate/severe CH patients. The OR and 95% CI for IFN-γ+874 AA compared with +874 TA in patients with mild CH, moderate/severe CH, and cirrhosis were 3.09 (1.51-6.33), 3.85 (1.70-8.70) and 3.14 (1.08-9.17), respectively. The IFN-γ+874 T/A alleles did not differ between any of the groups (Table 5).

The IL-10-1082 polymorphisms showed a significant association with the clinical outcome of persistent HBV

Table 5 Polymorphisms of IL-2-330, IFN-γ+874, IL-4-589, IL-10-1082 and -592 and clinical outcome *n* (%)

Genotype	Control (*n* = 74)	Mild CH (*n* = 122)	Moderate/severe CH (*n* = 57)	Cirrhosis (*n* = 24)	χ^2	*P*
IL-2-330						
TT	22 (29.7)	54 (44.3)	23 (40.4)	13 (54.2)		
GG	19 (25.7)	14 (11.4)	6 (10.5)	1 (4.2)	13.46	0.04
TG	33 (44.6)	54 (44.3)	28 (49.1)	10 (41.7)		
T	77 (52.0)	163 (66.8)	74 (64.9)	36 (75.0)	11.69	0.01
G	71 (48.0)	81 (33.2)	40 (35.0)	12 (25.0)		
IFN-γ+874						
TT	8 (10.8)	14 (11.5)	5 (8.8)	4 (16.7)		
AA	14 (18.9)	48 (39.3)	26 (45.6)	9 (37.5)	14.78	0.02
TA	52 (70.3)	60 (49.2)	26 (45.6)	11 (45.8)		
T	68 (45.9)	90 (36.9)	36 (31.6)	19 (39.6)	5.68	0.13
A	80 (54.1)	154 (63.1)	78 (68.4)	29 (60.4)		
IL-10-1082						
GG	1 (1.4)	1 (0.8)	1 (1.8)	0 (0.0)		
AA	16 (21.6)	44 (36.1)	24 (42.1)	13 (54.2)	11.40	0.07
GA	57 (77.0)	77 (63.1)	32 (56.1)	11 (45.8)		
G	59 (39.9)	79 (32.4)	34 (29.8)	11 (22.9)	5.85	0.12
A	89 (60.1)	165 (67.6)	80 (70.2)	37 (77.1)		
IL-10-592						
AA	34 (45.9)	51 (41.8)	22 (38.6)	11 (45.8)		
CC	9 (12.2)	11 (9.0)	8 (14.0)	2 (8.3)	2.50	0.87
AC	31 (41.9)	60 (49.2)	27 (47.4)	11 (45.8)		
A	98 (66.2)	163 (66.8)	71 (62.3)	33 (68.8)	0.92	0.82
C	50 (33.8)	81 (33.2)	43 (37.7)	15 (31.3)		
IL-4-589						
CC	3 (4.0)	3 (2.4)	3 (5.3)	0 (0.0)		
TT	38 (51.4)	69 (54.8)	24 (42.1)	11 (45.8)	4.58	0.60
CT	33 (44.6)	54 (42.9)	30 (52.6)	13 (54.2)		
C	39 (26.4)	58 (23.8)	36 (31.6)	13 (27.1)	2.46	0.48
T	109 (73.6)	186 (76.2)	78 (68.4)	35 (72.9)		

and/or HCV infection. IL-10-1082 AA was associated with an increased risk, but -1082 AG with a reduced risk of mild CH, moderate/severe CH, and cirrhosis. There was a significantly increased IL-10-1082 AA genotype frequency in cirrhosis compared with mild CH. IL-10-1082 AA showed a positive correlation with clinical outcome ($\gamma = 0.99$, $P = 0.00$), but the IL-10-1082 AG showed a negative correlation with clinical outcome ($\gamma = -0.99$, $P = 0.00$). The OR and the 95% CI for IL-10-1082 AA compared with -1082 AG in patients with mild CH, moderate/severe CH, and cirrhosis were 2.03 (1.03-3.98), 2.70 (1.24-5.88) and 4.26 (1.59-11.36), respectively. The IL-10-1082 G/A allele did not differ obviously in every group (Table 5).

The polymorphisms of IL-10-592 A/C and IL-4 -589 C/T did not differ significantly in every group (Table 5).

Association of IL-2-330, IFN-γ+874, IL-4-589 and IL-10-1082 and -592 polymorphisms with HCV replication and abnormal alanine aminotransferase (ALT) level

IL-10-1082 AA was associated with an increased risk, but -1082 AG was associated with a reduced risk of HCV RNA replication. The OR and 95% CI for IL-10-1082 AA compared with -1082 AG was 3.36 (1.67-6.76). IL-10-1082 A was associated with an increased risk, but -1082 G with a reduced risk of HCV RNA replication. The OR and 95% CI for IL-10-1082 A compared with -1082 G was 1.67 (1.08-2.57). The polymorphisms of IL-2-330, IFN-γ+874, IL-10-592 and IL-4-589 showed no association with HCV RNA replication (Table 6).

IL-10-592 AC showed an increased risk, but -592 AA showed a reduced risk of abnormal ALT. The OR and 95% CI for IL-10-592 AC compared with -592 AA was 2.83 (1.21-6.63). The IL-10-592 A/C alleles were not associated with abnormal ALT (Table 6).

The IL-4-589 CT/CC showed an increased risk, but -589 TT showed a reduced risk of abnormal ALT level. The OR and 95% CI for IL-10-589 CT and -589 CC compared with -589 TT genotype were 3.43 (1.47-8.00) and 8.25 (1.74-39.22), respectively, in patients with abnormal ALT. IL-4-589 C showed an increased risk, but -589 T showed a reduced risk of abnormal ALT level (Table 6). The OR and 95% CI for IL-10-589 C compared with -589 T was 2.31 (1.36-3.93).

The polymorphisms of IL-2-330, IFN-γ+874 and IL-10-1082 were not significantly associated with abnormal ALT (Table 6).

DISCUSSION

The natural outcome of HBV and HCV infection varies dramatically among individuals. HCV infection is self-limited in a fortunate minority, while the majority of subjects develop persistent (chronic) infection[1,2]. Although infection with HBV in adults progresses to the chronic phase in about 5%[3], among those individuals with persistent HBV or HCV infection, the majority develop

Table 6 Polymorphisms of IL-2-330, IFN-γ+874, IL-4 -589 and IL-10-1082 and -592 and HCV RNA and ALT level *n* (%)

	HCV RNA -	HCV RNA +	χ^2	*P*	ALT < 80 U/L	ALT ≥ 80 U/L	χ^2	*P*
IL-2-330								
TT	29 (37.2)	39 (37.1)			102 (41.8)	11 (33.3)		
GG	14 (17.9)	14 (13.3)	0.83	0.66	35 (14.3)	4 (12.1)	1.35	0.51
TG	35 (44.9)	52 (49.5)			107 (43.9)	18 (54.5)		
T	93 (59.6)	130 (61.9)	0.20	0.66	311 (63.7)	40 (60.0)	0.24	0.62
G	63 (40.4)	80 (38.1)			177 (36.3)	26 (39.4)		
IFN-γ+874								
TT	13 (16.7)	10 (9.5)			29 (11.9)	3 (9.1)		
AA	28 (35.9)	45 (42.9)	2.36	0.31	85 (34.8)	12 (36.4)	0.23	0.89
TA	37 (47.4)	50 (47.6)			130 (53.3)	18 (54.5)		
T	63 (40.4)	70 (33.3)	1.92	0.17	188 (38.5)	24 (36.4)	0.12	0.74
A	93 (59.6)	140 (66.7)			300 (61.5)	42 (63.6)		
IL-10-1082								
GG	1 (1.3)	2 (1.9)			3 (1.2)	0 (0.0)		
AA	14 (17.9)	44 (41.9)	12.27	0.00	81 (33.2)	16 (48.5)	3.25	0.20
GA	63 (80.8)	59 (56.2)			160 (65.6)	17 (51.5)		
G	91 (58.3)	147 (70.0)	5.36	0.02	166 (34.0)	17 (25.8)	1.79	0.18
A	65 (41.7)	63 (128)			322 (66.0)	49 (74.2)		
IL-10-592								
AA	41 (52.6)	44 (41.9)			110 (45.1)	8 (24.2)		
CC	6 (7.7)	11 (10.5)	2.10	0.35	27 (11.1)	3 (9.1)	6.32	0.04
AC	31 (39.7)	50 (47.6)			107 (43.9)	22 (66.7)		
A	113 (72.4)	138 (65.7)	1.88	0.17	327 (67.0)	38 (57.6)	2.30	0.09
C	43 (27.6)	72 (34.3)			161 (33.0)	28 (42.4)		
IL-4-589								
CC	3 (3.8)	5 (4.8)			6 (2.5)	3 (9.1)		
TT	48 (61.5)	55 (52.4)	1.53	0.47	132 (54.1)	8 (24.2)	12.46	0.02
CT	27 (34.6)	45 (42.9)			106 (43.4)	22 (66.7)		
C	33 (21.2)	55 (26.2)	1.24	0.27	118 (24.2)	28 (42.4)	9.97	0.00
T	123 (78.8)	155 (73.8)			370 (75.8)	38 (57.6)		

