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## Hepatitis C virus and type 2 diabetes

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### Abstract

This review focuses on the relationship between hepatitis C virus (HCV) infection and glucose metabolism derangements. Cross-sectional and longitudinal studies have shown that the chronic HCV infection is associated with an increased risk of developing insulin resistance (IR) and type 2 diabetes (T2D). The direct effect of HCV on the insulin signaling has been analyzed in experimental models. Although currently available data should be considered as preliminary, HCV seems to affect glucose metabolism *via* mechanisms that involve cellular pathways that have been implicated in the host innate immune response. IR and T2D not only accelerate the histological and clinical progression of chronic hepatitis C, but also reduce the early and sustained virological response to interferon- $\alpha$ -based therapy. Thus, a detailed knowledge of the mechanisms underlying the HCV-associated glucose metabolism derangements is warranted, in order to improve the clinical management of chronic hepatitis C patients.

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**Key words:** Hepatitis C; Fibrosis; Insulin resistance; Insulin signaling; Type 2 diabetes

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### INTRODUCTION

Hepatitis C virus (HCV) infection is a frequent cause of acute and chronic hepatitis, and leads to the development of cirrhosis and hepatocellular carcinoma. It is estimated that about 150 to 200 million people have been in contact with HCV worldwide, and approximately 85% are chronically infected. The spectrum of severity of liver disease associated with HCV varies widely, as does the rate of progression towards the cirrhotic stage. The latter seems to depend on several, mostly host-related cofactors, such as age, sex, level of alcohol consumption, overweight, immune status and co-infections<sup>[1,2]</sup>. One of these cofactors is type 2 diabetes (T2D), which has been recognized to modify the course of hepatitis C even at the stage of insulin resistance (IR), a condition that precedes the development of T2D<sup>[3,4]</sup>. Although individuals may develop IR independently of HCV, a considerable amount of clinical and experimental data suggest that HCV contributes to its pathogenesis. This aspect is important, because IR seems not only to accelerate the course of chronic hepatitis C, but also to influence the response to antiviral therapy<sup>[5]</sup>. The scope of this review is to discuss the current level of evidence in favor of a causal association between HCV and T2D/IR, its clinical impact, and some directions for management.

### ASSOCIATION BETWEEN HCV AND T2D

T2D is a common complication of all liver diseases, independently of the etiology, especially at the advanced stage. However, clinical and experimental data suggest a direct role of HCV in the perturbation of glucose metabolism. The first observation that cirrhotic patients infected with HCV may present with T2D more often than patients with cirrhosis of other

etiology was reported in 1994 by Allison *et al*<sup>[6]</sup>. Most studies using a cross-sectional design and comparing the prevalence of T2D in a population of chronic hepatitis C patients with that of a comparator group have confirmed these preliminary observations<sup>[7-14]</sup>, with rare exceptions<sup>[15]</sup>. Comparator groups have included patients with chronic liver disease<sup>[7-9,12-14]</sup>, drug users<sup>[10]</sup> or human immunodeficiency virus (HIV) mono-infected patients<sup>[11]</sup>. It can be argued that, in these studies, the two populations of patients-i.e. HCV-infected and uninfected-may have differed by relevant risk factors for T2D, notably age, gender distribution and stage of liver disease. It is noteworthy that, in the HIV-co-infected cohorts studied by Visnegarwala *et al*<sup>[11]</sup>, the association between HCV and T2D was significant, as assessed by multivariate analysis, only among subjects < 50 years old. Similarly, in the study by Lecube *et al*<sup>[12]</sup>, glucose abnormalities, including impaired fasting glucose (IFG), were significantly more prevalent (i.e. about three-fold) among HCV-positive patients attending a liver unit, compared to HCV-negative patients, only when they were at a pre-cirrhotic stage. These observations suggest that HCV interferes with glucose metabolism independently of age and stage of liver disease. At a later, cirrhotic stage, however, multiple factors contributing to IR may prevail and partially or completely mask the HCV-related effect. Further evidence has come *via* case-control studies in which all cases were represented by HCV-infected individuals<sup>[16-24]</sup>, although in most cases, the prevalence of T2D among HCV-infected individuals compared to matched controls was, in general, lower than that seen in previous studies.

Several investigators have approached this issue from a different point of view, i.e. measuring the prevalence of HCV markers among populations of diabetic patients<sup>[8,15,25-33]</sup>. Most controlled studies have suggested a significant association, the proportion of HCV-positive persons among diabetics being two- to seven-fold compared to controls<sup>[8,15,29,32]</sup>. The prevalence of HCV markers among patients with T2D reported by uncontrolled studies was also claimed to be higher than that observed in the general population taken as a reference<sup>[25,27,30]</sup>. However, the study by Sotiropoulos *et al*<sup>[26]</sup> reported a rather low HCV prevalence (1.65%), especially if one considers that a field survey in the Greek general population gave a HCV seroprevalence of 1.25%<sup>[34]</sup>. Other controlled studies from Italy<sup>[28]</sup>, Nigeria<sup>[31]</sup> and Turkey<sup>[33]</sup> have failed to find an excess prevalence of HCV infection among patients with T2D. The data have therefore proven inconclusive. It has been suggested that patients with T2D are at risk of blood-borne infections *via* repeated use of finger stick devices. However, a single study from France, evaluating the prevalence of HCV antibodies in 259 patients with T2D seen during 1998 at a diabetic unit, has failed to confirm this hypothesis<sup>[35]</sup>. One cannot exclude that iatrogenic transmission of HCV among diabetic patients may however have been significant in previous decades.

The potential ascertainment bias that may occur in

clinic-based studies that target a specific disease group has been overcome in a vast (and hitherto unsurpassed) study conducted in the general population, the Third National Health and Nutrition Examination Survey (NANHES-III)<sup>[36]</sup>. This study, which included 9841 subjects aged  $\geq 20$  years, showed that persons who were anti-HCV-positive and aged  $\geq 40$  years had an odds ratio of 3.77 (95% CI: 1.80-7.87), after adjusting for sex, body mass index (BMI) and ethnicity, of having T2D compared to anti-HCV-negative individuals.

Thus, clinic-based studies and the general population-based NANHES-III study came to similar conclusions, which reinforce the hypothesis of a causal association between HCV infection and T2D. As a result of the cross-sectional nature of all these surveys, however, as hinted before, a temporal relationship between HCV infection and T2D cannot be established. This issue, i.e. did the HCV infection come before the occurrence of T2D or *vice versa*, has been addressed by longitudinal studies. A prospective, case-cohort study, performed in the United States, analyzed whether persons who developed T2D were more likely to have had precedent HCV infection when enrolled in a community-based cohort of 1084 persons aged 44-65 years (the Atherosclerosis Risk in Communities Study)<sup>[37]</sup>. The prevalence of HCV in this population was 0.8%. A total of 548 subjects developed *de novo* T2D over 9 years of follow-up. Prior to entry, subjects had been categorized as low-risk or high-risk for T2D based on age and BMI. Among those at high risk for T2D, persons with HCV infection were more than 11 times as likely as those without HCV infection to develop T2D (relative hazard, 11.58; 95% CI: 1.39-96.6). Among those at low risk, the incidence of T2D was not increased among HCV-infected subjects. The conclusion of this important survey was that pre-existing HCV infection may increase the incidence of T2D in persons with known risk factors. The second study<sup>[38]</sup>, a community-based cohort survey performed in southern Taiwan, enrolled 4958 persons aged  $\geq 40$  years, without T2D at entry. This study included 3486 seronegative persons, 812 anti-HCV-positive patients, 544 individuals with the hepatitis B surface antigen (HBsAg) and 116 with hepatitis B virus (HBV)/HCV co-infection. Over a follow-up of 7 years, 474 cases of incident T2D were recorded: overall, 14.3% of anti-HCV-positive, 7.5% of HBsAg-positive, and 8.6% of seronegative individuals developed T2D during the study. Compared to anti-HCV-negative individuals, anti-HCV-positive persons had a higher cumulative incidence of T2D ( $P < 0.0001$ ). By multivariate analysis, the fact of being anti-HCV-positive, co-infection with HBV and HCV, overweight, obesity, and increasing age were all significantly associated with T2D, while sex and alcohol consumption, among other factors, were not. Interestingly, when patients were stratified by age and BMI, the risk of developing T2D among anti-HCV-positive individuals increased when age decreased and BMI levels increased. This study concluded that HCV infection is an independent predictor of T2D. The risk

was higher in patients with elevated BMI, but, at variance with the previous study, seemed to decrease with age.

Thus, cross-sectional and longitudinal studies both seem to converge towards the same conclusion, i.e. there exists an excess T2D risk in HCV-infected persons compared to controls infected with HBV, which suggests a direct role of HCV in inducing derangement of glucose metabolism. A recent, large meta-analysis, the first of this kind, has reached the same conclusion<sup>[39]</sup>.

An additional, strong case in favor of an association between HCV and T2D comes from longitudinal studies performed in patients having received a liver or kidney transplant. T2D is a common complication of liver transplantation (LT). Apart from isolated negative reports<sup>[40]</sup>, there is accumulating evidence that HCV is a strong predictor of new-onset T2D after LT<sup>[41]</sup>. A first study from Toronto, Canada<sup>[42]</sup>, analyzed the prevalence of T2D among 278 LT recipients, whose indication for transplantation was liver failure caused by HCV (110 patients), HBV (53 patients) or cholestatic liver disease (115 patients). Multivariate analysis revealed that HCV-related cirrhosis ( $P = 0.002$ ), pre-LT T2D ( $P < 0.0001$ ) and male gender ( $P = 0.019$ ) were independent predictors of the presence of T2D 1 year after LT. The high prevalence of T2D persisted among HCV-positive persons, with 41% being diabetic at 5 years. This observation was subsequently confirmed by other studies. In a series from Harvard<sup>[42]</sup>, which compared 47 HCV-positive to 111 HCV-negative cases, HCV infection was an independent risk factor for the development of T2D after LT (hazard ratio 2.5,  $P = 0.001$ ). These data were repeatedly confirmed by later studies<sup>[43-49]</sup>, with one exception from the [University of California, Los Angeles (UCLA)] series, in which the lack of association may have been a consequence of the excess representation of HCV-positive patients<sup>[50]</sup>. Several predisposing factors were identified across the studies: impaired fasting glucose and a maximum lifetime BMI over 25 kg/m<sup>2</sup><sup>[49]</sup>, age and male gender<sup>[48]</sup>, serum HCV RNA level after LT<sup>[51]</sup>, and use of tacrolimus<sup>[45]</sup> or steroid boluses<sup>[43]</sup>. On the other hand, use of cyclosporine<sup>[49]</sup> and rapid discontinuation of steroids<sup>[52]</sup> seem to reduce the incidence of T2D among HCV-positive persons.

A similarly increased risk of T2D has been reported after kidney transplantation (KT). After two early reports, underlining a rather strong association between ongoing HCV infection and post-KT T2D<sup>[53,54]</sup>, a major retrospective analysis on 427 kidney recipients without T2D before KT<sup>[55]</sup> showed that, by multivariate logistic regression, HCV (adjusted OR 5.58; 95% CI: 2.63-11.83;  $P = 0.0001$ ), weight at transplantation (adjusted OR 1.028; 95% CI: 1.00-1.05;  $P = 0.001$ ), and tacrolimus (adjusted OR 2.85; 95% CI: 1.01-5.28;  $P = 0.047$ ) were associated with newly onset T2D after KT. In this study, a significant interaction ( $P = 0.0001$ ) was found between presence of HCV and use of tacrolimus, since in the HCV-positive group, T2D occurred more often in tacrolimus-treated than cyclosporine A-treated patients (57.8% *vs* 7.7%;  $P < 0.0001$ )<sup>[55]</sup>. Most subsequent studies

confirmed this robust association<sup>[56-63]</sup>, with some exceptions<sup>[21,64-66]</sup>. Thus, in a recent meta-analysis of 10 studies, the pooled relative risk for post-KT T2D was 2.73 (95% CI: 1.94-3.83)<sup>[67]</sup>. When only two large studies were considered, the pooled relative risk was still 1.36 (95% CI: 1.21-1.54). The existing publication bias did not change the results in a meaningful way, after a sensitivity analysis was performed<sup>[67]</sup>. In addition to ongoing HCV infection, risk factors for developing T2D after KT are family history of T2D<sup>[55,60]</sup>, age<sup>[57,59,61,62]</sup>, use of tacrolimus<sup>[55,59,60,62,63]</sup>, smoking<sup>[61]</sup>, overweight/obesity<sup>[62,63]</sup>, African-American ethnicity<sup>[62]</sup> and pre-transplantation impaired fasting glucose<sup>[63]</sup>. Thus, there exists a significant increase of the risk of post-KT T2D in HCV-positive recipients, especially in the first 2 mo after transplantation<sup>[57]</sup>. Since T2D and its complications are a leading cause of mortality after KT, it is easy to understand that every effort should be made to clear HCV with antiviral therapy in the pre-KT period, whenever this is feasible.

Thus, HCV and T2D are associated more than just by chance, suggesting that HCV may alter glucose homeostasis by its direct action, or *via* indirect mechanisms such as through cytokine stimulation (see below). The association between HCV infection and glucose abnormalities holds true if, instead of looking at the occurrence of overt T2D, one considers pre-diabetes conditions, such as impaired glucose tolerance (IGT) or IR. The latter is defined as a condition in which higher than normal insulin concentration are needed to achieve normal metabolic responses or, alternatively, normal insulin concentration are unable to achieve normal metabolic responses<sup>[68]</sup>. It has to be stated clearly, however, that it is not clear whether IR associated with HCV infection invariably evolves towards T2D in all infected persons, especially those without other risk factors of T2D. There is a clear need of longitudinal studies that may clarify this issue.

In a classical paper, Hui and collaborators<sup>[4]</sup> compared fasting levels of serum insulin, C-peptide and IR [measured as homeostasis assessment (HOMA) score] in 121 HCV patients with stage 0 or 1 liver fibrosis and 137 healthy volunteers matched by sex, BMI, and waist-to-hip ratio. Results showed that such HCV-infected persons, notwithstanding their early stage of liver disease, had higher levels of insulin, C peptide, and HOMA scores compared with controls. Besides, this study was the first to suggest that genotype 3 may have significantly lower HOMA scores than other genotypes (which were comparable when adjusted for the remaining independent predictors of IR). Thus, this work showed how HCV may induce IR irrespective of the stage of advancement of the underlying liver disease, an effect that seemed to be genotype specific. In a similar, more recent paper, Moucari *et al.*<sup>[69]</sup> analyzed 600 consecutive patients (500 with chronic hepatitis C and 100 controls with chronic hepatitis B). IR was less frequent in chronic hepatitis B than in matched chronic hepatitis C cases (5% *vs* 35%, respectively,  $P < 0.001$ ), again irrespective of the stage of liver disease (patients were divided



according to the presence or absence of liver cirrhosis). Furthermore, IR was associated with genotypes 1 and 4 and high serum HCV RNA levels, even suggesting a trend, among patients without features of the metabolic syndrome, between HCV replication level and HOMA score. These data further corroborated the hypothesis that HCV may have a direct involvement in glucose metabolism derangement. A correlation between HCV RNA levels and HOMA score has been reported also by other studies<sup>[70-72]</sup>, especially in genotype 1<sup>[71]</sup> or after adjustment for age, gender and visceral adipose tissue area<sup>[72]</sup>. These results are not, however, confirmed by all investigators. In a recent paper, Anty *et al*<sup>[73]</sup> reported that lean patients with non-3 genotypes had higher glycemia and lower adiponectin levels than controls, at closer look it was evident that, considering only the 52 patients with F0/F1, then the HOMA scores were comparable to those of 22 controls ( $1.7 \pm 1.6$  vs  $1.4 \pm 1.5$ ,  $P = \text{NS}$ ). Negative results have also been reported from Japan, where two studies failed to identify HCV infection as independent predictor of IR<sup>[14,74]</sup>. Thus, further work is warranted in this field, and, more importantly, a thorough analysis, at the population level, of HCV sequences that may be directly involved in stimulating IR. Furthermore, it is impossible to determine whether HCV replication is responsible for increased IR or whether HCV replication is favored by hyperinsulinemia, as suggested by some *in vitro* data<sup>[75]</sup>, and/or by the increased serum levels of free fatty acids<sup>[76]</sup> typically observed in IR and T2D<sup>[77]</sup>. Finally, the poor correlation between HCV RNA levels and HOMA score may also be caused by the fact that the overall level of IR also depends on the contribution from the adipose tissue and muscle, two extrahepatic compartments not infected by HCV.

Finally, if HCV is increasing the level of IR or predisposes to the development of glucose metabolism disturbances, including T2D, in high-risk individuals, then curing HCV should result in amelioration of the HOMA score and in a decreased incidence of T2D after the end of therapy. Kawaguchi *et al*<sup>[78]</sup>, in their study on 89 patients, showed that eradication of HCV improved the HOMA score and the intrahepatic expression of the insulin receptor substrate (IRS) 1 and 2, two cellular transducers of the insulin signal (see below). Similar results have been reported in a cohort of 181 genotype 4 patients from Egypt<sup>[79]</sup>. Regarding the incidence of glucose metabolism derangements after sustained virological response (SVR), Romero-Gómez *et al*<sup>[80]</sup> assessed the effect of SVR and other host and viral factors on the incidence of impaired fasting glucose and T2D in 1059 patients with chronic hepatitis C treated with pegylated interferon (IFN)- $\alpha$ 2a and ribavirin. Their data show that SVR reduces by half the incidence of T2D and/or IFG during a post-therapy follow-up of  $27 \pm 17$  mo (range, 9.3-67 mo). Similar data have been reported in 234 patients followed in Barcelona for at least 3 years after the end of therapy<sup>[81]</sup>. However, in a cohort of 202 patients with a significantly longer follow-

up (8.0 years, range 5-16)<sup>[82]</sup>, the benefit of SVR (if any) was not observed, even after adjustment for several baseline risk factors of T2D.

In conclusion, HCV seems to increase the risk of incident T2D in predisposed individuals. As a result, the association between HCV and T2D is more evident among patients who are older and have higher BMI. When measuring IR before T2D has occurred, some HCV-infected patients are clearly less insulin sensitive than controls, matched for risk factors of T2D and stage of liver disease. This effect is probably associated with specific HCV sequences and/or subtypes, and shows some dose-dependence, i.e. may be correlated with HCV replication level. Curing HCV seems to have beneficial effects on the level of insulin sensitivity, although this may not be the rule. In the next chapter we will analyze the potential mechanisms of interference with the insulin signaling brought about by HCV.

## MECHANISMS OF HCV INTERFERENCE WITH INSULIN SIGNALING

Experimental data are compatible with direct interference of HCV with the insulin signaling cascade. This was first suggested by a study in which liver specimens obtained from 42 non-obese, non-diabetic, HCV-infected individuals and 10 non-HCV-infected subjects matched for age and BMI were exposed *ex vivo* to insulin, and examined for the contents and phosphorylation/activation status of some insulin signaling molecules<sup>[83]</sup>. Insulin-stimulated IRS-1 tyrosine phosphorylation was decreased by two-fold in HCV-infected patients compared to non-HCV-infected ones, and this was paralleled by significant reductions in IRS-1/p85 phosphatidylinositol 3 (PI3)-kinase association, IRS-1-associated PI3-kinase enzymatic activity and insulin-stimulated Akt phosphorylation<sup>[83]</sup>. It was concluded that, in patients with chronic hepatitis C, direct interactions between HCV and insulin signaling components occur that may result in IR, which in turn, may progress to T2D in at-risk individuals. In the transgenic mouse model<sup>[84]</sup>, the core-encoding region of HCV is sufficient to induce IR. This effect was reversed by treatment with anti-tumor necrosis factor (TNF)-antibodies, which suggested an increased level of serine phosphorylation of IRS-1 as induced by TNF- $\alpha$ . Thus, the core protein may induce IR indirectly *via* stimulation of the secretion of TNF- $\alpha$ . However, *in vitro* models suggest otherwise, hinting at a direct interaction of the core protein with the insulin signaling pathway. An increased proteasomal degradation of the IRS-1 and -2 *via* the activation of the suppressor of cytokine signaling (SOCS)-3 has been reported after transient expression of the core protein<sup>[85]</sup>. Direct but genotype-specific mechanisms have been advocated in another study<sup>[86]</sup>, in which down-regulation of peroxisome proliferator-activated receptor- $\gamma$  (PPAR- $\gamma$ ) and up-regulation of SOCS-7 was observed in cells transfected with the core protein of genotype 3,

whereas the core protein of genotype 1b activated the mammalian target of rapamycin, findings that were confirmed by using agonists for PPAR- $\gamma$  (rosiglitazone) or short interfering RNAs for SOCS-7<sup>[87]</sup>. Among the indirect mechanisms, an increased endoplasmic reticulum stress has also been described that may lead to IR<sup>[87]</sup>. More recently, the role of c-Jun N-terminal kinase (JNK) has been emphasized<sup>[88]</sup>. The HCV core protein-mediated Ser (312) phosphorylation of IRS-1 was inhibited by a JNK inhibitor in an *in vitro* infection assay using cell-culture-grown HCV<sup>[88]</sup>.

Studies on chronically infected patients have suggested that increased oxidative stress and intrahepatic inflammation may also play a role. Mitsuyoshi *et al*<sup>[89]</sup> evaluated 203 chronic hepatitis C patients with HCV genotypes 1 or 2 infection. HOMA and serum levels of thioredoxin, a marker of oxidative stress, were significantly correlated with each other, even after adjustment for BMI. However, in the human model, the indirect role of inflammatory mediators, such as TNF- $\alpha$ , seems more likely, in keeping with the transgenic mouse model. In fact, in chronic hepatitis C patients, an increased intrahepatic TNF- $\alpha$  response, which results in IR and a higher risk of developing T2D, has been described<sup>[90,91]</sup>. Further work is necessary in this field, and the availability of genotype-specific replicon assays may pave the way to more in-depth mechanistic analyses.

## CLINICAL CONSEQUENCES OF IR/T2D IN CHRONIC HEPATITIS C

The clinical consequences of IR and T2D on chronic hepatitis C are dual: accelerated fibrogenesis and reduced response to IFN-based therapy. Since one of the most frequent consequences of IR/T2D on the liver is steatosis, many data can be inferred indirectly looking at past studies in which the impact of non-virus- and non-alcohol-induced fatty liver on fibrosis progression was evaluated<sup>[2]</sup>. In fact, in these cases, the most likely cause of fatty liver was IR, and this, rather than steatosis, seems to predict the stage of fibrosis and its progression over time<sup>[4]</sup>. More generally, accelerated liver fibrogenesis should be considered in the complex of the consequences of the metabolic syndrome on the liver. This view allows one to consider several pathogenetic mechanisms other than IR, such as oxidative stress, increased secretion of pro-inflammatory adipokines and cytokines, and the peculiar susceptibility to apoptosis that has been associated with steatosis. High serum glucose<sup>[92]</sup>, hyperinsulinemia<sup>[93]</sup> and IR<sup>[4,71,94-97]</sup> are all associated with increased fibrosis in chronic hepatitis C, and more rapid progression of hepatitis C in diabetics has been reported also after LT<sup>[98]</sup> and KT<sup>[99]</sup>. However, claiming that the sole pathogenetic mechanism that underlies accelerated fibrogenesis in patients with chronic hepatitis C and IR is the hyperglycemic/hyperinsulinemic state is an oversimplification. First, it is not unknown whether patients with virus-induced

IR alone, i.e. without the other components of the metabolic syndrome (especially in the absence of the visceral obesity and the inflammatory state associated with it), share the same risk of increased liver disease progression compared to patients with overt metabolic syndrome. Second, patients with central obesity have not only increased IR but also altered levels of a whole array of pro-inflammatory cytokines and adipokines, which may exert their unwanted effects on the liver and other extra-adipose tissues independently of the action of insulin. The relative contribution of these cytokines to liver fibrosis in chronic hepatitis C is starting to be unraveled, but it is far from being fully understood.

In non-alcoholic steatohepatitis, hyperglycemia/hyperinsulinemia may be directly stimulating hepatic stellate cells to produce connective tissue growth factor (CTGF), which leads to increased collagen fiber deposition<sup>[100]</sup>. Increased intrahepatic levels of CTGF have been reported to occur in chronic hepatitis C<sup>[101]</sup>. The reduction of IR consequent to body weight reduction and increased physical activity may lead to reduced fibrosis score over time and a diminished number of activate hepatic stellate cells<sup>[102]</sup>.

Several pro-inflammatory cytokines and adipokines may be involved in the pathogenesis of liver injury in chronic hepatitis C. However, their relative contribution is under debate. A large, careful study has evaluated the role of TNF- $\alpha$ , interleukin 6, leptin and adiponectin in the pathogenesis of HCV-associated liver injury<sup>[103]</sup>. Only TNF-levels seemed to correlate with severity of portal and periportal inflammation, but none of the cytokines considered in this study were correlated with liver fibrosis. Several other studies have failed to pinpoint a clear correlation between the severity of fibrosis and serum levels of leptin<sup>[94,104-106]</sup>, with only one positive report<sup>[107]</sup>. The role of adiponectin is quite controversial<sup>[103]</sup>. In addition, recent data have suggested a potential involvement of resistin in the pathogenesis of liver fibrosis<sup>[108]</sup>. However, these latter data await independent confirmation. Finally, increased liver cell apoptosis has been reported to be correlated with steatosis<sup>[109]</sup>. Hepatocyte apoptosis can be measured by caspase activity in serum<sup>[110]</sup>. In the presence of steatosis, apoptosis is correlated with activation of stellate cells and increased stage of fibrosis, in keeping with the hypothesis that a steatotic liver is more vulnerable to liver injury, and suggesting another mechanism of liver disease progression in patients with fatty liver and the metabolic syndrome<sup>[109]</sup>.

Increasing levels of IR are associated with reduced rates of initial virological response<sup>[111-113]</sup> as well as SVR in chronic hepatitis C patients treated with a combination of pegylated IFN- $\alpha$  and ribavirin<sup>[114-119]</sup>. This negative association has been reported not only in patients infected with the HCV genotype 1<sup>[114,116,119]</sup>, but also in those with the so-called “easy-to-treat” genotypes 2 and 3<sup>[118]</sup>. Furthermore, the negative impact of IR on the early response to anti-HCV therapy has been

recently confirmed among HIV-infected patients<sup>[120]</sup>. The molecular link between IR and lack of responsiveness to IFN- $\alpha$  seems to lie in the increased levels of SOCS-3 in the liver<sup>[117,121]</sup>. Interestingly, SOCS-3, as stated above, is not only promoting the proteasomal degradation of IRS-1, which leads to impaired insulin signaling and IR<sup>[85]</sup>, but, together with other members of the SOCS family, is also a negative regulator in the transduction of the IFN- $\alpha$  signaling<sup>[122]</sup>. Thus, it is not too unlikely that HCV may have developed, from the evolutionary standpoint, the ability to activate SOCS-3 or other members<sup>[86]</sup> of the SOCS family as a mechanism to inhibit the IFN- $\alpha$  signaling, one of the main arms of the host innate immune response, simultaneously impairing the insulin signaling. This view seems to be supported by the recent finding that HCV may also activate the protein phosphatase 2A, again with the dual effect of interfering with the insulin<sup>[87]</sup> and IFN- $\alpha$ <sup>[123]</sup> signaling pathways. Whether these mechanisms may be exploited pharmacologically, i.e. with drugs aimed at reducing IR while improving the responsiveness to IFN- $\alpha$ , remains to be fully explored (see below).

## PERSPECTIVES FOR CLINICAL MANAGEMENT

The treatment of IR and T2D in chronic hepatitis C patients has two goals, as far as the underlying liver disease is concerned: to reduce fibrogenesis (hence liver disease progression) and to increase the response to IFN-based therapy. As pointed out above, it is not known whether IR invariably increases liver fibrosis, i.e. in the context of the metabolic syndrome or in cases of purely virus-induced IR, without the remaining constellation of cytokine changes that accompany the metabolic syndrome. This distinction is important also when antiviral therapy has to be undertaken, because here therapy should be aimed at correcting IR based on the underlying molecular mechanisms, which may differ according to the viral genotype and the presence or absence of metabolic syndrome. At present, however, the approach that is being followed is rather empirical.

A single study<sup>[102]</sup> has analyzed the biochemical and histological consequences of a 3-mo program that comprises body weight reduction and increased physical activity. In 19 subjects with steatosis and chronic hepatitis C, the weight loss was paralleled by progressive reduction of serum alanine aminotransferase levels and of mean fasting insulin. In patients with paired liver biopsies, steatosis decreased, together with the fibrosis score and the number of activated stellate cells, despite the persistence of HCV. The authors concluded that weight reduction may provide an important adjunct management strategy for patients with chronic hepatitis C<sup>[102]</sup>. Lifestyle changes are the single most important measure to reduce the incidence of T2D in those at risk<sup>[124]</sup> and of the metabolic syndrome in patients with IGT<sup>[125]</sup>. Moreover, the metabolic syndrome may even

regress following such intervention<sup>[125]</sup>, more often than among patients treated with metformin. Therefore, lifestyle changes (weight reduction and increased physical activity) should constitute the mainstay of the clinical management of patients with chronic hepatitis C and initial glucose metabolism derangements (IR and IGT), with the aim of reducing their progression to overt T2D and possibly, their impact on liver fibrogenesis.

Alternatively, insulin sensitizing agents have been tested with the specific aim of improving the rate of response to IFN- $\alpha$ -based therapy. As said above, IR reduces the rate of response to antivirals in chronic hepatitis C. Thus, it was suggested that IR should be corrected in patients with chronic hepatitis C not responding to IFN- $\alpha$ -based treatment, in order to improve response upon re-treatment. The modalities of this intervention, however, have not been established. In addition, the optimal HOMA score to be reached has not been identified. The preliminary data from four independent studies<sup>[126-129]</sup> have not been encouraging. A first prospective, multicenter study aimed at investigating the efficacy and safety of the insulin sensitizer pioglitazone, 15 mg *qd*, added to pegylated IFN-2a, 180 g *qw*/ribavirin, 1000-1200 mg *qd* combination therapy in chronic hepatitis C patients who were previously non-responders to a pegylated IFN- $\alpha$ /ribavirin combination<sup>[126]</sup>. All patients had a baseline HOMA > 2, because this was the threshold that discriminated responders from non-responders in previous studies<sup>[114,118]</sup>. None of the first five patients enrolled into the trial had a sufficient virological response after 12 wk to warrant continuation of the trial, which was therefore prematurely terminated. Data from three additional trials have been presented at the 2008 meeting of the American Association for the Study of Liver Diseases. In an interim analysis of one of them, 30 mg *qd* pioglitazone was given for 4 wk as monotherapy, and then added for the first 4 wk of standard therapy of treatment-naïve, non-diabetic, chronic hepatitis C patients. The authors showed that the triple regimen that contained pioglitazone increased significantly the rate of virological response after 4 wk therapy, compared to pegylated IFN- $\alpha$ /ribavirin combination alone<sup>[127]</sup>. However, long-term data are awaited before any conclusion can be drawn, and some caution is required. In fact, in another randomized, double-blind, placebo-controlled study, adding pioglitazone 30 mg *qd* simultaneously to standard care increased the early and end-of-treatment virological response, but failed to increase the SVR<sup>[128]</sup>. Further data are needed before insulin sensitizers can be added to the panoply of drugs to treat hepatitis C.

Furthermore, the effects of PPAR agonists on serum lipids and their potential consequences on the HCV life cycle should be investigated in more detail. It is also unclear whether the treatment with the insulin sensitizer should be started at the same time as the antiviral retreatment or precede it, in order to start the pegylated IFN- $\alpha$ /ribavirin combination only when the HOMA



score has decreased to a level predictive of an increased SVR<sup>[114,118]</sup>. It is not clear whether the best approach is to use a PPAR agonist (and at what dose) or a biguanide such as metformin, whose mechanism of action is specifically directed against the hepatic AMP-activated protein kinase<sup>[130]</sup>. The final results of the TRIC-1 study<sup>[129]</sup> show that adding metformin to pegylated IFN- $\alpha$ /ribavirin combination afforded a marginal, non-significant gain as to the SVR rate, despite an increased rapid virological response after 4 wk of triple therapy. Thus, further clinical trials aimed at reducing the IR in chronic hepatitis C *via* different pharmacological interventions are warranted.

## CONCLUSION

HCV and IR/T2D are associated to an extent that cannot be merely explained by chance, which suggests that HCV interferes directly (through one or more of its proteins) and/or indirectly (by modulating the production of specific cytokines, like TNF- $\alpha$ ) with glucose metabolism. Independently of the mechanism, IR and T2D have important effects on the hepatitis C progression and response to antivirals, which warrants specific and effective measures to correct such metabolic anomalies. Although lifestyle interventions are certainly indicated in patients with chronic hepatitis C and the metabolic syndrome, in order to reduce the cardiovascular morbidity and mortality, it remains to be fully explored whether these measures will also have an impact on the underlying liver disease. Insulin sensitizers are currently being evaluated in clinical trials, but available data do not warrant their use in all chronic hepatitis C patients with IR, with the specific aim of increasing response to antivirals, at least outside of clinical trials.

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EDITORIAL

## Efficacy of tricyclic antidepressants in irritable bowel syndrome: A meta-analysis

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### Abstract

We aimed to evaluate the efficacy of tricyclic antidepressants (TCAs) as a therapeutic option for irritable bowel syndrome (IBS) through meta-analysis of randomized controlled trials. For the years 1966 until September 2008, PubMed, Scopus, Web of Science, and Cochrane Central Register of Controlled Trials were searched for double-blind, placebo-controlled trials investigating the efficacy of TCAs in the management of IBS. Seven randomized, placebo-controlled clinical trials met our criteria and were included in the meta-analysis. TCAs used in the treatment arm of these trials included amitriptyline, imipramine, desipramine, doxepin and trimipramine. The pooled relative risk for clinical improvement with TCA therapy was 1.93 (95% CI: 1.44 to 2.6,  $P < 0.0001$ ). Effect size of TCAs *versus* placebo for mean change in abdominal pain score among the two studies was -44.15 (95% CI: -53.27 to -35.04,  $P < 0.0001$ ). It is concluded that low dose TCAs exhibit clinically and statistically significant control of IBS symptoms.

antidepressants; Irritable bowel syndrome; Efficacy; Clinical response; Abdominal pain

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### EPIDEMIOLOGY AND PATHOGENESIS OF IRRITABLE BOWEL SYNDROME (IBS)

IBS is a prevalent functional gastrointestinal (GI) disorder characterized by chronic or recurrent abdominal pain or discomfort associated with altered bowel habits<sup>[1]</sup>. Up to 20% of the North American population are affected by IBS<sup>[2]</sup>. A community-based study carried out in Birmingham, UK estimated the prevalence of IBS to be 10.5%<sup>[3]</sup>. IBS is commonly diagnosed between the ages of 15 and 45 years and affects women twice as often as men<sup>[3,4]</sup>. IBS places a significant burden on health economy in terms of using more health care services even for non-gastrointestinal symptoms comparing to general population<sup>[5]</sup>.

Environmental factors (psychological disturbances and stress), genetic links, recent infection, bacterial overgrowth, food intolerance, altered bowel motility and/or secretion, visceral hypersensitivity, altered central nervous system sensory processing, disturbed autonomic nervous system regulation, and serotonin dysregulation are all proposed as possible etiological factors for IBS<sup>[2-4,6,7]</sup>.

### MANAGEMENT OF IBS AND CURRENT PLACE OF TRICYCLIC ANTIDEPRESSANTS (TCAs)

In addition to non-pharmacological strategies such as diet and psychotherapy, various pharmacological agents are used for the management of IBS including bulking

agents, antidiarrheal agents, laxatives, antispasmodics, antidepressants, serotonergic agonists or antagonists, antibiotics and probiotics<sup>[8-12]</sup>. The rationale of using antidepressants in IBS is that these agents may alter pain perception by a central modulation of visceral afferents, treat comorbid psychologic symptoms, and alter GI transit. Different classes of antidepressants likely act by different combinations of mechanisms<sup>[1]</sup>. Two classes of antidepressants frequently used for the treatment of IBS are selective serotonin reuptake inhibitors (SSRIs) and TCAs. In a meta-analysis done in 2007<sup>[8]</sup>, the efficacy of SSRIs in IBS was reported. In the present paper, the efficacy of TCAs in IBS has been reviewed by meta-analysis of all randomized controlled trials.

## EVALUATION OF STUDIES

PubMed, Scopus, Web of Science, and Cochrane Central Register of Controlled Trials were searched for studies investigated the efficacy of TCAs in IBS. Data were collected for the years 1966 to 2008 (up to September). The search terms were: “tricyclic antidepressants”, “amitriptyline”, “amoxapine”, “clomipramine”, “desipramine”, “dothiepin”, “doxepine”, “imipramine”, “prindole”, “lofepramine”, “nortriptyline”, “opipramol”, “protriptyline”, or “trimipramine” and “irritable bowel”, “functional bowel diseases” or “irritable colon”. Search was restricted to English literature. Reference lists of the retrieved articles were also reviewed for additional applicable studies.

All controlled trials investigating the efficacy of TCAs in patients with IBS were considered. “Global improvement of symptoms” and “adequate relief of pain and discomfort” were the key outcomes of interest for assessment of efficacy. We evaluated all published studies as well as abstracts presented at meetings. Three reviewers independently examined the title and abstract of each article to eliminate duplicates, reviews, case studies, and uncontrolled trials. Trials were disqualified if they were not placebo-controlled or their outcomes did not consider efficacy. Reviewers independently extracted data on patients’ characteristics, therapeutic regimens, dosage, trial duration, and outcome measures. Disagreements, if any, were resolved by consensus.

Jadad score, which evaluates studies based on their description of randomization, blinding, and dropouts (withdrawals), was used to assess the methodological quality of the trials<sup>[13]</sup>. The quality scale ranges from 0 to 5 points with a low quality report of score 2 or less and a high quality report of score at least 3.

Data from selected studies were extracted into 2 × 2 tables. All included studies were weighted and pooled. Data analysis was done using StatsDirect (2.7.2). Relative risk (RR) and 95% confidence intervals (95% CI) were calculated using Mantel-Haenszel and effect size (weighted mean difference) meta-analysis was performed using Mulrow-Oxman method. The Cochran *Q* test was used to test heterogeneity. The event rate in the experimental (intervention) group against the event rate

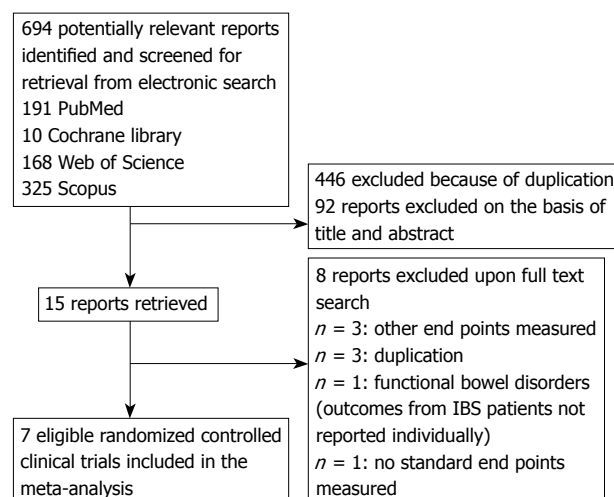


Figure 1 Flow diagram of the study selection process.

in the control group was calculated using L'Abbe plot as an aid to explore the heterogeneity of effect estimates. In case of homogeneity, fixed effect model was used for meta-analysis; otherwise random effect model was applied. In addition to Kendall's *t* test<sup>[14]</sup>, funnel plots were used as an indicator for publication bias<sup>[15]</sup>.

## FINDINGS

The electronic searches yielded 694 items; 191 from PubMed, 10 from Cochrane Central, 168 from Web of Science, and 325 from Scopus. Of these, 15 trials were scrutinized in full text and 7 trials<sup>[16-22]</sup> were included in the analysis (Figure 1). Of these 7 studies, 6<sup>[16-21]</sup> obtained a Jadad score of 3 or more and the remaining one<sup>[22]</sup> gained a Jadad score of 2 (Table 1). Regarding the Cochran *Q* test for heterogeneity, it was found that this study did not cause heterogeneity in our meta-analysis and thus, it was not excluded. Patients' characteristics, IBS subtype, TCA subclass, dosage, duration of treatment/follow up for each study are reported in Table 2. All subtypes of IBS (diarrhea-predominant, constipation-predominant and alternating) were incorporated in the included studies. This meta-analysis included 257 IBS patients randomized to receive either TCA or placebo. The efficacy of various TCAs has been investigated including amitriptyline (3 trials), imipramine (1 trial), desipramine (1 trial), doxepin (1 trial) and trimipramine (1 trial). Duration of treatment/follow up ranged between 4 and 12 wk. Definition of clinical response and mean change in abdominal pain score in each study are reported in Table 3.

Cochrane *Q* test suggested that the studies are homogeneous ( $P = 0.3284$ , Figure 2B) therefore, a fixed effect model was used for meta-analysis. Regression of normalized effect *versus* precision for all included studies for clinical response among TCAs *vs* placebo therapy was 2.40 (95% CI: -1.14 to 5.95,  $P = 0.14$ ). Funnel plot was suggestive of publication bias (Figure 2C); however, Kendall's *t* test was not indicative of such a bias ( $\tau = 0.05$ ,  $P > 0.9999$ ). Pooled RR for clinical response in 7 trials<sup>[16-22]</sup>



Table 1 Jadad quality score of randomized controlled trials included in the meta-analysis

Study	Factors and Jadad score			
	Randomization	Blinding	Withdrawals and dropouts	Total Jadad score
Vahedi <i>et al</i> <sup>[16]</sup> , 2008	1	1	1	3
Talley <i>et al</i> <sup>[17]</sup> , 2008	2	2	0	4
Morgan <i>et al</i> <sup>[18]</sup> , 2005	1	1	1	3
Rajagopalan <i>et al</i> <sup>[19]</sup> , 1998	1	2	0	3
Vij <i>et al</i> <sup>[20]</sup> , 1991	1	1	1	3
Greenbaum <i>et al</i> <sup>[21]</sup> , 1987	1	1	1	3
Tripathi <i>et al</i> <sup>[22]</sup> , 1983	1	1	0	2

Table 2 Characteristics of papers included in the meta-analysis

Study	Mean age	Sex		IBS subtype	Type of TCA	Daily dosage	Duration of treatment/follow up (wk)
		Female	Male				
Vahedi <i>et al</i> <sup>[16]</sup> , 2008	36	21	29	D-IBS	Amitriptyline	10 mg	8
Talley <i>et al</i> <sup>[17]</sup> , 2008	ND	21	13	D-IBS, C-IBS, Alt-IBS	Imipramine	2 wk: 25 mg; Thereafter to the end: 50 mg	12
Morgan <i>et al</i> <sup>[18]</sup> , 2005	39	22	0	D-IBS, C-IBS, Alt-IBS	Amitriptyline	First week: 25 mg; Thereafter to the end: 50 mg	4
Rajagopalan <i>et al</i> <sup>[19]</sup> , 1998	34.8	11	11	ND	Amitriptyline	First week: 25 mg; 2nd week: 50 mg; Thereafter to the end: 75 mg	12
Vij <i>et al</i> <sup>[20]</sup> , 1991	32.5	14	36	D-IBS, C-IBS, Alt-IBS	Doxepin	75 mg	6
Greenbaum <i>et al</i> <sup>[21]</sup> , 1987	45.2	18	11	D-IBS, C-IBS	Desipramine	First week: 50 mg; 2nd week: 100 mg; Thereafter to the end: 150 mg	6
Tripathi <i>et al</i> <sup>[22]</sup> , 1983	37	ND	ND	ND	Trimipramine	30 mg	5

IBS: Irritable bowel syndrome; D: Diarrhoea predominant; Alt: Alternating; C: Constipation predominant; TCA: Tricyclic antidepressant.

Table 3 Response to treatment

Study	Definition of response	Response		Change in abdominal pain score (No. of patients)	
		TCA	Placebo	TCA	Placebo
Vahedi <i>et al</i> <sup>[16]</sup> , 2008	Complete loss of symptoms (total score = 0) at the end of the study or at least two scores with a decrease in the number of symptoms	17/25	10/25	-	-
Talley <i>et al</i> <sup>[17]</sup> , 2008	Adequate relief of IBS symptoms over 50% of the weeks	10/18	9/16	-45.3 ± 26.3 (18)	-7.4 ± 46.9 (16)
Morgan <i>et al</i> <sup>[18]</sup> , 2005	Improvement of IBS symptoms determined by patients	13/22	5/22	-	-
Rajagopalan <i>et al</i> <sup>[19]</sup> , 1998	Global well-being: patients were asked to estimate at the post-treatment interview how much better or worse they were on the whole (in percentage) as compared to the pretrial period	7/11	3/11	-	-
Vij <i>et al</i> <sup>[20]</sup> , 1991	Improvement of 50% or above in IBS symptoms	11/21	5/23	-	-
Greenbaum <i>et al</i> <sup>[21]</sup> , 1987	Global assessment of improvement	15/28	5/28	-58.96 ± 19.37 (28)	-13.93 ± 17.76 (28)
Tripathi <i>et al</i> <sup>[22]</sup> , 1983	Improvement of 50% or above in IBS symptoms	7/25	4/25	-	-

was 1.93 (95% CI: 1.34 to 2.6,  $P < 0.0001$ , Figure 2A).

Studies that considered abdominal pain score as an outcome showed homogeneity using Cochrane  $Q$  test ( $P = 0.61$ ). Regression of normalized effect *vs* precision for all included studies for mean change in abdominal pain score could not be calculated because of too few strata.

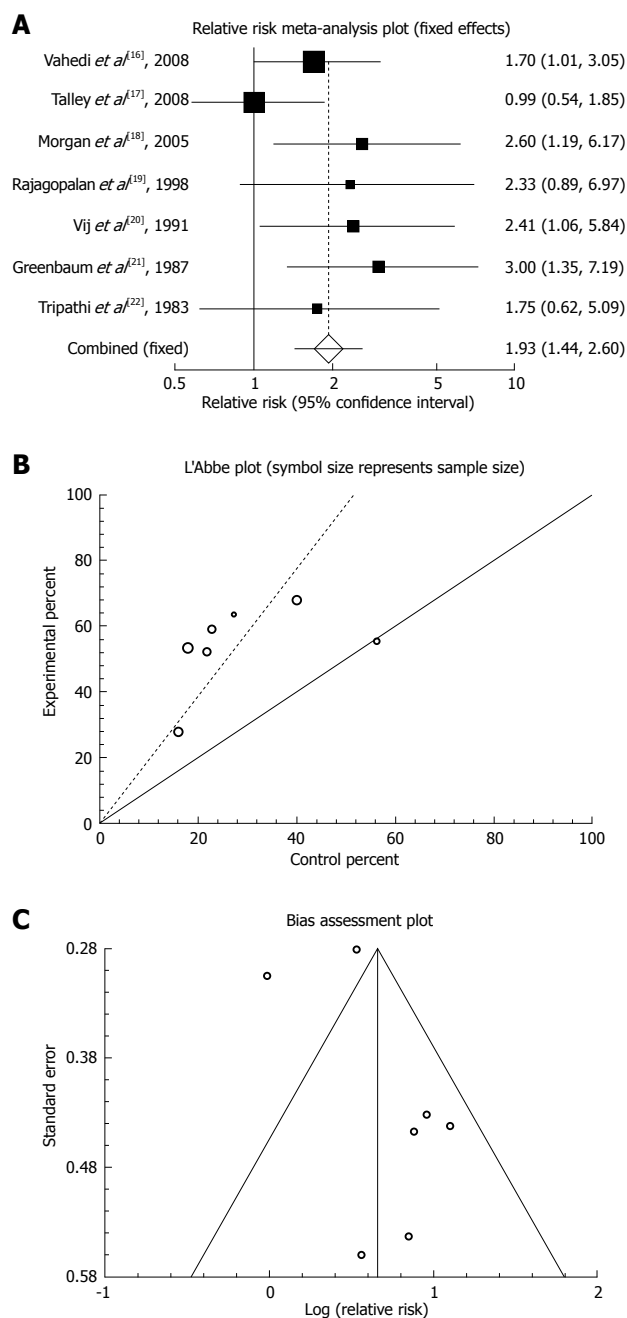
Using a fixed effect model, effect size of TCAs *versus* placebo for mean change in abdominal pain score among the two studies<sup>[17,21]</sup> was -44.15 (95% CI: -53.27 to -35.04,  $P < 0.0001$ , Figure 3).