CH, progressive fibrosis and even liver cancer[4,5,13]. However, some cases will never progress to any significant liver disease[6,7]. It remains unknown why the infection frequently turns into CH, and why the outcome with HBV and/or HCV infection varies dramatically. Cytokines play a crucial role in regulating the immune and inflammatory responses. Polymorphisms in the regulatory regions of the cytokine genes may influence their expression. Therefore, as genetic predictors of the disease susceptibility or clinical outcome, the polymorphisms of cytokine genes are potentially important.

IL-2 is a Th1 cytokine and has a powerful immunoregulatory effect on the stimulation of proliferation and activation of most T lymphocytes, natural killer cells, and B lymphocytes[14]. Some evidence has shown that the IL-2 levels of patients with moderate/severe CH and cirrhosis are decreased significantly more than those of healthy controls[15-17]. Other authors have observed that increased IL-2 expression is correlated with the grading/staging of chronic hepatitis C[18]. In this study, we found that the IL-2-330 SNP was associated with persistent HBV and/or HCV infection. The subjects with persistent HBV and/or HCV infections had significantly lower IL-2-330 GG genotype frequency and higher IL-2-330 TT genotype frequency than the controls. The subjects with IL-2-330 TT had a 3.46, 7.14 and 2.93 times higher risk of infection with HCV, HBV, or HBV/HCV. The subjects with chronic HCV and/or HBV infections had higher IL-2-330 T allele and lower -330 G allele frequencies, respectively. The patients with the -330 T allele had a 1.82, 2.26 and 1.73 times higher risk of infection with HCV, HBV and HBV/HCV, respectively. At the same time, we also found an association between IL-2-330 SNP and clinical outcome of mild CH, moderate/severe CH and cirrhosis. Compared with the controls, the patients with mild CH, moderate/severe CH and cirrhosis had significantly higher IL-2-330 TT genotype frequency and lower -330 GG genotype frequency. IL-2-330 GG showed a negative correlation with clinical outcome (γ = -0.92, P = 0.01). Individuals with -330 TT genotype had a 3.42, 3.29 and 11.11 times higher risk of developing into mild CH, moderate/severe CH, and cirrhosis, respectively. Subjects with IL-2-330 T had a 1.83, 1.70 and 2.75 times higher risk of developing into mild CH, moderate/severe CH, and cirrhosis, respectively. These results indicate that the IL-2-330 TT genotype and the -330 T allele are not only the potential susceptibility gene for HBV and/or HCV infections, but also the gene that potentially determines clinical outcome. Moreover, the clinical outcome of HBV and/or HCV infection is associated with IL-2 level, as determined by the IL-2-330 polymorphisms. A plausible explanation for the association of IL-2-330 TT genotype and T allele with persistent infection and clinical outcome is derived from experiments that have shown that patients with the IL-2-330 TT genotype and T allele, with lower IL-2 production[5], unsuccessfully eradicate the virus and become persistently viremic, which further results in susceptibility to HBV and/or HCV infection.

IFN-γ is a Th1 cytokine and plays an important role in modulating almost all the immune responses, such as T-cell differentiation, anti-proliferative, antitumor, and antiviral activities[19,20]. Pravica *et al*[21] have found that IFN-γ+874 T/T genotype is often regarded as indicating high IFN-γ production, and +874 A/A genotype as indicating low production. Some authors have found that plasma IFN-γ concentrations in chronic hepatitis C patients decrease significantly[22,23]. Other authors have documented a correlation between the increase of IFN-γ expression and grading in chronic hepatitis C infection[18,24,25]. In this study, compared with the controls, the IFN-γ+874 AA genotype was found more frequently and the +874 TA genotype was found less frequently in subjects with persistent HBV and/or HCV infection. Subjects with +874 AA genotype have a 3.23, 2.70 and 4.02 times higher risk of HCV infection, HBV infection, and HBV/HCV coinfection, respectively. No difference was found in the IFN-γ+874 T/A alleles among the groups. However, Liu *et al*[26] have found that the frequency of IFN-γ+874 A allele was significantly higher in patients with chronic HBV infection than in the controls (OR = 2.25, 95% CI = 1.69-2.99, $P < 0.0001$). Moreover, compared with the controls, the patients with mild CH, moderate/severe CH, and cirrhosis showed a higher IFN-γ+874 AA frequency and a lower IFN-γ+874 TA frequency. Individuals with IFN-γ+874 AA had a 3.09, 3.85 and 3.14 times higher risk of developing mild CH, moderate/severe CH, and cirrhosis, respectively. Compared with moderate/severe CH patients, cirrhosis patients showed a significantly decreased +874 AA genotype frequency. This probably resulted from the lower number of samples of cirrhosis. These results indicate that IFN-γ+874 AA genotype is not only the potential susceptibility gene for HBV and/or HCV infection, but also the gene that potentially determines clinical outcome. A plausible explanation is that IFN-γ+874 AA genotype produces a low level of IFN-γ, which results in the cytolytic defect that promotes viral persistence and hepatic persistent infection that determines the clinical outcome of HBV and/or HCV infection.

IL-10 is an important anti-inflammatory cytokine secreted from Th2 cells. The levels of IL-10 production determine immune regulation, and the balance between the inflammatory and humoral responses. There are three confirmed SNPs in the IL-10 gene promoter. The -1082 GG genotype is associated with high IL-10 production, the -1082 GA with intermediate production, and the -1082 AA with low production. Polymorphisms at position -819 and -592 have no independent influence on IL-10 production[27]. There are contradictory reports about the exact effect of IL-10 promoter polymorphisms on the natural outcome of HBV and HCV infection. Some authors have found that plasma IL-10 level is decreased significantly in chronic hepatitis C patients[22,28]. Others have reported that the level of IL-10 in patients with chronic hepatitis C is higher than that in healthy individuals ($P < 0.05$). The value of IL-10 showed a significant positive correlation with ALT[29,30]. In the present study, IL-10-1082 AA was associated with an increased risk, but -1082 AG was associated with a reduced risk of persistent HBV and/or HCV infection. Subjects with IL-10-1082 AA had a 2.34, 2.29 and 2.56 times higher risk of persistent HCV infection, HBV infection and HBV/HCV coinfection, respectively. At the same time, IL-10-1082 AA is associated with an increased risk, but -1082 AG is associated with a reduced risk of mild CH, moderate/severe CH, and cirrhosis. Also, IL-10-1082 AA showed a positive correlation with clinical outcome ($\gamma = 0.99$, $P < 0.01$), but -1082 AG showed a negative correlation with clinical outcome ($\gamma = -0.99$, $P < 0.01$). Individuals with the -1082 AA genotype had a 2.03, 2.70 and 4.26 times higher risk of developing mild CH, moderate/severe CH, and cirrhosis, respectively. These results indicate that the IL-10-1082 AA genotype is not only a potential susceptibility gene for HBV and/or HCV infection, but also potentially determines clinical outcome. IL-10-1082 AA and -1082 A were associated with an increased risk, but IL-10-1082 AG and -1082 G were associated with a reduced risk of HCV RNA replication (OR = 3.36, 1.67, respectively). This result indicates that IL-10-1082 AA and -1082 A are associated with HCV RNA replication. The evidence shows that during persistent HCV infection, IL-10 may compromise the cellular immune response to the virus[31-33]. A plausible explanation is that IL-10 is an important anti-inflammatory cytokine. IL-10-1082 AA and -1082 A lead to lower IL-10 production[27] and persistent infection, which determines the clinical outcome of chronic infection. The IL-10-1082 polymorphisms had no significant association with plasma ALT level, which agrees with the results of Abbas *et al*[34]. The IL-10-592 AC genotype frequency was significantly higher in patients with abnormal ALT (OR = 2.83). This result indicates that the IL-10-592 AC genotype is associated with abnormal ALT level and hepatocellular injury. Hepatocellular injury is probably caused by the decreased production of the anti-inflammatory cytokine IL-10, which results from linkage of the IL-10-592 and IL-10-1082 genes.

IL-4 is a Th2 cytokine and plays an important role in humoral immunity. IL-4 has the function of inhibiting the production of IFN-γ and downregulating the differentiation of Th1 cells[35]. IL-4 has the function of modulating inflammatory responses by downregulating production of pro-inflammatory mediators and preventing inflammatory injury of the liver[36,37]. It has been described that the IL-4-589 T compared with the -590 C allele increases the strength of the IL-4 promoter[38], which leads to high production of IL-4. The evidence has shown that IL-4 plays a key role in HBV and HCV infection pathogenesis. Some have reported that IL-4 levels are lower in patients infected with HCV[39]. Others have reported that IL-4 is significantly higher in chronic HCV infection with abnormal ALT than with normal ALT level[38,39]. In the present study, IL-4-589 polymorphisms were not significantly associated with persistent HBV and/or HCV infection or clinical outcome. However, we found that the IL-4-589 CT genotype and -589 CC genotype frequency was significantly higher than that

of -589 TT genotype frequency in patients with abnormal ALT level. Individuals with IL-4-589 CT and -589 CC genotype had a 3.43 and 8.25 times higher risk of abnormal ALT. Higher -589 C allele and lower -589 T allele frequencies in patients with abnormal ALT were observed; individuals with the -589 C allele had a 2.31 times higher risk of abnormal ALT. This result indicates that the IL-4-589 CC and -589 CT genotype and the -589 C allele are associated with liver inflammatory injury. Such injury is probably caused by the decreased production of anti-inflammatory cytokines such as IL-4 and IL-10.

In summary, the polymorphisms of IL-2-330, IFN-γ+874, IL-10-1082 and -592 and IL-4-589 showed a significant association with the outcome of chronic HBV and/or HCV infection. The IL-2-330 TT/T allele, IFN-γ+874 AA and IL-10-1082 AA were associated with increased risk, but IL-2-330 GG/G allele, IFN-γ+874 TA and IL-10-1082 AG were associated with reduced risk of persistent HBV and/or HCV infection, and of developing mild CH, moderate/severe CH, and cirrhosis. The IL-10-1082 AA/A allele was associated with an increased risk, but the IL-10-1082 AG/G allele was associated with a reduced risk of HCV RNA replication. IL-10-592 AC and IL-4-589 CT/CC showed increased risk, but IL-10-592 AA and IL-4-589 TT showed reduced risk of abnormal ALT.

COMMENTS

Background

The natural outcome in hepatitis B virus (HBV) and hepatitis C virus (HCV) infection varies dramatically among individuals. Some infections are self-limited; some individuals progress to persistent HBV or HCV infection; and some develop chronic hepatitis, fibrosis and even liver cancer. The mechanisms are unknown. Besides viral pathogenic factors, a more important factor is the different immune response to HBV or HCV infection between individuals. T helper (Th)1 and Th2 cytokines determine host immunity level, which is regulated by polymorphisms in the promoter region of the Th1 and Th2 cytokines. Interleukin (IL)-2 (-330), interferon (IFN)-γ (+874), IL-10 (-1082, -592), IL-4 (-589) should have some association with the outcome of HBV and/or HCV infection.

Research frontiers

It remains unknown why subjects infected with HBV and/or HCV frequently develop chronic infection, and why the outcome of infection varies dramatically. Among infections with HBV/HCV, some show a strong reduction in $CD4^+$ T-helper and $CD8^+$ cytotoxic-T-lymphocyte responses. This is likely to be crucial in clearance of acute viremia. It is thought that immune tolerance to HBV/HCV infection may explain the different susceptibility to HBV/HCV infection. Many manifestations show that the immunity level of the host correlates with relevant gene polymorphisms, especially with single nucleotide polymorphisms in the promoter region, which regulates gene expression. Furthermore, the gene polymorphisms probably determine the outcome of the infection.

Innovations and breakthroughs

The polymorphisms of IL-2-330, IFN-γ+874, IL-10-1082 and -592 and IL-4-589 showed a significant association with the outcome of chronic HBV and/or HCV infection. The IL-2-330 TT/T allele, IFN-γ+874 AA and IL-10-1082 AA were associated with increased risk, but the IL-2-330 GG/G allele, IFN-γ+874 TA and IL-10-1082 AG were associated with reduced risk of persistent HBV and/or HCV infection, and of developing mild chronic hepatitis (CH), moderate/severe CH, and cirrhosis. The IL-10-1082 AA/A allele was associated with increased risk, but the IL-10-1082 AG/G allele was associated with reduced risk of HCV RNA replication. IL-10-592 AC and IL-4-589 CT/CC showed increased risk, but IL-10-592 AA and IL-4-589 TT showed reduced risk of abnormal ALT.

Applications

These results suggest that polymorphisms in some cytokine genes influence persistent HBV and/or HCV infection, clinical outcome, HCV replication and liver damage. Some cytokines, including IL-2, IFN-γ and IL-10 might be beneficial to the clinical outcome of HBV and/or HCV infection. Some cytokines, including IL-4, might result in liver damage. These results provide a scientific basis for the further treatment of patients with chronic HBV and HCV infection and viral hepatitis.

Terminology

IL-2 is a Th1 cytokine and has a powerful immunoregulatory effect on the stimulation of proliferation and activation of most T lymphocytes, natural killer cells, and B lymphocytes. IFN-γ is a Th1 cytokine and plays an important role in modulating almost all the immune responses, such as T-cell differentiation, and anti-proliferative, antitumor, and antiviral activities. IL-10 is an important anti-inflammatory cytokine secreted from Th2 cells. The levels of IL-10 production determine immunoregulation, and the balance between the inflammatory and humoral responses. IL-4 is a Th2 cytokine and plays an important role in humoral immunity. IL-4 has the function of inhibiting the production of IFN-γ and downregulating differentiation of Th1 cells.

Peer review

This is interesting paper. These results suggest that the IL-10-1082 polymorphisms are associated with Hepatitis C viral replication and the IL-10-592 and IL-4-589 polymorphisms influence liver damage.

REFERENCES

1 **Alter MJ**, Margolis HS, Krawczynski K, Judson FN, Mares A, Alexander WJ, Hu PY, Miller JK, Gerber MA, Sampliner RE. The natural history of community-acquired hepatitis C in the United States. The Sentinel Counties Chronic non-A, non-B Hepatitis Study Team. *N Engl J Med* 1992; **327**: 1899-1905

2 **Seeff LB**. The natural history of chronic hepatitis C virus infection. *Clin Liver Dis* 1997; **1**: 587-602

3 **Vildózola Gonzales H**, Salinas JL. [Natural history of chronic hepatitis B virus infection] *Rev Gastroenterol Peru* 2009; **29**: 147-157

4 **Asselah T**, Boyer N, Ripault MP, Martinot M, Marcellin P. Management of chronic hepatitis C. *Minerva Gastroenterol Dietol* 2007; **53**: 9-23

5 **Hoffmann SC**, Stanley EM, Darrin Cox E, Craighead N, DiMercurio BS, Koziol DE, Harlan DM, Kirk AD, Blair PJ. Association of cytokine polymorphic inheritance and in vitro cytokine production in anti-CD3/CD28-stimulated peripheral blood lymphocytes. *Transplantation* 2001; **72**: 1444-1450

6 **Poynard T**, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. *Lancet* 1997; **349**: 825-832