## DISCUSSION

Visceral hypersensitivity and dysregulation of central pain perception in the brain-gut axis is considered to play a pivotal role in the pathophysiology of IBS. IBS

patients have a lower sensory threshold to colonic and rectal balloon distention and electrical stimulation<sup>[23]</sup>; therefore, beneficial effects of antidepressants can be explained by partial increment in central pain threshold. Other mechanisms by which antidepressants might express their effect include anticholinergic effects, regulation of GI transit and peripheral antineuropathic effects<sup>[24,25]</sup>. The results from the current meta-analysis show that TCAs induce clinical response and reduce abdominal pain score in patients with IBS.

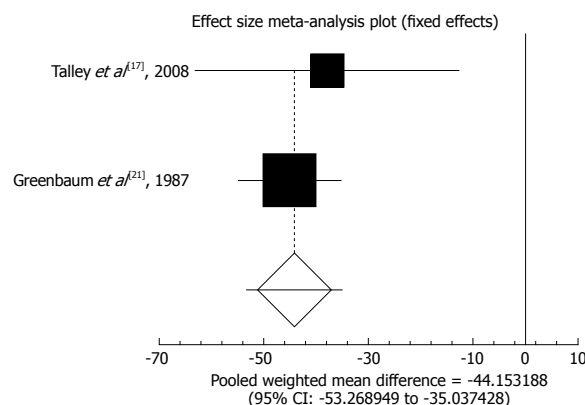
Other meta-analysis studies that considered the effects of antidepressants in functional gastrointestinal diseases have essential differences with the present study: O'Malley *et al*<sup>[26]</sup> pooled all functional diseases including IBS, functional dyspepsia, headache, fibromyalgia, and chronic fatigue. Jackson *et al*<sup>[27]</sup>



**Figure 2 Outcome of "clinical response" in the studies considering TCAs vs placebo therapy.** A: Individual and pooled relative risk; B: Heterogeneity indicators; C: Publication bias funnel plot.

included all the functional gastrointestinal disorders and found a statistically significant effect for TCAs (OR 4.2; 95% CI: 2.3 to 7.9). Quarero *et al*<sup>[8]</sup> included 4 studies for "global improvement of symptoms" and 2 studies for "abdominal pain" and demonstrated no benefit for antidepressants. Lesbros-Pantoflickova *et al*<sup>[28]</sup> demonstrated a favorable effect for antidepressants (OR 2.6; 95% CI: 1.9 to 3.5). Studies with visual analogue outcome<sup>[29]</sup> and functional dyspepsia patients were included in their analysis. None of these reviews meta-analyzed the newer evidence that has surfaced in the literature<sup>[16-18]</sup>.

Unfortunately, conduction of a randomized controlled trial in the field of antidepressants and IBS



**Figure 3 Pooled weighted mean difference for the outcome of "mean change in abdominal pain score" in the studies considering TCAs vs placebo.**

is challenging. High placebo response in IBS affects study trials and the stigma of antidepressants disturbs the compliance rate. Randomization is elusive as TCAs have immediate noticeable side effects for patients. As mentioned in Table 1, the majority of trials are of a medium quality. A well-designed trial has been conducted by Drossman *et al*<sup>[30]</sup> although not included in our study because of the recruitment of all functional bowel disorders. Drossman *et al*<sup>[30]</sup> conducted a large randomized 12-wk placebo-controlled trial evaluating the efficacy of desipramine in treating moderate to severe IBS (80% of the patients), functional constipation and functional chronic abdominal pain. Desipramine was shown to have statistically significant benefit over placebo in the per protocol analysis after non-compliant and drop-out patients were excluded (responder rate 73% *vs* 49%). 11% of the patients were proven to be non-adherent with non-detectable blood levels. This underlines the fact that previous studies might have underestimated the effect of antidepressants by the inclusion of non-adherent cases.

The choice of antidepressants in IBS patients remains controversial. Head to head trials comparing different classes or subclass formulations of antidepressants are lacking in the literature. In a recent meta-analysis<sup>[9]</sup>, we concluded that on current evidence, SSRIs do not improve abdominal pain, abdominal bloating or other IBS symptoms. Three studies have compared the effects of TCAs with SSRIs and all together they depict a non-conclusive picture<sup>[17,31,32]</sup>. Talley *et al*<sup>[17]</sup> compared imipramine with citalopram during a 12-wk trial. Clinical response was seen in 56% of imipramine group, 47% of citalopram group and 56% of placebo arm. Neither imipramine nor citalopram significantly improved global IBS endpoints over placebo. Forootan *et al*<sup>[31]</sup> compared the effects of nortriptyline, amitriptyline, and fluoxetine. The results demonstrated improvement of abdominal pain, flatulence, and general performance in all subgroups. Amitriptyline and nortriptyline improved frequency of defecation in both diarrhea- and constipation-predominant IBS, while fluoxetine improved GI transit of constipation- predominant IBS. Differential effects of amitriptyline and fluoxetine on

anorectal motility and visceral perception were assessed by Siproudhis *et al*<sup>[32]</sup> and the results demonstrated that both antidepressants similarly relax the internal anal sphincter, but only amitriptyline relaxed the external anal sphincter.

Generally, management of IBS requires lower doses of TCAs compared to doses used to treat depression; reflecting the fact that modulation of the brain-gut axis rather than treating concomitant depression is the target in IBS patients. Myren *et al*<sup>[29]</sup> in a large 8-wk trial demonstrated no dose-related response with various dosing regimens of trimipramine.

## CONCLUSION

This review has some limitations. Funnel plot is suggestive of publication bias with lack of negative small RCTs. However, a firm conclusion about bias is elusive to reach as the asymmetry of the funnel plot is minimal and Kendall's T is not suggestive of publication bias. In addition funnel plots can show asymmetry for various reasons other than publication bias<sup>[33]</sup>. Paucity of small negative or neutral studies has been brought to attention in other functional disorders<sup>[26]</sup> which might encompass IBS as well. Therefore, our pooled OR might be an overestimate of the true effect. Some other limitations that can be numbered for this meta-analysis are usage of various TCA formulations and doses, dissimilar duration of treatment, and different diagnostic criteria for IBS.

TCAs exhibit clinically and statistically significant control of IBS symptoms; however, given their abundant side effects they should be reserved for moderate to severe cases. Subjects should be started on subtherapeutic doses for depression and choice of drug should be tailored for each individual. We suggest using TCAs with the least anticholinergic effects (i.e. doxepin and desipramine) for elderly patients or constipation-predominant IBS and imipramine or amitriptyline for diarrhea-predominant IBS and patients with insomnia. Larger comparative trials with strict surveillance on compliance are needed to elaborate the role of antidepressants in standard practice.

In addition, new evidence suggests that IBS is very similar to IBD in pathogenesis but different in severity. Recent meta-analyses have indicated the benefit of antibiotics, probiotics, and anti-tumor necrosis factor agents<sup>[34-42]</sup> in IBD and thus the effects of these drugs on IBS remain to be elucidated in the future.

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## TOPIC HIGHLIGHT

Harry HX Xia, PhD, MD, Series Editor

# ***Clostridium difficile* associated infection, diarrhea and colitis**

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## Abstract

A new, hypervirulent strain of *Clostridium difficile*, called NAP1/BI/027, has been implicated in *C. difficile* outbreaks associated with increased morbidity and mortality since the early 2000s. The epidemic strain is resistant to fluoroquinolones *in vitro*, which was infrequent prior to 2001. The name of this strain reflects its characteristics, demonstrated by different typing methods: pulsed-field gel electrophoresis (NAP1), restriction endonuclease analysis (BI) and polymerase chain reaction (027). In 2004 and 2005, the US Centers for Disease Control and Prevention (CDC) emphasized that the risk of *C. difficile*-associated diarrhea (CDAD) is increased, not only by the usual factors, including antibiotic exposure, but also gastrointestinal surgery/manipulation, prolonged length of stay in a healthcare setting, serious underlying illness, immune-compromising conditions, and aging. Patients on proton pump inhibitors (PPIs) have an elevated risk, as do peripartum women and heart transplant recipients. Before 2002, toxic megacolon in *C. difficile*-associated colitis (CDAC), was rare, but its incidence has increased dramatically. Up to two-thirds of hospitalized patients may be infected with *C. difficile*. Asymptomatic carriers admitted to healthcare facilities can transmit the organism to other susceptible patients, thereby becoming vectors. Fulminant colitis is reported more frequently during outbreaks of *C. difficile* infection in patients with inflammatory bowel disease (IBD). *C. difficile* infection with IBD carries a higher mortality than without underlying IBD. This article

reviews the latest information on *C. difficile* infection, including presentation, vulnerable hosts and choice of antibiotics, alternative therapies, and probiotics and immunotherapy. We review contact precautions for patients with known or suspected *C. difficile*-associated disease. Healthcare institutions require accurate and rapid diagnosis for early detection of possible outbreaks, to initiate specific therapy and implement effective control measures. A comprehensive *C. difficile* infection control management rapid response team (RRT) is recommended for each health care facility. A communication network between RRTs is recommended, in coordination with each country's department of health. Our aim is to convey a comprehensive source of information and to guide healthcare professionals in the difficult decisions that they face when caring for these oftentimes very ill patients.

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## INTRODUCTION

In our two previous reviews<sup>[1,2]</sup>, we joined those who have written about the new more virulent strain of *Clostridium difficile* that was described in December 2005 in the National Institutes of Health (NIH)/Center for Disease Control and Prevention (CDC) Morbidity and Mortality Weekly Report. This CDC report emphasized that, in the past, *C. difficile*-associated diarrhea (CDAD) usually affected hospital inpatients, but now was appearing in relatively healthy adults, including some



who had not even been exposed to a hospital setting.

Loo *et al.*<sup>[3]</sup> and McDonald *et al.*<sup>[4]</sup> have indicated that, not only is the rate of disease associated with *C. difficile* increasing, but a previously uncommon strain of *C. difficile* has been identified. This strain of *C. difficile*, which has variations in its toxin genes, is more resistant to fluoroquinolones than prior strains. This newer and more virulent organism has emerged as a cause of geographically dispersed outbreaks of antibiotic-associated diarrhea (AAD), specifically *C. difficile* diseases, CDAD and *C. difficile*-associated colitis (CDAC).

CDAD has also become a more severe disease, and more often has progressed to toxic megacolon (TM). More severe CDAC and CDAD have started to increase in incidence and severity. *C. difficile* also accounts for an increasing percentage of community-acquired diarrhea cases. Fluoroquinolones, especially C-8-methoxy fluoroquinolones, such as moxifloxacin and gatifloxacin, have been incriminated in CDAD epidemics in different health care facilities. This current review attempts to provide an update on this new virulent organism that causes very severe CDAD and CDAC, and emphasizes the importance of early recognition of its complications and its treatment.

Typing of bacterial outbreaks characterize *C. difficile* as a Gram-positive, anaerobic, spore-forming bacillus that is spread indirectly *via* the fecal-oral route through spores left on surfaces. It produces two cytotoxins, which bind to receptors on intestinal epithelial cells, leading to inflammation and diarrhea. The toxins loosen the junctions of the epithelial cells that line the colon, allowing for penetration between epithelial cells<sup>[5]</sup>. This begins a cascade of tissue-damaging inflammatory processes that involve the release of destructive leukotrienes and cytokines.

Colonization of *C. difficile* is facilitated by the disruption of normal intestinal flora as a result of antimicrobial therapy. The antibiotics most frequently implicated in CDAD are clindamycin, penicillins, cephalosporins and fluoroquinolones<sup>[6]</sup>.

There has been a dramatic increase in the frequency, severity and refractoriness of *C. difficile* as seen in multiple outbreaks, not only in North America, but around the world. These factors are attributed to this hypervirulent strain, NAP1/BI/027.

Bartlett documented that, over the first 5 years in which CDAD was acknowledged to exist, 1978 to 1983, the most common cause of CDAD was previous use of clindamycin<sup>[7]</sup>. The standard diagnostic test was a cytotoxin assay. Standard management was to withdraw the implicated antibiotic and treat with oral vancomycin. Most patients responded well, but 25% relapsed when vancomycin was withdrawn.

Over the next 20 years (1983-2003), the most commonly implicated antibiotics were the cephalosporins, which reflected their increased rates of use. Fluoroquinolones now are the major inducing agents, along with cephalosporins, a phenomenon which presumably reflects newly-acquired *in vitro* resistance and the escalating rates of use<sup>[8]</sup>.

Between 2003 and 2006, *C. difficile* has become more frequent, more severe, more refractory to standard therapy, and more likely to relapse than in previous years. This pattern has been seen throughout the United States, Canada and Europe, and is now attributed to a new strain of *C. difficile*, alternatively designated as BI, NAP1, or ribotype 027 toxinotype III (all synonymous terms). Although this strain had been isolated as far back as 1984, it has recently emerged as a public concern with the development of fluoroquinolone resistance in our current era of widespread fluoroquinolone use.

The emergence of this hypervirulent *C. difficile* strain has vastly altered the face of the disease, with increased nosocomial outbreaks and concomitant morbidity. In 2007, Blossom and McDonald<sup>[9]</sup> reported on the increasing incidence and severity of *C. difficile*-associated disease attributable to this hypervirulent strain. This strain produces increased levels of toxins A and B, as well as an extra toxin, known as 'binary toxin', which accounts for its increased toxicity. This previously uncommon strain now has become epidemic, and has been reported in populations that previously had been thought to be at low risk, including peripartum women and healthy persons living in the community. Individuals with low or undetectable levels of antibody against *C. difficile* toxins are more likely to develop diarrhea than those with detectable antibody against the toxin. Careful adherence to infection control policies is critical to the control of *C. difficile*, especially at nursing facilities, long-term care and rehabilitation facilities and hospitals, as well as in the community. CDAD primarily occurs in hospitals, where exposure to antimicrobial drugs (the major risk factor for CDAD) and environmental contamination by *C. difficile* spores are more common<sup>[10]</sup>.

Outbreaks of CDAD due to the new, highly-virulent strain of *C. difficile* have been recognized throughout European health care facilities, including 75 hospitals in England, 16 hospitals in the Netherlands, 13 healthcare facilities in Belgium, and nine healthcare facilities in France. In Germany, the first cases of the highly-virulent *C. difficile* strain, reported in 2007 and characterized as PCR ribotype 027, were associated with high mortality<sup>[11]</sup>. Larger outbreaks of *C. difficile* have been reported in northern France in particular<sup>[12]</sup>. These outbreaks are very difficult to control, and preliminary results from case-control studies indicate a correlation with the administration of fluoroquinolones and cephalosporins.

Seroprevalence increased in Denmark with increasing age in both 1990 and 1998. Unfortunately, the increase was about four times higher in 1998 than in 1990, which suggests a higher rate of exposure to *C. difficile* in the general Danish adult population<sup>[13]</sup>.

In Dublin, Ireland, *C. difficile* is a major cause of infectious diarrhea in hospitalized patients<sup>[14]</sup>. Between August 2003 and January 2004, there was an appreciable increase in the incidence of *C. difficile*-associated disease, peaking at 21 cases per 1000 patient admissions. Of the *C. difficile* isolates recovered, 85 (95%) were identical toxin A-negative and toxin B-positive strains, corresponding to

toxintype VIII and PCR ribotype 017. All clonal isolates were resistant to multiple antibiotics, including ofloxacin, ciprofloxacin, levofloxacin, moxifloxacin and gatifloxacin [minimum inhibitory concentrations (MICs) > 32 µg/mL] and erythromycin, clarithromycin and clindamycin (MICs > 256 µg/mL). Recurrent *C. difficile*-associated disease occurred in 26 (36%) of the patients. At least 10 of these 26 patients (14%) developed *C. difficile* colitis. The authors found that careful attention to improving infection control interventions was the most important means of controlling this nosocomial pathogen.

Reported mortality rates from *C. difficile*-associated disease in the United States increased from 5.7 per million population in 1999 to 23.7 per million in 2004. These increased rates also may be caused by the emergence of a highly virulent strain of *C. difficile*. *C. difficile* infection, according to Schroeder<sup>[15]</sup>, is now responsible for approximately 3 million cases of diarrhea and colitis annually in the United States, and has a mortality rate of 1%-2.5%. Zilberberg *et al*<sup>[16]</sup> have reviewed a sample of more than 36 million annual discharges from non-governmental US hospitals, and have concluded that 2.3% of the cases of *C. difficile*-related disease were fatal, amounting for roughly 5500 deaths. That was nearly double the percentage that resulted in death in 2000.

In Canada, Pépin *et al*<sup>[17,18]</sup> have documented that, since 2002, an epidemic of CDAD caused by the same hypervirulent strain previously found in the United States, the United Kingdom and the Netherlands, has spread to as many as 30 hospitals in Quebec. More than half (55%) of the patients with CDAD at the investigators' own hospital had received fluoroquinolones within the preceding 2 mo. Moreover, the excessive use of proton pump inhibitors might have facilitated this epidemic. This CDAD was associated with a very high case-fatality rate and with a 30-d mortality rate of 23.0% (37/161) compared with 7.0% (46/656) of matched control subjects ( $P < 0.001$ ). Twelve months after diagnosis, mortality was 37.3% (60/161) among patients with CDAD *vs* 20.6% (135/656) among controls ( $P < 0.001$ ), for a cumulative absolute attributable mortality of 16.7% [95% confidence interval (CI) 8.6%-25.2%]. Each case of nosocomial CDAD led, on average, to 10.7 additional hospital days. These investigators documented especially high attributable mortality among elderly patients with CDAD, mostly caused by this hypervirulent strain, which represents a dramatic change in the severity of this infection. Kuijper *et al*<sup>[19]</sup> have estimated that the financial impact of CDAD on the healthcare system is 5-15000 Euros/case in England and \$1.1 billion/year total expenditures in the USA. Assuming a European Union (EU) population of 457 million, the potential cost of CDAD in the EU can be estimated to be 3 billion Euros/year, and this is expected to almost double over the next four decades.

In Zimbabwe, *C. difficile* was isolated from 29.0% of 100 chicken feces samples and from 22.0% of 100 soil samples. Some of the *C. difficile* isolates from chickens (89.7%) and soil (95.5%) were toxigenic. All

of the isolates were resistant to cefotaxime, gentamicin, ciprofloxacin, norfloxacin and nalidixic acid. The results of this study suggest that broiler chickens sold at marketplaces can be an important source of *C. difficile*, and may infect humans through consumption<sup>[20]</sup>.

The incidence of CDAD in Singapore has remained relatively low, with isolates remaining susceptible to metronidazole and vancomycin<sup>[21]</sup>.

## CHARACTERISTICS OF AN INCREASINGLY PATHOGENIC *C. DIFFICILE*

The new, hypervirulent strain, NAP1/BI/027, has been implicated as the responsible pathogen in selected *C. difficile* outbreaks since the early 2000s. The epidemic strain is resistant to fluoroquinolones *in vitro*, a characteristic which was an infrequent observation in *C. difficile* strains prior to 2001. Five main characteristics of this strain contribute to the clinical and epidemiological observations. (1) The epidemic strain produces a binary toxin, an additional toxin that is not present in other *C. difficile* strains<sup>[22,23]</sup>. (2) Binary toxin is related to the iota-toxin found in *Clostridium perfringens*, and its role in *C. difficile* pathogenesis is not fully understood<sup>[24,25]</sup>. (3) The epidemic strain produces substantially larger quantities of toxins A and B *in vitro* than other *C. difficile* strains<sup>[26]</sup>. (4) Toxin production by an emerging strain of *C. difficile* has been associated with outbreaks of severe disease in North America and Europe<sup>[27]</sup>. The epidemic strain is toxinotype III; most other *C. difficile* strains are toxinotype 0<sup>[28]</sup>. Toxinotyping is based on analysis of the pathogenic locus (PaLoc) of the *C. difficile* genome, the region that includes the genes for toxin A (tcdA), toxin B (tcdB), and neighboring regulatory genes. (5) The epidemic strain has a partial deletion of tcdC, a gene in PaLoc that is responsible for down-regulation of toxin production<sup>[29]</sup>.

*C. difficile* produces at least two distinct toxins<sup>[30]</sup>. These have been labeled toxin A and toxin B. Although initially thought to have distinctive actions, both now appear to be cytotoxic and enteropathic. Previous animal experiments have suggested that only toxin A mediates diarrhea and enterocolitis, even though *C. difficile* releases two structurally similar exotoxins. But when toxin A-negative/toxin B-positive strains of *C. difficile* are isolated from patients with AAD and colitis, this indicated that toxin B also may also be pathogenic in humans. *C. difficile* toxin B, like toxin A, has been found to be a potent inflammatory enterotoxin in the human intestine<sup>[31]</sup>.

Both toxins disrupt the actin cytoskeleton of intestinal epithelial cells by uridine diphosphate-glucose dependent glycosylation of Rho and Ras proteins<sup>[32]</sup>. Stabler *et al*<sup>[33]</sup> have reported that toxin B from 027 strains may have a different binding capacity than their less-virulent counterparts and may, in addition to the mutated tcdC regulator, be responsible for the increased virulence of the 027 strains.

The most widely used laboratory assays for *C. difficile* infection involve toxin A and/or toxin B detection, and

both are usually detected if diarrhea is present. Atypical toxin variant strains that may cause symptoms have also been described in Asia<sup>[34]</sup>.

Kuijper *et al.*<sup>[19]</sup> have claimed that *C. difficile* has more than 150 PCR ribotypes and 24 toxinotypes, and has a PaLoc with genes that encode for enterotoxin A (tcdA) and cytotoxin B (tcdB). Genes for the binary toxin are located outside the PaLoc. The recently completed genome sequence of *C. difficile* 630 has revealed a large proportion (11%) of mobile genetic elements, mainly in the form of conjugative transposons.

Drudy *et al.*<sup>[35]</sup> have reported on several *C. difficile* outbreaks due to PCR ribotype 027 (PCR-027) associated with a mutation in *gyrA* that is associated with high-level resistance to fluoroquinolones. This strain type, which contains genes for the binary toxin, has an 18-bp deletion and a frameshift mutation in *tcdC*, which results in deregulated expression of toxins A and B. These strains can produce up to 16 times more toxin A and 23 times more toxin B *in vitro* than toxinotype 0 strains. The strain demonstrates universally high-level resistance to fluoroquinolones, in contrast to PCR 027 isolates that were collected before 2001. Mutations at the active site or the quinolone resistance determining region of DNA gyrase and topoisomerase IV have been associated with increased resistance to fluoroquinolones in several bacteria. In *Escherichia coli*, amino acid substitutions that occur at Ser-83 in *gyrA* have also been associated with fluoroquinolone resistance. Thus, the emergence of the hypervirulent NAP1/O27 *C. difficile* strain, also known as BI NAP1, has vastly altered the face of the disease, with increased nosocomial outbreaks and concomitant morbidity in countries worldwide.

In an epidemic of *C. difficile*-associated disease in the Canadian province of Quebec, Warny *et al.*<sup>[26]</sup> documented that the dominant strain produced higher amounts of toxins A and B than those produced by non-epidemic strains. The epidemic strain was characterized as toxinotype III, North American PFGE type 1, and PCR-ribotype 027 (NAP1/027). This strain carried the binary toxin gene *cdtB* and an 18-bp deletion in *tcdC*. The authors isolated this strain from 72 patients with *C. difficile*-associated disease. Peak median (IQR) toxin A and toxin B concentrations produced *in vitro* by NAP1/027 were 16 and 23 times higher, respectively, than those measured in isolates representing 12 different PFGE types, known as toxinotype 0 [toxin A, median 848 µg/L (IQR 504-1022) *vs* 54 µg/L (23-203); toxin B, 180 µg/L (137-210) *vs* 8 µg/L (5-25);  $P < 0.0001$  for both toxins]. Thus, the severity of *C. difficile*-associated disease caused by NAP1/027 appears to be the result of hyper-production of toxins A and B. The dissemination of this strain across North America and Europe has led to dangerous changes in the epidemiology of *C. difficile*-associated disease.

A nationwide epidemiological study conducted in Korea has revealed that tcdA(-)tcdB(+) *C. difficile* strains already have spread extensively throughout the country. The use of enzyme immunoassays capable of detecting

TcdA and TcdB is strongly recommended for the diagnosis of CDAD in microbiology laboratories, in order to control the spread of the tcdA(-)tcdB(+) strains of *C. difficile*<sup>[36]</sup>. Sixty to 80% of *C. difficile* isolates in Korea have been reported to be toxigenic. Endoscopy, performed on 55/106 patients, revealed 29 with pseudomembranous colitis (PMC), five with colitis, 14 with other colon diseases, and seven normal colons. Among the 29 PMC cases, 21 (72.4%) were associated with tcdA-tcdB + strains ( $P = 0.0016$ ). These results reveal the emergence of tcdA-tcdB+ *C. difficile* strains in Korea, and these variant strains could evoke a higher rate of PMC than tcdA + tcdB + strains<sup>[37]</sup>.

### Toxin damage

*C. difficile* toxin A elicits intestinal fluid secretion and neutrophil infiltration by both mast cell-dependent and -independent pathways, and substance P participates in both pathways<sup>[38]</sup>.

Extensive mitochondrial damage occurs within 15 min in cells exposed to toxin A. Diminished ATP concentrations and increased oxygen radicals contribute to cytotoxicity from this bacterial toxin<sup>[39]</sup>.

The toxins damage the tight junctions of the intestinal epithelium. Tight junctions are crucial determinants of barrier function in intestinal epithelia, and are regulated by Rho guanosine triphosphatase. Rho kinase (ROCK) is a downstream effector of Rho. ROCK inhibition in calcium switch assays has shown that ROCK is necessary for the assembly of tight and adherens junctions. ROCK also is critical for assembly of apical junctional proteins and F-actin cytoskeleton organization during junctional formation<sup>[40]</sup>.

### *C. difficile* toxicity and the immune response processes

*C. difficile* toxins A and B are glucosyltransferases, which catalyze the inactivation of Rho proteins. *C. difficile* toxins act *via* translocation into target cells, and do their damage through autocatalytic processes by inactivating low-molecular-mass GTP-binding proteins of the Rho GTPase family involved in cellular signaling. This leads to cytotoxicity, including depolymerization of the target cell's actin cytoskeleton. Thus, these toxins glycosylate members of the Rho GTPase family, and this GTPase inactivation leads to depolymerization of the cell's actin cytoskeleton and, ultimately, cell death<sup>[41]</sup>. In addition, the *C. difficile* toxins further damage the intestine's target cells by initiating massive cellular immune responses; i.e. neutrophilic infiltration with up-regulation and release of cytokines, such as interleukin (IL)-8, IL-6, IL-1β, leukotrienes B<sub>4</sub> and interferon-γ.

Part of the mammalian immune response falls to the innate immune system called defensins and, specifically, human α-defensins produced by leukocytes, mucosal epithelial cells, and skin. Defensins, one of evolution's major groups of antibiotic peptides, have broad-spectrum antibiotic activity against Gram-positive and Gram-negative bacteria, fungi, and viruses<sup>[42-44]</sup>. Defensins are characterized by a conserved 6-cysteine array. Each cysteine has intra-molecular disulfide bonds

that are essential to protection against proteolysis<sup>[45]</sup>. Defensins also are known to contribute to wound healing, chemotaxis, and cytokine function<sup>[46,47]</sup>. Defensins are part of two major groups of antimicrobial peptides: defensins and cathelicidins. These groups of human defensins consist in part of alpha, beta and omega defensins, human neutrophil protein (HNP)-1, HNP-3, cathelicidin LL-37 and enteric human defensin (HD)-5. These peptides play a role in the innate immune response, by deactivating various microbial pathogens, as well as specific bacterial exotoxins.

The antibiotic activity of both HNPs and HD5 is well documented in host defenses against enteric pathogens<sup>[48,49]</sup>. HD5 and HD6 are produced and stored in Paneth cell secretory granules<sup>[50]</sup>, along with a variety of additional Paneth cell products demonstrated to have antimicrobial and immune activity<sup>[51-55]</sup>. The impact of defensins on *C. difficile* disease has been described by Giesemann *et al*<sup>[56]</sup> and others<sup>[57-60]</sup>.

Giesemann *et al*<sup>[56]</sup> have studied the effects of  $\alpha$ -defensin HNP-1, HNP-3, and enteric HD-5 on the activity of *C. difficile* toxins A and B. They found that the treatment of cells with human  $\alpha$ -defensins caused a loss of cytotoxicity of toxin B, but not of toxin A. In this study, only  $\alpha$ -defensins, but not  $\beta$ -defensin-1 or cathelicidin LL-37, inhibited toxin B-catalyzed *in vitro* glucosylation of Rho GTPases in a competitive manner. This indicates that human  $\alpha$ -defensins interact with high affinity for *C. difficile* toxin B. Defensins thereby provide a defense mechanism against clostridial glucosylating cytotoxins. At high concentrations, defensins (HNP-1  $\geq 2$   $\mu$ mol/L) also cause high-molecular-mass aggregates of *C. difficile* toxins, thus further decreasing their toxic effects on target cells.

*C. difficile* has been found in approximately 3% of normal adults and up to 40% of hospitalized patients<sup>[7]</sup>. However, as Salzman emphasizes: "only about one third of patients harboring *C. difficile* develop colitis, whereas the rest remain asymptomatic<sup>[61]</sup>". Giesemann *et al*<sup>[56]</sup> have shown that  $\alpha$ -defensins inhibit *C. difficile* toxin B, which offers insight into the possibility of different inflammatory responses in patients who develop CDAC *versus* others who do not. Salzman feels that " $\alpha$ -defensins show an additional antitoxin activity, in which HD5 is more effective; i.e. the stimulation of toxin aggregation." Giesemann has shown that HD5, used at concentrations that normally can be found in the small intestine, is effective at causing aggregation of toxin B, thus effectively preventing the toxin's ability to enter cells and interact with its target. These findings suggest an additional mechanism of antitoxin activity by  $\alpha$ -defensin HD5.

This ability of HD5 to cause toxin B aggregation may provide an explanation for both the asymptomatic carriage of this pathogen and the frequency of patient relapse following antibiotic treatment, especially if the small intestine is a reservoir for *C. difficile* carriage in the gut. Salzman postulated that *C. difficile* is able to maintain colonization of the small intestine, but unable to cause colitis, because the high concentration of HD5 at this site neutralizes the secreted exotoxin.

In summary, Salzman feels that, in the small intestine, high concentrations of HD5 result in toxin B aggregation and therefore, the prevention of intoxication. While, in the large intestine, inadequate amounts of  $\alpha$ -defensin are present to aggregate or inhibit toxin B, resulting in epithelial intoxication, inflammation, and neutrophilic infiltration.

Usually, *C. difficile* that transits through the large bowel will be prevented from finding a niche by the normal colonic microbiota. Yet, if the microbial ecology of the colon is disrupted, perhaps through antibiotic treatment, *C. difficile* can colonize the large intestine. Salzman postulates that, under these conditions, HD5 concentration is reduced by diffusion and dilution; thus, *C. difficile* exotoxins become free to interact with colonic enterocytes, resulting in intoxication, inflammatory responses, and infectious colitis.

### The carrier state

Many patients are colonized with *C. difficile*, but have no symptoms. Perhaps *C. difficile* is harbored in the small intestine, where its toxic effects are well neutralized. Lawrence has claimed that about 20% of hospitalized adults are *C. difficile* carriers; and, in LTCFs, the carriage rate may approach 50%<sup>[62]</sup>. Although asymptomatic, these individuals shed pathogenic organisms and serve as a reservoir for environmental contamination. About 3% of healthy adults and 20%-40% of hospitalized patients are colonized with *C. difficile*, which in healthy persons is metabolically inactive in the spore form. Many patients have *C. difficile* as an asymptomatic organism in their intestine on hospital admission, and it only becomes a problem after they are treated with antibiotics, if, in fact, it ever induces symptoms. Exposure to antibiotics that disrupt the colonic microbial flora appears to be the most important risk factor for CDAD.

### Treatment of asymptomatic carriers

Asymptomatic colonized patients can act as a reservoir for the transmission of CDAD. Data, however, are limited regarding whether the treatment of these asymptomatic carriers leads to a decrease in the nosocomial transmission of *C. difficile*. Thirty asymptomatic *C. difficile* carriers were randomly assigned to one of three treatment groups: oral vancomycin 125 mg four times daily; metronidazole 500 mg orally twice daily; or placebo. Johnson *et al*<sup>[63]</sup> have found that nine of 10 patients receiving vancomycin became culture-negative during and immediately after treatment, compared to three of 10 receiving metronidazole and two of 10 receiving placebo. However, this decolonization was transient, as most patients became re-colonized within weeks. Thus, metronidazole does not appear to be effective for the treatment of asymptomatic carriers. In the setting of a hospital outbreak in which temporary elimination of the organism is felt necessary to reduce horizontal transmission, vancomycin may be a useful tool<sup>[63]</sup>.

Riggs *et al*<sup>[64]</sup> have reported on molecular typing of *C. difficile* performed on asymptomatic carriers using pulsed-field gel electrophoresis. They found that 35 (51%)



of 68 asymptomatic patients were carriers of toxigenic *C. difficile*, and 13 (37%) of these patients carried epidemic strains. They have also reported that 87% of isolates found in skin samples and 58% of isolates found in environmental samples were identical to concurrent isolates found in stool samples. Spores on the skin of asymptomatic patients were transferred easily to the investigators' hands, again accounting for spread to persons in contact. This might be an explanation for the McFarland *et al*<sup>[65]</sup> observation that nosocomial CDAD frequently is transmitted between hospitalized patients, and that the organism often is present on the hands of hospital personnel caring for such patients. Kyne *et al*<sup>[66]</sup> have studied prospectively *C. difficile* infections in hospitalized patients who were receiving antibiotics, and identified no evidence of immune protection against repeat colonization by *C. difficile*. However, after colonization, there is an association between a systemic anamnestic response to toxin A, as demonstrated by increased serum levels of IgG antibody against toxin A, and asymptomatic carriage of *C. difficile*.

## PRESENTATION OF *C. DIFFICILE* INFECTION

*C. difficile* infection causes diarrhea, often watery, rather than bloody, and it generally develops within 48-72 h of infection. In some, the symptoms may be delayed for 2-3 mo, usually after an antimicrobial agent has been administered. In some, only a single antibiotic tablet may lead to severe disease. Over time, the clinical spectrum has become better appreciated, with illness severity noted to be broad-ranging, from an asymptomatic carrier state (without detectable toxin) to severe and life-threatening pseudomembranous colitis with toxic megacolon<sup>[67]</sup>.

The clinician must be ever on the alert to make an early diagnosis of *C. difficile*-related disease in the setting of new-onset loose stools or symptoms of abdominal distension and/or leukocytosis, since unexplained leukocytosis in hospitalized patients, even in the absence of diarrhea, may reflect underlying *C. difficile* infection<sup>[68]</sup>. In a prospective study, Bulusu *et al*<sup>[69]</sup> found that, of 60 patients with unexplained leukocytosis (with a white blood cell count > 15000/ $\mu$ L), a positive stool *C. difficile* toxin was observed more frequently in cases than in controls (58% *versus* 12%, respectively). Age over 75 years and immunosuppression were associated with a poor outcome. Earlier surgical consultation is warranted in severe cases to consider potentially life-saving colectomy, as well as alterations in the hospital-based standard of care for prevention.

Usually, the disease affects the colon and, in many cases, is made evident by the presence of colonic pseudomembranes. However, in patients with underlying Crohn's or ulcerative colitis, pseudomembranous changes may not occur; therefore, typical endoscopic findings of *C. difficile* may not be present, and the colonic mucosa will reflect only the underlying inflammatory bowel disease.

*C. difficile* infection may present with an acute abdomen but either absent or mild diarrhea, as described by

Triadafilopoulos and Hallstone<sup>[70]</sup> in 1991. Plain abdominal radiographs revealed megacolon in these patients. This was combined with small and large bowel dilation in one who exhibited a volvulus-like pattern, and isolated small-bowel ileus in another. Diagnosis was revealed by emergency colonoscopy. All patients had positive results for *C. difficile*, and two tested positive for cytotoxicity. All were treated with IV metronidazole, resulting in the resolution of all symptoms and abdominal findings.

An unusual manifestation of CDAC was described in 1981 by Dansinger *et al*<sup>[71]</sup>. They reported that up to half of patients with indolent *C. difficile* infection develop manifestations of protein-losing enteropathy, including ascites, peripheral edema, and hypoalbuminemia. Inflammation of the bowel may allow leakage of albumin into the lumen, causing colonic loss of albumin with inadequate compensatory hepatic synthesis. As a result, serum albumin levels may drop below 20 g/L (20 g/L)<sup>[71,72]</sup>. Older patients may present with pedal edema, and be mistakenly diagnosed with CHF.

Rubin *et al*<sup>[73]</sup> studied patients who had developed a more aggressive form of CDAD *versus* those who developed milder disease. They found that 21 of 710 patients (3%) either required intensive care unit (ICU) admission or died as a result of their infection. The factors predisposing to the development of severe *C. difficile* colitis included concurrent malignancy, chronic obstructive pulmonary disease, immunosuppressive or anti-peristaltic medications, renal failure, and the administration of clindamycin ( $P < 0.05$  for all). Patients with severe *C. difficile* colitis were more likely to have abdominal pain, tenderness and distention, peritonitis, hemoconcentration (> 5 points), hypoalbuminemia (< 30 mg/L), and an elevated (> 25000) or suppressed (< 1500) white blood cell count ( $P < 0.05$  for all). Therefore, we must initiate aggressive diagnostic and therapeutic modalities in this patient group.

Extra-colonic features may occur in CDAD patients<sup>[74]</sup>. These include small bowel involvement in those patients with previous small bowel surgery, and visceral abscesses, primarily in the spleen, and less commonly in the pancreas. Other features include a reactive polyarticular arthritis, cellulitis, necrotizing fasciitis, osteomyelitis, and prosthetic device infections. Arthritis after *C. difficile* was further characterized by Birnbaum as being an asymmetric oligoarthritis<sup>[74]</sup>. *C. difficile* colitis has also been reported associated with intra abdominal hypertension and abdominal compartment syndrome<sup>[75]</sup>.

## POPULATIONS AT INCREASED RISK

In 2004 and 2005, the CDC emphasized that the risk of CDAD is increased in certain susceptible populations (Table 1).

### Drug exposure

Although the antibiotics most frequently implicated in predisposition to *C. difficile* infection are fluoroquinolones, clindamycin, cephalosporins and penicillins, virtually all

**Table 1** Populations at increased risk for *C. difficile*

Patients who take the following drugs
Antibiotics
Proton pump inhibitors
Valacyclovir
Patient characteristics
IBD
Serous underlying illness-comorbidities
Gastrointestinal surgery/manipulations
Advanced age
Immune-compromising conditions (post transplantation)
Peri-partum
Environment
Prolonged stay in health-care settings
Laboratory factors
Hypoalbuminemia
Low levels of anti-toxin and B antibodies

antibiotics, including metronidazole and vancomycin, can predispose to *C. difficile*. De Andrés *et al*<sup>[76]</sup> reported a case of *C. difficile* colitis associated with valacyclovir treatment.

The risk of CDAD in hospitalized patients receiving antibiotics may be compounded by co-existing disorders that require treatment with PPI therapy, which inhibits one's defenses against ingested bacteria by virtually eliminating gastric acid<sup>[77]</sup>. Dial *et al*<sup>[78]</sup> estimated an adjusted risk ratio for *C. difficile*-associated disease with the current use of PPIs as 2.9 (95% CI: 2.4-3.4); and with H2-receptor antagonists, the rate ratio was 2.0 (95% CI: 1.6-2.7). The authors also uncovered an elevated rate of CDAD in patients on non-steroidal anti-inflammatory drugs (rate ratio, 1.3; 95% CI: 1.2-1.5). Thus, the consumption of drugs other than antibiotics may put one at increased risk for community-acquired *C. difficile*.

PPI therapy is also associated with an increased risk of recurrent *C. difficile* colitis. Patients receiving PPIs have been found to be 4.17 times as likely to have recurrence as their counterparts not receiving them<sup>[79]</sup>. This relationship between PPI therapy and *C. difficile* was elucidated by Jump who found that the survival of vegetative *C. difficile* in gastric contents obtained from patients receiving PPIs was also increased at a pH of > 5<sup>[80]</sup>.

### Peripartum

The incidence of severe CDAD is increasing in peripartum women. A PubMed search identified 24 recorded cases of peripartum *C. difficile* infection. Most patients (91%) had received prophylactic antibiotics during delivery or for treatment of bacterial infections. Two cases without known risk factors were found, by polymerase chain reaction analysis, to be infected with an epidemic and hypervirulent *C. difficile* strain. These cases demonstrate the need for clinicians to consider *C. difficile* infection in pregnant and peripartum patients with diarrhea, even if they do not have the traditional risk factors for *C. difficile* infection, such as antibiotic use or concurrent hospitalizations<sup>[81]</sup>.

### Co-morbidities

The Agency for Healthcare Research and Quality

(AHRQ) is the lead US Federal agency charged with improving the quality, safety, efficiency, and effectiveness of health care. AHRQ data make clear that one of the challenges in accurately diagnosing CDAD is that it is not unusual for patients who acquire *C. difficile* to have multiple co-morbidities. Thus, multiple co-morbidities put patients at risk for *C. difficile* infection. AHRQ found that hospitalized patients with CDAD had over 10 diagnoses, *versus* six diagnoses among patients without CDAD<sup>[82]</sup>. According to recent AHRQ data, four out of the top 20 most common principle diagnoses observed with CDAD are infections (sepsis, pneumonia, urinary tract infection, and skin infection), where antibiotic use would be difficult to avoid<sup>[82]</sup>.

### Post-transplantation patients

Sixteen patients, representing an incidence rate of 0.16%, developed a *C. difficile* infection after total joint arthroplasty (TJA) at one institution. Those at risk for developing CDAD after TJA were patients with deteriorated physical status and those who had received more than one antibiotic postoperatively<sup>[83]</sup>.

In addition, *C. difficile* is now considered to be a significant cause of diarrhea in heart transplant recipients, and the post-transplantation period is now considered one of greater risk<sup>[84]</sup>. With *C. difficile* infection, CDAC prior to 2000 was a rare complication in this patient group; but 38 of the 43 reported cases of CDAC in these patients occurred after 2000. Therefore, *C. difficile* is now also one of the most common causes of diarrhea in patients who have undergone solid organ transplantation<sup>[85]</sup>. Another group of patients at increased risk are post orthotopic liver transplant patients. Testing for *C. difficile* toxins among orthotopic liver transplant patients with nosocomial diarrhea revealed that 63% of samples are toxin-positive<sup>[86]</sup>.

The development of life-threatening toxic megacolon secondary to CDAC now must be considered in solid organ recipients. Toxic megacolon was reported in five patients by Stelzmueller *et al*<sup>[85]</sup>.

### Post-surgery

The risk of *C. difficile* infection was 14.9 cases per 1000 surgical procedures among patients who received preoperative prophylaxis (PAP) during the period 2003-2005, which is a significant increase compared with 0.7 cases per 1000 surgical procedures during the period 1999-2002 ( $P < 0.001$ ). Independent risk factors associated with *C. difficile* infection in patients given PAP alone, were older age, the administration of cefoxitin (rather than cefazolin) alone or in combination with another drug, and the year of surgery. Thus, in the context of a large epidemic of *C. difficile* infection associated with the emergence of a novel strain of organism, 1.5% of patients who had received PAP as their sole antibiotic treatment developed *C. difficile* infection. In situations in which the only purpose of PAP is to prevent infrequent and relatively benign infections, the risks of PAP may outweigh its benefits, especially in elderly patients<sup>[87]</sup>.

Unfortunately, the incidence of *C. difficile* infection is increasing in US surgical patients even without PAP, and infection with *C. difficile* is most prevalent after emergency operations and among patients who have undergone intestinal tract resections<sup>[88]</sup>.

### **IBD as a risk factor for CDAC**

IBD patients are at greater risk than the general population for acquiring *C. difficile* infection<sup>[89]</sup>. Issa *et al*<sup>[90]</sup> performed a retrospective, observational study in IBD patients to evaluate the impact of *C. difficile*. They found that the rate of *C. difficile* infection had increased from 1.8% of IBD patients in 2004 to 4.6% in 2005 ( $P < 0.01$ ). The proportion of IBD patients within the total number of *C. difficile* infections at their institution increased from 7% in 2004 to 16% in 2005 ( $P < 0.01$ ). In 2005, IBD colonic involvement was found in the vast majority (91%) of *C. difficile*-infected patients, a clear majority (76%) had contracted infection as an outpatient, and antibiotic exposure was identified in 61% of IBD patients with *C. difficile* infection. Over the period 2004-2005, more than half of the infected IBD patients required hospitalization, and 20% required colectomy. Univariate and multivariate analyses identified maintenance immunomodulator use and colonic involvement as independent risk factors for *C. difficile* infection in IBD. The authors also reported a nationwide doubling in the rate of *C. difficile* infection among hospitalized UC patients between 1998 and 2004. The pathologic/endoscopic features of pseudomembranous colitis CDAC varies as a spectrum, with some patients exhibiting only mild inflammatory changes confined to the superficial epithelium, and typical pseudomembranes and crypt abscesses may not be present. The more severe cases demonstrate marked mucin secretion, and more intense inflammation. Intense necrosis of the full thickness of the mucosa, with a confluent pseudo-membrane, can become more prominent as disease severity increases.

The association between IBD and *C. difficile* may be due to a variety of factors, including antibiotic use for treatment of other gastrointestinal pathogens and frequent hospitalizations for the management of IBD flares. Many of these patients are taking immunosuppressive medications that may confer additional risk of *C. difficile* infection. *C. difficile*, and specifically its toxins, have been implicated as a risk factor for the exacerbation of the inflammatory process in up to 5% of patients with ulcerative colitis or Crohn's disease. A severe clinical course may result from *C. difficile* infection superimposed on IBD, including the precipitation of toxic colitis and toxic megacolon.

CDAC in patients with IBD carries a higher mortality than in patients with *C. difficile* without underlying IBD. On multivariate analysis, patients in the *C. difficile*-IBD group had a four times greater mortality than patients admitted to hospital for IBD alone (AOR = 4.7, 95% CI: 2.9 to 7.9) or *C. difficile* alone (AOR = 2.2, 95% CI: 1.4 to 3.4), and stayed in the hospital for 3 d longer (95% CI: 2.3 to 3.7 d). Significantly higher mortality, endoscopy and surgery rates were found in patients with ulcerative colitis compared

with Crohn's disease ( $P < 0.05$ ) who had associated *C. difficile*<sup>[91]</sup>. The median times from admission to a positive *C. difficile* test result for non-IBD was much longer than in Crohn's disease and ulcerative colitis patients (4.0, 0.8, and 0.5 d, respectively). *C. difficile* infections in IBD are confirmed predominantly within 48 h of admission, suggesting most were acquired before hospitalization. CDAD rates approximately doubled in Crohn's disease (9.5 to 22.3/1000 admissions) and tripled in ulcerative colitis (18.4 to 57.6/1000). Length of stay was similar among the groups. For all years combined, the adjusted odds ratios for CDAD in all IBD, Crohn's disease, and ulcerative colitis admissions were 2.9 (95% CI: 2.1-4.1), 2.1 (1.3-3.4), and 4.0 (2.4-6.6), respectively<sup>[92]</sup>.

Patients with severe *C. difficile* infection, especially IBD patients, require prompt diagnosis and management, since failure to diagnose the infection can lead to inappropriate treatment with glucocorticoids or immunosuppressive therapy. Furthermore, *C. difficile* may be difficult to distinguish from an IBD relapse, given the similar symptoms of diarrhea, abdominal pain, and low-grade fever. Thus, a high index of suspicion is required when evaluating IBD patients with apparent flares, especially those who recently have received antibiotics and/or have been hospitalized.

Thus, speedy diagnosis largely requires the use of laboratory tools, since endoscopy may not be helpful early, because IBD patients may not develop pseudomembranes. Given the underlying colonic pathology, patients with IBD who develop *C. difficile* colitis frequently require colectomy (20 percent in one series)<sup>[90]</sup>.

## **CLINICAL DIAGNOSIS**

Delays in both diagnosing and treating both initial and recurrent CDAD<sup>[93]</sup> are due to the fact that CDAD can mimic the more common 'benign' antibiotic-associated diarrhea that is not caused by *C. difficile*<sup>[94]</sup>. Thus, the diarrhea from *C. difficile* will be ascribed to other causes; e.g. food poisoning, viral infection, or other causes. *Klebsiella pneumoniae*, *Candida* species and *Staphylococcus aureus* have been identified as potential causative organisms in *C. difficile* negative AAD patients<sup>[95]</sup>.

Patients can be infected with this microorganism and may have no symptoms of colitis. They, therefore, may not be tested for *C. difficile* infection (see section on presentation). These asymptomatic carriers, who are admitted to healthcare facilities and hospitals, become vectors during outbreaks and can transmit the organism to other susceptible patients. Most cases of CDAD occur at 4-9 d after discontinuation of antibiotic therapy, according to Schroeder<sup>[15]</sup>; however, CDAD can occur up to 8 wk after the discontinuation of antibiotics.

### **Sigmoidoscopy/colonoscopy for the diagnosis of CDAD**

Lower endoscopy is a useful tool for the diagnosis of *C. difficile*. This is especially when: (1) there is a high level of clinical suspicion for *C. difficile*, despite a negative laboratory assay; (2) prompt *C. difficile* diagnosis is needed before laboratory results can be obtained; (3) *C. difficile*

infection fails to respond to antibiotic therapy; or (4) when there is an atypical disease presentation, and *C. difficile* is suspected, as with ileus, acute abdomen, leukocytosis or diarrhea.

Endoscopy is not indicated in patients with classic clinical findings and a positive stool toxin assay. Conversely, endoscopy may be contra-indicated, especially in the setting of fulminant colitis, due to the risk of perforation.

**Endoscopic findings:** Pseudomembranes are pathognomonic for CDAC, but are not found in all areas of the colon, even in severe cases; thus, findings may be patchy. Pseudomembranes may be absent in the rectosigmoid area, but may be visualized more proximally with colonoscopy. This is true in patients with co-existing IBD. Pseudomembranes are raised yellow or off-white plaques, up to 2 cm in diameter, which are randomly scattered over the colorectal mucosa with normal intervening mucosa, and that cannot be removed by lavage. The pseudomembranes form when *C. difficile* toxin-induced cytoskeleton disruption causes shallow ulcerations on the intestinal mucosal surface. It is postulated that ulcer formation allows for the release of serum proteins, mucus, and inflammatory cells, which appear grossly on the colorectal mucosal surface as pseudomembranes. Light and scanning electron microscopy after exposure to either of the *C. difficile* toxins reveal patchy damage and exfoliation of superficial epithelial cells, while crypt epithelium remains intact. Fluorescent microscopy of phalloidin-stained sections shows that both toxins cause the disruption and condensation of cellular F-actin<sup>[96]</sup>.

Other colonic mucosal findings include bowel-wall edema, erythema, friability, and inflammation, with or without pseudomembranes. This manifests on the abdominal CT scan as thickening of the colonic wall.

Colonoscopic findings among 16 patients with histologically-proven antibiotic-associated PMC or CDAC were described by Seppälä *et al*<sup>[97]</sup>. Pseudomembranes were found in only five of 16 (31%) patients by sigmoidoscopy, but were found in 11 of 13 patients (85%) in whom colonoscopy also was performed. These findings suggest the importance of colonoscopy in the early diagnosis of CDAC, because the typical endoscopic changes of pseudomembranes are limited to the colon above the rectosigmoid area in most patients. Consequently, colonoscopy should be performed, instead of sigmoidoscopy, at least in clinically suspected CDAC cases<sup>[98]</sup>.

### Complications of *C. difficile* colitis

*C. difficile* colitis is usually associated with a mild/moderate course, but may progress to fulminant colitis. Fulminant colitis develops in 3%-8% of patients. The manifestations of fulminant colitis typically include severe lower quadrant or diffuse abdominal pain, diarrhea, abdominal distention, fever, hypovolemia, lactic acidosis, and marked leukocytosis (up to 40 000 white blood cells/microL or higher). Diarrhea may be

less prominent in patients with prolonged ileus, due to pooling of secretions in the dilated, atonic colon. Other potential complications of fulminant colitis include toxic megacolon and bowel perforation<sup>[73]</sup>.

Toxic megacolon is a clinical diagnosis based upon the finding of colonic dilatation (> 7 cm in its greatest diameter) accompanied by severe systemic toxicity. Abdominal plain films also may demonstrate small-bowel dilatation, air-fluid levels (mimicking an intestinal obstruction or ischemia), and 'thumb printing' (scalloping of the bowel wall) due to submucosal edema. Toxic megacolon may be complicated by bowel perforation.

This latter complication presents with abdominal rigidity, involuntary guarding, diminished bowel sounds, rebound tenderness, and severe localized tenderness in the left or right lower quadrants. Abdominal radiographs may demonstrate free intra-abdominal air. Thus, patients with toxic megacolon must be followed with daily upright abdominal X-rays to ascertain if perforation has occurred. Patients with toxic megacolon should be evaluated for surgical resection. Once fulminant colitis is diagnosed, subtotal colectomy with ileostomy usually is required. In these patients who develop a marked leukocytosis or bandemia, surgery is advisable, because the leukocytosis frequently precedes hypotension. The requirement for vasopressor therapy carries a poor prognosis, according to Shen *et al*<sup>[99]</sup>.

Lamontagne *et al*<sup>[100]</sup> has documented that emergency colectomy reduces mortality in patients with fulminant CDAD. The independent predictors of 30-d mortality in their study were leukocytosis  $\geq 50 \times 10^9/L$  (AOR, 18.6; 95% CI: 3.7-94.7); serum lactate  $\geq 5$  mmol/L (AOR, 12.4; 95% CI: 2.4-63.7); age  $\geq 75$  years (AOR, 6.5; 95% CI: 1.7-24.3); immunosuppression (AOR, 7.9; 95% CI: 2.3-27.2); and shock requiring vasopressor therapy (AOR, 3.4; 95% CI: 1.3-8.7). After adjusting for these confounders, patients who had an emergency colectomy were less likely to die than those treated medically. Colectomy also seemed more beneficial in patients 65 years or older; in those who were immune-competent; and those with leukocytosis  $\geq 20 \times 10^9/L$  or a serum lactate level between 2.2 and 4.9 mmol/L.

Small-bowel involvement with *C. difficile* enteritis is unusual<sup>[101]</sup>. Potential risk factors for small-bowel involvement with *C. difficile* enteritis include prior gastrointestinal surgery (including colonic resection) and advanced age<sup>[102]</sup>. Such patients may present with ileitis and high ileostomy output and may be at increased risk for fulminant disease.

Small-bowel involvement with *C. difficile* infection enteritis has been described increasingly since 2000. Usually, this occurs in patients with a history of a prior colectomy or total procto-colectomy for severe and extensive IBD. The ileal mucosa appears to be at increased risk for inflammatory disease in the specific subset of patients who have undergone a prior colectomy<sup>[67]</sup>. Serious post-colectomy concerns, like severe ileostomy dysfunction with high ileostomy volumes and marked diarrhea, have been known to occur after pan-procto-



colectomy and restorative ileo-anal pouch formation. They are almost always due to a non-*C. difficile* enteritis. This non-CDAD post colectomy enteritis can be life threatening; fortunately it is steroid/ immunosuppressive responsive, according to Gooding *et al*<sup>[103]</sup>. This picture can be mimicked by *C. difficile* infection.

Lundeen *et al*<sup>[104]</sup> reported that high ileostomy volumes may result from *C. difficile* enteritis in patients who have undergone colectomy for ulcerative colitis. All of the ileostomy output was positive for *C. difficile* toxins. These patients responded to metronidazole and/or vancomycin, in contrast to subjects with the former, non-CDAD entity.

Refractory or treatment-resistant pouchitis also may occur with *C. difficile* infection<sup>[105]</sup>. *C. difficile* infection involving ileal pouch-anal anastomosis is common, and occurs with or without the previous receipt of antibiotics<sup>[99]</sup>. Diagnosing recurrent *C. difficile* infection can be difficult in this group of patients, especially in the 20% without diarrhea.

### Laboratory confirmation

All health care facilities must develop rapid communication between the laboratory and the treating physician. At the Mayo Medical School, the time between electronic medical record reporting of a positive result for a test for *C. difficile* toxin in stool and the ordering of antimicrobial therapy was compared during consecutive periods when results were not telephoned ( $n = 274$ ) and when results were telephoned ( $n = 90$ ) to the clinical service<sup>[106]</sup>. The mean times to the ordering of antimicrobial therapy were 11.9 and 3.6 h, respectively ( $P < 0.001$ ). The clinical implications of this 8-h delay may be important, especially in patients with severe disease. Early recognition of CDAD caused by NAP1/027, followed by the initiation of rapid treatment, can help to prevent complications and further spread of the bacterium<sup>[107]</sup>.

Current laboratory testing lacks a single assay that is sensitive, specific, and rapid. Peterson *et al*<sup>[108]</sup> used clinical criteria that required at least three loose stools in one day, as part of the reference standard for a positive test result supporting CDAD (Table 2). They found that real-time PCR and anaerobic culture assays were significantly more sensitive than the enzyme immunoassay ( $P < 0.01$  to  $P < 0.05$ ). Real-time PCR has an assay turnaround time of  $< 4$  h, and is both more sensitive than, and as rapid as enzyme immunoassay. They feel that it is a feasible laboratory option to replace enzyme immunoassay for toxigenic *C. difficile* detection in clinical practice, as well as for use during the development of new therapeutic agents.

Tests for the presence of *C. difficile* and its toxins are imperfect, and false positives and false negatives are not uncommon. McFarland<sup>[30]</sup> found that false-negative results occur in 29%-56% of cases. False-negative results may occur when specimens are not promptly tested or not kept refrigerated until testing is performed. Also, there is a relatively high false-negative rate, due to the fact that 100-1000 pg of toxin must be present for an EIA test to be positive. Utilizing up to three serial EIA tests may increase the diagnostic yield by as much

Table 2 Laboratory diagnosis of *C. difficile*

Test	Sensitivity (%)	Specificity (%)	PPV	NPV
Enzyme immunoassay	73	98	73	98
Real-time PCR	93	97	76	99
Cell culture assay	77	97	70	98
Anaerobic culture for toxigenic <i>C. difficile</i> strains	100	96	68	100

Peterson LR, Manson RU, Paule SM, Hacek DM, Robicsek A, Thomson RB Jr, Kaul KL. Detection of toxigenic *Clostridium difficile* in stool samples by real-time polymerase chain reaction for the diagnosis of *C. difficile*-associated diarrhea. *Clin Infect Dis* 2007; 45(9): 1152-1160.

Table 3 Diagnosis of *C. difficile*

Enzyme immunoassay for toxins A & B - 80% sensitive Use 3 samples Cytotoxicity assay-more sensitive and specific, but takes 24-48 h
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as 10 percent, if the initial test is negative. If CDAD is suspected, despite negative initial testing, submission of multiple specimens and verifying that the laboratory is testing for both the A and B toxins is mandatory (Table 3).

Enzyme immunoassays are labor-intensive tests, requiring several hours of technician time and an assay reader. The batching of specimens increases cost efficiency, but may delay the reporting of results, especially if tests are not done every day. Rapid enzyme immunoassay is more costly for each test performed but, for laboratories that process only occasional samples, it appears to provide prompt, reliable, and cost-effective results.

Enzyme immunoassay rapid cards have been evaluated, in terms of their ability to detect *C. difficile* toxins A and B. For one such card, the EIAPrem, the positive predictive value (PPV) was 75/85 samples (88.2%; CI: 79% to 94%) and the negative predictive value (NPV) was 360/361 samples (99.7%; CI: 98% to 99%). For a review of all card performances, see references<sup>[109-112]</sup>.

Killgore *et al*<sup>[113]</sup> compared the results of analyses done with seven *C. difficile* typing techniques: multi-locus variable-number tandem-repeat analysis (MLVA); amplified fragment length polymorphism; surface layer protein A gene sequence typing; PCR-ribotyping; restriction endonuclease analysis (REA); multi-locus sequence typing; and pulsed-field gel electrophoresis (PFGE). All techniques appeared to be capable of detecting outbreak strains; but only REA and MLVA exhibited sufficient discrimination to distinguish strains from different outbreaks.

### Rapid laboratory tests

Comparison of four enzyme immunoassays (Bartels Prima System *C. difficile* Toxin A EIA, Cambridge Biotech Cytoclone A+B EIA, Meridian Diagnostics Premier *C. difficile* Toxin A EIA, and TechLab *C. difficile* Tox-A Test EIA) found that, although enzyme immunoassays

were less sensitive than cytotoxin assay, they provide same-day results and may be useful in laboratories without tissue culture facilities<sup>[114]</sup>.

ELISA Toxin A+B is a reliable method with 100% specificity and sensitivity in the rapid diagnosis of *C. difficile*. Its results can be utilized until culture results are obtained. The specificity of the Toxin A latex test is 100%; however, its use alone as a primary rapid diagnostic test is not recommended, because of its low (30.7%) sensitivity. This was shown when all of the culture positive samples underwent testing by ELISA Toxin A+B method and were found to be 100% positive, but only four of these positive culture samples (30.7%) yielded positive results with the Toxin A latex test<sup>[115]</sup>.

Overall, the new-generation assays still are less sensitive than the cytotoxin assay; however, their advantages are that they provide same-day results; they can be used as a screening test; and they may be useful in laboratories without tissue-culture facilities. Results from a study by Vanpoucke *et al*<sup>[111]</sup> could not recommend one single assay over the other for the diagnosis of CDAD.

Therefore, the cytotoxin assay test (CYTA) is highly sensitive and specific, but it is difficult to perform, and results are not available for 24-48 h<sup>[15]</sup>. What further complicates efforts to determine if toxin was present on admission is that *C. difficile* toxin is very unstable. The toxin degrades at room temperature and may be undetectable within 2 h after collection of a stool specimen. Given the cost and complexity of culture and cytotoxicity assays, most laboratories rely on tests for toxin A detection only. Moreover, enzyme immunoassays generally are available at lower cost and provide more rapid results, usually within 4 h. Their sensitivity generally ranges from 60% to 90%, and specificity from 75% to 100%. Testing of a single diarrheal stool generally is sufficient to make the diagnosis of CDAD; but unfortunately, doing so misses a substantial proportion of cases. Therefore, testing only should be performed on three loose stool specimens.

The cytotoxin assay test, though the 'gold standard' for assaying *C. difficile* toxins A and B, is labor-intensive, requires tissue-cultured cells and an inverted microscope, and needs overnight incubation before results can be read.

## TREATMENT OF THE NEW VIRULENT STRAIN OF CDAD

Recent experience has not altered the principles of management for the individual patient, but it does serve to emphasize the need for: (1) recognition of clinical characteristics that indicate severe CDAD (Table 4); (2) early recognition of *C. difficile*; (3) improved methods to manage severe relapsing disease; and (4) greater attention to infection control and antibiotic restraint. Previously published *C. difficile* infection management is available: [Fekety "Guidelines for the diagnosis and management of *Clostridium difficile*-associated diarrhea and colitis" *Am J Gastroenterol*, 1997; 92(5): 739-750] and the CDC's own

Table 4 CDAD severe disease

Patient characteristics
Older patients (> 65 yr)
Presence of comorbid conditions
Immune compromising conditions
Systemic immune response syndrome
Organ failure
Renal
Respiratory
Hypotension
Laboratory markers
Marked leukocytosis > 15 000
Renal failure Cr > 2.3 mg/L
Hypoalbuminemia
Extent of disease
Pancolitis by imaging modalities
Complications
Ileus
Toxic megacolon
Intestinal perforation

Any one of the above calls for classification as 'severe disease', using the authors' approach.

guidelines found at [http://www.cdc.gov/ncidod/dhqp/id\\_CdiffFAQ\\_HCP.html](http://www.cdc.gov/ncidod/dhqp/id_CdiffFAQ_HCP.html) and at <http://www.cdc.gov/ncidod/dhqp/pdf/isolation2007.pdf>.

The efficacy of metronidazole or vancomycin prophylaxis to prevent *C. difficile* infection in patients who are receiving other antimicrobials is unproven, and treatment with these agents is ineffective against *C. difficile* in asymptomatic carriers<sup>[116]</sup>.

The usual treatment for *C. difficile*-associated disease has been to stop antibiotics being given for other purposes and immediately start treatment with metronidazole or vancomycin. Patients who remain on antibiotics while undergoing treatment of CDAD have a high likelihood of treatment failure with metronidazole<sup>[117]</sup>.

In 1983, before the virulent *C. difficile* epidemics, metronidazole and vancomycin were shown to have equivalent efficacy and relapse rates, and to be tolerated to a similar extent by patients with *C. difficile*-related diarrhea and colitis, but metronidazole was considerably more economical. Metronidazole was favored because the pharmacy cost for the dosage used was \$387.48 to \$520.00 for vancomycin and \$11.84 for metronidazole<sup>[118]</sup>.

Findings from another study suggest that metronidazole and vancomycin are equally effective for the treatment of mild CDAD, but that vancomycin is superior for treating patients with severe CDAD. Among the patients with mild CDAD, treatment with metronidazole or vancomycin resulted in clinical cure in 90% and 98% of the patients, respectively ( $P = 0.36$ ). On the other hand, among the patients with severe CDAD, treatment with metronidazole or vancomycin resulted in clinical cure in 76% and 97% of the patients, respectively ( $P = 0.02$ ). Clinical symptoms recurred in 15% of the patients treated with metronidazole and 14% of those treated with vancomycin<sup>[119]</sup>.

In order to reduce vancomycin resistance, current guidelines still recommend the first-line use of

metronidazole over vancomycin. However, the new strain of *C. difficile* may not respond as well to treatment with metronidazole, despite the absence of laboratory evidence of metronidazole resistance.

Comparison of the clinical and microbiological effects of vancomycin and metronidazole reveal that vancomycin-treated patients are more likely to develop undetectable levels of *C. difficile* (adjusted hazard ratio, 3.99; 95% CI: 1.41-11.3;  $P = 0.009$ ) and to have resolution of diarrhea (adjusted hazard ratio, 4.17; 95% CI: 1.53-11.40;  $P = 0.005$ ) during the first 5 d of therapy<sup>[120]</sup>.

Recent studies demonstrate a high rate of failure of metronidazole, due either to infection with NAP-1 or to the presence, in hospitals, of older and sicker adults who previously have been treated with many broad-spectrum antibiotics. This raises the question as to what drug should be used as the initial therapy of *C. difficile* infection. The standard of care seems to be shifting towards using vancomycin first, if one is facing either a virulent organism or if risk factors for severe disease or several risk factors are present, like advanced age, immune deficiency, or pre-existing IBD (Table 4).

In addition, the cure rate seems to be significantly higher with vancomycin than metronidazole (97% *versus* 76%). In clinical practice, there is a shift toward using oral vancomycin as initial therapy for severe CDAD; and some clinicians are endorsing vancomycin as the preferred therapy for moderate to severe disease caused by this new epidemic strain. Currently, the treatment for hypervirulent *C. difficile* strains appears to be no different than for other *C. difficile* infections, and includes oral vancomycin<sup>[121]</sup>.

Failure with metronidazole treatment may be attributable to a slower and less consistent microbiological response than that with oral the next sentence is deleted because it is repeated exactly from a previous paragraph. Vancomycin-treated patients are more likely to develop undetectable levels of *C. difficile* (adjusted hazard ratio, 3.99; 95% CI: 1.41-11.3;  $P = 0.009$ ) and to have resolution of diarrhea (adjusted hazard ratio, 4.17; 95% CI: 1.53-11.40;  $P = 0.005$ ) during the first 5 d of therapy<sup>[120]</sup>.

Freeman *et al.*<sup>[122]</sup> found that duration of cytotoxin production by *C. difficile* ribotype 027 markedly exceeds that of ribotype 001. These findings may help to explain the increased severity of symptoms and higher case-fatality ratio associated with infections with *C. difficile* ribotype 027. The authors also found that sub-optimal gut concentrations of metronidazole, possibly due to inactivation by components of normal gut flora, are associated with continued toxin production. The persistence of *C. difficile* spores suggests that additional strategies to restore the normal colonic microflora also may be beneficial<sup>[123]</sup>. However we must take this paradigm change from metronidazole to vancomycin as initial therapy with caution. Pépin *et al.*<sup>[17,18]</sup> reported a large epidemic of CDAD in Quebec that included large numbers of patients with severe and complicated disease. They examined the relative efficacy of metronidazole and vancomycin in the wake of this hypervirulent strain. Pépin *et al.*<sup>[17,18]</sup> described a greater incidence of severe

**Table 5** Therapeutic approach to patients with severe *C. difficile* infection

Oral vancomycin, 500 mg <i>q.i.d</i>
Substitute intracolonic vancomycin infusion if ileus and add metronidazole 500 mg <i>q.i.d.</i> , IV
Consider IV immunoglobulin therapy (400 mg/kg)
Surgical evaluation for acute abdomen

complications associated with CDAD (defined as 30-d mortality, sepsis, toxic megacolon, emergent colectomy, or intestinal perforation) with the coincident emergence of NAP1/027 in Quebec in 2003. They observed an overall 79% decrease in progression to severe complicated CDAD in patients initially treated with vancomycin, rather than metronidazole, between 1991 and 2003. They also noted that marked leukocytosis or renal failure predicted a significant risk of complications and mortality. In 2004, this led to a change in guidelines in Quebec, which recommended that oral vancomycin be used as initial treatment in patients with these markers of severity. In some cases, rectal vancomycin (0.5-1 g dissolved in 1-2 L of isotonic saline) can be given as a single 60-min retention enema every 4-12 h. Rifaximin administered as a 'chaser', after control of acute *C. difficile* infection with a standard 10-14-d course of vancomycin, appeared to prevent recurrence in seven of eight patients, even though they were rifaximin resistant<sup>[124]</sup>.

An albumin level < 2.5 g/L and ICU stay are predictors of failure of metronidazole therapy for CDAD. These patients may benefit from oral vancomycin therapy at the outset<sup>[125]</sup>.

Regardless of what therapy is used, patients should be monitored carefully to ensure that they are responding to therapy, and not developing complications. If deterioration is suspected, or if the patient fits the criteria for very severe disease or is immunosuppressed or elderly, it may be wise to utilize vancomycin initially (Table 5). Our approach to patients with suspected or known *C. difficile* infection is based on the severity of their illness (Figures 1 and 2).

### Recurrent *C. difficile* infection

Twenty percent of *C. difficile* infection patients relapse, despite adequate therapy. Risk factors for relapse are presented in Table 6. Diagnosing recurrent *C. difficile* infection can be difficult, especially in the 20% without diarrhea. The usual treatment for recurrent *C. difficile* infection is a repeat course of metronidazole, unless the patient has severe disease. Tapered and pulsed dosing schedules of vancomycin have been investigated for the treatment of *C. difficile* infection that recurs after an initial course of vancomycin (Table 7). An example of an oral vancomycin taper schedule is as follows: 125 mg *qid* × 10-14 d; 125 mg *bid* × 7 d; 125 mg daily × 7 d; 125 mg once every 2 d × 8 d; and 125 mg once every 3 d × 15 d<sup>[126]</sup>. The treatment of recurrent *C. difficile* infection with various vancomycin daily doses (2 g/d, 1 g/d, and 500 mg/d) and administration schedules (daily vancomycin followed by tapered or pulsed dose



**Table 6** Risk factors for relapse (occurs in 10%-25% of cases<sup>1</sup>)

Prolonged antibiotic usage  
Prolonged hospitalization  
Age > 65 yr  
Diverticulosis  
Comorbid medical condition(s)

<sup>1</sup>Increased risk of relapse with increased number of relapses. Kelly CP, Lamont JT. Up-to-date May 2008.

**Table 7** Therapeutic approach to patients with recurrent *C. difficile* infection

Second course of initial antibiotic, if the patient has mild/moderate disease; if severe disease, begin vancomycin  
If recurrence after vancomycin, re-evaluate and treat with oral vancomycin and add tapering vancomycin regime and *S. boulardii*  
If recurrence despite above, consider  
Rifampicin  
Cholestyramine  
Fecal bacteriotherapy

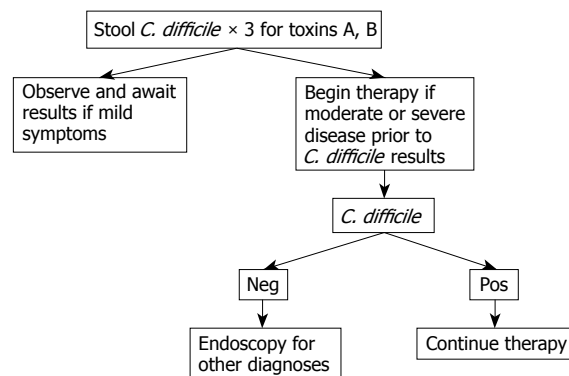
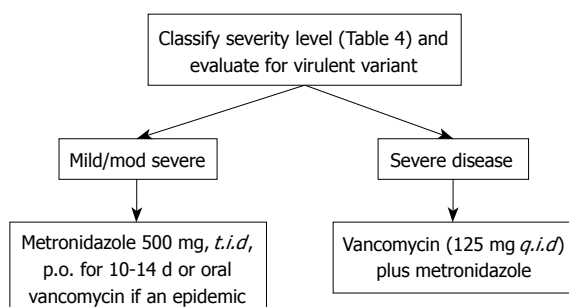
vancomycin therapy) was reported by McFarland *et al*<sup>[123]</sup>. They found that tapered and pulsed dosing schedules of vancomycin result in significantly better *C. difficile* infection cure rates than traditional vancomycin dosing.

Wenisch *et al*<sup>[127]</sup> conducted a prospective, randomized study to compare the efficacy of the oral drugs fusidic acid, metronidazole, vancomycin, and teicoplanin in the treatment of CDAD. Treatment resulted in clinical cure greater than 90% with all the agents: 94% vancomycin, 96% teicoplanin, 93% fusidic acid, and 94% metronidazole. However, recurrent clinical symptoms occurred in 16% of patients treated with vancomycin or metronidazole, 7% of those treated with teicoplanin, and 28% of those treated with fusidic acid. There was asymptomatic carriage of *C. difficile* toxin in 13% of patients treated with vancomycin, 16% with metronidazole, 4% with teicoplanin and 24% with fusidic acid. No adverse effects related to therapy were observed with vancomycin or teicoplanin. Considering the costs of treatment, their findings suggest that metronidazole is the drug of choice for CDAD, and that glycopeptides should be reserved for patients who cannot tolerate metronidazole or who do not respond to treatment with this drug.

### Probiotics

Studies on probiotics for *C. difficile* infection have been inconclusive and conflicting, with respect to treatment benefit. Nonetheless, the use of probiotics is becoming more widespread.

Pillai and Nelson conducted a meta-analysis to assess the potential therapeutic effects of probiotics for *C. difficile* infection<sup>[128]</sup>. Randomized, prospective studies (1966-2007) using probiotics alone or in conjunction with conventional antibiotics for the treatment of documented *C. difficile* colitis were eligible for inclusion. Ultimately, four studies met the inclusion criteria and were included in the review. The four studies examined the use of probiotics

**Figure 1** Approach to patients with suspected *C. difficile* infection.**Figure 2** Initial therapeutic approach to patients with *C. difficile* infection.

in conjunction with conventional antibiotics (vancomycin or metronidazole) for the treatment of recurrence or the initial episode of *C. difficile* colitis in adults. All of the studies were small and had methodological issues. A statistically-significant benefit of probiotics combined with antibiotics was detected in only one study. The authors concluded that, overall, there is insufficient evidence to recommend probiotic therapy as an adjunct to antibiotic therapy for *C. difficile* colitis. There also is no evidence to support the use of probiotics alone in the treatment of *C. difficile* colitis.

In 1994, McFarland *et al*<sup>[129]</sup> reported that patients receiving *Saccharomyces boulardii* were significantly less likely than patients receiving placebo to experience a recurrence of *C. difficile* diarrhea (RR 0.59; 95% CI: 0.35 to 0.98). Consequently, in a later meta-analysis, he compared the efficacy of probiotics for the prevention of AAD and the treatment of CDAD. Across 25 randomized controlled trials (RCTs), probiotics significantly reduced the relative risk of AAD (RR 0.43, 95% CI: 0.31 to 0.58,  $P < 0.001$ )<sup>[130]</sup>. Across six randomized trials, probiotics had significant efficacy for CDAD (RR 0.59, 95% CI: 0.41 to 0.85,  $P = 0.005$ ).

This time, McFarland *et al*<sup>[129]</sup> concluded that a variety of different types of probiotic show promise as effective therapies for these two diseases. Again using meta-analysis, three types of probiotics (*S. boulardii*, *Lactobacillus rhamnosus* GG, and probiotic mixtures) were found to significantly reduce the development of AAD. Only *S. boulardii* was effective for CDAD.

Treatment of recurrent *C. difficile* infection with high-dose vancomycin plus *S. boulardii* is the only treatment



combination that has been evaluated in a prospective, randomized, controlled trial and found to generate a significant trend toward reduced recurrent *C. difficile* infection<sup>[131]</sup>. *Lactobacillus* spp. have been evaluated for use in recurrent *C. difficile* infection, but data on regimens containing these organisms are poorly derived and conflicting. Fungemia with its administration has been reported in immunocompromised hosts. Therefore, its use is not appropriate in this group<sup>[132]</sup>.

### **Fecal bacteriotherapy especially for relapsing (recurrent) CDAD**

Relapse of *C. difficile* occurs in 10%-25% of patients treated with metronidazole or vancomycin. Furthermore, multiple relapses may occur in the same patient. An alternative approach to patients with recurrent CDAD involves the administration of the entire fecal flora from a healthy individual, which is referred to as fecal bacteriotherapy. Borody *et al*<sup>[133]</sup> reviewed 84 fecal transplantation therapies for severe cases of relapsing, or recurrent *C. difficile* infection (*via* various routes of administration). They found that 80% resulted in a good clinical response, resolution, or cure<sup>[133]</sup>. A review of eight reports on the infusion of feces or fecal bacteria revealed an optimistic cure rate, without recurrence in most patients<sup>[134]</sup>. In a study involving 18 patients treated with healthy donor stools *via* a nasogastric tube, 15 patients were recurrence-free at 90 d (two died of unrelated causes and one experienced recurrence)<sup>[135]</sup>. The patients described in these reports<sup>[133,136-144]</sup> included those with symptomatic relapse after receiving multiple courses of antibiotics; e.g. vancomycin, and/or metronidazole, and/or rifampicin together with cholestyramine. Case series have suggested a clinical benefit of fecal bacteriotherapy in patients with severe or recurrent CDAD who have failed to respond to standard approaches. Although the data are limited to case series, fecal bacteriotherapy has been used successfully to treat relapsing *C. difficile* infection. The precise mechanisms for the benefits of fecal bacteriotherapy are unclear. The reappearance of *Bacteroides* species after treatment suggests that *Bacteroides* species may be involved in the restoration of the presumably antibiotic-damaged flora in the colon.

Successful treatment with two or more fecal enemas has been described in other reports, according to Borody, Leis, & Gerald Pang (www.Up-to-date.com2008), involving a total of 23 patients with PMC who were refractory to antibiotic therapy, or who had experienced multiple relapses. In one study of 16 patients with severe, refractory disease treated over an 18-year period, 13 responded dramatically with decreases in diarrhea, temperature and leukocytosis. In a report describing nine patients, the single administration of a fecal enema (5-10 gm homogenized stool in pasteurized cow's milk) was effective in seven. In another case report, according to Borody, Leis, & Gerald Pang (www.Up-to-date.com), the one-time administration of bacteriotherapy was effective when 500 mL of fecal infusion in saline was delivered throughout the colon *via* a colonoscope. The authors hypothesized that the greater area of re-colonization by

fecal bacteria created a greater capacity to inhibit spore formation proximal to the splenic flexure. The use of the colonoscope to deliver fecal bacteria has an added theoretical advantage of permitting delivery of the active flora components to the distal small bowel, where *C. difficile* can reside. In addition, the colonoscope may permit the proximal delivery of flora in patients with a dilated colon, although colonoscopy must be performed extremely cautiously in this setting, because of the risk of perforation. One of the current authors (JSB) has utilized this modality with similar results.

Aas *et al*<sup>[135]</sup> reported on 18 subjects who received donor stool by nasogastric tube for recurrent *C. difficile* infection over a 9-year period at a single institution. During the period between the initial diagnosis of *C. difficile* colitis and the stool treatments, the 18 subjects received a total of 64 courses of antimicrobials (range, 2-7 courses; median, three courses). During the first 90 d after receipt of treatment with stool, two patients died of unrelated illnesses. Only one of the 16 survivors experienced a single recurrence of *C. difficile* colitis over the 90-d follow-up. No adverse effects associated with stool treatment were observed. Patients with recurrent *C. difficile* colitis may benefit from the introduction of stool from healthy donors *via* a nasogastric tube.

Lund-Tønnesen *et al*<sup>[140]</sup> reported on 18 patients with CDAD who were given homologous feces from one healthy donor. In 17 patients, feces were instilled *via* a colonoscope, and in one patient *via* a gastric stoma. Fifteen patients were clinically cured, and no relapses were observed; however, it is important to note that three patients with severe colitis did not respond to the treatment.

In recalcitrant, recurrent *C. difficile* infection, one should attempt initially to use probiotics that have been shown to be effective in published studies. Subsequently, in patients who remain seriously ill from recurrent *C. difficile* infection, fecal bacteriotherapy may be used when other approaches have been unsuccessful<sup>[133,145]</sup>. The above-mentioned study by Lund-Tønnesen *et al*<sup>[140]</sup>, in which three patients with severe colitis did not respond to the treatment, while only the remaining less-severely ill patients were clinically cured, and no relapses were observed may indicate that this may serve as rescue therapy for patients with recurrent *C. difficile*. But its role in patients with severe *C. difficile* infection remains unproven.

**Suggested protocol for fecal bacteriotherapy:** Barody's protocol is as follows. (a) Donor stool and blood are screened for pathogens and viruses before infusion. CBC, serological testing for hepatitis A, B, and C; HIV-1 and HIV-2 and syphilis, stool culture for enteric bacterial pathogens, and light microscopy examination of stool sample for parasites and ova are performed. (b) The donor is clinically well, with the passage of normal, daily stools, and has had no intake of antibiotics for the last 6 mo. (c) The donor should not be a close relative, living in the same household such as a husband or child, theoretically to avoid use of flora from a silent carrier

of the same pathogen. (d) The recipient is evaluated for HIV and hepatitis markers to avoid future questions about transmission. (e) Oral vancomycin (500 mg twice daily for 7 d) is administered, and then followed by a single oral lavage with 3-4 L of polyethylene glycol with electrolyte purgative (such as GoLYTELY). (f) Although the lavage is skipped in patients too ill to tolerate it, vancomycin pretreatment is used, whenever possible. (g) 200-300 gm of donor stool suspended in 200-300 mL of sterile normal saline (homogenized briefly in a kitchen blender to a liquid consistency) is administered *via* an enema within 10 min of preparation, and this is repeated daily for 5 d. (h) Initial infusion may be filtered and infused *via* colonoscopy, preferably into the terminal ileum to address known ileal presence of *C. difficile*. (i) At least five consecutive days of rectal enemas are administered, using donor stools. (j) The enema should be retained for at least 6 h (loperamide pretreatment may help), followed by a high-fiber meal and overall diet. (k) Although some patients are unable to retain the enema initially for prolonged periods, it appears that coating of the mucosa by the infusate is adequate. (l) Adverse effects have been transient and mild, and have consisted primarily of abdominal gurgling, gas and borborygmi-expected post-enema symptoms. Recurrence has not been observed with follow-up of 1-3 years in most patients, even though a number of patients subsequently have required antibiotics for unrelated infections.

In summary, patients who develop a second episode of *C. difficile* infection after successful treatment of the first episode may be at increased risk for developing complications. Although different drugs and regimens have been used, vancomycin may be the best option; and the combination of high-dose vancomycin plus *S. boulardii* is the only treatment combination that has been evaluated in a prospective, randomized, controlled trial to demonstrate a significant trend toward reduced recurrent *C. difficile* infection. Fecal bacteriotherapy seems promising and is undergoing further testing at this time

## NEWER ALTERNATIVE THERAPIES

Other therapeutic options for CDAD are being developed, and drugs used for other infections are being studied as alternatives to metronidazole and vancomycin.

Nitazoxanide, a nitrothiazolide and metabolic precursor of tizoxanide, has broad-spectrum activity against helminths and protozoa, as well as bacterial enteric pathogens, including *C. difficile*. It is marketed in the US and has been widely used throughout the world to treat parasitic diseases of the gastrointestinal tract; several million children have been treated with this drug over the past decade. Nitazoxanide is a US FDA approved drug that is used as an anti-protozoal agent for oral administration in pediatric patients, aged 1-11 years, with diarrhea. The drug acts by interfering with anaerobic metabolic pathways, and it has been shown to have excellent *in vitro* activity against *C. difficile*. An ongoing double-blind study comparing metronidazole with nitazoxanide for *C. difficile* infection involved the treatment

of 16 patients. The response rate for nitazoxanide was recently shown to be comparable to metronidazole for CDAD treatment in a prospective, randomized, double-blinded clinical trial<sup>[146-148]</sup>. It is associated with fewer side effects than metronidazole, which should improve compliance.

Tinidazole is a structural analogue of metronidazole, with similar bioavailability (100%) and fewer drug-related adverse effects, but similar *in vitro* activity against *C. difficile*<sup>[149,150]</sup>.

OPT-80, previously known as tiacumicin B, and with the proposed name difimicin, is a novel 18-membered macrocycle antibiotic. It has little or no systemic absorption after oral administration, and a narrow activity spectrum against Gram-positive aerobic and anaerobic bacteria, and has tested well in patients with *C. difficile* infection<sup>[151,152]</sup>.

Rifalazil and rifaximin are rifamycin derivatives. Rifalazil is an orally-absorbed systemic antibiotic with a broad spectrum of activity that has been shown to prevent and treat CDAD recurrence in a hamster model. Rifaximin, a non-systemic antibiotic approved by the US FDA for travelers' diarrhea, currently is under evaluation for the treatment of CDAD<sup>[153,154]</sup>.

In unresponsive cases (e.g. those who have had no improvement after 3 d on metronidazole), one should add oral vancomycin, 500 g four times daily and intracolonic vancomycin (500 mg of IV vancomycin in 100 mL of normal saline per rectal Foley catheter, clamping for 60 min, repeating every 6 h). In addition, if there is an ileus, metronidazole can be given intravenously. While there is still no significant experience with nitazoxanide or rifaximin, these would be reasonable choices. As well, Pullman *et al*<sup>[155]</sup> report that ramoplanin, a poorly-absorbed glycolipodepsipeptide that has been evaluated for the prevention of vancomycin-resistant enterococci, has good *in vitro* activity against *C. difficile*.

Teicoplanin may be a good choice, because some empirical evidence suggests that it is better than vancomycin for bacteriologic cure. It has borderline superior effectiveness in terms of symptomatic cure, but it is not readily available in the United States.

Therefore, in addition to nitazoxanide, bacitracin, teicoplanin, and fusidic acid, agents that have published efficacy, are several drugs, like rifaximin and PAR-101, which currently are under investigation. Other therapies, including polymers that bind *C. difficile* toxin, monoclonal antibodies to toxins, and preventative measures like toxoid vaccines, also are under study.

### A role for monoclonal antibodies?

Taylor *et al*<sup>[156]</sup> examined the safety and pharmacokinetics of a novel neutralizing human monoclonal antibody against *C. difficile* toxin A (CDA1) in 30 healthy adults whose median age was 27.5 years. While there were no serious adverse events related to its use, 21 of 30 reported non-serious adverse events were possibly related to CDA1. These included transient blood pressure changes requiring no treatment, nasal congestion, headache, abdominal cramps, nausea, and self-limited diarrhea. The authors concluded that, at least in healthy subjects, the

administration of CDA1 as a single intravenous infusion is safe and well tolerated.

### Anion-binding resins

The importance of toxin production in the pathophysiology of *C. difficile* diarrhea has prompted consideration of anion-binding resins as a possible alternative to antimicrobial therapy. An advantage of resin therapy is that the bowel flora is not altered, as occurs with antibiotics (e.g. vancomycin or metronidazole), which may allow for more rapid reconstitution of normal colonic flora. Anion-exchange resins bind vancomycin as well as toxins; thus, the resin must be taken at least 2 h or 3 h apart from the vancomycin. Suggested regimens are colestipol (5 g every 12 h) or cholestyramine (4 g three or four times daily) for 1-2 wk, usually in conjunction with vancomycin.

Tolvamer, a novel toxin-binding polymer, has been developed to ameliorate *C. difficile*-associated disease without adversely affecting normal flora. Tolvamer has been tested for its ability to neutralize clostridial toxins produced by the epidemic BI/027 strains, thereby preventing toxin-mediated tissue culture cell rounding. The titers of toxin-containing *C. difficile* culture supernatants were determined using confluent cell monolayers, and then the supernatants were used in assays containing dilutions of tolvamer to determine the lowest concentration of drug that prevented  $\geq 90\%$  cytotoxicity. Tolvamer neutralized toxins in the supernatants of all *C. difficile* strains tested. Specific antibodies against the large clostridial toxins TcdA and TcdB also neutralized the cytopathic effect, suggesting that tolvamer specifically neutralizes these toxins, and that the binary toxin (whose genes are carried by the BI/027 strains) is not a significant source of cytopathology against tissue culture cells *in vitro*<sup>[157]</sup>.

However, tolvamer is not FDA approved or commercially available, to date. Castanospermine has been identified as an inhibitor of the Rho/Ras-glucosylating *Clostridium sordellii* lethal toxin and *C. difficile* toxin B. Microinjection of castanospermine into embryonic bovine lung cells prevents the cytotoxic effects of toxins. The inhibitor binds in a conformation that brings its four hydroxyl groups and its N atom almost exactly into the positions of the four hydroxyls and the ring oxygen of the glucosyl moiety of UDP-glucose, respectively<sup>[158]</sup>. It is in its early stage of development.

### Vaccination

Testing the feasibility of active vaccination against *C. difficile* and its toxins in high-risk individuals currently is ongoing<sup>[159]</sup>. *C. difficile* toxoid vaccine has induced immune responses to toxins A and B in patients with CDAD, and has been associated with resolution of recurrent diarrhea. This parenteral *C. difficile* vaccine, which contains toxoid A and toxoid B, has been reported to be safe and immunogenic in healthy volunteers. Three patients with multiple episodes of recurrent CDAD were vaccinated. Two of the three exhibited an increase in serum IgG antitoxin A antibodies (three- and four-fold

increases), and in serum IgG antitoxin B antibodies (52 and 20-fold). Both individuals also developed cytotoxin-neutralizing activity against toxins A and B. Prior to vaccination, the subjects had required nearly continuous treatment with oral vancomycin for 7, 9, and 22 mo, respectively, to treat recurrent episodes of CDAD. After vaccination, all three subjects discontinued treatment with oral vancomycin without any further recurrence. Thus, *C. difficile* toxoid vaccine induced immune responses to toxins A and B in patients with CDAD, and was associated with resolution of recurrent diarrhea.

Vaccination with a partially-purified preparation of inactivated toxins A and B is also undergoing current study. Several studies have shown that the humoral immune response of the host to *C. difficile* toxins A and B influences the clinical course of CDAD, as well as the risk of relapse<sup>[160-162]</sup>.

Another vaccine, containing toxoids A and B, has been shown to induce adequate antibody responses in healthy volunteers<sup>[163]</sup>. The efficacy of this vaccine subsequently was evaluated in an open-label study involving three patients with recurrent *C. difficile* colitis<sup>[159]</sup>. Following four intramuscular inoculations over an 8-wk period, all three patients discontinued antibiotic treatment without further recurrence over 6 mo of follow-up.

### Immunoglobulin therapies

A retrospective review was performed on 264 *C. difficile* toxin-positive patients (November 2003-January 2005), which documented 14 patients with severe, refractory, recurrent *C. difficile* diarrhea who were treated with intravenous immunoglobulin (Flebogamma, 150-400 mg/kg)<sup>[164]</sup>. Patients received a median of three (range, 1-5 g/L) courses of vancomycin or metronidazole before receiving intravenous immunoglobulin. All had hypoalbuminemia (median, 22 g/L; range, 18-33 g/L) and raised C-reactive protein (median, 47 mg/L; range, 25-255 g/L) at the time of infusion. The median white cell count was  $15.3 \times 10^9$ /L (range, 4-24 g/L). Eight patients had evidence of pancolitis on abdominal imaging, suggesting severe *C. difficile* diarrhea. All patients tolerated intravenous immunoglobulin without side effects. Nine (64%) responded with bowel habits normalizing in a median of 10 (range, 2-26) d; one patient received two doses. One patient had a partial response from two doses, but died 2 mo later after a recurrence. Thus, intravenous immunoglobulin may be effective for severe, refractory, or recurrent *C. difficile* diarrhea after failed conventional treatment.

### Surgery

In patients with *C. difficile* colitis, a progressive, systemic inflammatory state may develop that is unresponsive to medical therapy; some cases ultimately will progress to colectomy or death. *C. difficile* colitis is a significant and increasingly common cause of death. Surgical treatment of *C. difficile* colitis has a high death rate once the fulminant expression of the disease is present<sup>[165]</sup>. These authors reviewed 2334 hospitalized patients with *C. difficile* colitis from January 1989 to December 2000.

In the setting of CDAD before the predominance of the hypervirulent strain, 64 patients died or underwent colectomy for pathology-proven *C. difficile* colitis. Unfortunately, those patients who underwent colectomy for *C. difficile* colitis had an overall death rate of 57%. Significant predictors of death after colectomy were preoperative vasopressor requirements and older age.

Fulminant *C. difficile* colitis is associated with a high mortality rate. As in the former study, Hall *et al*<sup>[166]</sup> found that the development of a vasopressor requirement and the need for intubation are ominous signs which should lead to rapid surgical intervention. From 1998 to 2006, they studied a total of 3237 consecutive patients with *C. difficile* cytotoxin-positive stool samples. Commonly referenced indicators for surgical intervention were gathered on the day of surgery. The preoperative characteristics of patients surviving subtotal colectomy were compared with those who did not survive. They found that 36 patients underwent colectomy. Twenty-three patients (64%) were discharged from the hospital alive. Preoperative intubation and vasopressor requirement were risk factors for in-hospital mortality (OR: 7.15; 95% CI: 1.28-39.8 and OR: 6.0; 95% CI: 1.08-33, respectively). Patients who had a recent surgical procedure experienced a lower in-hospital mortality rate (OR: 0.11; 95% CI: 0.02-0.52).

In the setting of CDAD due to the hypervirulent strain, some patients have progressed from severe disease to death in less than 48 h. Emergency colectomy has prevented mortality in some patients with fulminant CDAD. The decision to perform an emergency colectomy remains largely empirical<sup>[100]</sup>. In a retrospective observational cohort study of 165 cases of CDAD, among those patients who required ICU admission or prolongation of ICU stay between January 2003 and June 2005 at two tertiary care hospitals in Quebec, 53% died within 30 d of ICU admission, and almost half (44%) within 48 h of ICU admission. The independent predictors of 30-d mortality were: leukocytosis  $\geq 50 \times 10^9/L$  (AOR: 18.6; 95% CI: 3.7-94.7), lactate  $\geq 5$  mmol/L (AOR: 12.4; 95% CI: 2.4-63.7), age  $\geq 75$  years (AOR: 6.5; 95% CI: 1.7-24.3), immunosuppression (AOR: 7.9; 95% CI: 2.3-27.2) and shock requiring vasopressors (AOR: 3.4; 95% CI: 1.3-8.7). After adjustment for these confounders, patients who had an emergency colectomy were less likely to die (AOR: 0.22; 95% CI: 0.07-0.67,  $P = 0.008$ ) than those treated medically. Surgical intervention is indicated in the setting of peritoneal signs, severe ileus, or toxic megacolon; but colectomy also seems more beneficial in patients aged 65 years or older, in the immune competent, and in those with a leukocytosis  $\geq 20 \times 10^9/L$  or serum lactate between 2.2 and 4.9 mmol/L.