7 **Kenny-Walsh E**. Clinical outcomes after hepatitis C infection from contaminated anti-D immune globulin. Irish Hepatology Research Group. *N Engl J Med* 1999; **340**: 1228-1233

8 **Diepolder HM**, Zachoval R, Hoffmann RM, Jung MC, Gerlach T, Pape GR. The role of hepatitis C virus specific CD4+ T lymphocytes in acute and chronic hepatitis C. *J Mol Med* 1996; **74**: 583-588

9 **Cramp ME**, Carucci P, Underhill J, Naoumov NV, Williams R, Donaldson PT. Association between HLA class II genotype and spontaneous clearance of hepatitis C viraemia. *J Hepatol* 1998; **29**: 207-213

10 **Knapp S**, Hennig BJ, Frodsham AJ, Zhang L, Hellier S, Wright M, Goldin R, Hill AV, Thomas HC, Thursz MR. Interleukin-10 promoter polymorphisms and the outcome of hepatitis C virus infection. *Immunogenetics* 2003; **55**: 362-369

11 **Chen YP**, Feng XR, Dai L, Zhang L, Hou JL. Non-invasive diagnostic screening of hepatic fibrosis in patients with chronic hepatitis B. *Chin Med J* (Engl) 2004; **117**: 1109-1112

12 **Chang JJ**, Zhang SH, Li L. [Comparing and evaluating six methods of extracting human genomic DNA from whole blood] *Fayixue Zazhi* 2009; **25**: 109-111, 114

13 **Terrault NA**, Im K, Boylan R, Bacchetti P, Kleiner DE, Fontana RJ, Hoofnagle JH, Belle SH. Fibrosis progression in African Americans and Caucasian Americans with chronic hepatitis C. *Clin Gastroenterol Hepatol* 2008; **6**: 1403-1411

14 **Seder RA**, Paul WE. Acquisition of lymphokine-producing phenotype by CD4+ T cells. *Annu Rev Immunol* 1994; **12**: 635-673

15 **Sakaguchi E**, Kayano K, Segawa M, Aoyagi M, Sakaida I, Okita K. [Th1/Th2 imbalance in HCV-related liver cirrhosis] *Nippon Rinsho* 2001; **59**: 1259-1263

16 **Eckels DD**, Tabatabail N, Bian TH, Wang H, Muheisen SS, Rice CM, Yoshizawa K, Gill J. In vitro human Th-cell responses to a recombinant hepatitis C virus antigen: failure in IL-2 production despite proliferation. *Hum Immunol* 1999; **60**: 187-199

17 **Pawlowska M**, Halota W, Smukalska E, Grabczewska E. [Serum IL-2 and sIL-2R concentration in children with chronic hepatitis B] *Pol Merkur Lekarski* 2005; **18**: 33-35

18 **Napoli J**, Bishop GA, McGuinness PH, Painter DM, McCaughan GW. Progressive liver injury in chronic hepatitis C infection correlates with increased intrahepatic expression of Th1-associated cytokines. *Hepatology* 1996; **24**: 759-765

19 **Belardelli F**. Role of interferons and other cytokines in the regulation of the immune response. *APMIS* 1995; **103**: 161-179

20 **Muller U**, Steinhoff U, Reis LF, Hemmi S, Pavlovic J, Zinkernagel RM, Aguet M. Functional role of type I and type II interferons in antiviral defense. *Science* 1994; **264**: 1918-1921

21 **Pravica V**, Perrey C, Stevens A, Lee JH, Hutchinson IV. A single nucleotide polymorphism in the first intron of the human IFN-gamma gene: absolute correlation with a polymorphic CA microsatellite marker of high IFN-gamma production. *Hum Immunol* 2000; **61**: 863-866

22 **Zhang P**, Chen Z, Chen F, Li MW, Fan J, Zhou HM, Liu JH, Huang Z. Expression of IFN-gamma and its receptor alpha in the peripheral blood of patients with chronic hepatitis C. *Chin Med J* (Engl) 2004; **117**: 79-82

23 **Osna N**, Silonova G, Vilgert N, Hagina E, Kuse V, Giedraitis V, Zvirbliene A, Mauricas M, Sochnev A. Chronic hepatitis C: T-helper1/T-helper2 imbalance could cause virus persistence in peripheral blood. *Scand J Clin Lab Invest* 1997; **57**: 703-710

24 **Bertoletti A**, D'Elios MM, Boni C, De Carli M, Zignego AL, Durazzo M, Missale G, Penna A, Fiaccadori F, Del Prete G, Ferrari C. Different cytokine profiles of intraphepatic T cells in chronic hepatitis B and hepatitis C virus infections. *Gastroenterology* 1997; **112**: 193-199

25 **Paul S**, Tabassum S, Islam MN. Interferon-gamma (IFN-gamma) response to different hepatitis B virus antigens in hepatitis B virus infection. *Bangladesh Med Res Counc Bull* 2004; **30**: 71-77

26 **Liu M**, Cao B, Zhang H, Dai Y, Liu X, Xu C. Association of interferon-gamma gene haplotype in the Chinese population with hepatitis B virus infection. *Immunogenetics* 2006; **58**: 859-864

27 **Turner DM**, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV. An investigation of polymorphism in the interleukin-10 gene promoter. *Eur J Immunogenet* 1997; **24**: 1-8

28 **Gramenzi A**, Andreone P, Loggi E, Foschi FG, Cursaro C, Margotti M, Biselli M, Bernardi M. Cytokine profile of peripheral blood mononuclear cells from patients with different outcomes of hepatitis C virus infection. *J Viral Hepat* 2005; **12**: 525-530

29 **Jia HY**, Du J, Zhu SH, Ma YJ, Chen HY, Yang BS, Cai HF. The roles of serum IL-18, IL-10, TNF-alpha and sIL-2R in patients with chronic hepatitis C. *Hepatobiliary Pancreat Dis Int* 2002; **1**: 378-382

30 **Cacciarelli TV**, Martinez OM, Gish RG, Villanueva JC, Krams SM. Immunoregulatory cytokines in chronic hepatitis C virus infection: pre- and posttreatment with interferon alfa. *Hepatology* 1996; **24**: 6-9

31 **Eskdale J**, Gallagher G, Verweij CL, Keijsers V, Westendorp RG, Huizinga TW. Interleukin 10 secretion in relation to human IL-10 locus haplotypes. *Proc Natl Acad Sci USA* 1998; **95**: 9465-9470

32 **Edwards-Smith CJ**, Jonsson JR, Purdie DM, Bansal A, Shorthouse C, Powell EE. Interleukin-10 promoter polymorphism predicts initial response of chronic hepatitis C to interferon alfa. *Hepatology* 1999; **30**: 526-530

33 **Reuss E**, Fimmers R, Kruger A, Becker C, Rittner C, Hohler T. Differential regulation of interleukin-10 production by genetic and environmental factors--a twin study. *Genes Immun* 2002; **3**: 407-413

34 **Abbas Z**, Moatter T. Interleukin (IL) 1beta and IL-10 gene polymorphism in chronic hepatitis C patients with normal or elevated alanine aminotransferase levels. *J Pak Med Assoc* 2003; **53**: 59-62

35 **Vercelli D**, De Monte L, Monticelli S, Di Bartolo C, Agresti A. To E or not to E? Can an IL-4-induced B cell choose between IgE and IgG4? *Int Arch Allergy Immunol* 1998; **116**: 1-4

36 **Kato A**, Yoshidome H, Edwards MJ, Lentsch AB. Reduced hepatic ischemia/reperfusion injury by IL-4: potential anti-inflammatory role of STAT6. *Inflamm Res* 2000; **49**: 275-279

37 **Yoshidome H**, Kato A, Miyazaki M, Edwards MJ, Lentsch AB. IL-13 activates STAT6 and inhibits liver injury induced by ischemia/reperfusion. *Am J Pathol* 1999; **155**: 1059-1064

38 **Noguchi E**, Nukaga-Nishio Y, Jian Z, Yokouchi Y, Kamioka M, Yamakawa-Kobayashi K, Hamaguchi H, Matsui A, Shibasaki M, Arinami T. Haplotypes of the 5′ region of the IL-4 gene and SNPs in the intergene sequence between the IL-4 and IL-13 genes are associated with atopic asthma. *Hum Immunol* 2001; **62**: 1251-1257