The standard of care for patients undergoing emergency surgical intervention for CDAD is a total colectomy (with preservation of the rectum) and ileostomy, since primary anastomosis is not feasible acutely due to the pancolitis associated with severe disease. However, after colonic inflammation has subsided,

Table 8 Indications for emergency colectomy

Based upon
30-d mortality
Leukocytosis $\geq 20 \times 10^9/L$
Lactate $\geq 5$ mmol/L
Age $\geq 75$ yr
Immunosuppression
Shock requiring vasopressors
Especially in the presence of:
Toxic megacolon
Multi-organ system failure

Kelly CP, Lamont JT. Up-to-date May 2008.

closure of the ileostomy and ileorectal anastomosis can be performed (Table 8).

## CONCLUSION

CDAD has increased in frequency and severity throughout North America and Europe over the last several years, largely due to the emergence of the NAP1 epidemic strain. This transformation of a formerly mild disease into one that can cause severe morbidity and mortality within a few days has challenged the entire approach to this suddenly serious infection. Institutions require accurate and rapid diagnostics for early detection of cases and possible outbreaks, in order to initiate specific therapy and implement early and effective infection control<sup>[167]</sup>.

Aggressive diagnostic and therapeutic interventions are warranted in the setting of *C. difficile* infection. Bedside sigmoidoscopy or colonoscopy may be performed to make a presumptive diagnosis of *C. difficile* infection, by evaluating for the presence of pseudomembranes. Given the risk of perforation, care should be taken to introduce minimal amounts of air to avoid exacerbating ileus or distention. The choice of initial drug therapy depends on severity of illness, co-morbidities, and strain suspicion. Prompt surgical consultation is warranted to assess the requirement for colectomy<sup>[100]</sup>.

*C. difficile* infection is a global problem. A comprehensive *C. difficile* infection control management rapid response team (RRT) is recommended for each health care facility throughout the world. A communications network between RRTs also is recommended, in coordination with each country's Department of Health. It is only through the implementation of the new approaches to its diagnosis, therapy and presentation that we can help to reduce the morbidity and mortality caused by this infection.

## ADDENDUM I

### Contact precautions: For patients with known or suspected *C. difficile*-associated disease

We must address environmental reservoirs to help to limit transmission. *C. difficile* has been cultured not only from patient bathrooms and bedpans, but from stethoscopes, blood pressure cuffs, and hospital furniture.

The initial step is identifying possible *C. difficile* patients, especially in long-term care facilities (LTCFs). Quinn *et al.*<sup>[168]</sup> determined that only 111 facilities (42.2%) had a protocol to identify residents with *C. difficile* infection, and most (77.5%) did not test for *C. difficile* unless a resident had severe diarrhea. Only 58.5% of the facilities placed residents with *C. difficile* infection in private rooms, and 60.9% cohorted residents infected with *C. difficile* with other residents with *C. difficile* colonization or infection. Only 66 facilities (25.1%) had a program to control the use of antimicrobial agents.

Findings suggest that asymptomatic carriers of epidemic and non-epidemic *C. difficile* strains have the potential to contribute significantly to disease transmission in long-term care facilities. Thirty-five (51%) of 68 asymptomatic patients were carriers of toxigenic *C. difficile*, and 13 (37%) of these patients carried epidemic strains. Compared with non-carriers, asymptomatic carriers had higher percentages of skin (61% *vs* 19%;  $P = 0.001$ ) and environmental contamination (59% *vs* 24%;  $P = 0.004$ ). Eighty-seven percent of isolates found in skin samples and 58% of isolates found in environmental samples were identical to concurrent isolates found in stool samples. Spores on the skin of asymptomatic patients were transferred easily to investigators' hands. Previous *C. difficile*-associated disease ( $P < 0.001$ ) and previous antibiotic use ( $P = 0.017$ ) were associated with asymptomatic carriage, and the combination of these two variables was predictive of asymptomatic carriage (sensitivity, 77%; specificity, 58%; PPV, 66%; NPV, 70%)<sup>[64]</sup>.

In a prospective study of 27 patients with *C. difficile*-associated disease, it was found that *C. difficile* frequently contaminated multiple skin sites, including groin, chest, abdomen, forearms, and hands, and was easily acquired on investigators' hands. Skin contamination often persisted on patients' chest and abdomen after resolution of diarrhea. Thus, skin contact of the patient by a health-care worker is a means of *C. difficile* transmission<sup>[169]</sup>.

It is important to emphasize that asymptomatic fecal excretion of *C. difficile* is transient in most patients, and treatment with metronidazole is not effective. Although treatment with vancomycin is temporarily effective in asymptomatic carriers, it is also associated with a significantly higher rate of *C. difficile* carriage 2 mo after treatment and, therefore, is not recommended<sup>[63]</sup>.

An increase in hospital-acquired *C. difficile* infection rate was found at the University of Pittsburgh Medical Center. A comprehensive *C. difficile* infection control 'bundle' was implemented by hospital personnel to control the outbreak of *C. difficile* infection. This *C. difficile* infection control bundle consisted of education, increased and early case-finding, expanded infection-control measures, the development of a *C. difficile* infection management team, and antimicrobial management. Process measures, antimicrobial usage, and hospital-acquired *C. difficile* infection rates were analyzed, and *C. difficile* infection isolates were typed. The rates of compliance with hand hygiene and isolation were 75% and 68%, respectively.

The *C. difficile* infection management team evaluated a mean 31 patients per month (11% were evaluated for moderate or severe disease). The use of antimicrobial

therapy associated with increased *C. difficile* infection risk decreased by 41% during the period 2003-2005. The aggregate rate of *C. difficile* infection during the period 2001-2006 decreased to 4.8 infections per 1000 HDs; and, by 2006, it had decreased to 3.0 infections per 1000 HDs, a rate reduction of 71%. During the period 2000-2001, the proportion of severe *C. difficile* infection cases peaked at 9.4% (37 of 393 *C. difficile* infections were severe); this rate decreased to 3.1% in 2002 and further decreased to 1.0% in 2006, a 78% overall reduction. In 2005, 13% of *C. difficile* isolates were type BI (20% were hospital acquired), which represented a significant reduction from 2001. These authors concluded that the outbreak of *C. difficile* infection with the BI strain in hospital was controlled after implementing this infection control 'bundle'. Thus, early identification, coupled with appropriate control measures, reduces the rate of *C. difficile* infection and the frequency of adverse events. However, it requires a multipronged approach.

**Methods of contact precautions and control:** (1) Place patients with *C. difficile* in private rooms. (2) If private rooms are not available, place these patients in rooms with other patients who have *C. difficile*-associated disease. (3) Perform hand hygiene procedures preferably using soap and water-not alcohol. To reduce the transmission of *C. difficile* spores, environmental disinfection with 10% sodium hypochlorite and hand-washing with soap and water can be effective at removing the spores from hands and surfaces.

Strict antiseptic procedures should be followed by health care workers in contact with the patient, and these procedures should include the use of disposable gloves, and a mask and gown. Because alcohol is ineffective at killing *C. difficile* spores, health care workers must frequently wash their hands with soap and water, rather than with alcohol-based waterless hand sanitizers, especially when caring for CDAD patients. Patient-care equipment (e.g. blood-pressure cuffs, stethoscopes and thermometers) should either be used only for the infected patient or cleaned well before they are used with another patient.

Enhanced environmental cleaning following a regular schedule with dilute bleach should be used to eliminate *C. difficile* spores from all patient contact surface areas. These spores may remain on infected surface areas for months or even years.

In addition, note the ability of the vegetative form of *C. difficile* to survive on moist surfaces. On dry surfaces, vegetative *C. difficile* cells die rapidly, whereas they remained viable for up to 6 h on moist surfaces in room air. This illustrates the importance of washing and drying room surfaces when cleaning contaminated rooms.

A very important method of controlling outbreaks of *C. difficile*-associated disease should be restricting the use of antimicrobial agents that have been implicated as risk factors for the disease, as recommended by Gerding *et al.*<sup>[116]</sup>. Davey *et al.*<sup>[170]</sup> documented that interventions to improve antibiotic prescribing practices to hospital inpatients can be successful, and that they can reduce antimicrobial resistance and the rates of hospital-acquired infections.



### Control of fluoroquinolone use

Effective surveillance of antibiotic-resistant bacteria and CDAD must be intensified in every healthcare setting, but especially in long-term care and rehabilitation facilities. All these facilities must have easy laboratory access for prompt and active surveillance culturing and *C. difficile* cyto-toxin testing, for both A and B, at the earliest indication of any infection or CDAD. In addition, Furuno *et al*<sup>[171]</sup> advise that those patients at higher risk for carriage of antibiotic-resistant bacteria should be identified early for active surveillance targeting-culturing for methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant enterococci (VRE); e.g. patients who report having had antibiotics or prior hospital admissions within the past year. The authors found that this was very cost-effective, saving a projected \$19 000-\$26 000 relative to non-directed hospital wide screening for resistant organisms (MRSA and VRE), during the 8-mo study period at their tertiary care facility. They also found that there often is a significant delay between the onset of CDAD symptoms and the full implementation of CDC contact precautions.

## ADDENDUM II

Note that current Proper Hand Hygiene techniques for *C. difficile* differ from previous 2002 CDC Guidelines for hand hygiene in health-care settings, which were as follows.

IV.A.1. During the delivery of healthcare, avoid unnecessary touching of surfaces in close proximity to the patient to prevent both contamination of clean hands from environmental surfaces and transmission of pathogens from contaminated hands to surfaces.

IV.A.2. When hands are visibly dirty, contaminated with proteinaceous material, or visibly soiled with blood or body fluids, wash hands with either a non-antimicrobial soap and water or an antimicrobial soap and water.

IV.A.4. Wash hands with non-antimicrobial soap and water or with antimicrobial soap and water if contact with spores (e.g. *C. difficile* or *Bacillus anthracis*) is likely to have occurred. The physical action of washing and rinsing hands under such circumstances is recommended, because alcohols, chlorhexidine, iodophors, and other antiseptic agents have poor activity against spores.

Alcohol-containing hand disinfection products were recommended over soap and water in the control of most organisms of epidemiological importance<sup>[172]</sup>. Alcohol, however, does not eradicate *C. difficile* spores, maintain both Bettin *et al*<sup>[173]</sup> and Boyce *et al*<sup>[174]</sup>. Thus, what hand cleaning method to use in the presence of *C. difficile* infection is controversial. Papers conflict on the subject of alcohol eradicating *C. difficile* spores.

There even has been concern that the widespread use of alcohol-based hand sanitizers (instead of hand washing) has played a role in recent *C. difficile* outbreaks. Furthermore, because soap and water hand hygiene requires more time than alcohol-based hand hygiene, there is concern that alcohol-based hand hygiene may decrease overall effective hand hygiene compliance.

These concerns remain unproven. Overall CDAD rates have tended to decrease or remain the same after the introduction and increased use of alcohol-based sanitizers as the primary mode of hand hygiene<sup>[175,176]</sup>. There is a lack of rigorous evidence, however, linking specific hand hygiene interventions with the prevention of health care associated infections (HCAIs). The varied nature of the interventions used and the diverse factors affecting the acquisition of HCAIs make it difficult to show any specific effect of hand hygiene alone. The most frequent methodologies currently used in this research area have been before-and-after observational studies without a control comparison group<sup>[177]</sup>. However, the CDC recommends soap and water hand hygiene when caring for patients with CDAD.

In summary, if a facility is experiencing a *C. difficile* outbreak, it is prudent to emphasize that health care workers should frequently wash their hands with soap and water, in addition to using an alcohol-based hand sanitizer<sup>[178]</sup>.

The 2008 recommendations have been ambivalent, as seen below.

If your institution experiences an outbreak of *C. difficile*, consider using only soap and water for hand hygiene when caring for patients with *C. difficile*-associated disease; alcohol-based hand rubs are not as effective against spore-forming bacteria.

Current (Reviewed 3/08) CDC hand hygiene guidelines, available at <http://www.cdc.gov/handhygiene/> [7A] Accredited organizations are required to provide health care workers with a readily accessible alcohol-based hand rub product (CDC recommendations 8 C&D). However, use of an alcohol-based hand rub cleaner by any individual health care worker is not required. If you choose not to use it, then soap and water should be used instead.

In addition, use gloves when entering patients' rooms and during patient care; use gowns if soiling of clothes is likely; dedicate equipment, whenever possible.

Implement an environmental cleaning and disinfection strategy. Ensure adequate cleaning and disinfection of environmental surfaces and reusable devices, especially items likely to be contaminated with feces and surfaces that are touched frequently. Use an Environmental Protection Agency (EPA)-registered hypochlorite-based disinfectant for environmental surface disinfection after cleaning, in accordance with label instructions; generic sources of hypochlorite (e.g. household chlorine bleach) also may be appropriately diluted and used. Follow the manufacturer's instructions for the disinfection of endoscopes and other devices. Infection control practices in long-term care and home health settings are similar to those practices taken in traditional health-care settings.

How to clean and disinfect surfaces and devices according to the CDC's evidence-based guidelines for the prevention of CDAD (as reported at [http://www.cdc.gov/ncidod/dhqp/id\\_CdiffFAQ\\_HCP.html](http://www.cdc.gov/ncidod/dhqp/id_CdiffFAQ_HCP.html)). (1) Surfaces should be kept clean, and body substance spills should be managed promptly, as outlined in the CDC's 'Guidelines for Environmental Infection Control in Health-Care Facilities'. (2) Hospital cleaning products can be used for

routine cleaning. (3) Hypochlorite-based disinfectants have been used with some success for environmental surface disinfection in those patient-care areas where surveillance and epidemiology indicate ongoing transmission of *C. difficile*. (4) Consult the aforementioned guidelines for the use conditions for generic sources of hypochlorite-based products (e.g. household chlorine bleach) for disinfection of environmental surfaces. Note: EPA-registered hospital disinfectants are recommended for general use, whenever possible, in patient-care areas. At present, there are no EPA-registered products with specific claims for inactivating *C. difficile* spores, but there are a number of registered products that contain hypochlorite.

If an EPA-registered proprietary hypochlorite product is used, consult the label instructions for proper and safe use conditions. The literature supports the role of environmental disinfection with unbuffered hypochlorite solutions (diluted 1:10)<sup>[179]</sup>.

Fawley *et al.*<sup>[180]</sup> studied the differences between the activity of various cleaning agents and germicides against *C. difficile* spores and the potential for some of these products to promote sporulation. When used at recommended working concentrations, only chlorine-based germicides were able to deactivate *C. difficile* spores. *C. difficile* epidemic strains had a greater sporulation rate than non-epidemic strains. The mean sporulation rate, expressed as the proportion of a cell population that is in spore form, was 13% for all strains not exposed to any cleaning agent or germicide, and it was significantly increased by exposure to cleaning agents or germicides containing detergent alone (34%), a combination of detergent and hypochlorite (24%), or hydrogen peroxide (33%). By contrast, the mean sporulation rate did not change substantially after exposure to germicides that contain either a combination of detergent and dichloroisocyanurate (9%) or dichloroisocyanurate alone (15%).

A study by White *et al.*<sup>[181]</sup> revealed that all floor cleaning methods reduce the overall microbial load, though high counts and bacterial pathogens occasionally persist despite cleaning. Spray cleaning yielded marginally better results than traditional mopping and vacuuming. Wet scrubbing significantly reduced levels of coagulase-positive staphylococci ( $P = 0.03$ ), which, in combination with routine methods, produced an effect that persisted for at least a week. Any sudden change in CDAD incidence in any medical institution should be reported immediately to public health officials.

The use of copper surfaces within the clinical environment and the application of a germination solution in infection control procedures may offer a novel way by which to eliminate *C. difficile* from contaminated surfaces and reducing CDAD<sup>[182]</sup>.

Three novel copper-based biocidal formulations, but not their components (copper sulfate and inorganic binders), were found by Gant *et al.*<sup>[183]</sup> to have potent activity against organisms highly relevant to healthcare-associated infections, and all were active against *C. difficile* spores. This biocidal activity was not achieved by copper sulfate or the inorganic binders used in the formulations.

**Table 9** Strength of recommendation and quality of evidence

Category/grade	Definition
Strength of recommendation	
A	Good evidence to support a recommendation for use
B	Moderate evidence to support a recommendation for use
C	Poor evidence to support a recommendation
Quality of evidence	
I	Evidence from $\geq 1$ properly randomized, controlled trial
II	Evidence from $\geq 1$ well-designed clinical trial, without randomization; from cohort or case-control analytic studies (preferably from $> 1$ center); from multiple time series; or from dramatic results from uncontrolled experiments
III	Evidence from opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees

Adapted from Canadian Task Force on the Periodic Health Examination. *Can Med Assoc J* 1979; 121(9): 1193-1254.

All three copper-based formulations completely decontaminated UMF cloths containing MRSA, ACCB or *C. difficile* spores, suggesting that any of these copper-based formulations could be highly beneficial in the healthcare environment. None of the three copper-based formulations or copper sulfate was cytotoxic to human epithelial cells, up to concentrations of 100-200 ppm.

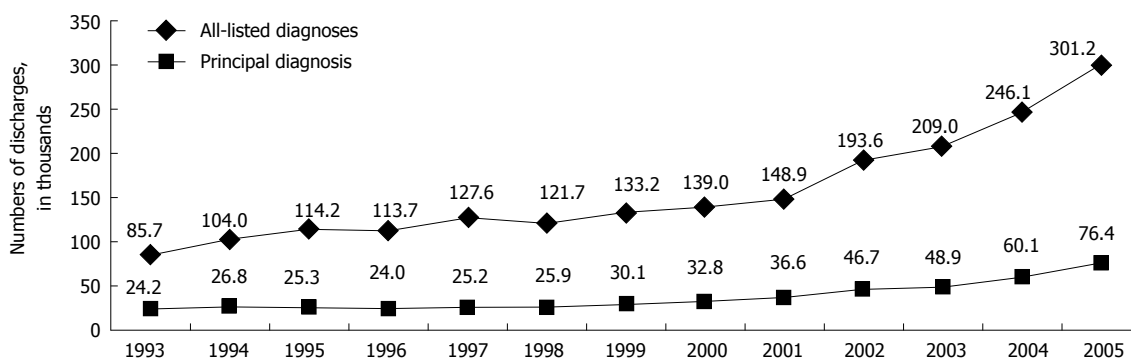
### ADDENDUM III

*C. difficile* infection rates are rising (Figures 3 and 4). The most recent guidelines on *C. difficile* infection based on the strength of recommendations and quality of evidence were issued on 9 October 2008. This was issued as part of the latest updated guidelines for hospital acquired infections by the American Hospital Association, the Joint Commission on Accreditation of Health Organizations, the Infectious Diseases Society of America, the Society for Healthcare Epidemiology of America, and the Association for Professionals in Infection Control and Epidemiology in-“A Compendium of Strategies to Prevent Healthcare-Associated Infections in Acute Care Hospitals.” *Infect Control Hosp Epidemiol* 2008; 29: S12-S21 (Authors-Yokoe, Mermel, Anderson, Arias, Burstin, Calfee, Coffin, Dubberke, Fraser, Gerding, Griffin, Gross, Kaye, Klompas, Lo, Marschall, Nicolle, Pegues, Perl).

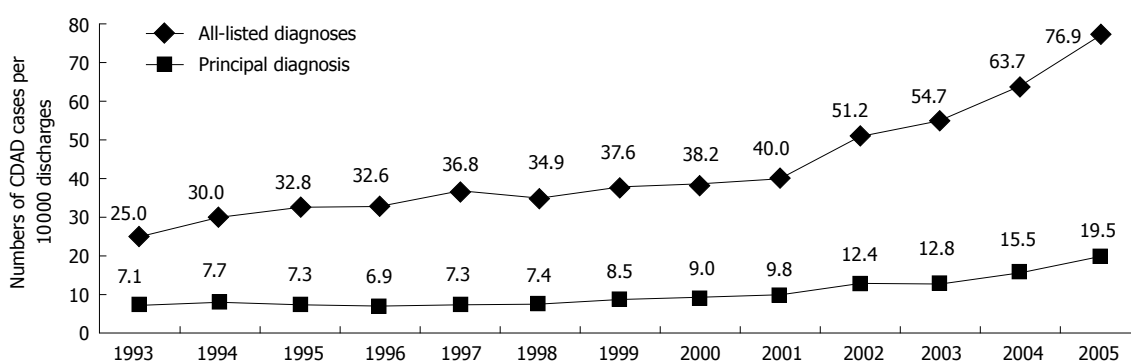
#### (I) Basic practices for prevention and monitoring of *C. difficile* infection

Recommended for all acute care hospitals using strength of recommendations and quality of evidence from Table 9.

**A. Components of a *C. difficile* infection prevention program:** (1) Use contact precautions for infected patients, with a single-patient room preferred (A-II for hand hygiene, A-I for gloves, B-III for gowns, and B-III for single-patient room). (2) Ensure cleaning and disinfection of equipment and the environment (B-III



**Figure 3 Trends in hospital stays associated with *C. difficile*-associated disease, 1993-2005.** (From Elixhauser and Jhung<sup>[82]</sup>) shows the trend in CDAD from 1993 through 2005. During the 8-year period from 1993 until 2001, the total number of hospital discharges with CDAD increased from approximately 85 700 to 148 900 per year, 74% increase. However, during the following 4-year period, from 2001 to 2005, the rate of increase for CDAD escalated, when the numbers of cases more than doubled to 301 200 (a 102 percent increase in 4 years). There were a total of 2 037 900 hospital discharges with CDAD over this 12-year period.



**Figure 4 Discharge rate for *C. difficile*-associated disease, per 10 000 hospital discharges, 1993-2005.** (From Elixhauser and Jhung<sup>[82]</sup>) shows the number of CDAD discharges per 10 000 hospital discharges from 1993 through 2005. The findings are similar to those of the previous figure. From 1993 to 2001, the rate of CDAD per 10 000 discharges increased by 60% while the rate of increase from 2001 to 2005 was considerably steeper, 92%. Thus, the recent sharp rise in CDAD was not attributable solely to an increase in the number of hospital discharges.

for equipment and B-II for the environment). (3) Implement a laboratory-based alert system to provide immediate notification to infection prevention and control personnel and clinical personnel about patients with newly diagnosed *C. difficile* infection (B-III). (4) Conduct *C. difficile* infection surveillance and analyze and report *C. difficile* infection data (B-III). (5) Educate healthcare personnel, housekeeping personnel, and hospital administration about *C. difficile* infection (B-III). (6) Educate patients and their families about *C. difficile* infection, as appropriate (B-III). (7) Measure compliance with CDC or World Health Organization hand-hygiene and contact precaution recommendations (B-III).

## (II) Special approaches for the prevention of *C. difficile* infection

Perform a *C. difficile* infection risk assessment. These special approaches are recommended for use in locations and/or populations within the hospital for which outcome data and/or risk assessment suggest lack of effective control despite implementation of basic practices.

**A. Approaches to minimize *C. difficile* transmission by healthcare personnel:** (1) Intensify the assessment of compliance with process measures (B-III). (2) Perform hand hygiene with soap and water as the preferred method

before exiting the room of a patient with *C. difficile* infection (B-III). (3) Place patients with diarrhea under contact precautions while *C. difficile* test results are pending (B-III). (4) Prolong the duration of contact precautions after the patient becomes asymptomatic until hospital discharge (B-III).

**B. Approaches to minimize *C. difficile* infection transmission from the environment:** (1) Assess the adequacy of room cleaning (B-III). (2) Use sodium hypochlorite (bleach)-containing cleaning agents for environmental cleaning. Implement a system to coordinate with the housekeeping department if it is determined that sodium hypochlorite is needed for environmental disinfection (B-II).

**C. Approaches to reduce the risk of *C. difficile* infection acquisition:** Initiate an antimicrobial stewardship program (A-II).

## (III) Approaches that should not be considered a routine part of *C. difficile* infection prevention

(1) Do not test patients without signs or symptoms of *C. difficile* infection for *C. difficile* (B-II). (2) Do not repeat *C. difficile* testing at the end of successful therapy for a patient recently treated for *C. difficile* infection (B-III).

## ADDENDUM IV

### *C. difficile* infection as a unique infectious problem

In the US, The Deficit Reduction Act of 2005 (P.L. 109-171) requires the Centers for Medicare & Medicaid Services (CMS), the US federal agency which administers Medicare, Medicaid, and the State Children's Health Insurance Program to deny the assignment of a case to a higher DRG (payment to a health care facility) based on the occurrence of one of a selected number of hospital-acquired conditions, if that condition was acquired during the hospitalization. This rule is named CMS-1390-P: Medicare Program; Proposed Changes to the Hospital Inpatient Prospective Payment Systems and Fiscal Year 2009 Rate - Provisions on Preventable Hospital-Acquired Conditions Including Infections. The US Congress requires CMS to select conditions that are high cost, high volume, or both; assigned to a higher paying DRG when present as a secondary diagnosis; and reasonably preventable through the application of evidence-based guidelines.

In its original ruling, CMS had proposed adding nine hospital-acquired conditions, including *C. difficile* to the list of hospital acquired infections for which it proposed to deny payment. This would have taken effect on 1 October 2008. In July 2008, however, in response to an April 2008 letter sent by all three of the US major gastroenterology organizations, CMS, in a final rule setting policies and payment rates for the hospital setting, decided not to add *C. difficile* to the list of 'Hospital-Acquired Conditions for Which It Will Deny Payment'.

The gastroenterology organizations in its April 2008 letter to CMS focused on the last criterion necessary for 'non-reimbursement'-reasonably preventable through the application of evidence-based guidelines.

US gastroenterology organizations, in the letter to CMS, made much about the alleged fact that alcohol-based products are effective against the majority of microorganisms other than (i.e. not for) *C. difficile*, with the statement that "Alcohol-based products, in compliance with CDC guidelines, have played a significant role in potentially complicating efforts to avoid the spread of CDAD."

"Indeed", stated the letter, quoting Shen *et al*<sup>[184]</sup> "the proportion of all hand hygiene episodes performed with soap and water dropped from 90% to 15%, three years after the introduction of alcohol hand gels in one U.S. teaching hospital". The letter continued by stating "that the trade-off of higher overall compliance against more focused use of soap and water is one that CMS must consider given that 'CDC guidelines have played a significant role in potentially complicating efforts to avoid the spread of CDAD'. "In conclusion", ended the letter to CMS, "the ACG, AGA and ASGE urge CMS not to add *C. difficile* to its list of hospital-acquired conditions for which additional payment as a complicating condition would not be available. We strongly believe that the disease is not reasonably preventable. Adding it to the list would create a very expensive and unworkable situation for CMS, hospitals,

physicians and patients."

However, as we have seen in our above review of *C. difficile* infection, the fact is that the hand washing issue is controversial and basically not objectively ascertained with good RCTs. Nevertheless, one can agree that hand washing at least may be superior secondary to the mechanical shedding of *C. difficile* spores with vigorous hand washing.

One can agree with the arguments against adding *C. difficile* infection to the list of non-reimbursable hospital services because of its variable incubation period. Complicating the accurate diagnosis of CDAD is that, while symptoms typically occur within 48 h of infection, patients infected in the hospital with *C. difficile* usually become infected within 3 wk of admission. However, the onset of symptoms can be delayed by 2-3 mo<sup>[30]</sup>. Also, although most cases of CDAD occur on days 4-9 of antibiotic therapy<sup>[15]</sup>, the subsequent diagnosis of CDAD is not always possible upon admission to the hospital, due to a variable incubation period. Therefore, one can agree that it is not reasonable to hold an inpatient hospital liable for a condition acquired in a different setting, one which is not even always detectable upon the patient's admission into their setting.

The CMS accepted these arguments against including *C. difficile* infection in those nosocomial infections that are not reimbursable. However, that still does not alleviate the responsibility of each healthcare facility to make aggressive attempts to counteract this problem. One can look to successful efforts made by others.

The University of Pittsburgh Medical Center developed a program, as mentioned in our review, consisting of education, increased early case finding, expanded infection-control measures, development of a *C. difficile* infection management team, and microbial management. The aggregate rate of *C. difficile* infection decreased from 7.2 infections per 1000 (9.4 during a peak) hospital discharges to 4.8 infections per 1000 hospital discharges, and later, was 3.0 infections per 1000 hospital discharges. The rates of compliance with hand hygiene and isolation were 75% and 68%, respectively<sup>[185]</sup>.

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## Malignancy in adult celiac disease

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### Abstract

Prior studies have suggested that the incidence of some neoplastic disorders, particularly malignant lymphoma and small intestinal adenocarcinoma, are increased in celiac disease. Earlier studies from the United Kingdom have also suggested a link between celiac disease and esophageal carcinoma, although this has not been confirmed in North America. The risk of other gastrointestinal cancers seems to be limited. Gastric cancer does not appear to be detected more frequently, although direct endoscopic visualization of the upper gastrointestinal tract is now very common in patients with celiac disease. Colon cancer also appears to be limited in celiac disease, even in patients first diagnosed with celiac disease late in life. This has led to the hypothesis that untreated celiac disease may be protective, possibly owing to impaired absorption of fat or fat-soluble agents, including hydrocarbons and putative co-carcinogens implicated in the pathogenesis of colon cancer, which may be poorly absorbed and rapidly excreted.

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**Key words:** Adult; Celiac disease; Adenocarcinoma; Lymphoma; T cell enteropathy

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### INTRODUCTION

Malignant disease is a serious concern in celiac disease<sup>[1]</sup> and recently has been reviewed in detail<sup>[2,3]</sup>. Some patients may even present with lymphoma<sup>[4,5]</sup> or a small-intestinal adenocarcinoma<sup>[6]</sup>, and the celiac disease is only detected later. In others, malignancy, particularly lymphoma, complicates the clinical course of well established celiac disease, but may be especially difficult to diagnose<sup>[7]</sup>. The precise risk of malignant disease in adult celiac disease is difficult to evaluate, but about 8%-10% with severe biopsy changes develop lymphoma<sup>[8]</sup>, and this figure has remained remarkably constant over several years<sup>[9]</sup>. Age of first diagnosis of celiac disease seems to be a critical factor. In those first diagnosed late in life (and presumably, initiating a protective gluten-free diet much later), detection of lymphoma may be much higher<sup>[8]</sup>.

Mechanisms involved in development of malignant disease in celiac disease require elucidation. Significantly, however, the small-intestinal mucosa that is involved, with changes caused by celiac disease, may still pathologically respond to a gluten-free diet, even after lymphoma is detected<sup>[4,5]</sup>. There are likely to be many potential confounding variables that alter the pathogenesis of lymphoma in a celiac population and influence risk measurements for various malignancies in different populations. These include genetic, geographic, infectious, and other epidemiological factors. Finally, the duration of gluten restriction and the degree of compliance with the gluten-free diet are specific factors that may be difficult to measure precisely, but seem crucial to malignant change in celiac disease.

### DIAGNOSTIC DIFFICULTIES

Most lymphomas are detected in the small intestine, usually the jejunum, but ileal localization may also occur<sup>[2]</sup>. Gastric or colonic lymphoma also occurs<sup>[2]</sup>. Lymphomas are usually ulcerating lesions, or stenosing and obstructing tumors<sup>[3]</sup>. Occasionally, the lymphoma may be multifocal or diffuse and localized only in the mucosa<sup>[5]</sup>. Often, concomitant nodal involvement is present<sup>[2]</sup>. The diagnosis may be especially difficult if small-intestinal (including duodenal) erosions and ulcers are present, as neoplastic cells may be more difficult to identify pathologically if significant superimposed inflammatory changes are present<sup>[4]</sup>. In some, benign

ulcers may lead to a mistaken diagnosis of Crohn's disease or a label of "ulcerative jejunoileitis"<sup>[4]</sup>. Some of these ulcers may contain frankly neoplastic lymphoma cells. Free perforation of the small intestine is a condition that should lead the clinician to a high level of suspicion of lymphoma in a patient with known or suspected celiac disease<sup>[10]</sup>. Even if there is a very high degree of suspicion, lymphoma may be notoriously difficult to diagnose, despite multiple endoscopic or suction small-intestinal biopsies<sup>[7]</sup>. In some patients that eventually prove to have lymphoma, even full thickness biopsies of the small intestine may not yield a definitive pathological diagnosis, especially if only mucosal disease is present. Additional tissue may be helpful for immunohistochemical labeling or PCR may be helpful in showing an altered binding pattern of antigen expression or a monoclonal cell population.

## TYPES OF LYMPHOMA

Lymphoma may be classified based on pathological and immunophenotypical features. B-cell and T-cell lymphomas both occur in celiac disease. However, detection of a T-cell type more often leads to suspicion of underlying celiac disease. Primary intestinal T-cell lymphoma is recognized under the WHO classification as enteropathy-associated T-cell lymphoma (ETL or EATL). They are very uncommon and represent an estimated 5% of all gastrointestinal lymphomas<sup>[3,11]</sup>. Previously, these were thought to be histiocytic in origin (and labeled malignant histiocytosis) but their origin now appears to be from T cells, specifically intra-epithelial lymphocytes<sup>[3,11]</sup>. In celiac disease (without lymphoma), the intra-epithelial lymphocytes express the following antigens (among others): surface CD3 and CD8. In a subset of patients that seem clinically refractory to a gluten-free diet, intra-epithelial lymphocytes have a different form of T-cell phenotypic expression: CD3 shows intra-cytoplasmic expression while CD8 expression is absent. Some believe this may reflect a specific form of refractory celiac disease (type 2) with a poor prognosis and a possible precursor lesion for the development of lymphoma<sup>[12-15]</sup>.

Even lymphomas with T-cell immunophenotypic features have been detected in extra-intestinal sites, which complicates the clinical course of celiac disease. These may be very rare and include lymphoma diffusely involving the liver and spleen (i.e. hepatosplenic type T-cell lymphoma) without evidence of small-intestinal involvement<sup>[16]</sup>, or lymphoma in other embryologically related or gut-derived sites, such as the thyroid gland or broncho-pulmonary and pleural sites<sup>[17]</sup>.

Recent studies have also provided evidence for an increased risk of other lymphoma types. In a pathological review of tumor materials from celiac disease patients, there was an apparent aggregation of autoimmune disorders, female sex and B-cell lymphoma<sup>[18]</sup>. More than double the risk for B-cell lymphoma was recorded, with

the most common type classified as a diffuse large B-cell lymphoma. In the same study, T-cell lymphomas had an approximately 50-fold risk along with a poorer prognosis (reflected in mean survival time after diagnosis and 5-year survival rate)<sup>[18]</sup>.

Recent studies have also evaluated risk of lymphoma in celiac disease. While the risk of lymphoma in celiac disease, especially of the T-cell type, is increased, the risk appears not to be as significant. The relative risk has been estimated to be close to 3 and likely is lower in clinically silent disease<sup>[19]</sup>.

## OTHER GASTROINTESTINAL CANCER

Also intriguing are studies related to malignant disease elsewhere in the gastrointestinal tract. Small-bowel adenocarcinoma is increased in celiac disease. Normally, this is a rare tumor. Some have suggested that this carcinoma may be related to an adenoma-carcinoma sequence<sup>[2]</sup>, but the risk of duodenal adenoma may not be increased in celiac disease<sup>[20]</sup>. Most patients appear to present with proximal small-intestinal localization, usually with small-bowel obstruction or bleeding. If complete surgical resection of a small-intestinal adenocarcinoma can be accomplished, the prognosis is better than if lymphoma is present<sup>[21]</sup>.

Some European studies have shown that there may be an increased risk of esophageal and pharyngeal carcinoma<sup>[1,22]</sup>. However, these findings have not been confirmed in America. In one report<sup>[8]</sup>, only a single terminal hypopharyngeal squamous cell carcinoma was detected in a celiac disease patient with lymphoma. No esophageal or gastric cancer was detected, despite repeated endoscopic studies during the course of diagnosis and treatment of celiac disease. Interestingly, however, Barrett's esophagus, a known precursor lesion of esophageal adenocarcinoma was frequently detected<sup>[8]</sup>. It may be that exposure to different environmental factors in different geographic areas or other confounding variables are important in cancer etiology and pathogenesis in celiac disease.

In a population-based cohort of celiac disease patients, overall colorectal cancer risk was marginally increased, owing to an increased risk in the ascending and transverse colon<sup>[23]</sup>, but not in dermatitis herpetiformis<sup>[23]</sup>. However, others have noted that colorectal cancer may not be increased<sup>[9]</sup>, especially in celiac disease patients with a diagnosis established late in life<sup>[9]</sup>. Possibly, untreated celiac disease is protective; dietary fat or fat soluble agents, including hydrocarbons or other putative co-carcinogens, may be implicated in the pathogenesis of colon cancer if poorly absorbed and rapidly excreted. Alternatively, immunological changes (e.g. increased intraepithelial lymphocytosis) in celiac disease may inhibit the development of epithelial malignancies at other gastrointestinal sites. Additional studies are needed to further clarify this information for celiac disease patients.

## TREATMENT

Treatment of lymphoma associated with celiac disease to date has not substantially differed from lymphoma in the absence of celiac disease, and generally involves a combination of surgical treatment, radiation and chemotherapy. Most believe that the best treatment results occur in those diagnosed early<sup>[24]</sup>. Biological agents are also being evaluated.

In newly diagnosed lymphoma patients with chronic diarrhea and weight loss, underlying celiac disease should be excluded, preferably prior to lymphoma treatment (since both radiation and chemotherapy may structurally alter the small intestine), because concomitant recognition of celiac disease may have important nutritional implications.

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REVIEW

## Capsule endoscopy

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### Abstract

Capsule endoscopy (CE) is a simple, safe, non-invasive, reliable technique, well accepted and tolerated by the patients, which allows complete exploration of the small intestine. The advent of CE in 2000 has dramatically changed the diagnosis and management of many diseases of the small intestine, such as obscure gastrointestinal bleeding, Crohn's disease, small bowel tumors, polyposis syndromes, *etc.* CE has become the gold standard for the diagnosis of most diseases of the small bowel. Lately this technique has also been used for esophageal and colonic diseases.

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**Key words:** Capsule endoscopy; Small intestine; Gastrointestinal hemorrhage; Crohn's disease; Gastrointestinal neoplasms; Intestinal polyposis

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### INTRODUCTION

A few years ago, the assessment of small bowel pathology was a major dilemma, especially when it came to the management of obscure gastrointestinal bleeding. Evaluation of the patients was frequently unsatisfactory because of the inability to completely visualize the small bowel mucosa with the available endoscopic and

radiological techniques. Capsule endoscopy (CE) was launched at the beginning of this millennium and since then has had a very important impact on managing obscure gastrointestinal bleeding and many other small bowel diseases.

### CAPSULE ENDOSCOPY

Until a few years ago, the small bowel was an organ which was very difficult to explore with the available endoscopic, radiological and nuclear medicine techniques. In routine practice, only the last few centimeters of the ileum were accessible to retrograde visualization by ileocolonoscopy. Exploration from the proximal side by push, sonde or intraoperative enteroscopy were invasive procedures that did not always allow us to visualize the lesions in the small bowel<sup>[1]</sup>. Sonde enteroscopy had been abandoned in the 90's because it was a tedious technique (long duration of the procedure) and it had several technical limitations. Push enteroscopy is limited by the depth of insertion of the scope and is poorly tolerated. Intraoperative enteroscopy is the most effective of these techniques, but it is the most invasive with a significant percentage of adverse side effects<sup>[2]</sup>.

With wireless CE we can provide a simple, safe, non-invasive, reliable procedure, well accepted and tolerated by the patient, which has revolutionized the study of the small bowel. This technique evaluates endoscopically, with high resolution images, the whole small bowel, avoiding any sedation, surgery or radiation exposure<sup>[2]</sup>.

Currently, CE is recommended as a third stage examination, after negative gastroscopy and colonoscopy in patients with obscure gastrointestinal bleeding. Also many studies have established, with a growing body of evidence, that this technique is cost-effective in other clinical situations, such as detection of small bowel lesions in Crohn's disease in patients in which other methods have failed to provide a diagnosis, non steroidal anti-inflammatory drug enteropathies, celiac disease, small bowel polyposis syndromes and small bowel tumors<sup>[2]</sup>. Other possible indications are HIV patients with gastrointestinal symptoms<sup>[3]</sup>, malabsorptive syndromes other than celiac disease<sup>[4]</sup>, Henoch-Schonlein purpura<sup>[5]</sup>, patients with small bowel transplants<sup>[6]</sup> and with intestinal graft *versus* host disease, particularly in monitoring the response to immunosuppressive therapy<sup>[7]</sup>.

The acquired knowledge of the wide range of lesions that can be found in the small bowel, encouraged the



implementation of some diagnostic and therapeutic techniques, such as double balloon enteroscopy, MRI-enteroclysis and CT-enteroclysis<sup>[2]</sup>.

The capsule endoscope is a disposable, small, swallowable, wireless, miniature camera which allows us to get a direct visualization of the gastrointestinal mucosa<sup>[8]</sup>. The initial capsule endoscope was developed by Given Imaging (Yoqneam, Israel) and approved in Europe by the European Medicines Agency and in the United States by the Food and Drug Administration in 2001<sup>[8]</sup>. This technique is available in over 4500 gastrointestinal centers throughout the world.

The capsule which measures only 11 mm × 26 mm and weighs 3.7 g, holds a metal oxide semiconductor imaging-chip video camera, 6 white light-emitting diode illumination sources, 2 silver-oxide batteries and a radio telemetry transmitter. The image field is 140 degrees, magnification is × 8 and the depth of view is 1 to 30 mm<sup>[9,10]</sup>.

Before the capsule is swallowed, 8 skin antennas are taped to the patient's anterior abdominal wall and connected to the hard drive. After an overnight fast, the patient swallows the capsule with a few sips of water, then the capsule is passively moved along by peristalsis. Two hours after ingestion, the patient is allowed to drink, while eating is allowed after 4 h. During the procedure the patient may carry on with his daily activities<sup>[11]</sup>.

The camera is activated by removal of the capsule from its magnetic holder and takes 2 images per second and transmits these by means of radio frequency to a sensor array placed on the patient's abdomen and from here to a recording device in a belt that the patient wears for the duration of the battery life (8 h). The use of the real time viewer may shorten procedures, as the patient can be disconnected once the cecum is visualized<sup>[11]</sup>.

After those 8 h, the sensor array and recorded data are removed and the recorded images are downloaded to the computer. It takes on average 40-60 min to read these images<sup>[3,12]</sup> and since it is very time-consuming, one possible cost-effective strategy could be the use of expert nurse endoscopists to select images. Some studies have shown that highly motivated nurses and gastrointestinal residents trained to read CE can detect clinically significant lesions at a similar rate to physicians<sup>[13-15]</sup>. Since its development, additional support systems have been added to the software to assist the reader, such as localization capability, suspected blood indicator, a multi-viewing feature and quick view modality<sup>[3]</sup>.

The capsule is excreted with the feces, usually within 24 to 48 h<sup>[16]</sup>. CE is usually performed as an outpatient procedure. The presence of intestinal contents or a motility disorder may cause the incomplete visualization of the intestinal mucosa. Several studies have examined the possibilities of improving bowel cleanliness and shortening transit time by means of different medications and different fasting periods. Nevertheless, small bowel preparation is still a controversial issue<sup>[6]</sup>. We have participated in a prospective multicenter randomized trial which has shown that bowel preparation with different laxatives does not improve the visualization of the small intestine<sup>[17]</sup>.

The main contraindication to performing CE is the suspicion or knowledge of an obstruction in the gastrointestinal tract.

The retention of the device is the main complication of the procedure and is defined when CE remains in the digestive tract for a minimum of 2 wk<sup>[18]</sup>. The frequency of this problem varies, depending mostly on the clinical indication for CE, and ranges from 0% in healthy subjects, to 1.5% in patients with obscure gastrointestinal bleeding, to 5% in patients with suspected Crohn's disease<sup>[6]</sup> and to 21% in patients with intestinal obstruction<sup>[3]</sup>. At present CE has some technical limitations: it cannot be used to obtain biopsy specimens or for endoscopic treatment and it cannot be controlled remotely<sup>[8]</sup>. CE has also some clinical limitations which are problems in sizing and locating small bowel lesions<sup>[2]</sup>, a possible false-negative CE result, due to the fact that the global miss rate is about 11%, ranging from 0.5% for ulcerative lesions to 18.9% for neoplastic disease and the fact that sometimes we can get findings of uncertain relevance in healthy subjects<sup>[8]</sup>. Another drawback is that in almost 20% of procedures the capsule does not reach the cecum while it is active<sup>[11]</sup>.

Since its development, more than 650 000 capsules have been swallowed worldwide<sup>[19]</sup> and more than 1000 peer-reviewed publications have appeared in the medical literature. The most important gastrointestinal societies have published guidelines about its use (ASGE<sup>[20]</sup>, ESGE<sup>[21]</sup>, BSG<sup>[22]</sup>).

In latter years, breakthrough developments in CE technology have enabled the direct visualization of the upper<sup>[23,24]</sup> and lower segments<sup>[25,26]</sup> of the gut using specifically designed capsules.

In recent issues of this journal we have coordinated the publication of several papers, some covering the latest advances in this field, presenting the use of CE in diagnosis of gastrointestinal bleeding<sup>[27]</sup>, inflammatory bowel disease<sup>[28]</sup>, celiac disease<sup>[29]</sup>, neoplastic disease<sup>[30]</sup>, non-steroidal anti-inflammatory drugs-enteropathy and rare intestinal diseases<sup>[31]</sup>, and also its use in pediatric patients<sup>[32]</sup>. Others studied the value of intestinal preparation before CE<sup>[33]</sup>, CE use in the colon<sup>[34]</sup> and esophagus<sup>[35]</sup>, and the Patency and Agile<sup>[36]</sup> capsules, and finally another paper was about the future of the CE<sup>[37]</sup>.

I wish to emphasize here that we have been very successful in convincing some of the most important groups working in this area to write the above-mentioned papers.

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## Endoscopic ultrasound elastography for evaluation of lymph nodes and pancreatic masses: A multicenter study

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### Abstract

**AIM:** To evaluate the ability of endoscopic ultrasound (EUS) elastography to distinguish benign from malignant pancreatic masses and lymph nodes.

**METHODS:** A multicenter study was conducted and included 222 patients who underwent EUS examination with assessment of a pancreatic mass ( $n = 121$ ) or lymph node ( $n = 101$ ). The classification as benign

or malignant, based on the real time elastography pattern, was compared with the classification based on the B-mode EUS images and with the final diagnosis obtained by EUS-guided fine needle aspiration (EUS-FNA) and/or by surgical pathology. An interobserver study was performed.

**RESULTS:** The sensitivity and specificity of EUS elastography to differentiate benign from malignant pancreatic lesions are 92.3% and 80.0%, respectively, compared to 92.3% and 68.9%, respectively, for the conventional B-mode images. The sensitivity and specificity of EUS elastography to differentiate benign from malignant lymph nodes was 91.8% and 82.5%, respectively, compared to 78.6% and 50.0%, respectively, for the B-mode images. The kappa coefficient was 0.785 for the pancreatic masses and 0.657 for the lymph nodes.

**CONCLUSION:** EUS elastography is superior compared to conventional B-mode imaging and appears to be able to distinguish benign from malignant pancreatic masses and lymph nodes with a high sensitivity, specificity and accuracy. It might be reserved as a second line examination to help characterise pancreatic masses after negative EUS-FNA and might increase the yield of EUS-FNA for lymph nodes.

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**Key words:** Endoscopic ultrasound; Elasticity coefficient; Elastography; Pancreatic mass; Lymph node

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## INTRODUCTION

A major limitation of endoscopic ultrasound (EUS) examination is its limited capacity to determine the exact nature of a lesion. Differential diagnosis between benign and malignant lymph nodes and focal pancreatic masses based on the EUS appearance is difficult and frequently requires EUS-guided fine needle aspiration (EUS-FNA) for confirmation of malignancy<sup>[1-4]</sup>.

Elastography has recently been presented as a novel technique that can be applied during ultrasound (US) examination to assess and measure tissue elasticity. Knowing that malignant tissues are generally harder than normal surrounding tissue, elastography might provide interesting clinical information to help distinguish benign from malignant tissue based on their specific tissue consistency. Clinical research has shown promising results in differentiating between benign and malignant tissue in the thyroid gland<sup>[5]</sup>, breast<sup>[6-8]</sup>, prostate<sup>[9,10]</sup> and to assess liver fibrosis<sup>[11-14]</sup>. Recently, elastography has also been introduced during EUS examination<sup>[15-19]</sup>. The current study is a continuation of previous research<sup>[15]</sup> to validate the potential role of elastography in distinguishing benign from malignant lymph nodes and focal pancreatic lesions in a large retrospective trial. The aim of this multicenter study was to classify lymph nodes and pancreatic masses during EUS examination as benign or malignant based on the real time (qualitative) elastography patterns and to compare the results with a classification based on the conventional B-mode EUS images and with the final diagnosis obtained by EUS-FNA and/or by surgical pathology.

## MATERIALS AND METHODS

### *Patients, procedure and examination technique*

Every patient ( $n = 222$ ) who underwent EUS examination with evaluation of a pancreatic mass ( $n = 121$ ) or lymph nodes ( $n = 101$ ), between October 2006 and February 2007, was included. The study was conducted in seven different centers throughout Europe. Only one lesion per patient was examined and by one single endoscopist per center. Each center started the study after six months experience of EUS elastography. The EUS examinations were performed with conventional linear EUS probes (Pentax EG38-UT and EG38-70UTK, Hamburg, Germany). The examined lesion was first classified as benign or malignant based on the conventional B-mode images. Subsequently, elastography was carried out in real time using a commercially available module incorporated into the Hitachi EUB-8500 system (Hitachi Medical Systems Europe, Zug, Switzerland). The technology measures the degree of tissue deformation after compression as an indicator for the stiffness of tissue. This compression during EUS examination is naturally obtained by arterial pulsations and respiratory movements. Detailed reviews on the technical aspects of elastography have been previously published<sup>[15,20,21]</sup>. The sample area was adjusted to the region of interest and the suitability of the elastographic signal was indicated

by a numeric scale within the image. Tissue elasticity was shown superimposed on the conventional B-mode EUS image by colors reflective of stiffness. Hard tissue areas were marked with blue, intermediate areas with green, medium soft areas with yellow and soft areas with red. Elastographic and B-mode images were displayed simultaneously side by side. The complete spectrum from blue to red was applied to each elastographic image and represented the graduation of relative elasticity within the sample area. Elastographic images were interpreted during the examination and a 60 s video loop was recorded for an interobserver study. After assessing the elastographic images, EUS-FNA was performed in all cases for clinical reasons using a 22-gauge needle (Wilson-Cook Medical, Winston-Salem, North Carolina). The technique of EUS-FNA is described elsewhere<sup>[22]</sup>. In all participating centers, the specimen were examined using the monolayer cytology technique<sup>[23]</sup>. An on-site pathologist was present in only four centers during the examination. In the remaining centers, the endoscopist assessed the sample to ensure sufficient tissue was obtained based on the presence of tissue filaments in the conservation solution and repeat punctures were performed if necessary. The pathologist was blinded to the elastography results. The final diagnosis was based on histology obtained by EUS-FNA and surgical specimen when available. If EUS-FNA was found to be negative (after at least one repeat examination), a 12 mo clinical and imaging follow-up was carried out in the absence of surgical specimens. The following parameters were recorded in a protocol: the classification as benign or malignant based on the B-mode images, the elastography score based on the elastographic pattern and the classification as benign or malignant based on this pattern and the final result based on histology.

### *Scoring system*

The elastographic images were scored according to elastographic patterns based on previous research<sup>[15]</sup>: a score equal to 1 was assigned when the image showed a homogenous soft tissue area (green) corresponding to normal tissue (Figure 1), a score equal to 2 when the image indicated heterogenous soft tissue (green, yellow, and red) corresponding to fibrosis or inflammatory tissue (Figure 2A and B), a score equal to 3 when the image displayed mixed colors or a honeycombed elastography pattern indicative of mixed hard and soft tissue making the interpretation difficult (Figure 3), a score equal to 4 when the image displayed a small soft (green) central area surrounded by mainly hard (blue) tissue corresponding to a malignant hypervascularized lesion (Figure 4) and a score equal to 5 was assigned to lesions representing mainly hard (blue) tissue with areas of heterogeneous soft tissue (green, red) representing zones of necrosis in an advanced malignant lesions (Figure 5). For the study purpose and to facilitate the use in clinical practice, we subsequently classified an elastography score equal to 1 and 2 as: (A) representing normal tissue or a benign



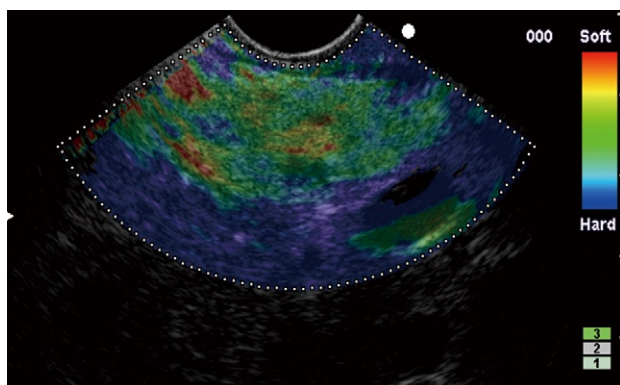


Figure 1 Elastographic image showing homogenous soft tissue corresponding to normal tissue.

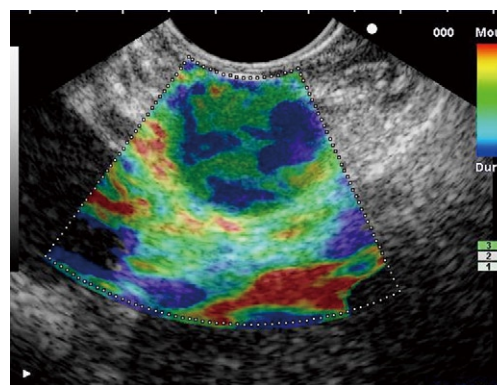


Figure 3 Elastographic image showing mixed hard and soft tissue ("honeycombed pattern") making the interpretation difficult.

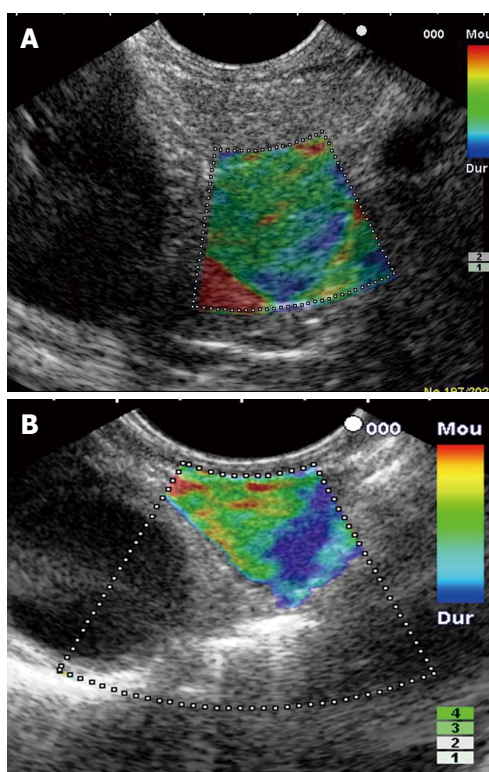


Figure 2 Elastographic image. A: Heterogenous soft tissue corresponding to fibrosis (benign nodule in patient who had an acute pancreatitis 2 mo before); B: Heterogenous soft tissue corresponding to inflammatory tissue (benign lymph node).

tumor; a score equal to 4 and 5 was classified as (C) representing a malignant lesion; and a score equal to 3 was classified as (B) which represented tissue difficult to classify as benign or malignant based on the elastographic pattern. However a score equal to (B) was considered as malignant for statistical analysis (Figure 6).

### Statistical analysis

The results are presented as means plus or minus standard deviation or as medians with ranges, depending on the data distribution. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were calculated as appropriate.

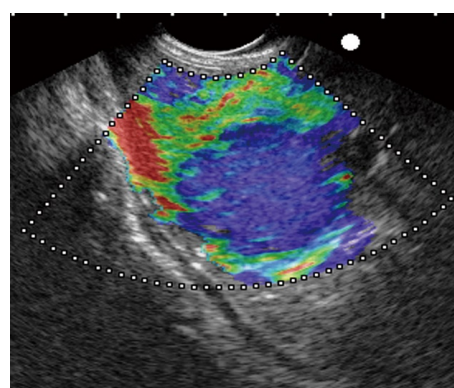


Figure 4 Elastographic image showing mainly hard tissue with a small soft central area corresponding to a malignant hypervascularized lesion (pancreatic neuroendocrine tumor).

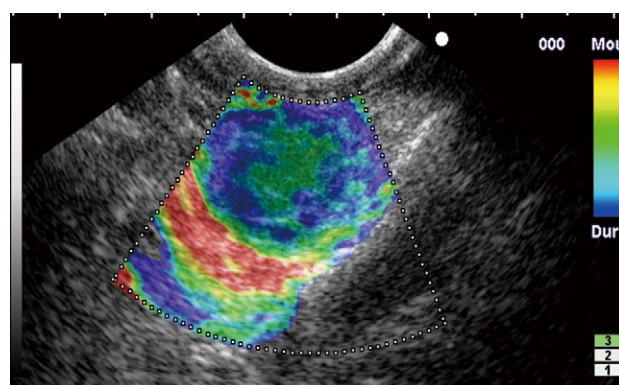


Figure 5 Elastographic image showing mainly hard tissue with areas of heterogeneous soft tissue corresponding to an advanced malignant lesion with necrotic areas (pancreatic adenocarcinoma).

An interobserver study was performed on a statistically representative and blinded selection of 30 videos (15 of a pancreatic mass and 15 of a lymph node). These videos were each evaluated by five endoscopists experienced in EUS and elastography. The elastographic images were scored with a whole number from 1 to 5 using the previous described criteria, constituting an ordered variable. The agreement between two different examiners was measured by an adapted kappa coefficient. The



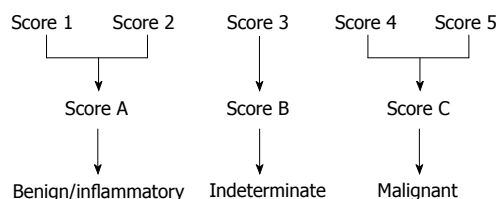


Figure 6 Elastography score.

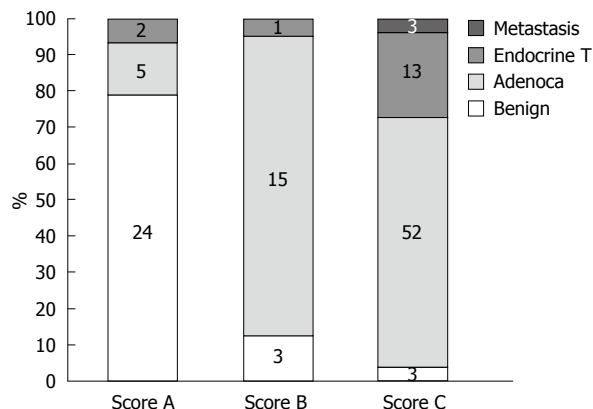


Figure 7 Pancreatic masses: elastography score and final histology.

interobserver study also evaluated the agreement between two examiners to classify the examined tissue as benign or malignant. The result is a binary variable (“malignant” or “benign”). The results being “inconclusive” were considered as missing data for the calculation of the agreement. This agreement was measured by the Cohen kappa coefficient.

## RESULTS

### Pancreatic masses

One hundred and twenty-one patients (77 M and 44 F, mean age 63 years) underwent EUS examination with elastography for evaluation of a pancreatic mass (mean diameter 29.5 mm, range 7-80 mm). The masses were located in the pancreatic head ( $n = 48$ ), isthmus ( $n = 17$ ), body ( $n = 29$ ), tail ( $n = 13$ ) and uncinate process ( $n = 14$ ). No complications occurred during the study. The final histological assessment was based on the FNA results in 82 cases and on surgical pathology in 39 cases. The final diagnosis of the pancreatic masses included pancreatic adenocarcinoma ( $n = 72$ ), malignant endocrine tumor ( $n = 16$ ), benign endocrine tumor ( $n = 2$ ), benign chronic pancreatitis related nodules ( $n = 28$ ) and pancreatic metastasis ( $n = 3$ ) (Figure 7). The elastographic images were interpreted as benign (score 1 + 2 = A) in 31 cases, indeterminate (score 3 = B) in 19 cases and malignant (score 4 + 5 = C) in 71 cases. Considering the “indeterminate” result equal to score (B) as malignant, the calculated sensitivity, specificity, positive and negative predictive values of EUS elastography to differentiate benign from malignant pancreatic masses were, respectively, 92.3%, 80.0%, 93.3% and 77.4% with a global accuracy of this new technology of 89.2%. The

Table 1 Pancreatic masses: classification as benign or malignant based on EUS elastography, conventional B-mode imaging and the final diagnosis based on histology

		Histology	
		Malignant (n)	Benign (n)
Elastography/ conventional B-mode	Malignant	84/85	6/27
	Benign	7/7	24/2

Elastography: sensitivity =  $84/91 = 92.3\%$ , specificity =  $24/30 = 80\%$ , accuracy =  $108/121 = 89.2\%$ ; Conventional B-mode: sensitivity =  $85/92 = 92.3\%$ , specificity =  $2/29 = 68.9\%$ , accuracy =  $87/121 = 71.9\%$ .

calculated sensitivity, specificity, positive and negative predictive values of conventional B-mode images to differentiate benign from malignant pancreatic masses were, respectively, 92.3%, 68.9%, 75.8% and 22.2% with an accuracy of 71.9% (Table 1).

### Lymph nodes

One hundred and one patients (56 M and 45 F, mean age 61.1 years) underwent EUS examination of a lymph node for staging of lung cancer ( $n = 25$ ), oesophageal carcinoma ( $n = 25$ ), gastric cancer ( $n = 13$ ), pancreatic cancer ( $n = 13$ ), for suspicion of lymph node relapse of kidney cancer ( $n = 2$ ) and of breast cancer ( $n = 8$ ). EUS examination was also performed for evaluation of isolated lymph nodes ( $n = 15$ ). Lymph nodes (mean diameter 20.1 mm, range 7-50 mm) were located in the mediastinum ( $n = 51$ ), in the cervical area ( $n = 4$ ), in the celiac or mesenteric area ( $n = 44$ ), and in the perirectal space ( $n = 2$ ). No complications occurred during the study. The final histological assessment was based on FNA and classified the lymph nodes as malignant in 57 cases, including metastasis of an adenocarcinoma ( $n = 35$ ), metastasis of a squamous cell carcinoma ( $n = 13$ ), metastasis of an endocrine tumor ( $n = 3$ ), metastasis of a melanoma ( $n = 1$ ), lymphomas ( $n = 5$ ), and benign in 44 cases (including three cases of sarcoidosis) (Figure 8). The elastographic images were interpreted as benign (score 1 + 2 = A) in 38 cases, indeterminate (score 3 = B) in 10 cases and malignant (score 4 + 5 = C) in 53 cases. Considering the “indeterminate” result equal to score (B) as malignant, the calculated sensitivity, specificity, positive and negative predictive values were, respectively, 91.8%, 82.5%, 88.8% and 86.8% with a global accuracy of this new technology of 88.1%. The calculated sensitivity, specificity, positive and negative predictive values for the conventional B-mode images were respectively 78.6%, 50.0%, 70.5% and 60.6% with an accuracy of 67.3% (Table 2).

### Inter-observer study

The kappa coefficient of the sonoelastography score for pancreatic masses was 0.524, for the lymph nodes 0.519, and 0.520 for all cases confound.

The kappa coefficient for the differentiation between benign and malignant tissue was 0.785 for the pancreatic masses, 0.657 for the lymph nodes and 0.725 for all cases confound.

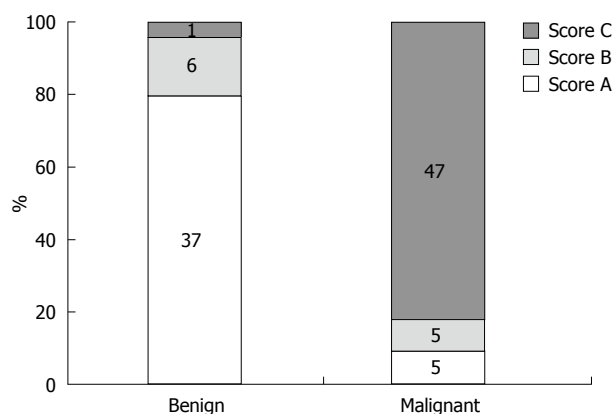


Figure 8 Lymph nodes: elastography score and final histology.

Table 2 Lymph nodes: classification as benign or malignant based on EUS elastography, conventional B-mode imaging and the final diagnosis based on histology

		Histology	
		Malignant (n)	Benign (n)
Elastography/ conventional B-mode	Malignant	51/48	2/20
	Benign	10/13	38/20

Elastography: sensitivity =  $51/61 = 83.6\%$ , specificity =  $38/40 = 95\%$ , accuracy =  $89/101 = 88.1\%$ ; Conventional B-mode: sensitivity =  $48/61 = 78.6\%$ , specificity =  $20/40 = 50\%$ , accuracy =  $68/101 = 67.3\%$ .

## DISCUSSION

The aim of this multicenter study was to evaluate the ability of EUS elastography to distinguish benign from malignant focal pancreatic masses and lymph nodes and to compare the results with the conventional B-mode images and final histology.

Our study shows that EUS elastography has high sensitivity, specificity and accuracy and a much higher specificity than conventional B-mode images to differentiate between benign and malignant focal pancreatic lesions. Using our current scoring system, 15.7% of the cases still obtain an elastography score equal to 3 indicating tissue difficult to classify as benign or malignant. However, 84% of these cases with an elastography score equal to 3 turned out to be malignant and we believe that the soft tissue parts of these focal lesions on elastography represent necrotic areas in an adenocarcinoma ( $n = 15$ ) or a hypervascularised area in an endocrine tumor ( $n = 1$ ). Hence, an elastography score equal to 3 should be considered as malignant, in our opinion.

There were seven false negative cases (five adenocarcinoma and two neuroendocrine tumors) that may be explained in a similar way: the presence of abundant necrotic or vascular tissue resulted in an elastographic pattern mainly consisting of soft tissue. By contrast, the false positive cases in our study ( $n = 6$ ) might represent patients with (early) chronic pancreatitis having areas of hard fibrotic nodules. Unfortunately, lack of surgical specimens in these patients cannot confirm

this hypothesis. However, in a recent publication by Janssen *et al.*<sup>[16]</sup>, the elastographic patterns of the normal pancreas and the pancreas affected by inflammatory or focal disease were studied. They concluded that elastography does not distinguish between chronic pancreatitis and tumors because of their similar fibrous structure. This implies that EUS elastography will not be able to help target suspicious lesions and improve the rather low accuracy of EUS-FNA in patients with chronic pancreatitis.

In distinguishing benign from malignant focal pancreatic lesions, EUS elastography does not replace tissue confirmation and we believe that EUS elastography should not be used as a first line examination in the evaluation of focal pancreatic lesions. However, when facing (repeated) negative EUS-FNA or technical problems in performing EUS-FNA, the interpretation of the EUS elastographic images could help orientate the diagnosis and influence the decision making for surgery when the lesion is suspicious on elastography, or justify a follow-up when the elastographic images are in favour of a benign lesion.

Our data also shows that EUS elastography has high sensitivity, specificity and accuracy in distinguishing benign from malignant lymph nodes and seems to be superior to conventional B-mode images. Whether the false negative and false positive cases in this study are due to the presence of necrotic and fibrotic areas in lymph nodes, respectively, is less certain. Our results confirm comparable results obtained by Săftoiu *et al.*<sup>[18]</sup> using similar elastography pattern criteria to differentiate benign from malignant lymph nodes in 42 patients with a reported sensitivity, specificity and accuracy of, respectively, 91.7%, 94.4% and 92.86%. The role of EUS elastography to distinguish benign from malignant lymph nodes should be considered as complementary to other imaging techniques rather than a replacement for tissue confirmation. Based on a high PPV, EUS elastography might help in selecting more suspicious lymph nodes for tissue sampling, especially in patients presenting multiple lymph nodes, such as in oesophageal or lung cancer. Based on a high NPV, it might be used to reduce the number of unnecessary biopsies. As for focal pancreatic lesions, EUS elastography might offer an alternative for differential diagnosis in the case of negative EUS-FNA of a lymph node, as well as in situations where EUS-FNA is not possible (technical problems, interposed malignant tissue or interposed vascular structure).

The current results are different from the results obtained during our previous research<sup>[15]</sup>. In this previous study, EUS elastography was shown to have a sensitivity of 100% and specificities of 67% and 50% for diagnosing malignant pancreatic masses and lymph nodes, respectively. Although false positive results in both study groups were reported, it should be recalled that the number of benign lesions in the previous study was relatively small.

For both pancreatic masses and lymph nodes, EUS elastography might also help in guiding the puncture in a non necrotic part of the suspicious lesion when necrotic

tissue is present, as in advanced cancer.

One of the main criticisms of EUS elastography is the variability of the elastographic images and the difficulty of interpretation<sup>[19]</sup>. However, our interobserver study showed a satisfying interobserver concordance for the differentiation between benign and malignant pancreatic masses and lymph nodes ( $\kappa = 0.725$ ).

In the absence of pathologic assesment of surgical specimens, we considered the EUS-FNA result as a gold standard. Although the specificity of EUS-FNA is close to 100%<sup>[24-28]</sup>, it has the potential to miss micro-invasion of malignancy into lymph nodes or to give false negative results for a necrotic pancreatic lesion. However, we consider it as representative of daily practice, particularly when it is combined with an adequate clinical and imaging follow-up period.

To overcome the difficulty in classifying the EUS elastography score equal to 3 or (B) as benign or malignant, we are currently evaluating the next generation of elastography software. This new software provides a quantitative histogram analysis of the elastographic images and has already proven to be useful in the evaluation of lymph nodes<sup>[18]</sup>.

The potential role of EUS elastography to help detect and differentiate submucosal tumors as well as any other solid masses situated nearby the gastrointestinal tract has still to be evaluated. The exact role of EUS elastography in patients manifesting symptoms suggestive of chronic pancreatitis with equivocal EUS (3 features or fewer) has still to be validated<sup>[29]</sup>.

EUS elastography is a new application in the field of the endosonography and seems to be able to differentiate benign from malignant lymph nodes and pancreatic lesions with a high sensitivity, specificity and accuracy. EUS elastography is superior compared to conventional B-mode imaging and the interobserver reproducibility is satisfying. The goal is not to replace tissue confirmation. Instead, the information obtained by EUS elastography should be considered as complementary to the conventional EUS imaging. It should be reserved as a second line examination to orientate further decision making after repeat negative EUS-FNA for pancreatic lesions. It may increase the yield of FNA and reduce the number of unnecessary biopsies when assessing lymph nodes. However, further research is necessary to improve our current elastography scoring system. The second generation of elastography software providing quantitative analysis of tissue elasticity might be able to increase the accuracy of this technique.

## COMMENTS

### Background

Elastography has recently been presented as a novel technique that can be applied during ultrasound examination to assess and measure tissue elasticity. Clinical research has shown promising results in differentiating between benign and malignant tissue in the thyroid gland, breast, prostate and to assess liver fibrosis.

### Research frontiers

endoscopic ultrasound (EUS) elastography is a new application in the field of the endosonography and seems to be able to differentiate benign from

malignant lymph nodes and pancreatic lesions with a high sensitivity, specificity and accuracy. EUS elastography is superior compared to conventional B-mode imaging and the interobserver reproducibility is satisfying.

### Innovations and breakthroughs

In distinguishing benign from malignant focal pancreatic lesions, EUS elastography does not replace tissue confirmation and should not be used as a first line examination in the evaluation of focal pancreatic lesions. However, when facing (repeated) negative EUS-guided fine needle aspiration (EUS-FNA) or technical problems to perform EUS-FNA, the interpretation of the EUS elastographic images could help orientate the diagnosis and influence the decision making for surgery when the lesion is suspicious on elastography, or justify a follow-up when the elastographic images are in favour of a benign lesion.

### Applications

The goal is not to replace tissue confirmation. Instead, the information obtained by EUS elastography should be considered as complementary to the conventional EUS imaging. It should be reserved as a second line examination to orientate further decision making after repeat negative EUS-FNA for pancreatic lesions. It might increase the yield of FNA and reduce the number of unnecessary biopsies when assessing lymph nodes.

### Peer review

The importance of the research and the significance of the research contents are high. Presentation and readability of the manuscript is highly acceptable.

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BRIEF ARTICLES

## Use of mycophenolate mofetil in inflammatory bowel disease

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therapy, without the need for dose escalation. Further evaluation of MMF comparing it to conventional immunosuppressants is required.

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**Key words:** Inflammatory bowel disease; Mycophenolate mofetil; Therapy; Crohn's disease; Ulcerative colitis

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### Abstract

**AIM:** To assess the efficacy and safety of mycophenolate mofetil (MMF) prospectively in inflammatory bowel disease (IBD) patients intolerant or refractory to conventional medical therapy.

**METHODS:** Crohn's disease (CD) or ulcerative colitis/IBD unclassified (UC/IBDU) patients intolerant or refractory to conventional medical therapy received MMF (500-2000 mg *bid*). Clinical response was assessed by the Harvey Bradshaw index (HBI) or colitis activity index (CAI) after 2, 6 and 12 mo of therapy, as were steroid usage and adverse effects.

**RESULTS:** Fourteen patients (9 CD/5 UC/IBDU; 8M/6F; mean age 50.4 years, range 28-67 years) were treated and prospectively assessed for their response to oral MMF. Of the 11 patients who were not in remission on commencing MMF, 7/11 (63.6%) achieved remission by 8 wk. All 3 patients in remission on commencing MMF maintained their remission. Ten patients were still on MMF at 6 mo with 9/14 (64.3%) in remission, while of 12 patients followed for 12 mo, 8 were in remission without dose escalation (66.7%). Three patients were withdrawn from the MMF due to drug intolerance. There were no serious adverse events attributed due to the medication.

**CONCLUSION:** MMF demonstrated efficacy in the management of difficult IBD. MMF appeared safe, well tolerated and efficacious for both short and long-term

### INTRODUCTION

The natural history of both forms of the inflammatory bowel diseases (IBDs), Crohn's disease (CD) and ulcerative colitis (UC), is characterized by a lifelong course of remissions and relapses and a proportion of these patients are steroid refractory or develop steroid dependence requiring maintenance immunosuppression. The most commonly used immunomodulatory medications are azathioprine (AZA), or its metabolite 6-mercaptopurine (6MP). Approximately 10% of patients, however, will be intolerant of these drugs, resulting in their withdrawal and the need for an alternative immunomodulator<sup>[1]</sup>. Up to 50% of CD and 20% of UC patients will also develop a severe acute episode of their disease requiring hospitalization<sup>[2]</sup> and almost half of these patients will require rescue therapy or surgery<sup>[2,3]</sup>. In severe steroid refractory UC, remission may be achieved through the use of cyclosporine or infliximab, but despite continued maintenance therapy for these patients with AZA/6MP, over 65% will relapse by 12 mo and 30% will require colectomy<sup>[4]</sup>. Thus, despite the advent of new biological agents, used in combination with AZA/6MP, efficacy is not universal so the need for other immunomodulatory medications remains imperative.

Mycophenolate mofetil (MMF) is a powerful



immunosuppressant primarily indicated for prevention of solid organ transplantation rejection. It is an anti-metabolite with pharmacodynamic properties similar to AZA. MMF appears to be very safe and efficacious for this indication and is used as a first-line anti-rejection drug in many transplant centers. More recently, however, this immunosuppressant has been employed in the management of difficult IBD cases<sup>[5,6]</sup>. Its efficacy has primarily been assessed in small, uncontrolled case series with only a few small randomized trials<sup>[7-9]</sup>. They indicate that MMF may be effective in IBD, but its role is controversial. The problem arises primarily from observations that despite clinical remission and response achieved early in the course of treatment, a large proportion of patients ultimately flare and require biological agents or surgery. There is also a suggestion that the MMF dose needs to be increased over time in order to maintain an effect and some studies also suggest non-superiority of MMF to conventional immunosuppressants such as AZA<sup>[10,11]</sup>.

Most of the early studies on MMF were undertaken in patients with chronic active CD who failed, or were intolerant to, AZA, and demonstrated good efficacy<sup>[7,9]</sup>. These findings, however, were not supported by later studies, with either a low response or high relapse rate<sup>[5,8,12]</sup>. The rate of treatment discontinuation due to side effects was also high<sup>[6,12,13]</sup> and studies comparing MMF to AZA yielded conflicting and inconsistent results<sup>[7,10]</sup>. In one study MMF was identified to be more likely to be effective in AZA intolerant, rather than refractory patients, and was not inferior to AZA in the management of UC for the induction or maintenance of remission at 6 mo<sup>[11]</sup>. Another study with longer term outcomes evaluating MMF in a cohort of AZA resistant/intolerant patients, however, observed that although MMF was initially effective, relapses were common<sup>[14]</sup>.

This study presents our experience in the use of MMF in the treatment of IBD patients in the short and long term. We prospectively assessed the efficacy of MMF in both the induction and maintenance of remission in patients who were intolerant of AZA/6MP and had previously failed courses of either methotrexate (MTX), antibiotics and/or infliximab. We particularly examined the need for dose escalation over time in patients who initially responded to the MMF as this has been a criticism of its long-term efficacy.

## MATERIALS AND METHODS

All subjects were patients at the Centre for Inflammatory Bowel Diseases, Fremantle Hospital, which is a specialist IBD unit in a 450 bed tertiary institution that services the southern metropolitan region of Perth, Australia. Patients with IBD were classified as CD or ulcerative colitis/IBD unclassified (UC/IBDU) according to the "Montreal Classification" (a modification of the Vienna Classification). The diagnosis of IBD had to be definite, and was made in accordance with previously established criteria based upon clinical, endoscopic, histopathological

and radiological findings. The diagnoses of CD or UC/IBDU were exclusive of infective enterocolitis (excluded by stool microscopy and culture, bacterial and amoebic serology, acid-fast staining of biopsies and mycobacterial cultures), Behcet's disease and microscopic colitis. Patient demographics, disease status, infusion number, response and remission rates and adverse effects were recorded.

All patients were treated between Jan 2003 and July 2008. Patients treated with MMF received between 500 mg and 2000 mg twice a day with the dose optimized to maintain the white cell count (WCC) between 4 and  $6 \times 10^9/L$ , neutrophil count  $> 2.0 \times 10^9/L$  and lymphocyte count at or just below the normal range of  $1.1 \times 10^9/L$  without side effects. A clinical response to the MMF in CD patients was determined by a reduction in the Harvey Bradshaw index (HBI) of greater than or equal to 3, with a remission defined as a HBI less than 5 off steroids. A clinical response to the MMF in the UC/IBDU patients was defined as a reduction of 4 or more points in the colitis activity index (CAI) and remission was considered to be CAI of less than or equal to 4 off steroids. The response and remission rates after 8 wk of therapy and long-term response to treatment with MMF were assessed.

Serious adverse effects (SAE) were analyzed. Serious adverse effects are defined as any adverse drug experience occurring that results in death, life-threatening adverse event, persistent or significant disability/incapacity, required in-patient hospitalization, or prolonged hospitalization or congenital anomaly or birth defect.

## RESULTS

The primary indications for treatment were either steroid refractoriness, or dependence, and allergy, or intolerance, to AZA/6MP therapy. Patients were steroid dependant if they were unable to be withdrawn from steroids without a disease flare and patients were steroid refractory if they continued to suffer active inflammation whilst on steroids of 20 mg or greater per day. All patients with active disease were considered for treatment with MMF only after demonstrating failure of disease control or steroid dependency. Two CD and 1 UC/IBDU patients were in clinical remission at the time of commencing the MMF. One of these patients suffered from severe psoriasis in addition to her CD and was changed to MMF in consultation with the dermatologists in an attempt to control both the psoriasis and the CD. The second patient had undergone 3 terminal ileal resections with recurrent severe ileal inflammation occurring within 1 to 2 years after each surgery, but was allergic to AZA/6MP, while MTX and infliximab were ineffective. The third patient required 6MP to maintain remission, but was intolerant of this medication due to severe alopecia.

### Patient demographics

Fourteen patients (9 male, 5 female) were treated with MMF during the study period (Table 1). The ages at time of commencing the MMF ranged from 28-67 years (mean age  $50.4 \pm 12.9$  years). Nine patients suffered from CD

**Table 1** Demographics of the IBD patients using the Montreal classification

	CD patients <i>n</i> = 9	UC/IBDU patients <i>n</i> = 5
Gender: male	57.1% (8/14)	57.1% (8/14)
Age at diagnosis		
Mean $\pm$ SE (range)	38.6 $\pm$ 13.3 yr (19-54)	44.0 $\pm$ 12.7 yr (30-63)
A1- $\leq$ 16	0% (0/9)	0% (0/5)
A2-17-40	44.4% (4/9)	40% (2/5)
A3- > 40	55.6% (5/9)	60% (3/5)
Disease duration		
Mean (range)	10.4 yr (1-26)	9.8 yr (1-28)
Crohn's disease		
L1-terminal ileum	22.2% (2/9)	
L2-colon	33.3% (3/9)	
L3-ileocolonic	44.4% (4/9)	
L4-upper GI	11.1% (1/9)	
P-perianal	22.2% (2/9)	
B1-inflammatory	44.4% (4/9)	
B2-stricturing	33.3% (3/9)	
B3-perforating	22.2% (2/9)	
Ulcerative colitis/IBDU		
E1-proctitis		20% (1/5)
E2-left sided		40% (2/5)
E3-extensive		40% (2/5)
Raised CRP	55.6% (5/9)	80.0% (4/5)

and 5 had UC/IBDU. Of the CD patients, 77.8% (7/9) suffered from colonic inflammation, 22.2% (2/9) had ileal involvement alone, 11.1% (1/9) had jejunal CD, and 22.2% (2/9) suffered from perianal disease. Of the UC/IBDU patients, 2 had extensive colitis, while 2 suffered left-sided colitis and 1 patient had proctitis. The age of diagnosis was lower in the CD patients (mean 38.6  $\pm$  13.3 years, range 19-54 years) compared to the UC/IBDU patients (mean 44.0  $\pm$  12.7 years, range 30-63 years), but this was not statistically significant. Both the CD and UC/IBDU patient groups had similar disease duration at the time of the MMF therapy (mean 10.4 years and 9.8 years respectively). Four (44.5%) of the CD patients had previously undergone at least one surgery (1 subtotal colectomy, 2 small bowel resections and 1 total colectomy and ileal surgery). C-reactive protein (CRP) levels were also elevated in 5 of the 9 CD patients and 4 of 5 UC/IBDU patients prior to commencement of the MMF.

#### Current and previous medical therapy

Conventional therapies had been tried in all patients (Table 2). Surgical options had been discussed in detail and were considered to be either medically inappropriate at that stage, or were declined by the patient. Of the 9 CD patients, 88.9% (8/9) were on 5-aminosalicylic acid (5ASA) and 77.8% (7/9) were dependent on, or intolerant to, oral steroids. Antibiotic therapy with metronidazole and ciprofloxacin had been tried and was unsuccessful in 44.4% (4/9) of CD patients. All but 1 (88.9%) of the CD patients were allergic or intolerant to the use of AZA/6MP (drug fevers, severe vomiting requiring hospitalization, severe alopecia and hepatotoxicity). MTX was ineffective in 33.3% (3/9) with 44.4% (4/9) intolerant to, or refusing to take, this medication. Infliximab was ineffective and not continued

**Table 2** Medications taken by study patients at time of the commencement of MMF therapy

	CD patients <i>n</i> = 9	UC/IBDU patients <i>n</i> = 5
5-ASA		
Current	88.9% (8/9)	100% (5/5)
Steroids		
Current	55.5% (5/9)	100% (5/5)
Intolerant	22.2% (2/9)	0%
AZA/6MP		
Intolerant	88.8% (8/9)	100% (5/5)
Methotrexate		
Ineffective	33.3% (3/9)	N/A
Intolerant	11.1% (1/9)	
Refused	33.3% (3/9)	
Antibiotics		
Ineffective	33.3% (3/9)	N/A
Intolerant	11.1% (1/9)	
Infliximab		
Current	0%	0%
Ineffective	44.4% (4/9)	40% (2/5)
Intolerant	22.2% (2/9)	0%

N/A: Not applicable.

**Table 3** Response and remission rates at 8 wk and CRP levels with MMF therapy

	CD patients <i>n</i> = 9	UC/IBDU patients <i>n</i> = 5
Remission	66.7% (6/9)	80% (4/5)
Response	66.7% (6/9)	80% (4/5)
Intolerant	22.2% (2/9)	20% (1/5)
Ineffective	11.1% (1/9)	0% (0/5)
Raised CRP		
In responders	0% (0/6)	0% (4/5)
In non responders	33.3% (1/3)	100% (1/1)

in 44.4% (4/9) and 22.2% (2/9) were intolerant to its use (anaphylaxis and serum sickness).

Of the UC/IBDU patients, all were currently on both 5ASA and oral steroid therapy. All of these patients were intolerant to AZA/6MP therapy, while infliximab had been tried and was ineffective in 2 patients.

#### Efficacy at 8 wk

After 8 wk of MMF therapy, 63.6% (7/11) of patients (3 CD and 4 UC/IBDU) who were not in remission at commencement of MMF responded and went into remission, while 71.4% (10/14) went into remission or maintained clinical remission (Tables 3 and 4) as determined either by the HBI or CAI. The 2 patients who had AZA/6MP ceased and MMF commenced due to concurrent severe psoriasis and severe alopecia, maintained their disease remission. The other CD patient who was placed on MMF to prevent post-surgical recurrence was still in remission. All patients in remission at 8 wk also had normal CRP levels. Three of the 14 patients (2 CD and 1 UC/IBDU) were intolerant to MMF and took the medication for 1 mo or less. In only 1 patient was MMF ineffective after 8 wk of therapy, with the patient undergoing surgery 6 mo after commencing the MMF. The surgical pathology

Table 4 Individual patient data of disease extent, age at treatment, duration of MMF therapy and response

	Sex	Diagnosis	Age at diagnosis	Age at MMF	Indication for MMF	Disease extent	Duration of MMF (mo)	Response after 8 wk	Steroids continued at 8 wk
1	M	CD	27	28	Steroid dependant	Pancolitis	30	Remission	Ceased
2	M	CD	54	55	Steroid dependant	L Sided colitis/fistula	12	Remission	Ceased
3	F	CD	50	67	Severe Psoriasis	Ileocolonic disease	48	Remission	N/A
4	M	CD	41	41	Steroid dependant	Ileocolonic disease	12	Remission	Ceased
5	M	CD	53	63	Recurrent TI resections	Recurrent ileal disease	15	Remission	N/A
6	F	CD	32	58	Steroid intolerant	Colectomy/2x TI resection/ recurrent ileal disease	< 1	Intolerant	Continued
7	F	CD	19	28	Steroid dependant	Subtotal colectomy/recurrent ileal disease/fistula	< 1	Intolerant	Continued
8	M	CD	21	48	Steroid dependant	Ileal disease	6	Ineffective	Surgery
9	F	UC/IBDU	43	50	Steroid dependant	Pancolitis	12	Remission	Ceased
10	M	UC/IBDU	31	33	Steroid dependant	L Sided colitis	9	Remission	Ceased
11	M	UC/IBDU	53	64	Steroid dependant	L Sided colitis	22	Remission	Ceased
12	F	CD	50	50	Recurrent flares	Pancolitis	12	Remission	Ceased
13	M	UC/IBDU	63	63	Steroid dependant	Pancolitis	8	Remission	Ceased
14	F	UC/IBDU	30	58	Steroid dependant	Subtotal colectomy/proctitis	< 1	Intolerant	Continued

demonstrated chronic active inflammation and fibrosis of the previous ileocolonic anastomosis.

#### Efficacy at 6 mo

Of the 10 patients on MMF who responded or were in remission at 8 wk, all were still on MMF at 6 mo. Only one patient suffered a disease flare in that 6-mo period. This patient flared 10 wk after commencing the MMF and required further steroids and a single dose of infliximab to induce remission, but continued on the MMF with subsequent successful withdrawal of the steroids and no further need for infliximab therapy. None of the other patients required an increase in their dose of MMF over the 6-mo period in order to maintain their remission. The patient who was on the MMF for recurrent inflammation following previous terminal ileal resections for uncontrolled CD inflammation underwent a colonoscopy at 6 mo, which demonstrated no ileal or colonic CD inflammation.

#### Efficacy at 12 mo

Ten patients (Table 4) had been on MMF for more than 6 mo (mean  $18.1 \pm 12.1$  mo, max 48 mo) with 8 patients taking MMF for 12 mo or more. Of the 10 patients, 1 flared at 8 mo and was withdrawn from MMF due to lack of efficacy. One of the patients died 12 mo after commencing the MMF from an unrelated cause while in remission from his CD. One patient who flared after 30 mo of MMF was withdrawn and commenced on adalimumab with good effect. A total of 12 patients were followed for 12 mo or more and of these 8 were in remission (66.7%). All the 8 patients on MMF maintained their remission without the need for dose escalation.

#### Adverse effects

There was one serious adverse event (SAE) in this patient cohort. This patient died from decompensated alcoholic liver disease. He had previously denied any significant alcohol consumption on numerous occasions

and had been on a stable dose of MMF for over 10 mo. The patient presented to hospital jaundiced with ascites and blood results consistent with an acute hepatitis. The MMF was ceased and the patient was subsequently diagnosed with acute severe alcohol-induced hepatitis. His condition deteriorated over a 2-wk period and he died from liver failure. This SAE was considered to be 'unlikely related' to the MMF use. Adverse events that resulted in cessation of the medication occurred in 3 (21.4%) patients (2 CD and 1 UC/IBDU). These were GI disturbances (nausea and vomiting) and severe headaches. There were no other adverse events that required modification of the MMF dose.

## DISCUSSION

The treatment of refractory IBD has always been one of the most challenging aspects in the clinical practice of luminal gastroenterology. MTX has been the primary alternative therapy for CD patients who are treatment refractory or intolerant to AZA/6MP. Although MTX has demonstrated efficacy in CD, the rate of adverse events at the higher doses often required to achieve clinical response/remissions has limited its use<sup>[15]</sup>. At low doses, however, MTX is often ineffective<sup>[15]</sup> and definitely less effective than AZA/6MP<sup>[16]</sup> with longer-term studies demonstrating a frequent loss of efficacy over time<sup>[17]</sup>. A systematic review of 5 trials identified only one large randomized trial that recommended high dose parenteral MTX to induce clinical remission<sup>[18]</sup>. The remaining studies using oral forms have disappointing results<sup>[15]</sup> and because of its route of administration MTX is not acceptable to many patients. Despite some evidence justifying the use of MTX in UC<sup>[19]</sup>, and fistulising CD<sup>[20]</sup>, data remains limited and confined to retrospective chart reviews. AZA/6MP, therefore, has been the mainstay of immunosuppressive maintenance therapy in IBD. The use of MMF has, therefore, been proposed as an alternative immunosuppressive therapy for patients who either are refractory or intolerant to AZA/6MP.

The aim of our study was to prospectively evaluate the short and long-term efficacy and safety of MMF in patients who were either steroid refractory, or dependent, as well as intolerant or allergic to AZA/6MP therapy. We also wanted to examine the need for dose escalation of MMF over time as this has been suggested as a problem with the use of MMF by some studies<sup>[13]</sup>. As with many of the other published data on MMF, ours was a small cohort of IBD patients with open-label use of MMF. Our patients, however, were assessed at numerous time points and were followed for over a year. The patients in our cohort were also medication resistant, with two thirds failing anti-TNF- $\alpha$  therapy, suggesting a more difficult-to-treat population of patients compared to some other studies. Despite this the results were encouraging. Overall the response rate observed was 71% of patients achieving or maintaining a complete clinical remission after 8 wk of therapy. Excluding the 3 patients who were in remission and off steroids at the time of commencing the MMF, the response/remission rates were still 63.6% at 8 wk. These findings are in contrast with current literature, which reports short-term response rates of only between 25%-40%<sup>[8,13,14]</sup>.

A proportion of MMF-treatment failures in previous studies have been attributed to discontinuation secondary to significant adverse effects. In our study MMF was generally well tolerated, but discontinuation of the MMF secondary to adverse effects was still 21.4%, similar to the 30% observed in other studies. This does not explain the difference, however, in the overall response rates and the reasons behind the difference remains unclear. Relapses over time have also been previously reported as common<sup>[8,13,14]</sup>. Early relapse in our cohort, however, was not commonly observed and even after 12 mo of MMF therapy, 57.1% (8/14) of our IBD patients were still in remission. Of particular note is the lack of dose escalation required over time in our patients responding to MMF. None of the 8 patients on MMF in remission at 12 mo had their dose of MMF increased in the previous 6 mo.

In our experience, the efficacy of MMF appears to differ in some aspects to the published data. Our data demonstrate that MMF can be efficacious and well tolerated in treating refractory IBD patients who are intolerant to AZA/6MP. Problems of lack of long-term efficacy and early disease flare as well as the need for dose escalation over time did not eventuate. Our findings support the use of MMF in the management algorithm of resistant IBD, but its role needs further clarification in larger randomized, double-blind studies comparing it to conventional immunosuppressants. Long-term efficacy would appear to be demonstrated in our study and our current experience suggests that MMF can and should be considered in patients who have failed conventional immunosuppressive therapy.

## COMMENTS

### Background

Treatment for patients with inflammatory bowel disease (IBD) refractory, or intolerant, to conventional immunosuppressive therapy such as azathioprine/6-

mercaptopurine and methotrexate is difficult. The advent of biological therapies has alleviated this problem to a certain degree but there are still a proportion of patients who fail to respond to them or develop drug reactions. The need for alternative effective immunosuppressive agents in the management of IBD are thus required.

### Research frontiers

This study aimed to further define the role of mycophenolate mofetil (MMF) in the treatment of inflammatory bowel disease. The use of this immunosuppressant has been studied in patients refractory, or intolerant, to conventional treatments and results have varied with some studies showing a lack of efficacy or high rates of adverse events. The authors describe a single center experience in the use of MMF for difficult-to-treat IBD patients.

### Innovations and breakthroughs

In contrast to previous reports the study identified MMF to be a safe and efficacious choice in the treatment of difficult IBD and found that the agent to be well tolerated and the response to be sustained. The reported clinical remission rates also seem to be higher than those in previous studies.

### Applications

The findings of study supported the use of MMF in the treatment of patients with IBD who are refractory or intolerant to conventional therapies such as azathioprine/6-MP or methotrexate.

### Terminology

Inflammatory bowel disease is a group of chronic diseases involving the gastrointestinal tract particularly in the small and large bowel. It is divided into 2 groups: Crohn's disease and ulcerative colitis. Crohn's disease is characterized by transmural rather than superficial mucosal inflammation and often presents as a discontinuous disease involving the small or large intestine, or both. Ulcerative colitis/IBD unclassified is the Montreal classification of patients with IBD but without the features needed to diagnose Crohn's disease.

### Peer review

The authors examined the use of mycophenolate mofetil in the treatment of inflammatory bowel disease and found it to be a good alternative immunomodulator in those with IBD who have either failed or become intolerant to conventional therapy. The presence of a good response in those who previously failed biological agents suggests a possible role of MMF in the management of this subgroup of patients as well.

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BRIEF ARTICLES

## Oral allopurinol to prevent hyperamylasemia and acute pancreatitis after endoscopic retrograde cholangiopancreatography

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### Abstract

**AIM:** To assess the efficacy of allopurinol to prevent hyperamylasemia and pancreatitis after endoscopic retrograde cholangiopancreatography (PEP).

**METHODS:** One hundred and seventy patients were enrolled and randomized to two groups: a study group ( $n = 85$ ) who received 300 mg of oral allopurinol at 15 h and 3 h before endoscopic retrograde cholangiopancreatography (ERCP) and a control group ( $n = 85$ ) receiving an oral placebo at the same times. Main Outcome Measurements included serum amylase levels and the number severity of the episodes of

pancreatitis. Serum amylase levels were classified as normal ( $< 150$  IU/L) or hyperamylasemia ( $> 151$  IU/L). Episodes of PEP were classified following Ranson's criteria and CT severity index.

**RESULTS:** Gender distribution was similar between groups. Mean age was  $53.5 \pm 18.9$  years for study group and  $52.8 \pm 19.8$  years for controls. Also, the distribution of benign pathology was similar between groups. Hyperamylasemia was more common in the control group ( $P = 0.003$ ). Mild PEP developed in two patients from the study group (2.3%) and eight (9.4%) from control group ( $P = 0.04$ ), seven episodes were observed in high-risk patients of the control group (25%) and one in the allopurinol group (3.3%,  $P = 0.02$ ). Risk factors for PEP were precut sphincterotomy ( $P = 0.02$ ), pancreatic duct manipulation ( $P = 0.002$ ) and multiple procedures ( $P = 0.000$ ). There were no deaths or side effects.