39 **Cribier B**, Schmitt C, Rey D, Lang JM, Kirn A, Stoll-Keller F. Production of cytokines in patients infected by hepatitis C virus. *J Med Virol* 1998; **55**: 89-91

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CASE REPORT

Asymptomatic lymphangioma involving the spleen and retroperitoneum in adults

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Abstract

Lymphangioma, a benign neoplasm of the lymphatic system, is common in children but rare in adults. Its clinical manifestations include abdominal pain, nausea, vomiting and a palpable mass. However, abdominal sonography or abdominal computed tomography (CT) scan can also incidentally reveal lymphangioma. A larger or symptomatic lymphangioma is treated with total resection to prevent recurrence, infection, torsion and enlargement. Although lymphangioma rarely becomes malignant, its prognosis is generally good. We report a cystic lymphangioma of the spleen and retroperitoneum, which was incidentally found in a 56-year-old man who was hospitalized due to a colon mass. Physical examination showed no specific findings. Abdominal CT revealed a 5.7 cm, non-enhanced multilobulated cystic mass with multiple septa in the spleen and a 10 cm lobulated cystic mass in the para-aortic area. Splenectomy and retroperitoneal resection of the cystic mass were conducted. The endothelium of splenic and retroperitoneal cyst was immunohistochemically stained with D2-40 antibody. The patient was finally diagnosed with splenic cystic and retroperitoneal cavernous lymphangioma.

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Key words: Lymphangioma; Retroperitoneum; Spleen

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INTRODUCTION

Lymphangioma, a very uncommon neoplasm, is mainly found in children but rarely in adults[1,2]. In 1885, Frink reported the first lymphangioma in the spleen. Cases of splenic and retroperitoneum lymphangioma are rarely reported in adults. In our case, lymphangioma involved both the spleen and the retroperitoneum. Cases like ours, involving two organs, are extremely rare[3-5]. The clinical manifestations of lymphangioma include abdominal pain, fever, fatigue, weight loss and hematuria. However, it is sometimes asymptomatic[6,7]. We report, here, a case of a 56-year-old man who was hospitalized due to a colon mass. Lymphangioma in his spleen and retroperitoneum was incidentally found in abdominal computed tomography (CT). This case is very rare because lymphangioma was found both in his spleen and in his retroperitoneum.

CASE REPORT

A 56-year-old man was hospitalized at a private clinic due to a suspected colon cancer detected at colonoscopy. He

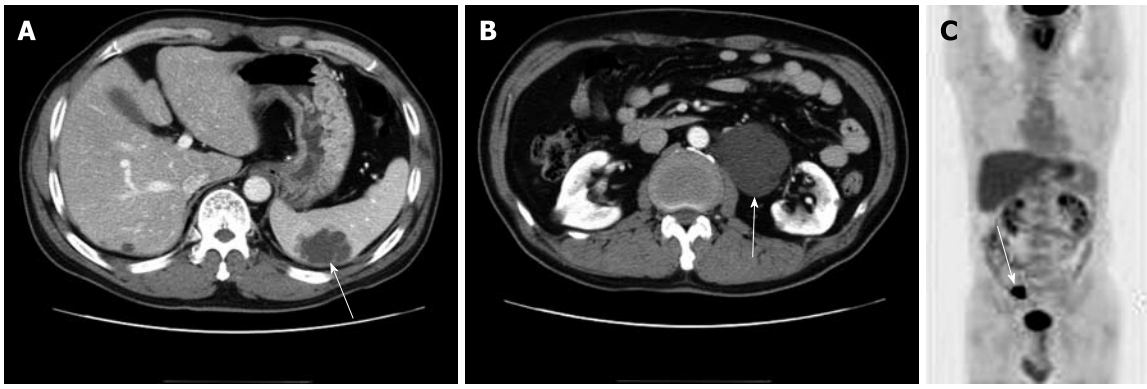

Figure 1 Enhanced abdominal CT and F-18 FDG Torso PET CT images of lymphangioma. A: A 5.7 cm lobulated & septated mass without enhancement in the spleen (white arrow); B: A 10 cm lobulated cystic mass in the paraaortic area (white arrow); C: A hypermetabolic lesion in the sigmoid colon mass (white arrow) and maxSUV 23 but no hypermetabolic lesion in the spleen and retroperitoneum.

had no history of any medical disease, and no weight loss, abdominal pain and dyspepsia. Physical examination revealed no palpable mass, tenderness or rebound tenderness in his abdomen with normal bowel sound. Laboratory studies had no specific findings except for creatinine (1.4 mg/dL). Abdominal X-ray did not reveal calcification, but abdominal CT showed a 5.7 cm non-enhanced cystic mass with septum in the spleen and a 10 cm lobulated cystic mass in the left paraaortic area (Figure 1A and B). PET CT revealed a hypermetabolic lesion in the sigmoid colon where the maxSUV was 23. However, no hypermetabolic lesion was found in the spleen or in the left paraaortic area (Figure 1C).

A 5 cm × 3 cm colon polyp with a neck and an intramural adenocarcinoma invading the lamina propria were found in the sigmoid colon. Splenectomy and resection of the retroperitoneal mass were conducted, during which a 4.5 cm × 3.5 cm bulging mass was exposed in the lower pole of the spleen (Figure 2A). The cut surface was composed of a multilobular cyst with a smooth inner surface. Histology showed thin fibrous walls of the cyst, which were different in size with no bleeding or necrosis (Figure 2B). In immunohistochemical study, the endothelium of splenic cystic mass was stained with D2-40 antibody (Figure 2C) and finally diagnosed as a cystic lymphangioma. The retroperitoneal mass was 4.5 cm × 4.5 cm × 2.5 cm in size, and its outer surface was lobular and yellow to brown in color (Figure 3A). The cut surface showed a sponge-like and ill-defined cyst.

Histology showed small cysts interspersed among fat, which were different in size, but had no calcification, bleeding, or necrosis (Figure 3B). In immunohistochemical study, the endothelium of retroperitoneal cystic mass was stained with D2-40 antibody (Figure 3C). The retroperitoneal mass was finally diagnosed as a cavernous lymphangioma. The patient was discharged 10 d after operation without complication.

DISCUSSION

Cystic lymphangioma is a benign neoplasm composed of a malformation of the lymphatic system[1,2]. The incidence of lymphangioma occurring after 20 years of age is very low. Lymphangioma generally occurs under the age of 2 years. There is no difference in the incidence between males and females[2]. The most commonly involved sites are the neck (75%) and axilla (20%). Lymphangioma is infrequently encountered in the mediastinum, adrenal gland, kidney, bone, omentum, gastrointestinal track, retroperitoneum, spleen, liver and pancreas[2,5]. There are two hypotheses for the causes and pathogenesis of lymphangioma, namely abnormal congenital development and bleeding or inflammation of the lymphatic system which causes an obstruction leading to additional lymphangiomas. The clinical manifestations of lymphangioma are left upper quadrant pain, abdominal distension, loss of appetite, nausea, vomiting and a palpable mass. However, lymphangioma can also occur without any symptoms[6,7]. Our case had no symptoms of lymphangioma and no palpable mass in the abdomen, and is unique because lymphangioma was found both in his spleen and in his retroperitoneum. Even if there are reports on cystic lymphangioma involving mesentery and retroperitoneum[2], no report is available on lymphangioma involving both the spleen and the retroperitoneum in an asymptomatic patient. Lymphangioma involving more than two abdominal organs is rarely reported[2]. In our case, renal vein penetrated through the retroperitoneal lymphangioma, but retroperitoneal lymphangioma could be resected without impairment of the kidneys. Abdominal sonography can show a lowly attenuated mass and a highly attenuated inner part of lymphangioma, while abdominal CT may demonstrate multiple lowly attenuated, thin-walled, well-demarcated cysts and calcification in the cyst wall. The cystic surface can be divided by a septum and multiple lobules[7]. MRI may reveal a highly attenuated space in multiple lobules. Only a radiological image may have limitations in its diagnosis[6,7]. In this case, abdominal CT revealed a 5.7 cm cystic mass in the spleen and a 10 cm cystic mass composed of lobules in the paraaortic area. PET CT did not show highly metabolized lesions in the spleen or in the retroperitoneum of our case. Generally, lymphangioma is divided into capillary, cavernous and cystic types, with the cystic type being the most common[3]. Histology can show dilated lymphatic vessels surrounded by flat endothelial cells between fat, fibrotic