**CONCLUSION:** Oral allopurinol before ERCP decreased the incidences of hyperamylasemia and pancreatitis in patients submitted to high-risk procedures.

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**Key words:** Endoscopic retrograde cholangiopancreatography; Hyperamylasemia; Acute pancreatitis; Oral allopurinol; Risk factors

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## INTRODUCTION

Pancreatitis is the most common complication of endoscopic retrograde cholangiopancreatography (ERCP)<sup>[1-4]</sup>, with the reported incidence ranging from 1.8% to 7.2% in most prospective series<sup>[5-9]</sup>. However, the reported incidence can be up to 30%, depending on the criteria used to diagnose pancreatitis, the type and duration of patient follow-up and the type of case mix<sup>[10]</sup>. More commonly, hyperamylasemia occurs in up to 30% of patients undergoing ERCP<sup>[11]</sup>.

The generally accepted criteria for the diagnosis of post-ERCP pancreatitis (PEP) were proposed in 1991 during a consensus workshop. These criteria include the new onset of pancreatic-type abdominal pain associated with at least a threefold increase in serum amylase or lipase occurring within 24 h after an ERCP. The pain symptoms need to be severe enough to require admission to a hospital or to extend the length of stay of patients who are already hospitalized<sup>[12]</sup>. Most of the episodes of acute pancreatitis are catalogued as mild. However, based on the presence of organ failure or local complications, acute severe pancreatitis occurs after 0.3% to 0.6% of ERCP procedures<sup>[10,13,14]</sup>.

Numerous attempts have been made to find a pharmacologic agent that could be used to reduce the incidence and severity of PEP. An ideal agent should be highly effective in reducing PEP, safe for the patient, well tolerated, relatively affordable and not require a prolonged administration time. Unfortunately, nearly all of the agents investigated have fallen short of these goals, but several agents have shown some promise<sup>[15,16]</sup>. An early step in the pathogenesis of acute pancreatitis is capillary endothelial injury manifested by an increase in capillary permeability<sup>[17,18]</sup>. Subsequent research has suggested that this capillary injury might be mediated by oxygen-derived free radicals<sup>[19-21]</sup>. Xanthine oxidase catalyzes the conversion of hypoxanthine to xanthine, which generates an oxygen-derived free radical. This catalyst is commonly derived from a ubiquitous inactive precursor, xanthine dehydrogenase, which is present in the pancreas and the intestinal mucosa. Xanthine dehydrogenase is converted to xanthine oxidase by the proteolytic cleavage of a peptide fragment. These findings have prompted attempts at the prevention of pancreatitis by treatment with free radical scavengers (e.g. superoxide dismutase, dimethyl sulfoxide or catalase), protease inhibitors (e.g. gabexate) or xanthine oxidase inhibitors (e.g. allopurinol)<sup>[22-25]</sup>.

The efficacy of oral allopurinol to reduce PEP has been investigated in an *in vivo* animal model<sup>[26]</sup>. Pretreatment was not only associated with a significant (sixfold) reduction in the incidence of pancreatitis, but when pancreatitis did occur it was less severe. Other animal models using pretreatment with allopurinol have demonstrated a significant reduction in the progression of histological pancreatic injury and in the severity of experimental pancreatitis in dog and rat models<sup>[27-29]</sup>. These findings in animals supported the need for human studies on the utility of allopurinol pretreatment to reduce the incidence of hyperamylasemia and PEP.

One randomized clinical trial has reported positive clinical results<sup>[30]</sup>, whereas three have reported negative outcomes<sup>[31-33]</sup>. Given the findings of these published clinical results, the beneficial animal data and the practical benefits of allopurinol's potential use for prevention of hyperamylasemia and PEP, we designed a randomized clinical trial to compare the rates of these symptoms seen with treatment using either allopurinol or a placebo.

## MATERIALS AND METHODS

### *Trial design and patient selection*

This was a randomized clinical trial carried out in patients who underwent ERCP within a six-month period (July through December 2007) at the Endoscopy and Gastroenterology Departments of the High Specialty Medical Unit, Specialties Hospital of the Western National Medical Center of the Mexican Institute of Social Security. From the 300 candidates for ERCP, only 170 met the trial criteria. Patients needed to be over 18 years old and undergoing ERCP due to suspected bile duct obstruction with intact papilla of Vater. No patients were enrolled in the study if they had clinically evident acute pancreatitis or hyperamylasemia ( $> 150$  IU/L) before the procedure or if they had ingested nonsteroid anti-inflammatory drugs (NSAIDs) within a week prior to assessment. Patients submitted to diagnostic, therapeutic or failed ERCP 12 mo before the inclusion in the study were not admitted nor were those who had undergone previous endoscopic or surgical sphincterotomy. Patients were also excluded if they were being treated with anticoagulants or platelet antiagregants, such as acetyl salicylic acid and placitaxel or with a prothrombin time with a difference of  $> 5$  s against the blind sample taken no earlier than 72 h before the study. We also eliminated patients who were allergic or hypersensitive to allopurinol or hydrosoluble contrast solutions or those with active hemorrhages of peptic origin. Additional exclusion criteria included a hemoglobin level of less than 8 g/dL; a platelet count of less than  $60 \times 10^9$ /L; relative neutropenia (absolute neutrophil count  $< 2.0 \times 10^9$ /L); significant renal dysfunction (serum creatinine level,  $> 200$   $\mu$ mol/L); decompensated cirrhosis; a known or suspected pregnancy or presence of lactation; current or recent use of allopurinol (within 48 h); current use of drugs with a known interaction with allopurinol, including cyclophosphamide, chlorpropamide, azathioprine/mercaptopurines, or probenecid and an inability to swallow or absorb oral medication.

### *Main outcome measurements*

We included 170 patients in this study. Randomization was performed at the Department of Gastrointestinal Endoscopy by using computer-generated random numbers. Allopurinol and the placebo were similar in presentation and packed in appropriate containers with the identification code. The drug or placebo was only administered after informed consent was obtained. Eighty-five patients were randomly assigned to the study

group receiving 600 mg of allopurinol divided in two oral doses before the procedure (300 mg at 15 h and 300 mg at 3 h before ERCP) and 85 patients were assigned to the control group receiving two doses of an oral placebo at the same time. Blood samples were drawn from all patients to determine serum amylase levels before the procedure and 2 h later and classified as normal level ( $< 150$  IU/L); or hyperamylasemia ( $> 151$  IU/L). If the amylase serum level was  $> 151$  IU/L and there was no evidence of acute pancreatitis (abdominal pain, nausea or vomiting), patients were started on a liquid diet and discharged 8 h to 24 h after the endoscopic procedure. If the serum amylase was above 600 UI/L or three times above the normal value and the patient had a sharp pain irradiating to the back and nausea or vomiting, the diagnosis of PEP was established in the absence of radiological evidence of a pneumoperitoneum or emphysema in the retroperitoneal space through a plain radiologic examination of the abdomen or CT scan. These patients were managed in the hospital with fasting, hydration with crystalloid solutions, antiemetics (metoclopramide) and analgesics. Pancreatitis episodes were classified according to Ranson's prognostic criteria and CT severity index<sup>[34]</sup>.

Details concerning the endoscopic procedure, specifying the difficulty for cannulation, number of pancreatic duct injections, sphincterotomies, characteristics of the bile duct, presence of choledocolithiasis, as well as defining whether the procedure was diagnostic or therapeutic (endoprosthesis placement or stone extraction). Patients were classified as low-risk for the development PEP or those male and older than 50 years old, when the procedure was diagnostic or therapeutic with sphincterotomy, biliary or pancreatic stenting and stone extraction or presence of chronic pancreatitis. Otherwise, patients were considered as high-risk for the development of PEP in the case of female gender and younger than 50 years old, those submitted to pancreatic duct manipulation or precut sphincterotomy, multiple endoscopic procedures, difficult or failed cannulation and patients with suspected sphincter of Oddi dysfunction<sup>[10,16]</sup>. Other complications such as perforation, bleeding and infection, were recorded.

### Statistical analysis

The results are shown as percentages and as means with standard deviations. Statistical inference was tested using chi-squared or Fisher's exact test for qualitative variables, while Student's *t* test was used for quantitative variables. To explore the risk factors, the relative risks and 95% confidence intervals were estimated.  $P < 0.05$  was considered statistically significant. Finally, the reduction in absolute risk (ARR), the reduction in relative risk (RRR) and number needed to treat (NNT) were analyzed to estimate factors needed to prevent an episode of pancreatitis.

### Ethical considerations

The research protocol was reviewed and approved by the Research and Ethics Committees of our Institution. All patients signed informed consent forms before

Table 1 Demographics and ERCP data

	Allopurinol <i>n</i> = 85	Placebo <i>n</i> = 85	<i>P</i>
Age	53.5 ± 18.9	52.8 ± 19.8	0.82
Gender M/F	36/49	34/51	0.86
Diagnosis			
Benign			
Choledocholithiasis	35	35	0.51
Iatrogenic injury of the biliary tract	11	14	0.48
Chronic pancreatitis	3	1	0.31
Chronic hepatopathy	2	2	0.60
Sphincter of oddi dysfunction	2	2	0.60
Mirizzi's syndrome	1	0	0.50
Malignant			
Pancreatic adenocarcinoma	11	12	0.82
Cholangiocarcinoma	4	5	0.50
Periampullary carcinoma	2	2	0.60
Gallbladder cancer	0	1	0.50
Normal cholangiography	8	5	0.48
Failed procedure	6	6	0.61
Total	85	85	

taking part in the study. The study was financed with funds from the Department of Gastroenterology, Gastrointestinal Endoscopy and the Medical Research Unit in Clinical Epidemiology of the Medical Center.

## RESULTS

The patients participating in the trial comprised 70 men (41.2%) and 100 women (58.8%). The study group had 36 men and 49 women; in the control group there were 34 men and 51 women. The average age for the study group was  $53.5 \pm 18.9$  years and for the control group it was  $52.8 \pm 19.8$  years. Basal amylase levels were  $50.8 \pm 19.3$  U/dL for the study group and  $46.9 \pm 16.1$  U/dL for the control group.

A benign diagnosis for both groups was reported in 108 patients (63.5%): 54 in the study group and 54 in the control group. Malignant diseases were diagnosed in 17 and 20 cases respectively (21.8%). Normal cholangiography was determined in eight and five cases respectively (7.6%) and difficult or failed ERCP occurred in six patients in each group (7%). The diagnoses reached are shown in Table 1. No significant statistical differences were found between groups, and there were no differences in the procedural details described in Table 2. Twenty-three patients developed hyperamylasemia ( $> 151$  IU/L), five (5.8%) from the study group and eighteen (21.1%) from the control group ( $P = 0.003$ ). Ten patients developed pancreatitis, two from the study group (2.3%) and eight from the control group (9.4%;  $P = 0.04$ ). In all cases amylase levels were above 600 IU/L (range 771 to 8886 IU/L). These patients were classified according to Ranson's criteria at admission and at 48 h by CT Severity index as having mild pancreatitis (less than 3 positive signs and Balthazar's A an B without necrosis, severity index of 0 to 1 points). They were handled conservatively and all did well. All patients were discharged within three days of starting the treatment.



**Table 2** Procedural details, endpoints and post-ERCP morbidity *n* (%)

	Allopurinol group <i>n</i> = 85	Placebo group <i>n</i> = 85	<i>P</i>
<b>Procedural details</b>			
Total procedural time (min)	37.8 ± 11.9	38.2 ± 12.4	0.82
Cannulation time (min)	15.4 ± 5.5	15.6 ± 5.6	0.81
Pancreatic cannulation and injection	24 (24.7)	18 (21.1)	0.18
Number of injections	1.23 ± 0.42	1.27 ± 0.44	0.60
Acinarization	9 (10.5)	9 (10.5)	0.58
<b>Invasive diagnostics</b>			
Cytology	15 (17.6)	17 (20)	0.42
Intrabiliary biopsy	2 (2.3)	2 (2.3)	0.69
<b>Therapeutics</b>			
Any Therapeutics	71 (83.5)	74 (87)	0.51
Precut sphincterotomy	15 (17.6)	18 (21.1)	0.56
Biliary sphincterotomy	20 (23.5)	17 (20)	0.57
Stone extraction	29 (34.1)	27 (31.7)	0.74
Biliary stenting	32 (37.6)	37 (43.5)	0.43
Pancreatic stenting	2 (2.3)	3 (3.5)	0.64
<b>End points</b>			
Hyperamylasemia	5 (5.8)	18 (21.1)	0.003
Pancreatitis	2 (2.3)	8 (9.4)	0.049
PEP in low-risk procedures	1/55 (1.8)	1/57 (1.7)	0.70
PEP in high-risk procedures	1/30 (3.3)	7/28 (25)	0.02
<b>ERCP morbidity</b>			
Bleeding	2 (2.3)	2 (2.3)	0.69
Perforation	1 (1.1)	0	0.50

The analysis of the risk factors for the development of PEP revealed that Gender ( $P = 0.52$ , RR, 0.83, CI 95% 0.24-3.1), age [younger or older than 50 years old ( $P = 0.31$ , RR, 0.38, CI 95% 0.04-3.12)] and etiology ( $P = 0.18$ , RR, 0.77, CI 95% 0.46-1.29) were not statistically different between groups. When sphincterotomy or biliary stenting was performed, no risk of developing acute pancreatitis was observed ( $P = 0.31$ , RR, 0.38, CI 95% 0.04-3.12). Otherwise, we observed a marked tendency to favor the development of acute pancreatitis if precut sphincterotomy was performed ( $P = 0.022$ , RR, 4.9, CI 95% 1.3-18.19), if there was instrumentation of the pancreatic duct ( $P = 0.002$ , RR 9.3, CI 95% 1.91-45.4) or if multiple endoscopic procedures such as pre-cut sphincterotomy plus pancreatic duct manipulation plus biliary stenting during the same ERCP were performed, ( $P = 0.000$ , RR 14.8, CI 95% 3.0-73.06). PEP was observed in eight patients submitted to high-risk procedures (Table 2), one (3.3%) corresponded in the allopurinol group and seven (25%) patients for the control group ( $P = 0.02$ ). In contrast, two episodes of PEP were observed in patients submitted to low-risk procedures, one (1.8%) from the allopurinol group and one (1.7%) from the control group ( $P = 0.70$ ).

We found an ARR of 21.7%, with an RRR of 86.8% and an NNT of 4.6 patients submitted to high-risk ERCP procedures to avoid a clinically evident episode of pancreatitis. Major complications were observed in four patients (two from each group) consisting of mild to moderate bleeding which required blood transfusion and resolved without surgical intervention and one perforation was observed in a patient of the study group

treated surgically without complications or mortality. No adverse events were recorded with the use of allopurinol or the placebo.

## DISCUSSION

Xanthine oxidase (XO) was first discovered in milk over a century ago and in rat serum nearly 70 years ago<sup>[35,36]</sup>. This enzyme is now known to be present in many different tissues and in a wide range of species from bacteria to humans<sup>[37,38]</sup>. It is a cytosolic metalloflavoprotein that is predominantly responsible for the oxidation of endogenous purines and exogenous ethanol<sup>[39-41]</sup>. Granger and colleagues demonstrated that XO was an important source of the oxidative stress associated with ischemia and reperfusion<sup>[38]</sup>. This enzyme has since been implicated in the pathogenesis of a wide spectrum of diseases<sup>[42]</sup>, and it is thought to be the most important source of oxygen-derived free radicals and cell damage during reoxygenation of hypoxic tissues and pancreatitis<sup>[40-44]</sup>. A number of studies in animal models conducted during the past two decades have highlighted the potential benefit of XO inhibition in a range of clinical settings. Thus, clinical studies have shown that it is safe and effective for the treatment of gout and tumor-lysis syndrome (a life-threatening constellation of metabolic abnormalities resulting from spontaneous or treatment-related tumor necrosis or fulminant apoptosis) and to reduce complications such as postoperative arrhythmias, myocardial infarction and associated mortality after cardiovascular surgery<sup>[39]</sup>.

Allopurinol has high oral bioavailability (80%-90%), a rapid onset of action (peak circulating level reached in 0.5-2 h) and a 70% hepatic transformation to a long-lasting active metabolite (oxypurinol, with a half-life of 15 h)<sup>[39]</sup>. These pharmacokinetic attributes mean a single oral dose of allopurinol before ERCP could conceivably prevent PEP, because the drug targets those changes that contribute to the initial triggering of pancreatitis<sup>[42,43]</sup>. Allopurinol is also an inexpensive generic drug with an excellent safety record and is not included in the catalog of drugs inducing pancreatitis<sup>[45]</sup>.

Four randomized clinical trials have been published in full to date (Table 3): a negative study from Budzyńska *et al*<sup>[31]</sup> ( $n = 300$ ), a positive study from Greece<sup>[30]</sup> ( $n = 250$ ), a negative study from the USA<sup>[32]</sup> ( $n = 701$ ) and the most recent study published by Romagnuolo *et al*<sup>[33]</sup>, from Canada ( $n = 586$ ) with negative results. In the present study, we demonstrated that the use of allopurinol led to a significant reduction in the incidence of hyperamylasemia (5.8% *vs* 21.1% in placebo-treated controls,  $P = 0.003$ ) and acute pancreatitis (2.3% *vs* 9.4%,  $P = 0.04$ ). According to the particular patient's conditions, type of endoscopic procedure or multiple procedures, patients were divided as low and high risk for the development of PEP. The incidence was similar between patients submitted to low-risk procedures. In contrast, the difference was statistically significant in high-risk procedures, favoring the use of allopurinol (incidence 3.3% in the study group *versus* 25%

Table 3 Summary of randomized trials using allopurinol to prevent post-ERCP pancreatitis

Study (year), SC vs MC, country	n	Dose, mg	Allopurinol vs placebo PEP rates	Percentage high risk <sup>1</sup>	Comment
Budzyńska <i>et al</i> <sup>[31]</sup> (2001) SC, Poland	300	400 <sup>2</sup>	12.1% vs 7.9%; 12 vs 8	0	3-arm study, with third arm (n = 100) given prednisone
Kastinelos <i>et al</i> <sup>[30]</sup> (2005) SC, Greece	250	1200 <sup>3</sup>	3.2% vs 17.8%; 4 vs 21	0	2 patients with suspected SOD
Mosler <i>et al</i> <sup>[32]</sup> (2005) MC, USA	701	900 <sup>4</sup>	13.0% vs 12.1%; 46 vs 42	70.2	4% absolute benefit in high-risk patients; 4% absolute harm in average risk
Romagnuolo <i>et al</i> <sup>[33]</sup> (2008) MC, Canada	586	300 <sup>5</sup>	5.5% vs 4.1%; 16 vs 12	11.3	Harm in average risk patients; benefit in high-risk patients
Current study (2009) SC, Mexico	170	600 <sup>6</sup>	2.3% vs 9.4%; 2 vs 8	34.1	21.7% absolute benefit in patients with high-risk procedures favoring allopurinol, no difference in low-risk procedures
Raw pooled	2007 (1008 vs 999)	-	7.9% vs 9.1%; 80 vs 91	-	1.2% difference (95% CI, 3.2% to 2.0%)

<sup>1</sup>As defined in this protocol, namely sphincter manometry and/or pancreatic therapy. Other higher-risk cases (e.g. precut sphincterotomy or suspected SOD) were not considered; <sup>2</sup>200 mg 15 h before, 200 mg 3 h before; <sup>3</sup>600 mg 15 h before, 600 mg 3 h before; <sup>4</sup>600 mg 4 h before, 300 mg 1 h before; <sup>5</sup>300 mg 1 h before; <sup>6</sup>300 mg 15 h before, 300 mg 1 h before; SC: Single centre; MC: Multicenter.

in the control group,  $P = 0.02$ ). Fortunately, all episodes of acute pancreatitis were catalogued as mild and there were no deaths.

There was variability in the doses used in the previous studies and in the baseline rates of PEP in the control (placebo) groups (some of which are out of the usual range reported), but these differences do not appear to completely explain the heterogeneity in the results. There remains a possibility for a threshold effect or a minimally effective dose for allopurinol, given that the positive study<sup>[30]</sup> used the highest dose (1200 mg); however, there does not seem to be a clear dose-response relationship as the larger negative studies<sup>[31-33]</sup> used different lower doses (300, 400 and 900 mg; Table 3). The four earlier studies all checked formally or informally for interactions, presenting the active treatment and placebo PEP rates in different subgroups. None found significant interactions between diagnostic and therapeutic procedures. The most detailed analyses of this type were found in the studies by Mosler *et al*<sup>[32]</sup> and Romagnuolo *et al*<sup>[33]</sup>. Both demonstrated a benefit in the reduction of the episodes of acute pancreatitis as well as the severity when analyzing high-risk patients or those requiring sphincter of Oddi manometry or planned pancreatic therapy. Mosler *et al*<sup>[32]</sup> demonstrated that allopurinol reduced the incidence of PEP from 27% to 23% in the high-risk group (4% absolute risk reduction) and also reduced harm (8% versus 12% PEP) in the non-high-risk group (Table 3). Romagnuolo *et al*<sup>[33]</sup> found that, for non-high-risk patients, the crude rate of PEP was 5.4% in the allopurinol group and 1.5% in the placebo group ( $P = 0.017$  favoring the placebo, indicating harm associated with allopurinol), whereas in the high-risk group the PEP rates were 6.3% in the allopurinol group and 23% in the placebo group ( $P = 0.050$  favoring allopurinol). It is also necessary to note that more patients in the allopurinol group (44% vs 34%  $P = 0.02$ ) required pancreatic duct injection as well as more injections (two versus one,  $P = 0.01$ ). However, confounding was not confirmed statistically, and correcting for pancreatic injection in a stratified model still showed a nonsignificant trend toward harm for allopurinol in the non-high-risk subgroup. If

allopurinol is truly harmful for non-high-risk patients undergoing ERCP (the adjusted subgroup OR was not significant), the mechanism responsible is unclear. It could be the result of an idiosyncratic reaction to the medicine itself; one study did suggest that medications with a history of inducing pancreatitis could increase the risk of PEP<sup>[46]</sup>.

Budzyńska *et al*<sup>[31]</sup>, also included primarily non-high-risk patients and showed a higher rate of PEP with allopurinol. In contrast, the patients in the study by Kastinelos *et al*<sup>[30]</sup> were also primarily non-high-risk patients and yet the study showed a significant benefit for allopurinol. In our results, using 600 mg of allopurinol we observed a significant reduction in the episodes of mild acute pancreatitis (2.3% vs 9.4,  $P = 0.04$ ), but the difference was attributed to a beneficial effect of allopurinol in patients submitted to high-risk procedures, since in low-risk procedures the difference was not statistically significant.

The debate still continues. In a recent meta-analysis just published in September, 2008, Bai *et al*<sup>[47]</sup> concluded that allopurinol may not be useful to prevent PEP. However, they recognized the limitations of their meta-analysis since it was a study-level analysis and the authors denoted the difficulties in stratifying high-risk patients and high-risk procedures because this information was not available in reviewed trials<sup>[30-33,47]</sup>. To overcome the limitations, they recommended the design of multicenter trials with appropriate numbers of high-risk patients and high-risk procedures.

In conclusion, extensive evidence supports a beneficial effect of allopurinol in the prevention and severity of experimental pancreatitis. Clinical evidence supports a favorable effect of oral allopurinol in the prevention of PEP in patients submitted to high-risk procedures. Our results establish a reduction of the incidence of asymptomatic hyperamylasemia and PEP, particularly in patients submitted to high-risk procedures. More clinical trials with different dosification and patient selection are required to definitively determine any positive or deleterious effect of oral allopurinol in the prevention of PEP.



## COMMENTS

### Background

Endoscopic retrograde cholangiopancreatography (ERCP) is a widely applied method for the diagnosis and treatment of pancreatobiliary disease. Post-ERCP pancreatitis is the most common postoperative complication of ERCP and its prevention has become an urgent clinical challenge.

### Research frontiers

ERCP is an indispensable method for the diagnosis and treatment of pancreatobiliary disease, and pancreatitis is the most common postoperative complication of it. There are some studies on drugs for preventing post-ERCP pancreatitis, but their results remain debatable. Therefore, most endoscopy centers do not give patients a conventional chemoprophylaxis.

### Innovations and breakthroughs

This trial revealed that oral allopurinol 300 mg 15 and 3 h (600 mg) before ERCP could reduce pancreatitis and hyperamylasemia.

### Applications

Oral allopurinol 300 mg 15 and 3 h (600 mg) before ERCP can prevent post-ERCP pancreatitis. Compared with other drugs, oral allopurinol is inexpensive, convenient and has very few side-effects, and can be used as a protective drug for preventing post-ERCP pancreatitis.

### Peer review

This paper is interesting since aiming to demonstrate the effect, and possible effectiveness, of allopurinol on the occurrence of post ERCP acute pancreatitis. The design is well organized and the conclusion is that this drug has a preventive effect on post ERCP - hyperamylasemia and pancreatitis, especially in high risk patients.

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# An autoregressive integrated moving average model for short-term prediction of hepatitis C virus seropositivity among male volunteer blood donors in Karachi, Pakistan

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## Abstract

**AIM:** To identify the stochastic autoregressive integrated moving average (ARIMA) model for short term forecasting of hepatitis C virus (HCV) seropositivity among volunteer blood donors in Karachi, Pakistan.

**METHODS:** Ninety-six months (1998-2005) data on HCV seropositive cases ( $1000^{-1} \times \text{month}^{-1}$ ) among male volunteer blood donors tested at four major blood banks in Karachi, Pakistan were subjected to ARIMA modeling. Subsequently, a fitted ARIMA model was used to forecast HCV seropositive donors for 91-96 mo to contrast with observed series of the same months. To assess the forecast accuracy, the mean absolute error rate (%) between the observed and predicted HCV seroprevalence was calculated. Finally, a fitted ARIMA model was used for short-term forecasts beyond the observed series.

**RESULTS:** The goodness-of-fit test of the optimum ARIMA (2,1,7) model showed non-significant autocorrelations in the residuals of the model. The forecasts by ARIMA for 91-96 mo closely followed the pattern of observed series for the same months, with mean monthly absolute forecast errors (%) over 6 mo of 6.5%. The short-term forecasts beyond the observed

series adequately captured the pattern in the data and showed increasing tendency of HCV seropositivity with a mean  $\pm$  SD HCV seroprevalence ( $1000^{-1} \times \text{month}^{-1}$ ) of  $24.3 \pm 1.4$  over the forecast interval.

**CONCLUSION:** To curtail HCV spread, public health authorities need to educate communities and health care providers about HCV transmission routes based on known HCV epidemiology in Pakistan and its neighboring countries. Future research may focus on factors associated with hyperendemic levels of HCV infection.

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**Key words:** Hepatitis C virus; Blood donor; Ecological analysis; Autoregressive integrated moving average model; Pakistan

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## INTRODUCTION

Hepatitis C virus (HCV) infection poses a major public health problem in developing countries, including Pakistan. However, the results of prevalence studies have shown variable estimates in select groups including 1.8% to 3.0% in volunteer blood donors<sup>[1,2]</sup> and 16% to 20.5% in familial contacts of infected patients<sup>[3,4]</sup>. A community-based study in Hafizabad, Punjab, found a 6.5% HCV seroprevalence<sup>[5]</sup>. Using these estimates, Pakistan has been grouped into intermediate category with respect to burden of HCV infection<sup>[6]</sup>. Several routes have been implicated for nosocomial and community acquired

HCV infection including unsafe injections, recycling of used syringes, inadequate sterilization of surgical and dental equipment, and facial shaving by barbers<sup>[2,7]</sup>.

Public health authorities in Pakistan intermittently run educational campaigns in electronic and print media to create awareness in the general population to halt HCV spread. However, in the absence of adequate HCV surveillance, the true impact of the HCV control efforts remains uncertain. Volunteer blood donors are generally considered to be the healthier segment of any community and the proportions of HCV seropositivity among them may be considered to mirror the situation in the general population<sup>[8]</sup>. We have previously reported a significant increase in HCV seroprevalence among volunteer blood donors over the past several years using data from two blood banks<sup>[2]</sup>. However, there is need to expand this HCV surveillance network countrywide to obtain more reliable and representative estimates.

Recently, mathematical models have been used to project the future HCV prevalence among intravenous drug users<sup>[9]</sup>, and its impact on the future development of HCV related morbidity and mortality<sup>[10]</sup>. Modeling and forecasting HCV seropositivity among volunteer blood donors in Pakistan, and perhaps in other neighboring countries, might provide useful information for allocating resources, and re-shaping and planning future control activities<sup>[11]</sup>. This study aimed to develop a univariate time series model for HCV seropositivity ( $1000^{-1} \times \text{month}^{-1}$ ) among volunteer blood donors attending four large blood banks. Specifically, the objective of this study was to identify the stochastic autoregressive integrated moving average (ARIMA) model for short term forecasting of HCV seropositivity ( $1000^{-1} \times \text{month}^{-1}$ ) among volunteer blood donors in Karachi, Pakistan.

## MATERIALS AND METHODS

### Setting

This study was conducted in Karachi-the largest cosmopolitan city and the hub of economic activity of Pakistan. It has an estimated population of 9.3 million, accounting for approximately 10% of the total population of the country. Forty three percent of the city's population is under the age of 15 years. The population of Karachi comprises several ethnic groups defined by mother tongue, including predominantly Urdu, Sindhi, Punjabi, Pushto, and Balochi. The healthcare facilities for the population include several small and tertiary care hospitals, both in the private and public sector.

### Data

Eight-year (1998-2005) data on monthly aggregates of number of donors attending four large blood banks (blood bank I-IV) in Karachi were available for this study. These blood banks receive blood donations only from non-remunerated volunteer blood donors. Blood bank I is part of a tertiary care hospital in the private sector and receives blood donations as replacements

from friends and relatives of inpatients requiring blood transfusions. Blood banks II-IV belong to non-governmental organizations and cater for the needs of those in Karachi who need blood transfusions, including the patients with leukemia, hemophilia, thalassemia and other blood related diseases. Blood banks II-IV also receive blood donations from volunteers on an exchange basis. Prior to blood donation, each blood donor is subjected to screening for known risk factors for transfusion transmissible infections. All the blood banks follow similar criteria to receive blood donations and exclude potential donors who admit known risk factors of transfusion transmissible infections or any medical or non-medical condition associated with high risk (e.g. use of narcotic drugs, history of jaundice in the past 5 years and recent hospitalization). All four blood banks in the study use commercially available enzyme-linked immunosorbant assay kits and results are interpreted according to the manufacturer's instructions.

As noted earlier, blood donations between January 1998 and December 2005 by men aged 18-64 years were included in this evaluation. HCV serological results of consecutive blood donations from these blood banks were available from variable starting dates depending on the completed records, to assess the proportions of HCV seropositive donors.

### Analytic approach

We used methods developed by Box and Jenkins to build an ARIMA time series model<sup>[12]</sup>. This model-building process is designed to take advantage of associations in the sequentially lagged relationships that usually exist in data collected periodically. The general form of the ARIMA model was

$$\Delta^d z_t = \Phi_1 z_{t-1} + \dots + \Phi_p z_{t-p} + a_t - \theta_1 a_{t-1} - \dots - \theta_q a_{t-q}$$

where:

$\Delta^d z_t$  = differenced series i.e.  $z_t - z_{t-1}$

$z_t$  = set of possible observations on the time-sequenced random variable

$a_t$  = random shock term at time  $t$

$\Phi_1 \dots \Phi_p$  = autoregressive parameters of order  $p$

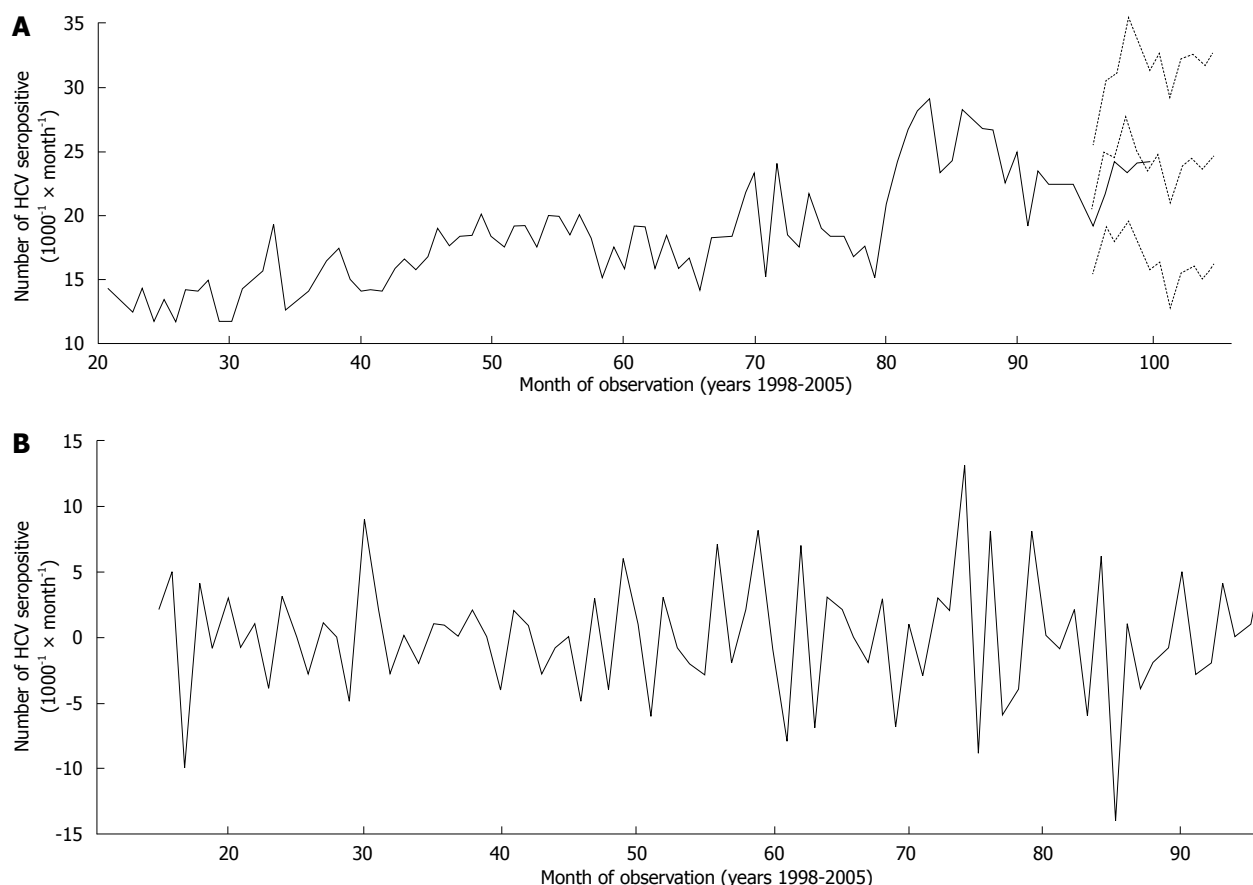
$\theta_1 \dots \theta_q$  = moving average parameters of order  $q$

The series was subjected to Box-Cox transformation<sup>[13]</sup>. The transformed series was then differenced at the non-seasonal level and mean corrected to induce stationarity. Sample autocorrelation and partial autocorrelation functions were used to identify the ARIMA model of the appropriate order. Estimates of the model's parameters were obtained by the maximum likelihood method. Diagnostic checking included residual analysis and the Akaike Information Criterion was used to compare goodness-of-fit among ARIMA models. The final model was a result of several iterations of the identification, estimation, and checking process, and met the conventional criteria for the adequacy of the model<sup>[14]</sup>.

### Assessment of forecast accuracy

The last 6 observations in the data set were used for validation of the forecast accuracy of the ARIMA





**Figure 1** Hepatitis C virus seroprevalence ( $1000^{-1} \times \text{month}^{-1}$ ) among volunteer male blood donors in Karachi, Pakistan 1998-2005. A: Observed data along with forecasts; B: Transformed series.

**Table 1** Hepatitis C virus seroprevalence ( $1000^{-1} \times \text{year}^{-1}$ ) among male volunteer blood donors at four large blood banks in Karachi (1998- 2005)

Yr	Mean	SD	95% CI for mean		Minimum	Maximum
			Lower limit	Upper limit		
1998	13.8	1.5	12.8	14.7	12	16
1999	16.3	2.2	14.8	17.7	13	21
2000	18.5	2.2	17.1	19.9	15	22
2001	19.8	2.0	18.6	21.1	16	22
2002	19.9	3.0	18.0	21.8	15	26
2003	20.3	3.2	18.2	22.3	16	27
2004	29.3	2.6	27.6	30.9	25	33
2005	24.8	2.1	23.4	26.1	21	27
Total	20.3	5.1	19.3	21.3	12	33

$F = 47.9$ ;  $df = 7, 88$ ;  $P < 0.001$ .

model. The fitted ARIMA model was used to forecast the HCV seroprevalence ( $1000^{-1} \times \text{month}^{-1}$ ) for 91-96 mo (June 2005 to December 2005) to contrast with the observed series of the same months. The average forecast error at prediction interval of  $m$  months ( $\bar{\epsilon}_m$ ) was calculated as:

$$(\bar{\epsilon}_m) = \left[ \sum_{i=1}^6 (y_{t+m} - \hat{y}_{t+m}) / 6 \right]^{1/2}$$

Where  $y_{t+m}$  and  $\hat{y}_{t+m}$  denote the observed and forecast values for month  $t + m$ . Finally, the fitted ARIMA model was used for short term (January 2006 to June 2006) forecasts along with their 95% confidence limits beyond the observed series.

## RESULTS

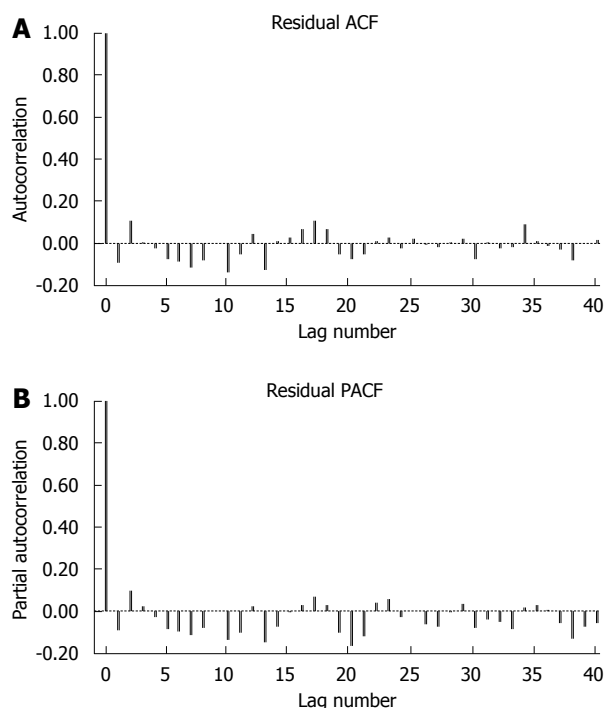
### Descriptive analysis

The crude HCV seropositivity ( $1000^{-1}$ ) among the male volunteer blood donors during the study period was 20.3 (12792/630134). The mean prevalence ( $1000^{-1} \times \text{month}^{-1}$ ) was 18.3 [95% confidence interval (CI): 16.8-19.9]. There was no statistically significant difference in HCV seropositivity ( $1000^{-1}$ ) across various months of the years ( $F = 0.201$ ;  $P = 0.997$ ) (data not shown). However, a substantial variation in HCV seroprevalence ( $1000^{-1}$ ) was observed across different calendar years ( $F = 47.895$ ;  $P < 0.001$ ) (Table 1). The observed and transformed series are presented in Figure 1.

### ARIMA model

The parameters' estimates for the optimum ARIMA (2,1,7) model for the series of monthly HCV seropositive donors ( $1000^{-1}$ ) are shown in Table 2. The autocorrelation and partial autocorrelation functions of the residuals showed good-fit (Figure 2). The residual plots showed small variations around the zero mean. None of these residuals had its magnitude larger than twice the standard deviation. Residuals' autocorrelations were not significantly different from zero as a set and had constant variance, thus confirming the adequacy of the model (Ljung-Box statistic = 20.4;  $P = 0.433$ ).

The forecasts by the ARIMA (2,1,7) model for 91-96 mo (June 2005 to December 2005) using the



**Figure 2** Residual plots for the final ARIMA (2,1,7) model of HCV seroprevalence ( $1000^{-1} \times \text{month}^{-1}$ ) among male volunteer blood donors in Karachi, Pakistan 1998-2005. A: Autocorrelation function; B: Partial autocorrelation function.

observed series of months 1-90, closely followed the pattern of observed series for the same months (Figure 1), with mean  $\pm$  SD and maximum monthly absolute forecast errors (%) over 6 mo interval being  $6.5\% \pm 3.4\%$  and  $10\%$ , respectively. Furthermore, the short term (January 2006 to June 2006) forecasts beyond the observed series adequately captured the pattern in the data (Figure 1) and showed evidence of increasing tendency of HCV seroprevalence ( $1000^{-1} \times \text{month}^{-1}$ ) with the mean  $\pm$  SD as  $24.3 \pm 1.4$  over the forecast interval.

## DISCUSSION

Epidemiological surveillance of communicable diseases is one of the more traditional public health activities. Time series analysis of surveillance data on prevalence and/or incidence of various infections may be helpful in developing hypotheses to explain and anticipate the dynamics of the observed phenomena and subsequently in the establishment of a quality control system and re-allocation of resources<sup>[15,16]</sup>. This method is an ecologic approach and takes advantage of the strong association in the sequentially lagged relationship that usually exists in the data collected periodically<sup>[17]</sup>.

During the study period, the overall HCV seroprevalence ( $1000^{-1}$ ) in volunteer blood donors was 20.3, which falls in the range of 14.9 to 38.9 known for first time blood donors in other developing countries. However, HCV seroprevalence ( $1000^{-1}$ ) in this study was much higher than the 2.1 reported for developed countries<sup>[18]</sup>. The low HCV seroprevalence in resource-

**Table 2** Autoregressive integrated moving average model (2,1,7) of hepatitis C virus seroprevalence ( $1000^{-1} \times \text{month}^{-1}$ ) among male volunteer donors in Karachi, Pakistan, (January 1998-December 2005)

Parameters	Estimate	Standard error	t-ratio
Autoregressive parameter ( $\Phi$ )			
$\Phi_2$	0.67	0.15	4.5
Moving average parameter ( $\theta$ )			
$\theta_2$	-0.59	0.18	3.3
$\theta_3$	0.49	0.15	3.3
$\theta_4$	-0.80	0.11	7.3
$\theta_6$	0.74	0.21	3.5
$\theta_7$	-0.37	0.17	2.2

White noise variance = 9.74; Ljung-Box Q statistic = 20.4 ( $P = 0.433$ ).

rich countries is attributed to safe blood transfusion, whereas, in poor regions of the world, several million people acquire HCV infection each year as a result of contaminated transfusions and the re-use of infected medical devices<sup>[18,19]</sup>. Therefore, public health practices adopted by the developed countries need to be strictly enforced in less developed countries to break the chain of transmission of HCV and other blood-borne pathogens.

Monitoring of HCV seropositivity among volunteer blood donors may provide clues about the effectiveness of control efforts of public health authorities and future trend of the proportion of HCV infected donors in Pakistan. In this paper, we used the ARIMA model on a time series of HCV seropositivity ( $1000^{-1}$ ) collected monthly over a period of 96 mo on asymptomatic male volunteer blood donors from four major blood banks in Karachi. The forecasts made in a prospective manner over six months demonstrated increasing tendency of HCV seropositivity among the blood donors in this cosmopolitan city. Such a predicted increase in HCV seropositivity might result from inconsistent and naïve HCV control efforts on the part of public health officials in Pakistan. Therapeutic injections in a health-care setting have consistently been shown as a strong risk factor for HCV infection in Pakistan<sup>[2,7,20,21]</sup>, and if concerted efforts by the public health authorities are not made, might continue to contribute to the increasing load of HCV infection in this and similar settings in the region. An increasing trend among first time US blood donors of 50 to 59 years of age from 1995 to 2002 has been demonstrated<sup>[22]</sup>. According to the authors, teenage children and young adults in 1960, and 1970s might have experimented with drug injection and were infected with HCV. These people entered into the 50 to 59 years age group during 1995 to 2002. However, in other age groups of donors in the same study and two other studies from US<sup>[23,24]</sup>, and from other developed countries (France<sup>[25,26]</sup> and Spain<sup>[27]</sup>) have shown a decreasing trend of residual risk of HCV infection in blood donors. According to these investigators different factors could have played a role in this reduction, for instance, increased awareness about the factors associated with increased risk of HCV infection, voluntary deferral by potential high risk

donors, improvement in donor recruitment, and /or an overall decrease in HCV infection level in the general population. Such factors need to be evaluated in our population in future studies.

Results from our previous study<sup>[2]</sup>, and those predicted by ARIMA model for 6 mo beyond the observed data exhibited a slightly increasing tendency of HCV seropositivity among male volunteer blood donors over the forecast period. This increasing pattern of HCV seroprevalence among these asymptomatic male volunteer donors merits further investigation of factors contributing to HCV seroprevalence in this population, which is thought to be a mirror image of the situation in the general population.

Some limitations of this study need to be taken into account when interpreting the results. Our HCV seroprevalence estimates are based on ELISA, which has sensitivity of more than 95%. However, these results do not reflect possible HCV infections that do not produce detectable seropositivity during the window period of HCV infection. The exact proportion of these HCV infected, but HCV seronegative, is not known, however, it has been argued that this figure must be very small given the use of current sero-assays<sup>[28]</sup>. Our HCV seroprevalence estimates in male volunteer donor population were based on data from a limited number of blood banks; we do not know whether they reflect the national average. The blood banks that participated in this study however, account for a substantial proportion of donations made annually in Karachi. These centers are located in large metropolitan areas where the prevalence and/or incidence of HCV may be higher than the national figures. Therefore, we think we are justified in making generalizations from our data. In conclusion, in the absence of comprehensive HCV surveillance in the general population in Pakistan and perhaps in other neighboring countries, further monitoring of HCV seropositivity in blood donors and the investigation of factors associated with hyperendemic HCV infection using multivariate ARIMA models might further expand our understanding about HCV epidemiology in this region. Furthermore, effective screening of all blood donors for HCV infection at all blood banks should be seriously considered, because one single HCV infected regular blood donor could transmit the infection to several recipients.

## ACKNOWLEDGMENTS

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## COMMENTS

### Background

Less developed countries such as Pakistan generally lack effective surveillance systems for communicable diseases. In this study, the authors used the Box-Jenkins approach to fit an autoregressive integrated moving average (ARIMA)

model that might be used to monitor and predict trends in hepatitis C virus (HCV) seroprevalence in the general population using volunteer blood donors as a sentinel group. This information may be helpful to facilitate early public health responses to minimize HCV related morbidity and mortality.

### Research frontiers

Developed countries have been able to control the HCV transmission in the general population by public health measures. However, such initiatives are practiced at sub-optimal level in resource-constrained countries. This problem is further compounded by the absence of effective surveillance of communicable diseases including blood-borne pathogens. Therefore, alternative methods to monitor and predict the burden of such infections are needed for rational allocation of resources.

### Innovations and breakthroughs

This is the first application of an ARIMA model to monitor and predict the HCV seroprevalence in volunteer blood donors at multiple blood banks. Such data on infections with HCV and other blood-borne pathogens may mirror the situation in a setting that lacks an effective surveillance system for these infections.

### Application

The fitted ARIMA model could be used for sentinel surveillance of blood-borne infections in volunteer blood donors. Therefore, the estimates for current and predicted future burden of these infections could be used by public health authorities for making rational policy decisions for control and prevention of HCV and other blood-borne pathogens in resource-constrained countries including Pakistan.

### Peer review

The authors describe an effective model to predict HCV seropositivity in Pakistan.

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## Pentoxifylline *versus* prednisolone for severe alcoholic hepatitis: A randomized controlled trial

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**Author contributions:** De BK conceptualized the study; De BK and Dutta D designed the research; Gangopadhyay S and Dutta D performed the research; Baksi SD randomized the patients; Pani A administered the drugs to the patients; Dutta D and Ghosh P analyzed the data; De BK and Dutta D wrote the manuscript.

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profile and renoprotective effects of pentoxifylline compared with prednisolone suggest that pentoxifylline is superior to prednisolone for treatment of severe alcoholic hepatitis.

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**Key words:** Alcoholic hepatitis; Pentoxifylline; Prednisolone; Maddrey discriminant function score; Model for end-stage liver disease score; Glasgow alcoholic hepatitis score

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De BK, Gangopadhyay S, Dutta D, Baksi SD, Pani A, Ghosh P. Pentoxifylline *versus* prednisolone for severe alcoholic hepatitis: A randomized controlled trial. *World J Gastroenterol* 2009; 15(13): 1613-1619 Available from: URL: <http://www.wjgnet.com/1007-9327/15/1613.asp> DOI: <http://dx.doi.org/10.3748/wjg.15.1613>

### Abstract

**AIM:** To compare the efficacy of pentoxifylline and prednisolone in the treatment of severe alcoholic hepatitis, and to evaluate the role of different liver function scores in predicting prognosis.

**METHODS:** Sixty-eight patients with severe alcoholic hepatitis (Maddrey score  $\geq 32$ ) received pentoxifylline ( $n = 34$ , group I) or prednisolone ( $n = 34$ , group II) for 28 d in a randomized double-blind controlled study, and subsequently in an open study (with a tapering dose of prednisolone) for a total of 3 mo, and were followed up over a period of 12 mo.

**RESULTS:** Twelve patients in group II died at the end of 3 mo in contrast to five patients in group I. The probability of dying at the end of 3 mo was higher in group II as compared to group I (35.29% *vs* 14.71%,  $P = 0.04$ ; log rank test). Six patients in group II developed hepatorenal syndrome as compared to none in group I. Pentoxifylline was associated with a significantly lower model for end-stage liver disease (MELD) score at the end of 28 d of therapy ( $15.53 \pm 3.63$  *vs*  $17.78 \pm 4.56$ ,  $P = 0.04$ ). Higher baseline Maddrey score was associated with increased mortality.

**CONCLUSION:** Reduced mortality, improved risk-benefit

### INTRODUCTION

Severe alcoholic hepatitis is an acute or acute-on-chronic hepatic inflammatory response syndrome, which is part of the spectrum of diseases that result from alcohol-induced liver injury, ranging from the most common asymptomatic fatty liver to fulminant hepatitis and cirrhosis in the long term. However, it is difficult to predict the clinical response in an individual patient, as only a minority of individuals consuming large amounts of alcohol develop alcoholic hepatitis<sup>[1,2]</sup>. The importance of acute alcoholic hepatitis lies in its significant morbidity and mortality, with a reported in-hospital mortality as high as 44%<sup>[3]</sup>. Large amounts of alcohol with binge drinking, malnutrition, and female sex, are some of the factors associated with more severe disease<sup>[4]</sup>. The presence of coexisting hepatitis C has been found to be associated with worse prognosis<sup>[5]</sup>. Recent studies have shown that impaired immune response, endoplasmic reticulum stress, mitochondrial dysfunction, and free-radical injury induced by alcohol and its acetaldehyde adduct metabolites, Kupffer cell activation and cytokine production, have an important role in accentuating the hepatocyte injury and disease

precipitation<sup>[6,7]</sup>. Serum level of cytokines such as tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-1, IL-6 and IL-8 are elevated in acute alcoholic hepatitis<sup>[8]</sup>. Studies have shown a linear relationship between TNF- $\alpha$  receptors and mortality from acute alcoholic hepatitis<sup>[9]</sup>.

Maddrey discriminant function (DF) has commonly been used in estimating mortality among patients with acute alcoholic hepatitis, with an elevated DF ( $> 32$ ) indicating an increased likelihood of death, and conversely, a low DF suggesting a generally favorable prognosis<sup>[10,11]</sup>. Recently, model for end-stage liver disease (MELD) score and glasgow alcoholic hepatitis score (GAHS) have also gained interest as predictors of disease outcome in patients with severe acute alcoholic hepatitis<sup>[12,13]</sup>.

Although prednisolone is used widely and considered the standard treatment for severe acute alcoholic hepatitis with DF score  $\geq 32$ , it is not free of adverse effects and has had its share of controversies<sup>[14]</sup>. Recently, pentoxifylline, a non-specific phosphodiesterase inhibitor, with combined anti-inflammatory (TNF- $\alpha$  inhibition) and antifibrogenic properties, has been found to be useful in patients with acute alcoholic hepatitis with DF  $\geq 32$ <sup>[15-17]</sup>. The beneficial effects are believed to occur through various mechanisms such as inhibition of phosphodiesterases, increased cAMP levels and down-regulation of TNF- $\alpha$ , IL-1, IL-6, transforming growth factor-beta (TGF- $\beta$ ), interferon-gamma (IFN- $\gamma$ ), stellate cell activation and procollagen- I mRNA expression<sup>[18]</sup>.

Although many individual studies are available on the efficacy of pentoxifylline and prednisolone in the treatment of severe alcoholic hepatitis, as far as we are aware, no study has compared the two drugs head to head in a randomized controlled study.

The present study compared the efficacy of pentoxifylline and prednisolone in the management of severe alcoholic hepatitis (DF  $\geq 32$ ), and their immediate and short-term outcomes. Also, we evaluated the GAHS and MELD score in patients with severe alcoholic hepatitis and compared them to traditional scores like DF and Child's score.

## MATERIALS AND METHODS

One hundred and fifty-eight chronic alcoholic patients attending the liver clinic, outpatient department or the emergency medical services of the Medical College and Hospitals Calcutta were initially considered. The study was carried out from July 2006 to September 2008. The patients were initially examined clinically, evaluated, and subsequently were admitted for the duration of the study. The study protocol was approved by the institutional ethical committee. All the patients underwent investigations for liver chemistry (liver function tests, prothrombin time), complete hemogram, random blood sugar level, urea, creatinine, electrolytes, viral markers such as hepatitis B surface antigen (HBsAg), anti-hepatitis C virus (HCV) antibody, hepatitis A virus IgM, hepatitis E virus IgM, serum ceruloplasmin, 24-h urinary copper (as and when required), and antinuclear antibody, upper gastrointestinal endoscopy, and Doppler abdominal

ultrasound, as and when required. Patients were included who had a history of chronic alcohol intake of more than 50 g/d<sup>[19]</sup> with clinical and biochemical features of severe alcoholic hepatitis [Maddrey DF  $\geq 32$  and aspartate aminotransferase: alanine aminotransferase (AST: ALT)  $> 2:1$ , with absolute values of AST  $< 500$  IU/L and ALT  $< 200$  IU/L]. Patients with any other potential etiology of liver injury (acute or chronic viral hepatitis, autoimmune liver disease, Wilson's disease) even in the background of chronic alcohol intake were excluded from the study. Also, patients with a history of abstinence from alcohol in the last month, or who were positive for human immunodeficiency virus antibodies were excluded. Patients with infection, sepsis or spontaneous bacterial peritonitis, gastrointestinal bleeding, hepatorenal syndrome, acute pancreatitis or any other severe associated disease (uncontrolled diabetes, hypertension, heart failure, pulmonary disease or malignancy) at the time of inclusion or in the previous 3 mo were also excluded.

MELD score, GAHS and Child's score were calculated for all the patients who were included in the study. Only those patients were considered for final study who gave a prior informed written consent for pharmacotherapy.

The included patients were then divided into two groups by a computer-generated randomization table: group I, patients receiving pentoxifylline, and group II, patients receiving prednisolone. The pharmacotherapy (pentoxifylline or prednisolone) was started within a week of admission.

Patients in group I received pentoxifylline (Trental tablets, Sanofi Aventis, Mumbai, India) at a dose of 400 mg thrice daily orally and a placebo tablet in the place of prednisolone for the first 4 wk. Patients in group II received prednisolone tablet (Wysolone, Wreath, Mumbai, India) at a dose of 40 mg once daily for 4 wk and a placebo tablet taken thrice daily in place of pentoxifylline for the same duration. During the study, concomitant treatments with salicylates, nonsteroidal anti-inflammatory drugs, budesonide, anti-TNF $\alpha$  agents, vitamin E, s-adenosyl methionine or ursodeoxycholic acid were not allowed. The investigators who allocated the patients to the groups, administered the drugs and collected the clinical and laboratory data, as well the statisticians, were all blinded regarding the nature of the pharmacotherapy. All the patients were admitted in the wards of the Department of Medicine, Medical College and Hospitals, Calcutta for the initial period of 4 wk. All investigations such as liver function tests, prothrombin time, electrolytes, renal profile and abdominal ultrasound were repeated after the initial 4 wk of pharmacotherapy. After the initial 4 wk, the study was opened and the patients allocated to the different groups were revealed. After 4 wk of initial therapy, the dose of prednisolone in group II was tapered by 5 mg/wk over a period of 7 wk and then stopped. Patients in group I (pentoxifylline) who tolerated the drug well, continued to receive the medication at the same dose for the next 8 wk, and then stopped.

Only those patients who were clinically stable at the end of 4 wk were discharged and later followed-up in the

liver clinic. All the patients were counseled for strict alcohol abstinence at the time of discharge from the hospital.

The patients were reviewed at least once a month in the liver clinic. During follow-up, all the patients were examined clinically, and asked about drug compliance, intake of alcohol or potential drug adverse effects. Liver function tests, prothrombin time, renal function test, electrolytes, and abdominal ultrasound were performed as and when required. Maddrey DF, MELD, GAHS and Child's scores were calculated for all the patients during follow-up. Patients who had any alcohol intake in the follow-up period were excluded thereafter from the study.

### Statistical analysis

Student's *t* test was used for analysis of continuous variables, Fisher's exact test for binary variables, and the  $\chi^2$  test was used for categorical variables. All results of continuous variables are expressed as mean  $\pm$  SD. Survival curves were estimated according to the Kaplan-Meier method and were compared using the log-rank test. Survival comparisons between groups were performed on an intent-to-treat basis. Results were considered statistically significant at  $P < 0.05$ .

## RESULTS

Of the 158 patients initially evaluated, 74 who fulfilled the inclusion criteria without any other potential etiology of liver injury or severe co-morbid states were considered. Two patients refused consent for the study and another two patients refused to be admitted for the duration of the study. Seventy patients who fulfilled the inclusion and exclusion criteria and who gave informed written consent were randomized and divided into two groups: group I (pentoxifylline) had 34 patients, and group II (prednisolone) had 36 patients. The total duration of follow-up was 12 mo, with the patients being examined and evaluated in the liver clinic on a monthly basis. Two patients in group II withdrew voluntarily from the study and were excluded.

A total of 68 patients, 34 in each group, were considered for the final analysis. The baseline clinical and biochemical parameters of the patients receiving pentoxifylline or prednisolone are elaborated in Table 1, and were found to be comparable.

In group I, pentoxifylline therapy had to be stopped prematurely (within 3 mo) in five patients because of the development of life-threatening complications, all of whom unfortunately succumbed to the disease. Two patients expired following massive gastrointestinal bleeding. Two patients were lost to progressive hepatic encephalopathy and one patient died of sepsis, not responding to conservative management. Out of the five patients lost, two patients succumbed in the first 4 wk and three expired between 4 wk and 3 mo of therapy.

In group II, prednisolone therapy was stopped prematurely (within 3 mo) in 13 patients because of development of life-threatening complications. Two patients developed sepsis and both of them died of septic

**Table 1** Comparison of baseline parameters of patients receiving pentoxifylline (group I) vs those receiving prednisolone (group II) in the treatment of severe alcoholic hepatitis (mean  $\pm$  SD)

Parameter	Group I (pentoxifylline) ( <i>n</i> = 34)	Group II (prednisolone) ( <i>n</i> = 34)	<i>P</i> value
Age (yr)	47.53 $\pm$ 11.16	46.47 $\pm$ 9.67	0.68
Male:female	34:0	33:1	-
Ascites	31	33	0.37
Encephalopathy	20	23	0.61
Varices	23	22	0.80
Maddrey DF score	54.25 $\pm$ 16.24	57.78 $\pm$ 17.08	0.39
MELD score	23.14 $\pm$ 3.97	22.65 $\pm$ 3.33	0.58
GAHS	8.23 $\pm$ 1.07	7.94 $\pm$ 0.95	0.24
Child's score	11.85 $\pm$ 1.62	12.15 $\pm$ 1.28	0.41
Mean TLC (/cm <sup>3</sup> )	13926.47 $\pm$ 3068.15	15225 $\pm$ 11836.18	0.5379
Serum Na (mEq/L)	135.26 $\pm$ 8.26	132.80 $\pm$ 6.90	0.1908
Serum K (mEq/L)	4.18 $\pm$ 0.72	4.293 $\pm$ 0.98	0.6207
Urea (mg/dL)	31.68 $\pm$ 27.63	25.74 $\pm$ 16.92	0.2889
Creatinine (mg/dL)	1.42 $\pm$ 0.61	1.19 $\pm$ 0.32	0.057
Bilirubin (mg/dL)	5.40 $\pm$ 2.50	6.604 $\pm$ 3.90	0.1345
Albumin (gm/dL)	3.19 $\pm$ 0.67	3.040 $\pm$ 0.75	0.3870
ALT (IU/L)	54.88 $\pm$ 23.25	57.38 $\pm$ 20.50	0.6397
INR	1.97 $\pm$ 0.34	2.04 $\pm$ 0.31	0.3493

$P < 0.05$  considered statistically significant. TLC: Total leucocyte count.

**Table 2** Causes of death in patients receiving pentoxifylline or prednisolone in the treatment of severe alcoholic hepatitis (*n* = 34)

Cause of death	Group I (pentoxifylline)	Group II (prednisolone)
Hepatorenal syndrome	0	6
Sepsis	1	2
Gastrointestinal bleed	2	2
Encephalopathy	2	1
Unknown	0	1
Total	5	12

shock. Two patients had upper gastrointestinal bleed and succumbed to hemodynamic failure. One patient developed acute pancreatitis 26 d after inclusion; prednisolone was stopped and the patient responded to conservative management who has been doing well till the end of this study. Six patients died of hepatorenal Syndrome, not responding to conservative management. This is in sharp contrast to Group- I where none of the included patients developed hepatorenal Syndrome. One patient died of progressive hepatic encephalopathy and the cause of death could not be determined in one of the patients. Out of the total of 12 patients who expired in group II, seven succumbed in the first 4 wk and five more were lost between 4 wk and 3 mo of therapy. The cause of death and the complication profile are shown in Tables 2 and 3. The mortality was significantly higher among patients receiving prednisolone (35.29%) as compared to 14.71% among those receiving pentoxifylline, as elaborated by Kaplan-Meier analysis shown in Figure 1 ( $P = 0.04$ ).

Thirty-two patients in group I and 27 in group II were evaluated in the liver clinic at the end of 4 wk. The study was opened at this point in time and the allotment

**Table 3** Morbidity/complication profile of patients receiving pentoxifylline (group I) or prednisolone (group II) in the treatment of severe alcoholic hepatitis

Complications	Duration of follow-up			
	0-3 mo		3 mo to 1 yr	
	Group I (n = 34)	Group II (n = 34)	Group I (n = 29)	Group II (n = 22)
Nausea	24	19	14	4
Vomiting	12	8	4	1
Dyspepsia	3	7	1	1
GI bleed	-	2	2	4
Oral thrush	-	6	-	-
Sepsis	2	2	-	3
Recurrent encephalopathy	2	-	5	-
Worsening ascites	-	2	-	2
Impaired glucose tolerance	-	2	-	2
Delayed wound healing	-	2	-	1
Deep vein thrombosis	-	1	-	-
Pancreatitis	-	1	-	-
Hepatorenal syndrome	-	6	-	-

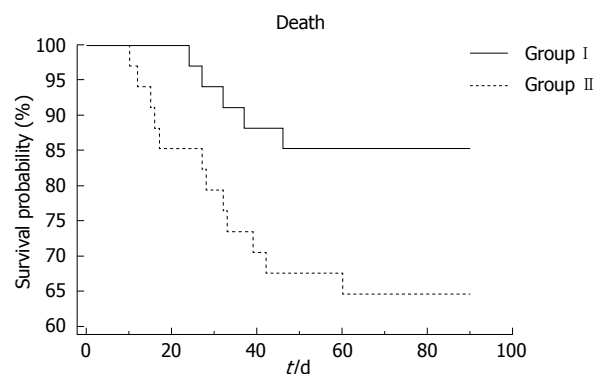
**Table 4** Comparison of baseline parameters of patients succumbing to various complications to those surviving at the end of the study (12 mo)

Parameter	Patients succumbing to complications (n = 17)	Surviving patients (n = 51)	P value
Age (yr)	44.53 ± 11.19	47.82 ± 10.07	0.26
Male:female	17:0	50:1	-
Ascites	17	47	0.23
Encephalopathy	8	35	0.19
Maddrey DF score	63.22 ± 18.58	53.61 ± 15.39	0.038
MELD score	23 ± 4.15	22.86 ± 3.50	0.89
GAHS	8.35 ± 0.99	8 ± 1.02	0.21
Child's score	12 ± 1.06	12 ± 1.57	1.00
Mean TLC (/cm <sup>3</sup> )	14008.82 ± 2804.14	14764.71 ± 9827.88	0.77
Serum Na (mEq/L)	131.76 ± 4.51	134.80 ± 8.35	0.16
Serum K (mEq/L)	4.22 ± 1.12	4.25 ± 0.76	0.90
Urea (mg/dL)	31.94 ± 27.95	27.63 ± 21.22	0.51
Creatinine (mg/dL)	1.19 ± 0.32	1.33 ± 0.55	0.34
Bilirubin (mg/dL)	6.88 ± 4.92	5.71 ± 2.56	0.21
Albumin (gm/dL)	2.97 ± 0.74	3.16 ± 0.70	0.32
ALT (IU/L)	52 ± 20.66	57.51 ± 22.18	0.37
INR	2.14 ± 0.32	1.96 ± 0.31	0.049

All values are expressed as mean ± SD.  $P < 0.05$  considered statistically significant.

of patients to the different groups was revealed. The investigations done at the baseline were repeated, and the patients were re-admitted if deemed necessary. The patients were followed-up on a monthly basis and the investigations were repeated at the end of 3 mo, 6 mo and 1 year. The patients did relatively well beyond 3 mo of follow-up, and no more patients succumbed to the disease. In group I (pentoxifylline), one patient resumed alcohol consumption and another was lost to follow-up after 5 mo, and they were excluded from further analysis. In group II (prednisolone), two patients resumed alcohol consumption, after 8 and 10 mo of follow-up, respectively, and both were excluded from further analysis.

The morbidity/complication profiles among the two groups were comparable (Table 3). Nausea followed by vomiting and dyspepsia were the most common adverse

**Figure 1** Survival curves (Kaplan-Meier life table analysis) of patients receiving pentoxifylline (group I) as compared to patients receiving prednisolone (group II), at the end of 3 mo of therapy.

effects encountered in both groups. Patients receiving pentoxifylline more frequently complained of nausea and vomiting, whereas dyspepsia was more common among those receiving prednisolone. Recurrent hepatic encephalopathy was only seen in group I (pentoxifylline), while oral thrush, worsening of ascites, impaired glucose tolerance, delayed wound healing and deep vein thrombosis were seen only in group II (Table 3). On follow-up, recurrent encephalopathy was observed among five patients in group- I (pentoxifylline) in contrast to none in group II. The summary of the trial and its design is shown in Figure 2.

Table 4 shows the baseline profile of patients who succumbed to various complications as compared to those surviving at the end of the study. It shows that baseline Maddrey DF score and international normalized ratio (INR) was significantly higher among patients who succumbed to the disease as compared to those who survived ( $P = 0.038$  and  $0.049$  respectively; Table 4). The baseline MELD score, GAHS and Child's score was not significantly different among the patients who expired as compared to those who survived (Table 4).

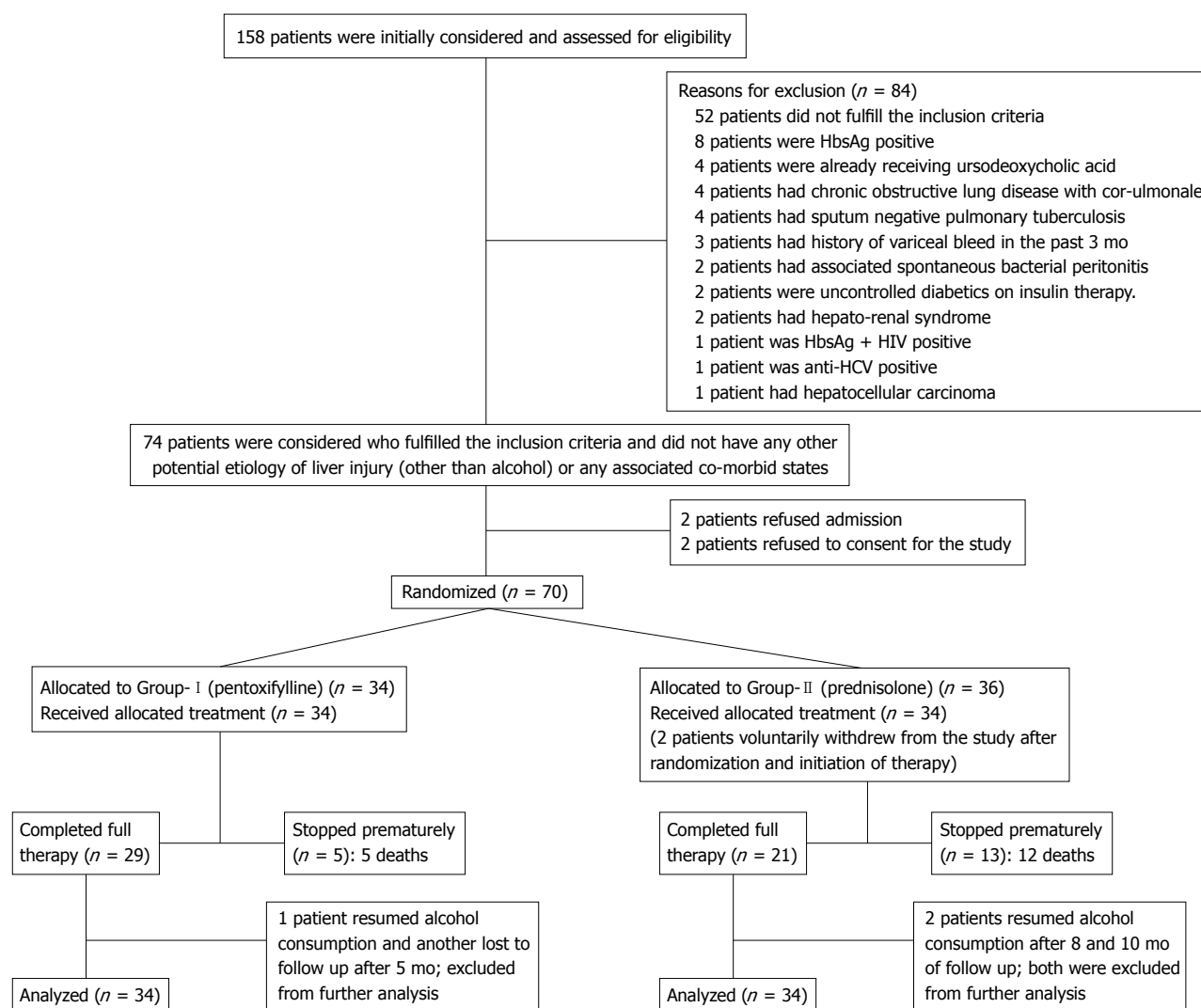
Table 5 shows the progression of GAHS, MELD, Child's and Maddrey DF score in the patients over 12 mo. The fall in Maddrey DF score and GAHS was comparable among the patients receiving pentoxifylline



**Table 5** Progression of scores evaluating the severity of liver disease of patients receiving pentoxifylline (group I) as compared to those receiving prednisolone (group II) in the treatment of severe alcoholic hepatitis (mean  $\pm$  SD)

Liver disease score	Baseline	Duration of follow-up			
		4 wk	3 mo	6 mo	1 yr
Maddrey DF score					
Group I <sup>1</sup>	54.25 $\pm$ 16.24	23.29 $\pm$ 12.07	14.3 $\pm$ 4.53	10.24 $\pm$ 4.27	7.79 $\pm$ 3.2
Group II <sup>2</sup>	57.78 $\pm$ 17.08	27.82 $\pm$ 11.73	15.60 $\pm$ 6.21	11.16 $\pm$ 3.70	7.27 $\pm$ 2.67
P value	0.39	0.15	0.39	0.43	0.94
MELD Score					
Group I <sup>1</sup>	23.14 $\pm$ 3.97	15.53 $\pm$ 3.63	12.41 $\pm$ 2.88	10.37 $\pm$ 2.32	9.18 $\pm$ 1.59
Group II <sup>2</sup>	22.65 $\pm$ 3.33	17.78 $\pm$ 4.56	13.45 $\pm$ 2.77	11.14 $\pm$ 1.83	9.4 $\pm$ 1.88
P value	0.58	0.04	0.20	0.21	0.67
GAHS					
Group I <sup>1</sup>	8.23 $\pm$ 1.07	6.37 $\pm$ 0.79	6.10 $\pm$ 0.77	5.96 $\pm$ 0.90	5.74 $\pm$ 0.66
Group II <sup>2</sup>	7.94 $\pm$ 0.95	6.52 $\pm$ 1.09	5.91 $\pm$ 0.61	5.91 $\pm$ 0.61	5.7 $\pm$ 0.57
P value	0.24	0.56	0.34	0.81	0.83
Child's score					
Group I <sup>1</sup>	11.85 $\pm$ 1.62	9.69 $\pm$ 2.57	7.14 $\pm$ 1.60	5.96 $\pm$ 1.09	5.78 $\pm$ 0.89
Group II <sup>2</sup>	12.15 $\pm$ 1.28	9.81 $\pm$ 2.08	7.59 $\pm$ 1.68	6.23 $\pm$ 0.97	5.9 $\pm$ 0.79
P value	0.41	0.84	0.33	0.38	0.63

$P < 0.05$  considered statistically significant. <sup>1</sup>In group I:  $n = 34$  at baseline,  $n = 32$  at 4 wk and  $n = 29$  at 3 mo,  $n = 27$  at 6 mo and 1 year. <sup>2</sup>In group II:  $n = 34$  at baseline,  $n = 27$  at 4 wk and  $n = 22$  at 3 mo, 6 mo and  $n = 20$  at 1 year.

**Figure 2** Summary of trial design and follow-up.

or prednisolone. MELD score was observed to be significantly lower among the patients receiving

pentoxifylline at the end of 4 wk, as compared to those receiving prednisolone.

## DISCUSSION

The pathogenesis of alcohol-induced liver injury has not yet been clearly elucidated. Oxidative and nitrosative stress are believed to have a key role in the pathogenesis of alcoholic liver disease, and greater emphasis has been given to the role of cytochrome P450 2E1 in mitochondrial stress and disruption<sup>[20]</sup>. Altered signaling pathways and involvement of extrahepatic mediators such as adiponectin may also have a key role<sup>[20]</sup>. Augmented TNF- $\alpha$  production by macrophages and Kupffer cells and signaling *via* the p55 TNF receptor have been shown to be critical in the development of steatosis and hepatitis following chronic alcohol intake<sup>[21]</sup>. Pentoxifylline, a non-specific phosphodiesterase inhibitor, with combined anti-inflammatory and antifibrogenic properties, has been shown to block the activation of hepatic stellate cells in culture<sup>[22]</sup>. It also has inhibitory effects on basic mechanisms of fibrogenesis such as cell proliferation and extracellular matrix synthesis<sup>[23]</sup>. Pentoxifylline has an added advantage of fewer adverse effects, such as gastrointestinal bleeding and renal shutdown, as compared to steroids. In the present study, none of the patients developed hepatorenal syndrome in the pentoxifylline group as compared to six in the prednisolone group. The MELD score in the pentoxifylline group was found to be significantly lower at the end of 4 wk of therapy (Table 5), as compared to the prednisolone group, confirming the renoprotective effects of pentoxifylline (as serum creatinine is a component of MELD score). Also gastrointestinal bleeding occurred more frequently in the prednisolone group as compared to the pentoxifylline group (Table 3).

The most important observation was the significantly reduced mortality among patients in the pentoxifylline group (14.71%) as compared to those receiving prednisolone (35.29%,  $P = 0.04$ , Figure 1). This reduced mortality in the pentoxifylline group was observed in spite of the increased occurrence of recurrent encephalopathy among patients in the pentoxifylline group. The patients with recurrent attacks of encephalopathy responded well to conservative management. This reduced mortality among patients in the pentoxifylline group can at least in part be explained by the renoprotective effects of pentoxifylline and the lower occurrence of gastrointestinal bleeding. In spite of the increased occurrence of nausea, and to a lesser extent vomiting, among patients in the pentoxifylline group, they were not severe enough to warrant stoppage of therapy. Also, with time, the occurrence of these complications was reduced (Table 3). Oral thrush, impaired glucose tolerance, poor wound healing, deep venous thrombosis and pancreatitis were some of the significant problems faced by the patients in the prednisolone group (Table 3).

Retrospectively, on analyzing the different liver function scores at the time of inclusion, only a higher Maddrey DF score was associated with the occurrence of increased mortality among patients with severe alcoholic hepatitis (Table 4). Thus Maddrey DF score remains the score of choice in determining prognosis

of patients with severe alcoholic hepatitis, even after the advent of newer scores like MELD and GAHS.

One of the limitations of this study is the absence of evidence of histological improvement and survival among patients receiving pentoxifylline or prednisolone, because of the lack of availability of transjugular liver biopsy. Also the assessment of immunological and inflammatory status (e.g. TNF- $\alpha$ ) of the patients was not possible. Nevertheless, a reduced mortality and more advantageous risk-benefit profile of pentoxifylline compared with prednisolone in patients with severe alcoholic hepatitis suggest that pentoxifylline is at least equivalent to prednisolone in the treatment of severe alcoholic hepatitis. However, further studies with a larger cohort of patients is warranted to decide if pentoxifylline is actually superior to the traditional drug prednisolone in the treatment of severe alcoholic hepatitis.

## COMMENTS

### Background

Severe alcoholic hepatitis is an acute, potential life-threatening manifestation of alcohol-induced liver injury, and forms part of the spectrum of liver disease, ranging from asymptomatic fatty liver to cirrhosis. The importance of severe alcoholic hepatitis lies in its significant morbidity and mortality, with a reported in-hospital mortality as high as 44%. Prednisolone has been used widely and is considered the standard treatment for severe acute alcoholic hepatitis with maddrey discriminant function (DF) score  $\geq 32$ . However, it is not free of adverse effects and has had its share of controversies.

### Research frontiers

Various other drugs have been tried in the treatment of alcoholic hepatitis, such as antioxidants, colchicines, calcium channel inhibitors, propylthiouracil and d-penicillamine, without much success. Augmented tumor necrosis factor (TNF)- $\alpha$  production by macrophages and Kupffer cells plays an important role in the pathogenesis of severe alcoholic hepatitis. However, infliximab, a human-mouse chimeric antibody to TNF- $\alpha$ , when used with prednisolone, has been found to be associated with severe infections, and is thus potentially harmful. The challenge is to find a drug whose efficacy is not only comparable to that of the standard drug prednisolone, but also safe and easy to administer over long periods of time.

### Innovations and breakthroughs

Recently, pentoxifylline, a non-specific phosphodiesterase inhibitor, with anti-inflammatory (TNF- $\alpha$  inhibition) and antifibrogenic properties has been found to be useful in patients with severe alcoholic hepatitis. The idea was to evaluate the efficacy of pentoxifylline and compare it to the standard drug prednisolone in the treatment of severe alcoholic hepatitis in a randomized controlled study, and to study the immediate and short term outcomes. Significantly reduced mortality, a more advantageous risk-benefit profile, and renoprotective effects of pentoxifylline compared with prednisolone in patients with severe alcoholic hepatitis may be considered as a breakthrough.

### Applications

The authors found that pentoxifylline was tolerated well in the treatment of severe alcoholic hepatitis, and was associated with significantly lower mortality, significantly lower model for end-stage liver disease (MELD) score at the end of 4 wk, and absence of hepatorenal syndrome. This should encourage the use of pentoxifylline in the treatment of severe alcoholic hepatitis. However, long-term prospective studies with a larger cohort of patients are needed to decide if pentoxifylline is actually superior to the traditional drug prednisolone in the treatment of severe alcoholic hepatitis.

### Terminology

MELD score is a measure of the severity of liver dysfunction and has been recently used in the assessment of patients with severe alcoholic hepatitis. However Maddrey DF score remains the standard for the assessment of patients with severe alcoholic hepatitis.

### Peer review

The experiments were planned and executed well and the manuscript is well written.

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BRIEF ARTICLES

## Altered spontaneous contractions of the ileum by anesthetic agents in rats exposed to peritonitis

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### Abstract

**AIM:** To investigate *in vitro* effects of propofol, midazolam and dexmedetomidine, which are commonly used anaesthetic or sedatives, on spontaneous contractions of the ileum both in normal rats and those exposed to hyperdynamic peritonitis.

**METHODS:** Spontaneous contractions of isolated ileum muscle segments from sham operated rats and those exposed to peritonitis, were studied *in vitro*. The amplitude and the frequency of spontaneous contractions of ileum muscle segments were studied after adding dexmedetomidine, propofol, and midazolam to the organ bath in a cumulative manner.

**RESULTS:** Both amplitude ( $85.2 \pm 6.6$  vs  $47.4 \pm 7.1$ ) and frequency ( $32.8 \pm 4.6$  vs  $20.2 \pm 3.9$ ) of spontaneous contractions in ileum smooth muscle segments were decreased significantly in the peritonitis group compared to the control group ( $P < 0.05$ ). Dexmedetomidine significantly increased the amplitude of spontaneous contractions ( $85.2 \pm 6.6$  vs  $152.0 \pm 5.4$ ,  $P < 0.05$ ) whereas, propofol ( $85.2 \pm 6.6$  vs  $49.6 \pm 4.8$ ,  $P < 0.05$ ) and midazolam ( $85.2 \pm 6.6$  vs  $39.2 \pm 4.5$ ,  $P < 0.05$ ) decreased it in both control and peritonitis groups. The frequency of spontaneous contractions were significantly decreased by propofol

in both control ( $32.8 \pm 4.6$  vs  $18.2 \pm 3.4$ ,  $P < 0.05$ ) and peritonitis groups ( $20.2 \pm 3.9$  vs  $11.6 \pm 3.2$ ,  $P < 0.05$ ). Dexmedetomidine and midazolam did not cause significant changes in the number of spontaneous contractions in both control and the peritonitis groups ( $P > 0.05$ ).

**CONCLUSION:** Propofol, midazolam and dexmedetomidine have various *in vitro* effects on spontaneous contractions of the rat ileum. While dexmedetomidine augments the spontaneous contraction of the rat ileum, propofol attenuates it. However, the effects of these compounds were parallel in both control and peritonitis groups.

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**Key words:** Ileum; Propofol; Midazolam; Dexmedetomidine; Peritonitis

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### INTRODUCTION

Dysmotility of the gastrointestinal tract is a major complication in critically ill patients in intensive care units. Most of the time, this dysmotility manifests itself as inhibition of gastrointestinal motility, and rarely as hypermotility<sup>[1]</sup>. Hypomotility can cause a functional, nonmechanical obstruction, most commonly an adynamic ileus. Impaired motility in critically ill patients can be caused by intestinal ischemia, electrolyte imbalances, peritoneal injury, abdominal surgery, lower-lobe pneumonia, pancreatitis, cholecystitis, intraabdominal abscesses, and medications (opiates, dopamine, diltiazem, verapamil, and anticholinergics)<sup>[2]</sup>. Sedatives such as propofol and midazolam further inhibit delayed intestinal transit in critically ill patients



in a dose dependent manner. The delay may cause complications such as intolerance to enteral feeding and overgrowth of bacteria in the gastrointestinal tract resulting in increase the incidence of aspiration pneumonitis<sup>[3]</sup>.

Abdominal sepsis or peritonitis is also a major cause of morbidity and mortality in surgical intensive care units. Gastrointestinal dysmotility commonly accompany peritonitis and those patients suffering peritonitis are also exposed to the additive effects of sedatives or anesthetics in surgical intensive care units. Koyluoglu *et al*<sup>[4]</sup> recently demonstrated that peritonitis induced a decrease in the amplitude and frequency of spontaneous contractions of ileum and jejunum segments from rats. Therefore, it is preferable to use a sedative or an anaesthetic that has few inhibitory effects on gastrointestinal transit in patients with peritonitis. However, there has been little study on this topic in the literature. In the present study, we aimed to investigate *in vitro* effects of propofol, midazolam and dexmedetomidine on spontaneous contractions of the ileum both in normal rats and those are exposed to hyperdynamic peritonitis.

## MATERIALS AND METHODS

### Animal preparation

Sixteen male Wistar albino rats each weighing approximately 280 g were used in this study. The study was approved by the ethics committee of Cumhuriyet University School of Medicine. Cecal ligation and puncture were used as the peritonitis model<sup>[5]</sup>. Animals were divided into two groups. The first group consisted of sham surgical controls that underwent the same procedure as the peritonitis group, such that laparotomy was performed under anesthesia, with manipulation of the cecum, but cecum ligation and puncture were not performed. Rats in the second group underwent cecal puncture and ligation as previously described by Martin *et al*<sup>[5]</sup>. Animals were anesthetized with intramuscular injections of 3 mg/kg xylazine (Rompun®, Bayer, Istanbul, Turkey) and 90 mg/kg ketamine (Ketalar®, Pfizer, Istanbul, Turkey), following which, laparotomy was performed *via* a 2 cm midline incision and the cecum was exposed. The cecum was ligated using 4/0 silk suture material just below the ileocecal valve, so that intestinal continuity was maintained. Then, the cecum was punctured using an 18-gauge needle in three locations, 1 cm apart, on the antimesenteric surface of the cecum, and cecum was gently compressed until feces were extruded. The cecum was replaced into the peritoneal cavity and the abdomen was then closed. When the animals were alert, they were transferred to single housing cages where they were left with *ad libitum* food and water. We then observed the rats in a recovery cage for 24 h.

A summary of the experimental treatments is presented below: Groups: Group I ( $n = 8$ ): Sham surgical controls; Group II ( $n = 8$ ): Peritonitis group.

At the second laparotomy, 24 h later, the rats were killed by cervical dislocation. The abdomen was opened with a midline incision and the ileum was removed and placed in previously aerated (95% O<sub>2</sub> and 5% CO<sub>2</sub>) Krebs-bicarbonate solution (composition in mmol/L: NaCl, 120; KCl, 4.6; CaCl<sub>2</sub>, 2.5; MgCl<sub>2</sub>, 1.2; NaHCO<sub>3</sub>, 22; NaH<sub>2</sub>PO<sub>4</sub> and glucose 11.5). Whole full-thickness segments of ileum were placed in circular direction in a 10 mL tissue baths, filled with pre-aerated Krebs-bicarbonate solution (KBS) at 37°C. The upper end of the preparation was tied to an isometric transducer (Grass FT 03, Quincy, MA, USA) and preloaded with 1-1.5 g. Tissues were allowed to equilibrate for 30 min.

### *In vitro* muscle contractility studies

Muscle segments from each group were contracted with 80 mmol/L KCl to ensure that they worked properly at the beginning and end of each experiment.

At the beginning of each experiment, 80 mmol/L KCl was added to the organ bath, and the contraction was considered as reference response. Subsequently, the amplitude of spontaneous contractions of the isolated ileum muscle segments were calculated as a percentage of the contraction induced by KCl (80 mmol/L) from both control and peritonitis groups. Changes in the frequency (number/min.) of spontaneous contractions were expressed as the number of contractions for 10 min intervals.

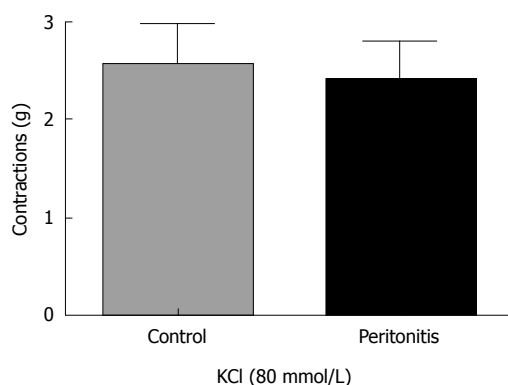
Following the KCl response, smooth muscle segments were allowed to equilibrate for 30 min before addition of cumulative doses of propofol ( $10^{-8}$ - $10^{-4}$  mol/L), midazolam ( $10^{-8}$ - $10^{-4}$  mol/L) and dexmedetomidine ( $10^{-8}$ - $10^{-4}$  mol/L). Amplitudes of the contractions induced by these compounds from both control and peritonitis groups were calculated as the percentage of the initial spontaneous contractions. Changes in the frequency of spontaneous contractions were expressed as the number of spontaneous contractions for 10 min after drug application. Isometric tensions were recorded on a Grass model 79 E polygraph. All experiments were performed in duplicate.

### Drugs

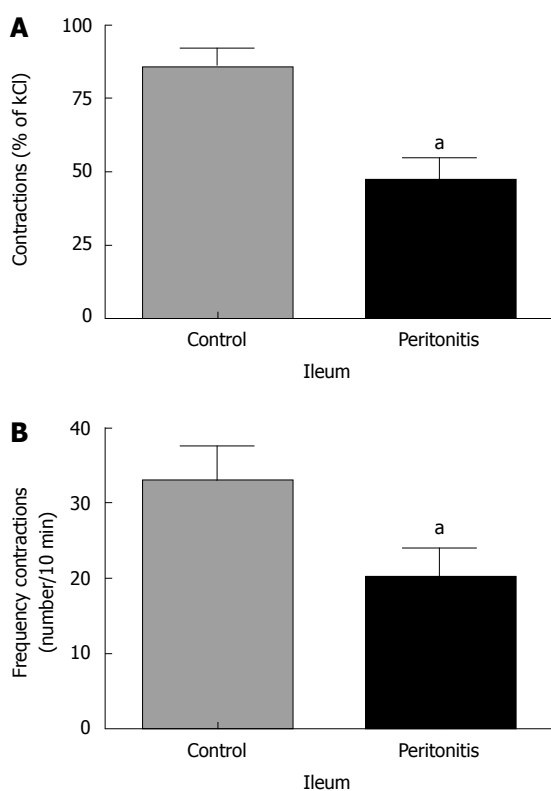
The following compounds were used: Propofol (2,6diisopropylphenol®, Aldrich Chemicals Co., USA), Midazolam (Midazolam hydrochloride®, Sigma, St Louis, USA), Dexmedetomidine (Abbot Laboratories, Abbot Park, IL, USA). All drugs were dissolved in distilled water. All drugs were freshly prepared on the day of the experiment.

### Data analysis

All data are expressed as mean  $\pm$  SD. Statistical comparisons between groups were performed using general linear models of analysis of variance (ANOVA) followed by the Newman-Keuls test and a *t* test when appropriate and *P*-values of less than 0.05 were considered to be statistically significant.



**Figure 1** KCl (80 mmol/L) induced contractions of isolated ileum muscle segments in control and peritonitis groups. No statistical difference was observed between groups ( $P > 0.05$ ).

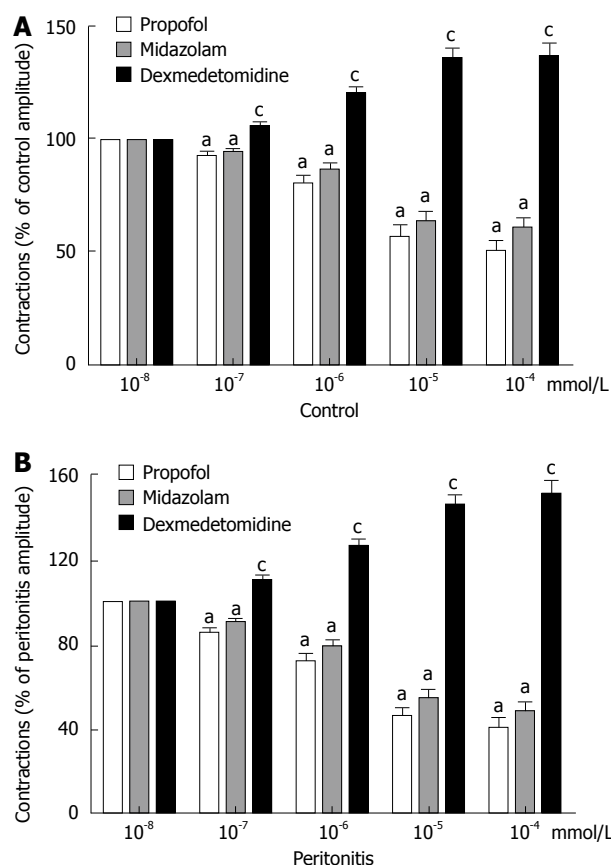


**Figure 2** Changes in the spontaneous contractions of the isolated ileum muscle segments. A: Amplitudes were calculated as a percentage of the contraction induced by KCl (80 mmol/L) from both control and peritonitis groups; B: Frequencies were expressed as the number of contractions for 10 min from both control and peritonitis groups. (<sup>a</sup> $P < 0.05$  vs control group; analysis of variance followed by Newman-Keuls test).

## RESULTS

Contractions induced by 80 mmol/L KCl were not significantly different between the peritonitis group and the control group in isolated ileum smooth muscle segments which indicated that muscle segments from both groups worked properly (Figure 1).

The mean amplitude of the spontaneous contractions was  $85.2 \pm 6.6$  in the control and  $47.4 \pm 7.1$  in the peritonitis group, respectively. The number of spontaneous contractions obtained in 10 min in the



**Figure 3** Amplitudes of the contractions induced by anaesthetic agents. A: Control group; B: Peritonitis group; both were calculated as the percentage of the initial contractions. (<sup>a</sup> $P < 0.05$  vs initial contractions, <sup>c</sup> $P < 0.05$  vs propofol and midazolam; analysis of variance followed by Newman-Keuls test).

peritonitis group was  $32.8 \pm 4.6$  and  $20.2 \pm 3.9$  in the control group. Both the amplitude and the frequency of spontaneous contractions of ileum smooth muscle segments were decreased significantly in the peritonitis group compared to the control group ( $P < 0.05$ ), (Figure 2A and B).

The amplitudes of spontaneous contractions of ileum muscle segments were studied after adding dexmedetomidine, propofol, and midazolam to the organ bath. Dexmedetomidine ( $10^{-8}$ - $10^{-4}$  mol/L) significantly increased the amplitude of spontaneous contractions starting from  $10^{-7}$  mol/L in isolated ileum muscle segments, in both the control and peritonitis groups, in a concentration-dependent manner. Propofol ( $10^{-8}$ - $10^{-4}$  mol/L) and midazolam ( $10^{-8}$ - $10^{-4}$  mol/L) decreased the amplitude of spontaneous contractions starting from  $10^{-7}$  mol/L as the molar concentrations of these drugs were increased ( $P < 0.05$ ). However, there was no statistical difference between the decreasing effects of propofol and midazolam ( $P > 0.05$ ), (Figure 3A and B), (Tables 1 and 2).