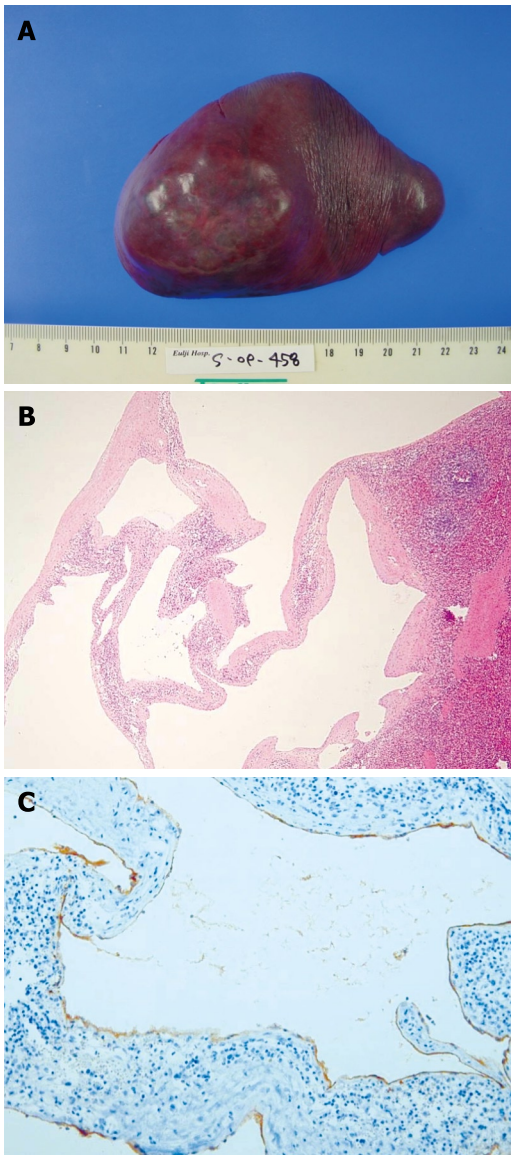

Figure 2 Gross and microscopic images of splenic lymphangioma. A: A 4.5 cm × 3.5 cm bulging cystic mass in lower pole; B: HE staining of splenic lymphangioma, × 20; C: Immunostaining with D2-40 antibody (× 200) for cystic lining cells.

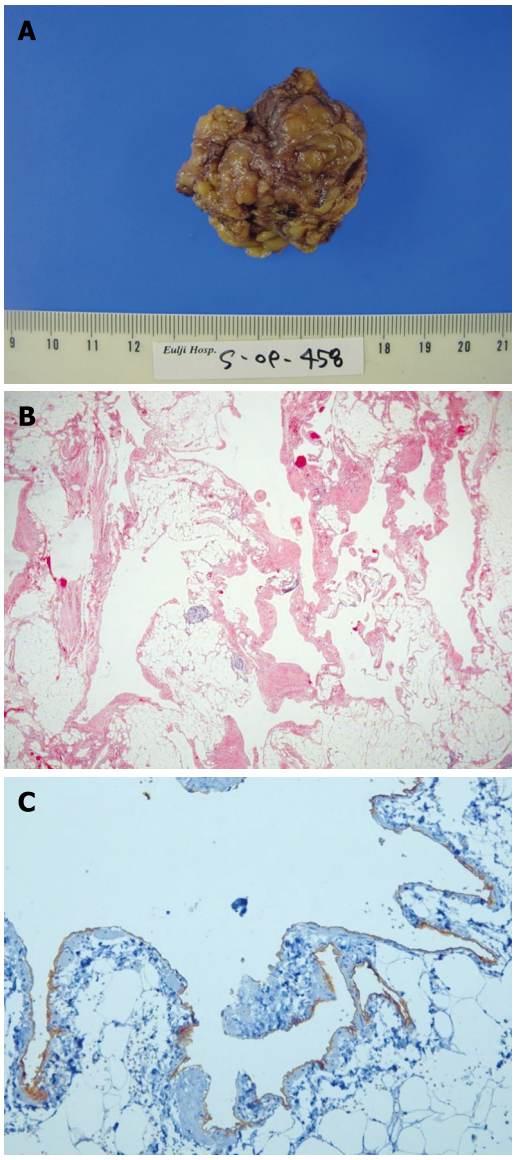

Figure 3 Gross and microscopic images of retroperitoneal lymphangioma. A: A 4.5 cm × 4.5 cm × 2.5 cm retroperitoneal mass; B: HE staining of retroperitoneal lymphangioma, × 20; C: Immunostaining with D2-40 antibody (× 200) for cystic lining cells.

and lymphatic structures. Immunohistochemical staining can show a lymphangioma which is positive for keratin and HBME, and is negative for FV Ⅲ-Rag, CD 31, CD 34 because it develops from the middle lobe[8]. Lymphatic vessel endothelial receptor-1, vascular endothelial growth factor-3, prox-1, and monoclonal antibody D2-40 are the markers for specific endothelial cells of lymphatic tissues, which are used in immunochemical studies of lymphangioma. In this case, the endothelium of splenic and retroperitoneal cystic mass was stained with D2-40 antibody and the splenic and retroperitoneal cystic mass was finally diagnosed as splenic cystic and retroperitoneal cavernous lymphangioma, based on the microscopic findings. Surgical treatment is needed to prevent recurrence, infection, rupture, and bleeding[5]. Conservative treatment modalities include aspiration, drainage and sclerosis, with a high risk of recurrence. However, an incidentally found small cyst needs not be resected, and the current treatment is a partial splenectomy to remove it[5]. The post-operative recurrence rate is low and the rate of transformation into malignancy is very low as well. Complications are peritonitis, bleeding, abscess and torsion[9]. The natural regression rate is very low. If multiple lymphangiomas progress rapidly and are invasive, their prognosis could be poor[10]. In this case, a 5.7 cm splenic lymphangioma and a 10 cm lymphangioma were surgically removed and the patient was discharged 10 d later without any complication. Our case may be the first reported case of lymphangioma involving both the spleen and the retroperitoneum in an asymptomatic patient.

REFERENCES

1 **de Perrot M**, Rostan O, Morel P, Le Coultre C. Abdominal lymphangioma in adults and children. *Br J Surg* 1998; **85**: 395-397

2 **Kim DH**, Byun JN, Jang JY. Cystic lymphangioma involving

the mesentery and the retroperitoneum: A case report. *J Korean Radiol Soc* 2005; **52**: 347-350
3 **Takayama A**, Nakashima O, Kobayashi K, Kojiro M. Splenic lymphangioma with papillary endothelial proliferation: a case report and review of the literature. *Pathol Int* 2003; **53**: 483-488
4 **Cherk M**, Nikfarjam M, Christophi C. Retroperitoneal lymphangioma. *Asian J Surg* 2006; **29**: 51-54
5 **Chang CH**, Hsieh CB, Yu JC, Jan CI. A case of lymphangioma of the spleen. *J Med Sci* 2004; **24**: 109-112
6 **Chan IYF**, Khoo J. Retroperitoneal lymphangioma in an adult. *J HK Coll Radiol* 2003; **6**: 94-96
7 **Kwon AH**, Inui H, Tsuji K, Takai S, Imamura A, Kamiyama Y. Laparoscopic splenectomy for a lymphangioma of the spleen: report of a case. *Surg Today* 2001; **31**: 258-261
8 **Kim MJ**, Cho KJ, Han EM, Lee YJ. Splenic lymphangioma: A report of three cases. *Korean J Pathol* 2002; **36**: 416-419
9 **Yang DM**, Jung DH, Kim H, Kang JH, Kim SH, Kim JH, Hwang HY. Retroperitoneal cystic masses: CT, clinical, and pathologic findings and literature review. *Radiographics* 2004; **24**: 1353-1365
10 **Wadsworth DT**, Newman B, Abramson SJ, Carpenter BL, Lorenzo RL. Splenic lymphangiomatosis in children. *Radiology* 1997; **202**: 173-176

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CASE REPORT

Acute myelogenous leukemia and acute leukemic appendicitis: A case report

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Abstract

Acute myelogenous leukemia (AML) can involve the gastrointestinal tract but rarely involves the appendix. We report a male patient who had 1 year partial remission from AML and who presented with apparent acute appendicitis as the initial manifestation of leukemia relapse. Pathological findings of the appendix revealed transmural infiltrates of myeloblasts, which indicated a diagnosis of leukemia. Unfortunately, the patient died from progression of the disease on the 19th d after admission. Although leukemic cell infiltration of the appendix is uncommon, patients with leukemia relapse can present with symptoms mimicking acute appendicitis.

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Key words: Acute myeloid leukemia; Appendicitis; Appendectomy; Granulocytic sarcoma

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INTRODUCTION

Acute myeloid leukemia (AML) affects middle-aged adults. When the disease involves soft tissue, it is called granulocytic sarcoma (GS). GS can present in the gastrointestinal tract but involvement of the appendix is uncommon. Furthermore, infiltration of the appendix by leukemic cells is also a rare manifestation of leukemia relapse. Herein, we report a 75-year-old man with AML-M2 who had been in partial remission for 1 year, and who presented with symptoms mimicking acute appendicitis as the initial manifestation of leukemia relapse. Subsequent pathological examination confirmed the diagnosis.

CASE REPORT

A 75-year-old man was admitted for evaluation of right lower quadrant abdominal pain and fever for 3 d. He had partial remission of AML-M2 for 1 year, after chemotherapy with low dose cytarabine. His past history included hypertensive cardiovascular disease with congestive heart failure, coronary artery disease, and chronic obstructive pulmonary disease. Physical examination showed rebound tenderness over the right lower quadrant. The leukocyte count was 35×10^3/L, with 15% neutrophils, 26% lymphocytes, 1% monocytes, 2% eosinophils, 0% basophils, and 56% immature cells. Hemoglobin and platelet counts were 9.9 g/dL and $64 \times 10^3/\mu$L, respectively. C-reactive protein was 7.98 mg/dL. Abdominal computed tomography showed thickening of the appendiceal wall and periappendicular fat stranding (Figure 1). The diagnosis of acute appendicitis was made, and appendectomy was performed immediately after admission. Grossly, the appendix was gray in color and soft in consistency. Microscopically, the sections showed transmural infiltrates of myeloblasts, which were positive for myeloperoxidase, CD43 and CD34 immunohistochemical stains (Figure 2). Hence, AML-M2 with involvement of the appendix was diagnosed. Thereafter the patient received chemotherapy with low-

Figure 1 Abdominal computed tomography reveals appendicular wall thickening and periappendicular fat stranding.

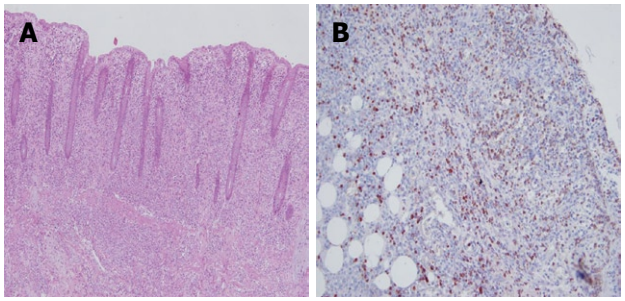

Figure 2 Cross-section of the appendix specimen. A: Leukemia involving the appendix is characterized by transmural infiltrates of myoloblast cells (HE, × 200); B: These tumor cells are immunoreactive to myeloperoxidase protein (HE, × 200).

dose cytarabine (20 mg/kg for 12 d). However, he died from progressive disease complicated by septic shock and acute respiratory failure on the 19th d after admission.

DISCUSSION

The incidence of GS is estimated to be 3% in living adult patients with AML and 4.7% in children[1,2]. Rappaport initially introduced the concept of acute lymphoblastic leukemia infiltrating the appendix[3]. In a review of the literature, AML involving the appendix has been described[1,3,4]. As a presentation of AML in adults, GS can involve various sites throughout the body but it is rare in the gallbladder and appendix. Bowel infiltration by leukemic cells, described initially in the 19th century, was thought to be a rare condition until autopsy studies in the 1960s and 1970s, which indicated a prevalence of this presentation in 10% to 53.3% of leukemia patients[5-8]. It has been reported that appendiceal involvement by leukemic cells occurs in approximately 3 of 36 patients (8.3%)[6]. Seven leukemia patients with involvement of the appendix, including our own, were identified in the literature. The survival time varied as shown in Table 1[1,4,6].

Surgical management of patients with leukemia and acute abdomen has not been advocated because of the high rate of operative mortality in the past[4,6]. However, there is some support for surgical management of appendicitis in acute leukemia as the most effective method of therapy[1,4,9]. Systemic chemotherapy is necessary in this setting for additional radiation or surgery in patients with GS[10].

In conclusion, we report a rare case of AML who had been in partial remission for 1 year and presented with symptoms of acute appendicitis as the initial manifestation of leukemia relapse. Although leukemic cell infiltration into the appendix is uncommon, our case highlights the importance of differential diagnosis of acute appendicitis including recognition of possible leukemic involvement. The physicians should be aware of these conditions.

Table 1 Seven cases of leukemia infiltration of the appendix described in the literature

No.	Sex/age	Type	Treatment	Survival time (d)
1	F/77	M3	Surgery	30
2	M/71	M2	Surgery	49
3-6	NA	NA	3 surgery	Hours to days
7 (Our case)	M/75	M2	Surgery	19

M: Male; F: Female; NA: Not available.

REFERENCES

1 **Müller G**, Dargent JL, Duwel V, D'Olne D, Vanvuchelen J, Haot J, Hustin J. Leukaemia and lymphoma of the appendix presenting as acute appendicitis or acute abdomen. Four case reports with a review of the literature. *J Cancer Res Clin Oncol* 1997; **123**: 560-564
2 **Pui MH**, Fletcher BD, Langston JW. Granulocytic sarcoma in childhood leukemia: imaging features. *Radiology* 1994; **190**: 698-702
3 **Rappaport H**. Tumors of the hematopoietic system, in Atlas of Tumor Pathology, Section III, Fascicle 8. Washington DC: Armed Forces Institute of Pathology, 1967: 241-247
4 **Toubai T**, Kondo Y, Ogawa T, Imai A, Kobayashi N, Ogasawara M, Kiyama Y, Higa T, Sato K, Miyokawa N, Tanaka J, Imamura M, Kasai M. A case of leukemia of the appendix presenting as acute appendicitis. *Acta Haematol* 2003; **109**: 199-201
5 **Askanazy M**. Über akute Leukämie und ihre Beziehung zu geschwürigen Prozessen im Verdauungskanal. *Arch Pathol Anat Physiol Klin Med* 1894; **137**: 1-24
6 **Prolla JC**, Kirsner JB. The gastrointestinal lesions and complications of the leukemias. *Ann Intern Med* 1964; **61**: 1084-1103
7 **Cornes JS**, Jones TG. Leukaemic lesions of the gastro-intestinal tract. *J Clin Pathol* 1962; **15**: 305-313
8 **Viadana E**, Bross ID, Pickren JW. An autopsy study of the metastatic patterns of human leukemias. *Oncology* 1978; **35**: 87-96
9 **Kim KU**, Kim JK, Won JH, Hong DS, Park HS. Acute appendicitis in patients with acute leukemia. *Korean J Intern Med* 1993; **8**: 40-45
10 **Shaikh BS**, Frantz E, Lookingbill DP. Histologically proven leukemia cutis carries a poor prognosis in acute nonlymphocytic leukemia. *Cutis* 1987; **39**: 57-60

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SCIENTIST'S STORY

So you aspire to be a Professor?

Nicholas J Talley

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"The truth is that life is delicious, horrible, charming, frightful, sweet, bitter, and that is everything." Anatole France (François-Anatole Thibault, 1844-1924).

When I was in medical school and early on in residency, I dreamed of undertaking research and becoming an academic but I did not really know how to start; in the end, I settled for trying to master internal medicine as I sweated to pass the formidable barrier examination for admission to Fellowship of the Royal Australasian College of Physicians (comprising both a written test and an even more challenging clinical "viva"). My entry into research was entirely fortuitous; having completed Basic Training I wanted a change (I like change), and I was lucky to learn from my father (a prominent Sydney gastroenterologist in private practice) that one of Australia's most famous academic gastroenterologists, Professor Douglas (Doug) Piper, was looking for someone to undertake a PhD with him. Remarkably few had expressed an interest in doing so; a PhD would require 3 years of ones life living on a National Health and Medical Research Council (NH&MRC) scholarship that would hardly keep body and soul together.

Figure 1 Nicholas J Talley, MD, PhD, FACP, FRACP, FRCP Chair.

Actually I was not sure I wanted to become a gastroenterologist (in fact, I favored neurology because my Dad was a gastroenterologist, and two Nicholas Talley's in the specialty seemed a formula for confusion). However, I saw working with Doug as a real opportunity, so I took the risk and said yes, one of the best decisions of my life (Figure 1).

Professor Doug Piper was particularly interested in peptic ulcer disease, and our initial NH&MRC grant application (developed before the observation of spiral bacteria in the stomach had been announced), aimed to determine the natural history of peptic ulcer disease. This grant failed to attract funding, which at the time seemed quite devastating. Remarkably, this apparent setback turned out to be very fortunate, because we then were forced to look at alternative projects. Almost by chance we fell upon a common clinical problem; it had been long observed that most patients with ulcer-like symptoms had no ulcer crater or other obvious explanation for their complaints. Little was known about this mysterious and vague entity, then called non-ulcer dyspepsia, and the topic seemed ripe for a comprehensive clinical research project that would fulfill the requirements for a higher degree in Australia. As a clinician I became quite fascinated by the problem (I still am). After reading the proceedings from a Scandinavian symposium pointing out many of the gaps in knowledge (published by the makers of cimetidine, Smith Kline), I decided to pursue the topic. I was blissfully unaware of how difficult such a task might be, but had the gift of youthful enthusiasm and energy.

And so my odyssey began; with the help of Doug's team I designed several protocols and set out to learn about the epidemiology, etiopathogenesis and treatment of non-ulcer dyspepsia. It was very hard the first year; there were the inevitable setbacks and it took a while to become used to research rather than full time practice. I

thought about quitting. But I learned that guts and grit count more than any other characteristic in the research setting. I undertook classes in clinical epidemiology and biostatistics which I loved, and learnt how to apply rigorous clinical research methods and avoid some of the obvious pitfalls. Momentum helps too; my first paper on the topic was published in 1985[1] and I caught the publishing bug (once you know how, it is great fun to collect, analyze and share your data with the world). The research efforts eventually spilled over into a two volume thesis entitled "Non-ulcer dyspepsia: A study of the psychosocial, environmental, clinical, therapeutic and prognostic aspects - with particular reference to a subgroup with dyspepsia of unknown cause (essential dyspepsia)." Doug used to say a thesis should be judged first by its weight and then by its quality, which I took to heart. The publication of several papers led to the offer of a fully paid Research Fellowship at Mayo Clinic in Rochester, Minnesota in the famous Gastroenterology Unit [headed then by a very prominent and brilliant expatriate Australian, Professor Sidney (Sid) Phillips]. Sid was an inspiring mentor who trained many of the most brilliant minds in motility research; I learned how to do physiological research and joined the staff during one of the happiest times of my life.

As my career trajectory exploded upwards, a further boost came after presenting new work as a very raw Assistant Professor of Medicine at Digestive Diseases Week. After the oral symposium, Douglas Drossman and Grant Thompson strode up to me to introduce themselves. I was invited to join a new consensus group that would meet in Rome; the idea was to develop diagnostic criteria for the first time for all of the functional gastrointestinal disorders, including non-ulcer dyspepsia (the precursor of the famous Rome criteria!). I said yes, and have been involved ever since with what is now known as the Rome Foundation (and the Rome Ⅰ, Ⅱ and Ⅲ criteria-Rome Ⅳ is currently being planned).

So if you want to eventually reach the rank of Professor and, more importantly, contribute to our field, the formula in my mind is uncomplicated. Try to choose a great mentor first and foremost; they are the ones eager to impart their knowledge, leave a legacy and develop the science (you can find most of the talented names on PubMed today with ease - look at how much and where they have published as a guide). Search for someone who thinks outside the box and has a strong team working with them; teams tend to perform best in the research world. The project is important too, but without the right mentor your chance of major success greatly diminishes. Second, in my humble view brains matter much less than drive. Be prepared to work hard and realize you will have to overcome all sorts of roadblocks to succeed; if you are prepared to be persistent and flexible, you will make it. Do not neglect to read the literature widely and deeply in your chosen topic area; critically appraise the science, know intimately all of the relevant papers, and look for the gaps (there are always many!). I tell my Research Fellows I know they will become experts by the time they complete their projects. Third, continuously develop your skill set. Learn how to not only do the science but also how to analyze, present and write it up. Submit and personally present your work at major international meetings whenever you have the opportunity; this is very good exposure, allows you to learn the state of the art and will promote making professional contacts around the world who will likely prove invaluable. Scientific presentation is a skill that can be learned and improves with practice; seek out ways to become a better public speaker. Above all, dedicate yourself to publishing any and all important data you generate (if your work is not published in a peer review journal, it essentially does not exist!). Serendipity helps too, but chance usually only plays a role if you are well prepared. If you follow these simple strategies, academic opportunities and promotion are almost guaranteed; it is straightforward to become a Professor once you learn the trade, and most importantly the journey is great fun too.

REFERENCES

1 **Talley NJ**, Piper DW. The association between non-ulcer dyspepsia and other gastrointestinal disorders. *Scand J Gastroenterol* 1985; **20**: 896-900

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Meetings

Events Calendar 2009

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Hyatt Regency San Francisco, San Francisco, CA
Mouse Models of Cancer

January 21-24, 2009
Westin San Diego Hotel, San Diego, CA
Advances in Prostate Cancer Research

February 3-6, 2009
Carefree Resort and Villas, Carefree, AZ (Greater Phoenix Area)
Second AACR Conference
The Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved

February 7-10, 2009
Hyatt Regency Boston, Boston, MA
Translation of the Cancer Genome

February 8-11, 2009
Westin New Orleans Canal Place, New Orleans, LA
Chemistry in Cancer Research: A Vital Partnership in Cancer Drug Discovery and Development

February 13-16, 2009
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19th Conference of the APASL
http://www.apasl2009hongkong.org/en/home.aspx

February 27-28, 2009
Orlando, Florida
AGAI/AASLD/ASGE/ACG Training Directors' Workshop

February 27-Mar 1, 2009
Vienna, Austria
EASL/AASLD Monothematic: Nuclear Receptors and Liver Disease
www.easl.ch/vienna2009

March 13-14, 2009
Phoenix, Arizona
AGAI/AASLD Academic Skills Workshop

March 20-24, 2009
Marriott Wardman Park Hotel Washington, DC
13th International Symposium on Viral Hepatitis and Liver Disease

March 23-26, 2009
Glasgow, Scotland
British Society of Gastroenterology (BSG) Annual Meeting
Email: bsg@mailbox.ulcc.ac.uk

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Silver Spring, Maryland
2009 Hepatotoxicity Special Interest Group Meeting

April 18-22, 2009
Colorado Convention Center, Denver, CO
AACR 100th Annual Meeting 2009

April 22-26, 2009
Copenhagen, Denmark
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Digestive Disease Week 2009

May 29-June 2, 2009
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45th ASCO Annual Meeting
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May 30, 2009
Chicago, Illinois
Endpoints Workshop: NASH

May 30-June 4, 2009
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June 17-19, 2009
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Accelerating Anticancer Agent Development

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Methods in Clinical Cancer Research (Europe)

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Snowmass, CO, United States
Pathobiology of Cancer: The Edward A. Smuckler Memorial Workshop

July 17-24, 2009
Aspen, CO, United States
Molecular Biology in Clinical Oncology

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Frontiers in Basic Cancer Research

October 13-16, 2009
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In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of

balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

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5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

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

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Issue with no volume

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Books

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Conference paper

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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/eid.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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