The frequency of spontaneous contractions of the ileum segments were significantly decreased by cumulative doses of propofol ( $10^{-8}$ - $10^{-4}$  mol/L) in both the control and peritonitis groups ( $P < 0.05$ ). Dexmedetomidine ( $10^{-8}$ - $10^{-4}$  mol/L) and midazolam ( $10^{-8}$ - $10^{-4}$  mol/L) did not cause a significant change in the number of spontaneous

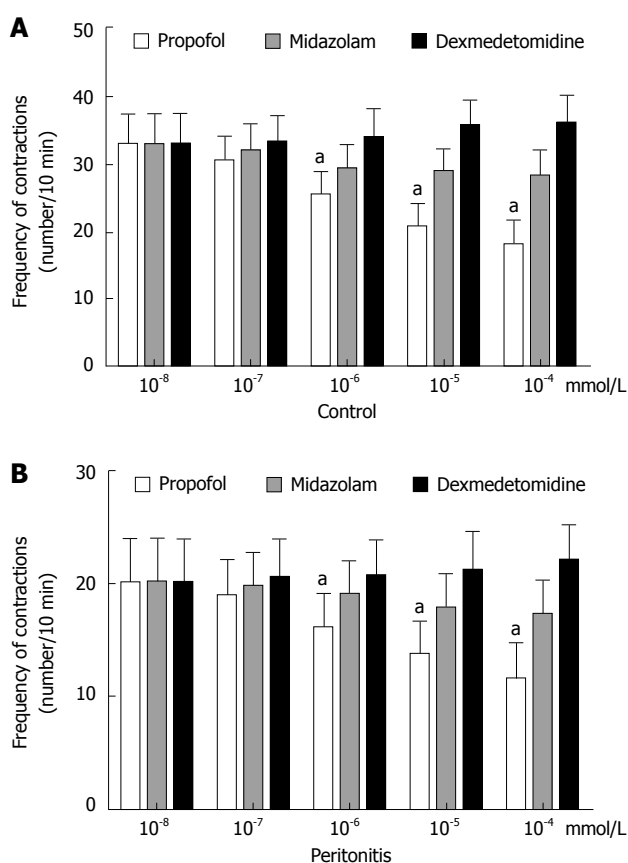
**Table 1** Effects of Propofol, midazolam and dexmedetomidine on spontaneous contractions in experiment groups

	Control	Peritonitis
Amplitude (%) <sup>1</sup>	85.2 ± 6.6 (100)	47.4 ± 7.1 <sup>a</sup> (100)
Propofol	49.6 ± 4.8 <sup>b</sup>	59.4 ± 5.2 <sup>b</sup>
Midazolam	39.2 ± 4.5 <sup>b</sup>	51.8 ± 5.0 <sup>b</sup>
Dexmedetomidine	136.6 ± 5.8 <sup>bc</sup>	152.0 ± 5.4 <sup>bc</sup>
Frequency (#/10 min)	32.8 ± 4.6	20.2 ± 3.9 <sup>a</sup>
Propofol	18.2 ± 3.4 <sup>b</sup>	11.6 ± 3.2 <sup>b</sup>
Midazolam	28.2 ± 3.8	17.4 ± 3.0
Dexmedetomidine	36.2 ± 4.0	22.2 ± 3.1

<sup>1</sup>Amplitudes of the contractions induced by anesthetic agents from both control and peritonitis groups were calculated as the percentage of the initial spontaneous contractions; <sup>a</sup> $P < 0.05$  vs control; <sup>b</sup> $P < 0.05$  vs initial spontaneous contractions; <sup>c</sup> $P < 0.05$  vs propofol and midazolam.

**Table 2** Effects of Propofol, midazolam and dexmedetomidine on amplitude and frequency of the spontaneous contractions

	Amplitude	Frequency
Propofol	Decreased	Decreased
Midazolam	Decreased	No significant change
Dexmedetomidine	Increased	No significant change



**Figure 4** Changes in the frequency of spontaneous contractions. A: Control group; B: Peritonitis group. Both were expressed as the number of contractions for 10 min. (<sup>a</sup> $P < 0.05$  vs initial contractions; analysis of variance followed by Newman-Keuls test).

contractions of the isolated ileum muscle segments in both the control and peritonitis groups ( $P > 0.05$ ) (Figure 4A and B) (Tables 1 and 2).

## DISCUSSION

The first finding of our study is that peritonitis altered the spontaneous activity of the rat ileum by decreasing both the amplitude and the frequency of the contractions in accordance with the previous study reported recently by Koyluoglu *et al*<sup>[4]</sup>. The main findings are that dexmedetomidine, a selective  $\alpha_2$ -adrenergic agonist, increased the amplitude, midazolam decreased the amplitude and propofol decreased both the amplitude and frequency of the spontaneous contractions in rat ileum in a concentration-dependent manner in both the control and peritonitis groups. Liu *et al*<sup>[6]</sup> reported that the  $\alpha_2$ -adrenoceptors inhibited neurogenic contractions of the rat ileum. Dexmedetomidine inhibited peristalsis in the guinea pig small intestine *in vitro* in a concentration dependent manner<sup>[7]</sup>. Dexmedetomidine strongly inhibited intestinal transit in the rat, however this inhibition was less potent than morphine<sup>[8]</sup>. However, in our study, dexmedetomidine, when applied in a concentration dependent manner significantly increased the amplitude but did not change the frequency of spontaneous contractions in the rat ileum *in vitro* both in the control and peritonitis groups. Our result seems to contradict previous reports. However, similarly to our results, Karaman *et al*<sup>[9]</sup> showed that dexmedetomidine *in vitro* caused a significant increase in the amplitude and frequency of spontaneous contractions in rat myometrium in a dose-dependent manner. The augmenting effect of this agent on spontaneous contractions needs to be investigated further.

Lee *et al*<sup>[10]</sup> demonstrated that propofol has an *in vitro* inhibitory effect on spontaneous contractile activity and causes acetylcholine induced contractions of human gastric and colonic smooth muscles at clinically relevant concentrations. It was demonstrated in a previous study that propofol, at concentrations of  $10^{-7}$  and  $10^{-6}$  mol/L, potentiated the guinea pig ileum contractile responses to  $\gamma$ -aminobutyric acid (GABA), but only at the lower dose range of applied GABA; at a concentration of  $10^{-5}$  mol/L, it inhibited the contractile effect over the entire dose range of applied GABA<sup>[11]</sup>. In the present study, propofol decreased both amplitude and frequency of contractions of rat ileum muscle segments in the control and peritonitis groups. Previous studies have reported the relaxant effects of propofol on other smooth muscle tissues such as vascular<sup>[12]</sup> and uterine smooth muscles<sup>[13]</sup>. The action of propofol involves a positive modulation of the inhibitory function of the neurotransmitter GABA through GABA A receptors<sup>[14]</sup>. Jensen *et al*<sup>[15]</sup> evaluated the influences of propofol, nitrous oxide and isoflurane and found that recovery and postoperative bowel function were not influenced by the anaesthetic technique after major gastrointestinal surgery. However, it might be different in case of a critically ill patient with peritonitis in an intensive care unit. The combined inhibitory effects of peritonitis and propofol might further slow intestinal transit and lead to an adynamic ileus.

Like other benzodiazepines, midazolam acts on the benzodiazepine binding site of GABA A receptors. When bound, it enhances the binding of GABA to the GABA A receptor, resulting in inhibitory effects on the central nervous system<sup>[16]</sup>. Castedal *et al*<sup>[17]</sup> reported in their manometric study that midazolam had relatively few effects on small bowel motility. In the present study, midazolam decreased the amplitude but caused very little change in the frequency of ileum contractions. Midazolam is often used for intravenous conscious sedation in endoscopic retrograde cholangiopancreatography (ERCP). Midazolam significantly altered the mobility of the sphincter of Oddi and caused a significant reduction in basal pressure of the sphincter of Oddi but did not affect the phasic frequency<sup>[18]</sup>.

In conclusion, dexmedetomidine, midazolam and propofol have various *in vitro* effects on spontaneous contractions of the rat ileum. While dexmedetomidine augments the spontaneous contraction of the rat ileum, propofol attenuates it. The effects of these agents were parallel in both control and peritonitis groups. The clinical implications of these findings need to be tested in surgical intensive care units, which might help in choosing the most appropriate drug for the sedation of patients with peritonitis.

## COMMENTS

### Background

Gastrointestinal dysmotility commonly accompanies peritonitis and those patients suffering peritonitis are also exposed to the additive effects of sedatives or anaesthetics in surgical intensive care units. Therefore, it is preferable to use a sedative or an anaesthetic that has few inhibitory effects on gastrointestinal transit in the patients with peritonitis. The *in vitro* effects of propofol, midazolam and dexmedetomidine, which are commonly used anaesthetic or sedatives, are worth investigating for that purpose.

### Research frontiers

The first finding of the study is that peritonitis altered the spontaneous activity of the rat ileum by decreasing both the amplitude and the frequency of the contractions. The main findings are that dexmedetomidine increased the amplitude, midazolam decreased the amplitude and propofol decreased both the amplitude and frequency of the spontaneous contractions of the rat ileum in a concentration-dependent manner in both the control and peritonitis groups.

### Innovations and breakthroughs

In this study, the *in vitro* effects of dexmedetomidine, midazolam and propofol on spontaneous contractions of ileum muscle in rats exposed to peritonitis were demonstrated.

### Applications

These findings will aid the selective use of sedatives or anaesthetics that have fewer inhibitory effects on gastrointestinal transit in patients with peritonitis.

### Peer review

The authors of the present study have compared the acute effects of different sedatives or anaesthetics on the spontaneous contractions in the ileum of sham-operated rats and rats with peritonitis. The amplitude and the frequency of the spontaneous contractions were affected to a different extent by the respective sedatives. This study was well performed.

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S- Editor Tian L L- Editor Stewart GJ E- Editor Yin DH



# Treatment of massive pancreaticojejunal anastomotic hemorrhage after pancreatoduodenectomy

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**Author contributions:** Liu C and Qiu YH contributed equally to this work; Qiu YH wrote the manuscript; Liu C designed and revised the manuscript; Jiang XQ, Yi B, Yu Y and Tan WF provided the collection of all the subjects material in addition to providing financial support for this work.

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modality for patients with acute hemorrhage after PDT. Vasography should be performed to locate the bleeding site.

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**Key words:** Pancreatoduodenectomy; Massive hemorrhage; Transcatheter artery embolization; Complication; Treatment

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## Abstract

**AIM:** To compare the treatment modalities for patients with massive pancreaticojejunal anastomotic hemorrhage after pancreatoduodenectomy (PDT).

**METHODS:** A retrospective study was undertaken to compare the outcomes of two major treatment modalities: transcatheter arterial embolization (TAE) and open surgical hemostasis. Seventeen patients with acute massive hemorrhage after PDT were recruited in this study. A comparison of two treatment modalities was based upon the clinicopathological characteristics and hospitalization stay, complications, and patient prognosis of the patients after surgery.

**RESULTS:** Of the 11 patients with massive hemorrhage after PDT treated with TAE, one died after discontinuing treatment, the other 10 stopped bleeding completely without recurrence of hemorrhage. All the 10 patients recovered well and were discharged, with a mean hospital stay of 10.45 d after hemostasis. The patients who underwent TAE had a re-operation rate of 18.2% and a mortality rate of 9.1%. Among the six patients who received open surgical hemostasis, two underwent another round of open surgical hemostasis. The mortality was 50%, and the recurrence of hemorrhage was 16.67%, with a mean hospital stay of 39.5 d.

**CONCLUSION:** TAE is a safe and effective treatment

## INTRODUCTION

Massive pancreaticojejunal anastomotic hemorrhage is the second most common complication of pancreatoduodenectomy (PDT). Blind open surgical hemostasis, however, poses additional risks and complications, which can be prevented by transcatheter arterial embolization (TAE). The present study was to evaluate the effectiveness of TAE and open surgical hemostasis. Between June 2005 and August 2008, a total of 308 patients underwent PDT in our hospital. Of these patients, 17 had massive pancreaticojejunal anastomotic hemorrhage following PDT. In this retrospective study, we summarized our clinical experiences with these patients in order to compare the safety and efficacy of TAE and open surgical hemostasis. The results may help determine the therapeutic approaches to massive pancreaticojejunal anastomotic hemorrhage after PDT.

## MATERIALS AND METHODS

### Patient information

A total of 17 subjects were enrolled in our study, including 13 men and five women, aged 42-68 years (mean  $60 \pm 2.45$  years). There were 10 cases of lower

common bile duct carcinoma, three cases of pancreatic head carcinoma and four cases of ampullary carcinoma. Obstructive jaundice was found in 12 patients. A catheter was inserted into the pancreatic duct in four cases, pancreatic duct exterior drainage was placed in two cases, and T-tube external drainage was placed in six cases, respectively. No T-tube or pancreatic duct drainage was placed in the remaining five patients. Post-surgical hemorrhage occurred in 10 cases from the gastroduodenal artery, from the posterior edge of the pancreatic stump in one case, and from the inferior pancreaticoduodenal artery in one case. Patients with upper gastrointestinal hemorrhage after PDT could be divided into early- and late-stage groups depending on the occurrence of hemorrhage within 5 d after PDT<sup>[1]</sup>. In our study, there were two cases of early-stage hemorrhage and 15 cases of late-stage hemorrhage. Pancreatic leakage was confirmed in five cases. One patient withdrew from the study because his family gave up the treatment. All patients enrolled in this study signed the informed consent.

#### **Inclusion criteria**

Patients who were diagnosed as lower common bile duct cancer, pancreatic head cancer, or digestive tract tumors such as ampullary carcinoma, with the need of pancreatoduodenectomy. Modified Child procedures were adopted in each patient, were included in this study. Digestive tract reconstruction was performed in order of pancreas-intestine, bile duct-intestine and stomach-intestine. An internal support tube was placed in the pancreatic duct and a cross-section of the pancreas was sutured to stop bleeding. Patients with massive pancreaticojejunal anastomotic hemorrhage after PDT and specific criteria are listed below.

#### **Exclusion criteria**

Patients not meeting the above diagnostic criteria or with accompanying mental disorders or severe primary diseases in cardiovascular, liver, kidney and hematopoietic systems, or those giving up treatment and withdrawing from the study were excluded, except for women in pregnancy or breast-feeding, or those going to be pregnant.

#### **Diagnosis of massive pancreaticojejunal anastomotic hemorrhage after PDT**

In our study, a modified Child's procedure was adopted for all subjects, namely digestive tract reconstruction was performed in order of pancreas-intestine, bile duct-intestine and stomach-intestine. An internal support tube was placed in the pancreatic duct and a cross-section of the pancreas was sutured to stop bleeding as previously described<sup>[2-4]</sup>. The diagnosis of massive pancreaticojejunal anastomotic hemorrhage after PDT was based on the literature<sup>[3]</sup>. The main diagnostic criteria for patients in our study were as follows. (1) Hemorrhage occurred within 1 mo of PDT (the last hemorrhage in our study occurred on day 23 post-surgery) and the presence of a

massive hemorrhage impacting vital signs was confirmed by angiography or gastroscopy (subjects whose origin of hemorrhage could not be found by angiography or those with acute ulcer hemorrhage were confirmed by gastroscopy). (2) Massive hemorrhage manifested as fresh blood effusing suddenly from the abdominal drainage tube or T-tube, or massive hematemesis or blood drainage from the gastric tube (> 200 mL). (3) Patients experienced hypovolemic shock accompanying a simultaneous decrease in hemoglobin (hemoglobin decreased more than 30 g/L in 24 h). Massive pancreaticojejunal anastomotic hemorrhage after PDT was diagnosed when the patients had hemorrhage and any other manifestations.

#### **Treatment of massive hemorrhage**

**TAE:** Of the 11 patients treated with TAE, eight underwent hepatic artery embolization, two underwent embolization of hepatic artery proper, and one refused any treatment upon initiating TAE. Hemorrhage was stopped in six cases after a single embolization procedure. Two patients underwent TAE twice for hemostasis (Figure 1). Emergency arteriography showed that the two patients had pancreatic stump artery bleeding into the jejunum. Hepatic artery was embolized during the first TAE, but recurrence of bleeding was found 4 h later. Arteriography showed that the pancreatic stump artery bled again, and microcoils embolizing the hepatic artery were displaced into the right hepatic artery. TAE was performed again with the common hepatic artery embolized using five microcoils, which stopped postoperative hemorrhage. Arteriography showed no obvious origin of hemorrhage in patient 3 during the first TAE. However, 7 h later, the patient received another arteriogram because of recurrence of bleeding. Duodenal stump bleeding was detected, and the common hepatic artery was then embolized to stop the hemorrhage. Arteriography revealed a crude edge on hepatic artery in patient 5, and the bleeding was stopped after embolization of the hepatic artery. A "vascular pool" image was observed in the inferior pancreaticoduodenal artery of patient 6, which was considered the origin of bleeding stopped by embolizing the common hepatic artery.

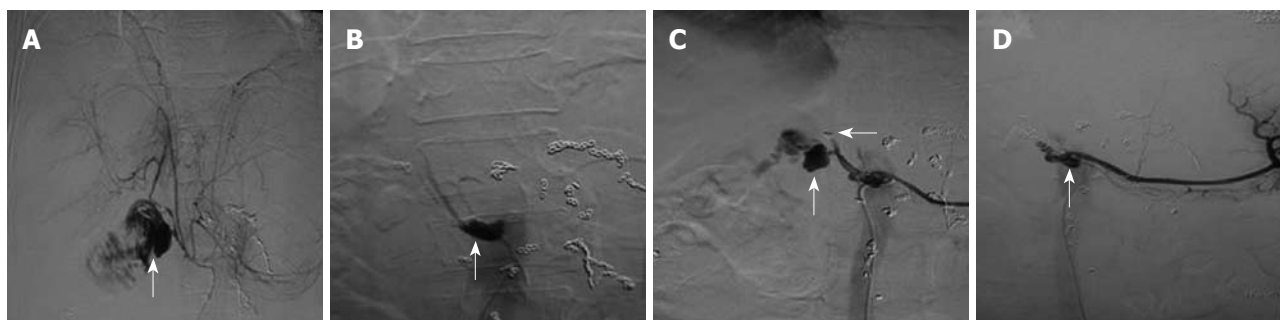
Complications such as hepatophyma and spleen necrosis may occur after TAE. In this study, patient 2 suffered from fever 2 mo after operation, with its peak at 39°C. Hepatophyma larger than 5 cm in diameter was seen in the right liver lobe. The patient recovered after paracentesis and anti-infective therapy. No such complications were found in the other patients.

**Open surgery:** A total of six patients received direct open surgical hemostasis for their hemorrhage.

## **RESULTS**

#### **Efficacy of TAE**

A total of 11 cases of massive hemorrhage after PDT



**Figure 1** Images from patient 2 (treated using TAE). A: Angiogram of the gastroduodenal stump bleeding before the first embolization. Arrow: Angiogram of the gastroduodenal stump bleeding; B: Angiogram of the gastroduodenal stump, which stopped bleeding after the first TAE. Arrow: Angiogram of the gastroduodenal stump); C: Angiogram of the stump prior to the second embolization. Angiography indicates that microcoils have entered the hepatic artery, causing renewed bleeding from the stump. Arrows: The second angiogram of the gastroduodenal stump bleeding; D: After the second embolization, angiography confirmed that the bleeding was stopped. Arrow: Angiogram of the gastroduodenal stump.

**Table 1** Overview of 11 patients with massive hemorrhage after PDT treated with TAE

Case	Time until bleeding	Symptom	Bleeding site	Embolization site	TAE	Hospital stay	Complications after TAE	Prognosis
1	21	T-tube bleeding	Pancreatic stump artery	Proper hepatic artery	1	9	High TB	Good
2	23	T-tube bleeding, hematemesis	Gastroduodenal stump	Common hepatic artery	2	14	High TB	Good
3	12	Double catheterization cannula, T-tube bleeding	Gastroduodenal stump	Common hepatic artery	2	14	High TB	Good
4	14	Hematemesis	Gastroduodenal stump	Common hepatic artery	1	5	Hyperpyrexia, pyemia	Dead
5	3	Double catheterization cannula bleeding	Proper hepatic artery	Proper hepatic artery	1	11	None	Good
6	5	Double catheterization cannula bleeding	Inferior pancreaticoduodenal artery	Common hepatic artery	1	8	High TB	Good
7	7	Single lumen cannula bleeding	Gastroduodenal Stump	Common hepatic artery	1	7	None	Good
8	9	Hematemesis	Gastroduodenal stump	Common hepatic artery	1	11	High TB	Good
9	10	Hematemesis	Gastroduodenal stump	Common hepatic artery	1	13	High TB	Good
10	7	Double catheterization cannula bleeding	Gastroduodenal stump	Common hepatic artery	1	13	High TB	Good
11	8	Double catheterization cannula bleeding	Gastroduodenal stump	Common hepatic artery	1	10	None	Good

TB: Total bilirubin.

underwent TAE which completely stopped their bleeding, except for one patient who died after refusing further treatment. Bleeding was stopped in eight patients after a single TAE procedure and in two patients after two treatments with TAE. All 10 patients recovered well and were discharged.

Total bilirubin (TB) exceeded the ULN level in seven out of the 10 patients and was as high as 178  $\mu\text{mol/L}$  in patient 2 after TAE, who was discharged with a full recovery of liver function after treatment. Patient 4 experienced a fever at 39.5°C after TAE and his TB level was 140  $\mu\text{mol/L}$ . Blood bacterial culture showed pyemia, and this patient abandoned treatment 5 d after TAE, and died 7 d after discharge. The other patients had a good prognosis after anti-infective and supportive treatment. Recurrence of hemorrhage was not found during a follow-up period of 2 mo and all 10 patients reported having an acceptable quality of life. The mean hospital stay was 10.45 d after hemostasis. Patients requiring a second operation accounted for 18.2%, and the overall mortality rate was 9.1% (Table 1).

### Efficacy of open surgery

Six patients underwent a second open surgical hemostasis for hemorrhage. Patients 1, 3 and 6 recovered and were discharged. However, patient 6 received an additional open surgical hemostasis, and patient 2 received emergency open surgical hemostasis again because of rebleeding 5 and 9 d after surgery, respectively. Patient 2 died of multiple organ failure. Liver and renal failure was observed in patients 4 and 5 after surgery. Both patients abandoned treatment, and one of them died 1 wk after discharge. The other patients had a good prognosis after anti-infective and supportive treatment. The mortality rate for patients undergoing open surgery was 50%, the recurrence rate of hemorrhage was 16.67%, and the mean hospital stay was 39.5 d (Table 2).

## DISCUSSION

Pancreatojejunal and choledochojejunal anastomotic internal drainage can effectively prevent biliary and pancreatic leakage. PDT is a common abdominal

Table 2 Overview of six patients with massive hemorrhage after PDT treated with open surgical hemostasis

Case	Time until bleeding	Symptom	Bleeding site	Hemostasis surgeries	Duration of hospital stay after hemostasis	Complications after surgery	Prognosis
1	17	T-tube bleeding	Gastroduodenal stump	1	59	None	Good
2	9	T-tube bleeding, hematemesis	Gastroduodenal stump	2	32	Multiple organ failure	Dead
3	10	T-tube bleeding	Gastroduodenal stump	1	63	None	Good
4	11	Hematemesis	Gastroduodenal stump	1	16	Liver failure	Dead
5	8	T-tube bleeding	Gastroduodenal stump	1	13	Renal failure	Dead
6	8	T-tube, pancreatic duct bleeding	Gastroduodenal stump	2	54	None	Good

operation. However, it is associated with the most common complications of hemorrhage and pancreatic leakage, with a relatively high risk<sup>[5]</sup>. The incidence of postoperative massive hemorrhage is 7.5%-12.4%. From June 2005 to August 2008, we performed 308 PDT, and massive pancreaticojejunal anastomotic hemorrhage occurred in 16 patients, accounting for 5.2% of its overall incidence rate, which is slightly lower than the reported rate<sup>[6]</sup>. Massive hemorrhage after PDT is difficult to stop, and if it is not stopped immediately, the mortality rate can be as high as 30%-58%<sup>[7,8]</sup>. Massive pancreaticojejunal anastomotic hemorrhage after PDT, mainly from the gastroduodenal stump, is often caused by transudatory digestive juices or corrosion of peripheral tissues by local fluid infection. Corrosion of the anastomotic vicinal vessels is especially common. In the present study, pancreatic leakage was confirmed in five of the 16 patients, and the number of leukocytes was increased before bleeding in six of them, and the number of WBC was  $20.5 \times 10^9/L$  in one patient. In addition, four of the six patients had a fever. Gastroduodenal arterial bleeding was detected by DSA in five of our patients, suggesting that it is particularly important to prevent intra-abdominal infection after PDT. Abdominal CT and B-ultrasound should be regularly performed to monitor the fever or blood pressure of such patients, to ensure that seroperitoneum is not compromised. If there is any indication of infection, puncture and drainage should be carried out to prevent further infection. During the surgery, arteries should be ligated twice or transfixed, with longer suturing ends as appropriate<sup>[9]</sup>.

Pancreaticojejunal anastomotic hemorrhage after PDT is difficult to treat. TAE with or without surgery might be a more effective procedure to stop bleeding. Since PDT may produce significant surgical trauma, and the site of hemorrhage is often difficult to locate, blind open surgical exploration often ends in failure. In addition, postoperative complications such as pancreatic leakage and intra-abdominal infection can result in more severe consequences than those caused by hemorrhage. Thus, better treatment modalities are needed for pancreaticojejunal hemorrhage after PDT. In our study, six patients underwent open surgical hemostasis again after PDT, and two of them received additional open surgical hemostasis because of recurrence of bleeding, which can be explained as follows. Namely, their overall poor condition following two major operations may have hindered recovery; the exact site of bleeding could

not be accurately located during the first operation for hemorrhage; intra-abdominal organ edema such as pancreatic edema was common and pancreatic leakage and intra-abdominal infections were not completely controlled. One patient with an intra-abdominal infection died of multiple organ failure. Liver or renal failure was also found in two patients after surgery. Both patients abandoned treatment, and one of them died 1 wk after discharge. Overall, the mortality rate for open surgery was 50%, the recurrence rate of bleeding was 16.67%, and the mean hospital stay was 39.5 d, suggesting that the prognosis of patients undergoing TAE is rather poor.

However, TAE can prevent re-operation risk and complications. In our study, 18.2% patients had a second TAE with a mortality of only 0.9%. In addition, the mean hospital stay after TAE was 10.45 d. Angiography should be performed as soon as hemorrhage is diagnosed to locate the site of bleeding. Then, embolization can be performed to stop hemorrhage<sup>[4,5]</sup>.

Angiography can diagnose hemorrhage and evaluate the efficacy of its treatment.

Angiography can locate the site of bleeding. TAE after PDT is needed for successful treatment of hemorrhage, and cooperation between surgeons is necessary to maximize the therapeutic efficacy and minimize the risk of TAE.

Since patients will be transported and allergy testing of contrast medium will be carried out during TAE, preoperative and intraoperative anti-hemorrhagic shock should be prevented. Any change in vital signs should be monitored while performing TAE. Larger microcoils should be selected for TAE because the common hepatic artery is large with a high-pressure blood flow.

After TAE, patients should be closely observed, and the outcome of hemostasis should be confirmed by arteriography. Patients without recurrence of bleeding can be sent to the intensive care unit for observation. The site of bleeding was found in eight of our 10 patients who underwent TAE. No recurrence of bleeding was observed at first in the other two patients who required additional surgery, which may have resulted from their poor condition (unresolved shock and low blood pressure), leading to reduced bleeding despite. Spastic contraction of blood vessels and/or obstruction of vessels or blood clots may have prevented accurate angiography. It was reported that gastroduodenal arterial stump bleeding is one of the main sources of pancreaticojejunal anastomotic hemorrhage after PDT<sup>[10]</sup>. If pancreaticojejunal



anastomotic hemorrhage is suspected but not dealt with, massive hemorrhage may occur. Common hepatic arterial embolization may be a better choice of treatment.

Nevertheless, we found that common hepatic artery embolization with TAE was likely to result in liver injury, which was manifested as elevated TB and aminotransferase level. In our study, TB level was higher in five patients than in normal controls after TAE, and the aminotransferase level was three-fold higher in four patients than in normal controls. However, if not accompanied with liver disease, liver failure rarely occurs after TAE. We provided supportive treatment for patients with liver injury by increasing the oxygen flow and concentration, extending oxygen absorption time, maintaining a high oxygen pressure and 100% oxygen saturation, and improving liver support. We also provided low-dose hormone therapy with prostaglandin E, growth hormone, and hepatocyte growth factor to increase the liver blood flow and promote liver cell regeneration. The patients received additional supportive treatment with plasma and human serum albumin when necessary.

In summary, TAE, which can avoid reoperation and complications of surgery, is an effective and safe treatment modality for acute postoperative hemorrhage after PDT. However, TAE may damage liver function due to hepatic artery embolization. Angiography can locate the site of bleeding, and if there are indications for surgery, TAE should be performed in time.

## COMMENTS

### Background

Massive pancreaticojejunal anastomotic hemorrhage after pancreaticoduodenectomy (PDT) is the second most common severe complication, only next to pancreaticojejunal anastomotic dehiscence. Blind open surgical hemostasis has more surgical risks and complications. However, transcatheter arterial embolization (TAE) can prevent reoperation risk and complications.

### Research frontiers

An internal support tube was placed in the pancreatic duct and a cross-section of pancreas was sutured to stop bleeding. If the bleeding site was located by selective angiography, embolization could be performed to stop bleeding.

## Applications

TAE is an effective and safe treatment modality for acute hemorrhage after PDT. Vasography should be performed in time to locate the site of bleeding. TAE can also prevent reoperation risk and complications.

## Peer review

The authors showed that TAE was an effective and safe treatment modality for acute hemorrhage after PDT. The initial observations are interesting.

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BRIEF ARTICLES

## Passage of bone-marrow-derived liver stem cells in a proliferating culture system

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**Author contributions:** Cai YF and Chen JS contributed equally to this work; Cai YF, Chen JS, Su SY, Zhen ZJ and Chen HW designed the research; Cai YF, Su SY, Zhen ZJ and Chen HW performed the research; Chen HW contributed new reagents/analytic tools; Cai YF, Chen JS and Zhen ZJ analyzed the data; Cai YF, Chen JS, Chen HW wrote the paper.

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### Abstract

**AIM:** To explore the feasibility of passage of bone-marrow-derived liver stem cells (BDLSCs) in culture systems that contain cholestatic serum.

**METHODS:** Whole bone marrow cells of rats were purified with conditioning selection media that contained 50 mL/L cholestatic serum. The selected BDLSCs were grown in a proliferating culture system and a differentiating culture system. The culture systems contained factors that stimulated the proliferation and differentiation of BDLSCs. Each passage of the proliferated stem cells was subjected to flow cytometry to detect stem cell markers. The morphology and phenotypic markers of BDLSCs were characterized using immunohistochemistry, reverse transcription polymerase chain reaction (RT-PCR) and electron microscopy. The metabolic functions of differentiated cells were also determined by glycogen staining and urea assay.

**RESULTS:** The conditioning selection medium isolated BDLSCs directly from cultured bone marrow cells. The selected BDLSCs could be proliferated for six passages and maintained stable markers in our proliferating system. When the culture system was changed to a differentiating system, hepatocyte-like colony-forming

units (H-CFUs) were formed. H-CFUs expressed markers of embryonic hepatocytes (alpha-fetoprotein, albumin and cytokeratin 8/18), biliary cells (cytokeratin 19), hepatocyte functional proteins (transferrin and cytochrome P450-2b1), and hepatocyte nuclear factors 1 $\alpha$  and -3 $\beta$ ). They also had glycogen storage and urea synthesis functions, two of the critical features of hepatocytes.

**CONCLUSION:** BDLSCs can be selected directly from bone marrow cells, and pure BDLSCs can be proliferated for six passages. The differentiated cells have hepatocyte-like phenotypes and functions. BDLSCs represent a new method to provide a readily available alternate source of cells for clinical hepatocyte therapy.

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**Key words:** Liver stem cells; Bone marrow; Cell separation; Cell proliferation; Cell differentiation

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Cai YF, Chen JS, Su SY, Zhen ZJ, Chen HW. Passage of bone marrow-derived liver stem cells with a proliferating culture system. *World J Gastroenterol* 2009; 15(13): 1630-1635 Available from: URL: <http://www.wjgnet.com/1007-9327/15/1630.asp> DOI: <http://dx.doi.org/10.3748/wjg.15.1630>

### INTRODUCTION

About a decade ago, many reports highlighted the broad developmental potential of bone-marrow-derived stem cells<sup>[1]</sup>. This gave new hope for cell therapy using autologous bone marrow cells, which has few ethical problems and has been applied to severe liver diseases<sup>[2]</sup>. However, little progress has been made in recent years because of the difficulties of selection and proliferation of this specific cell population<sup>[3]</sup>. It is necessary to find a new way to isolate and purify bone-marrow-derived liver stem cells (BDLSCs). Following the principle that cells in culture can survive only when they become accommodated to the surrounding environment, we developed a culture system to isolate BDLSCs from

bone marrow cells. Within this system, only BDLSCs could survive, while the other bone marrow cells could not<sup>[4]</sup>. This method could provide pure BDLSCs, but still could not harvest large numbers of cells, and the efficiency of differentiation was insufficient for therapeutic application. Here, we developed a proliferating culture system to passage BDLSCs and a differentiating system to yield hepatocyte-like cells, by modified culture environments.

## MATERIALS AND METHODS

### **Selection, proliferation and differentiation of BDLSCs**

#### **Preparation of conditioning selection medium:**

Common bile duct ligation and transection were carried out under general (ether) anesthesia in Sprague-Dawley rats weighing 200-250 g to induce cholestasis. After 10 d, whole blood was collected from each rat, and serum was separated. Cholestatic serum (50 mL/L) was added to Dulbecco's Modified Eagle's Medium (DMEM; Gibco) that contained 20 mmol/L HEPES (Sigma),  $10^{-7}$  mol/L dexamethasone (Sigma), and antibiotics, to act as the conditioning selection medium (See our previous study)<sup>[4]</sup>.

**Culture of bone marrow cells:** Rat bone marrow cells were obtained by flushing the femurs. About  $3 \times 10^8$  bone marrow cells were retrieved from one rat. Bone marrow cells were suspended in DMEM and plated at a density of  $1 \times 10^9$  cells/L onto culture dishes. DMEM enriched with 100 mL/L fetal bovine serum (FBS; HyClone), 20 mmol/L HEPES,  $10^{-7}$  mol/L dexamethasone, and antibiotics was used. Dishes were placed in a humidified incubator containing 50 mL/L CO<sub>2</sub> and 950 mL/L O<sub>2</sub> at 37°C.

**Selection and passage of liver stem cells:** Three days after culture, the medium and suspended cells were discarded and replaced by conditioning selection medium. Pure liver stem cells were selected by this medium. The cells were then proliferated with a new proliferating culture system, which contained 5% cholestatic serum, 10% FBS (Gibco), 10 mmol/L nicotinamide (Sigma), 1 mmol/L 2-phosphate ascorbic (Sigma), 1 mg/mL galactose (Sigma), 30 µg/mL praline (Sigma), insulin/transferrin/selenite mixture (Gibco), 10 ng/mL epidermal growth factor (EGF; Pepro Tech) and 10 ng/mL hepatocyte growth factor (HGF; Pepro Tech). When the cells developed into the proliferating stage, 10 ng/mL leukocyte inhibitory factor (LIF; Cytolab) was added to inhibit the differentiation of the cells. Passaging was carried out when the cells overlapped in the plate.

**Differentiation of liver stem cells:** Each passage of the stem cells was cultured in the differentiating system (i.e. the above culture system containing 20 ng/mL EGF, 25 ng/mL HGF, 1% DMSO (Sigma) and 20 ng/mL interleukin (IL)-3 (Pepro Tech), and LIF was discarded at this time. Differentiated cells were harvested.

### **Growth curve of passaged BDLSCs**

The passaged BDLSCs were digested and re-cultured on 24-well dishes at a concentration of  $1 \times 10^7$ /L. The mean number of cells in every three wells were counted daily for 8 d. The cell growth curve was then drawn according to these numbers.

### **Flow cytometry detection of the stability of stem cell markers of cell passages**

After each passage, the stem cells and the differentiated cells were digested to prepare single-cell suspensions. The cell surface markers beta-2 microglobulin (β<sub>2m</sub>), Thy-1, CD34, Flt-3, c-kit and IL-3R were detected with cytometry. Each marker was detected six times in every passage, and mean values were determined. The stability of stem cell surface marker expression was determined, and the expression before and after differentiation was compared.

### **Morphological and phenotypic markers of differentiated cells**

**Immunohistochemistry:** Stem cells of each passage were cultured in the differentiating system in six-well dishes with cover glasses. When hepatocyte-like cells came into being, cover glasses and the differentiated BDLSCs were removed and fixed for immunohistochemistry. The primary antibodies were goat anti-rat albumin, alpha-fetoprotein (AFP), and cytokeratin -8/18 (CK8/18) polyclonal antibodies. (see our previous study)<sup>[4]</sup>.

**Electron microscopy:** Culture dishes of differentiated cells were washed with PBS, and fixed in glutaraldehyde for 48 h. After fixation, cells were curetted and centrifuged to form aggregates. After post-fixation in osmium tetroxide, cells were dehydrated in graded alcohols and embedded in low-viscosity epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate and viewed under an electron microscope (see our previous study)<sup>[4]</sup>.

**Reverse transcription polymerase chain reaction (RT-PCR):** Total RNA was extracted from the differentiated cells and mRNA transcription of hepatocyte nuclear factor (HNF)-1α, HNF-3β, CK18, CK19, albumin, AFP, transthyretin (TTR) and cytochrome P450-2b1 (CYP2b1) were detected by RT-PCR (see our previous study)<sup>[4]</sup>.

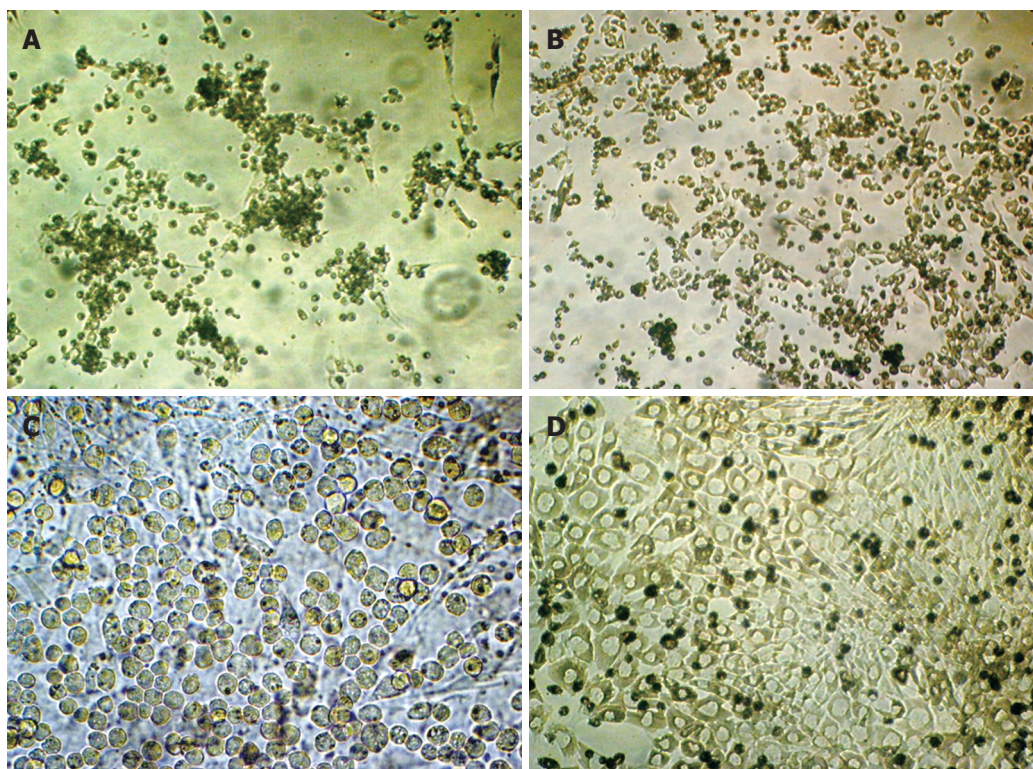
### **Function tests of differentiated cells**

Periodic acid-Schiff (PAS) staining for glycogen and urea assay of the differentiated cells were also conducted to confirm their hepatocyte-like function (see our previous study)<sup>[4]</sup>.

### **Statistical analysis**

Data were presented as mean ± SD. All the data were analyzed using the SPSS statistical package 13.0. Differences in means were tested by the unpaired Student's *t* test. All tests were considered statistically significant at *P* < 0.05.





**Figure 1** Morphological evidence of BDLSC differentiation. A: BDLSC clone selected from bone marrow cells, phase-contrast microscope (200 ×); B: Proliferation of liver stem cell clone-phase-contrast microscope (200 ×); C: Differentiation-prohibited passage stem cells, phase-contrast microscope (400 ×); D: Hepatocyte-like cells after differentiation, phase-contrast microscope (400 ×).

## RESULTS

### Morphological evidence of BDLSC differentiation

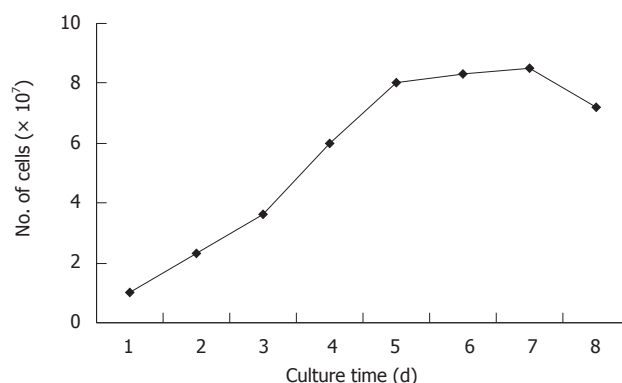
During the first 3 d, many colonies appeared in the conditioning cholestatic serum. These colonies were composed of small, undifferentiated cells in the center, and epithelioid cells at the periphery (Figure 1A). After replacing with the proliferating system, the colonies enlarged, and the cells proliferated rapidly in about 4 d (Figure 1B). With the addition of LIF, mature differentiation was inhibited, and colonies of small round cells with large nuclei, little endochylema and high nuclear-to-cytoplasmic ratio appeared (Figure 1C). The colonies maintained the ability of proliferation and passage was required in 5-7 d. The original aim of six passages could be achieved. After six passages, however, the proliferation was difficult to maintain and fibroblast-like cells appeared. After replacing with the differentiating system, hepatocyte-like colony-forming units (H-CFUs) started to appear. The H-CFUs were composed of small, undifferentiated cells in the center, and large cells with regular multilateral contours, low nuclear-to-cytoplasmic ratio, and single round nuclei at the periphery. The differentiated cells formed cords or trabeculae that resembled the hepatocyte cords in hepatic lobules (Figure 1D).

### Growth curve of passaged BDLSCs

The curve showed that the number of cells increased as the culture time passed, and rapid proliferation appeared from day 2 to day 5 (Figure 2).

### Flow cytometry detecting stability of stem cell markers of cell passage

Flow cytometry detecting the cell surface markers of



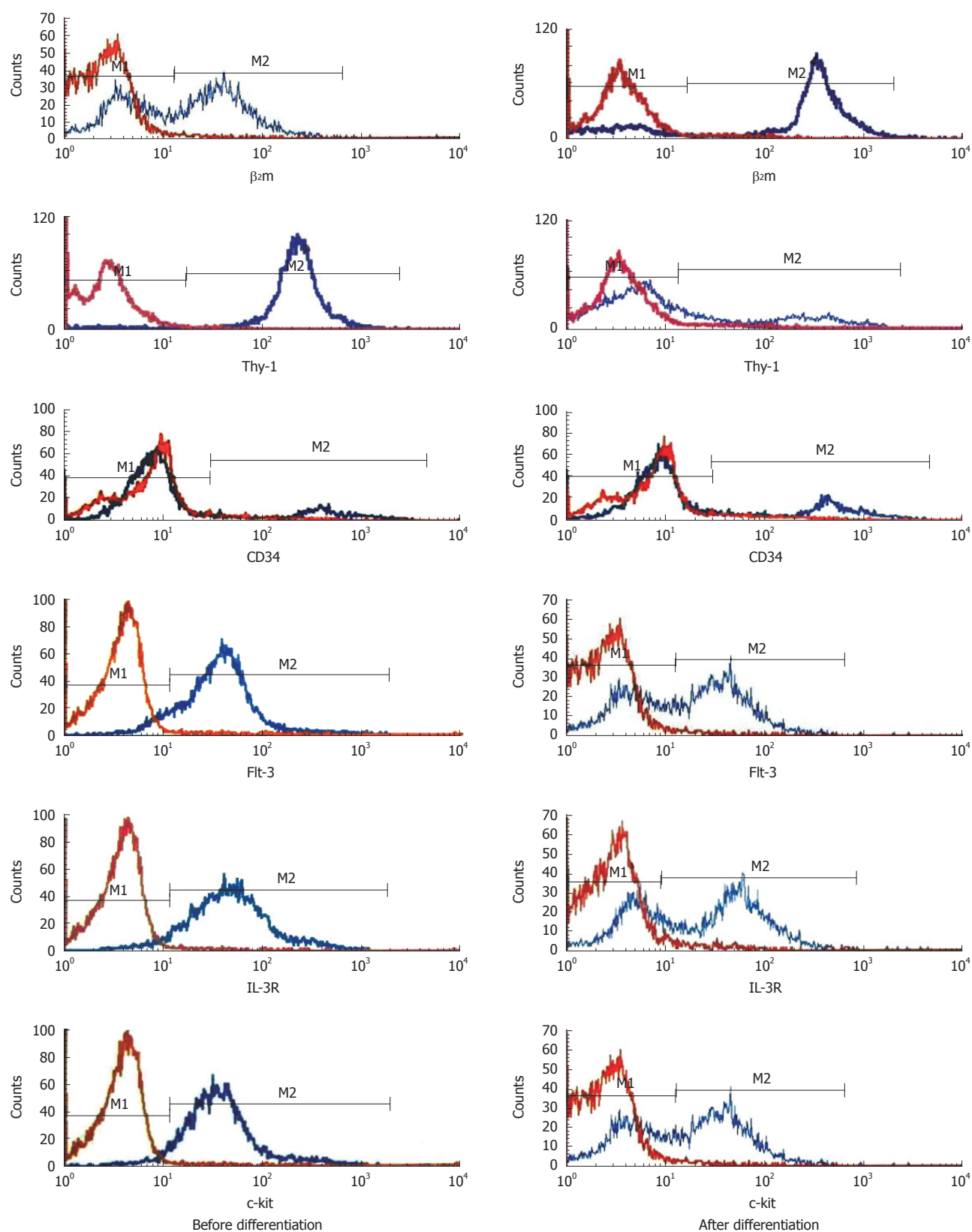
**Figure 2** Cell growth curve of passaged BDLSC.

each passage showed that the undifferentiating cells were relatively stable  $\beta_2m^{low}/Thy-1^+/CD34^{low}/c-kit^+$  cells. The markers changed after differentiation, with significant differences ( $P < 0.05$ ) in all of the detected markers except CD34 (Figures 3 and 4).

### Phenotypic markers of differentiated cells

The differentiated cells from each passage were analyzed for biochemical evidence of hepatocytic differentiation, in order to confirm their characteristics. Just as in our previous study<sup>[4]</sup>, immunohistochemistry was performed on differentiated cells grown on cover glasses to determine the presence of albumin, AFP and CK8/18, the characteristic proteins expressed during hepatocyte development, which revealed diffuse cytoplasmic staining for these proteins. RT-PCR further confirmed the hepatocytic characteristics of differentiated cells as the results showed that there were mRNA transcripts of HNF-1 $\alpha$ , HNF-3 $\beta$ , albumin, AFP, CK-18, CK-19, TTR,





**Figure 3** Differences of stem cell markers before and after differentiation by flow cytometry. Red curves: Negative control, M1: Negative part, M2: Positive part.

and CYP2b1, all of which were hepatocyte specific.

Ultrastructurally, the differentiated cells were rich in endoplasmic reticulum and ribosomes and contained abundant ellipsoid mitochondria, which were typical features of adult hepatocytes.

#### Function tests of differentiated cells

We also found glycogen storage in the cytoplasm of hepatocyte-like differentiated cells by PAS staining, and urea production and secretion by urea assay of the culture medium<sup>[4]</sup>.

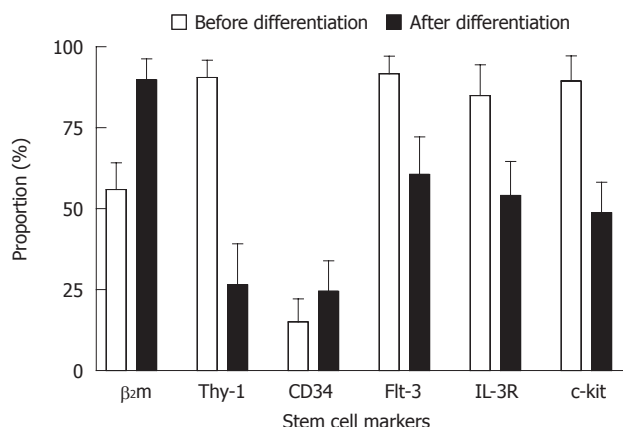


Figure 4 Expression of stem cell markers before and after differentiation.

## DISCUSSION

Bone-marrow-derived stem cells are able to transdifferentiate into hepatic cells as shown in cross-sex and cross-strain bone marrow and whole liver transplantation experiments<sup>[5]</sup>. Great interest has been aroused in the identification and isolation of BDLSCs<sup>[6]</sup>. However, the characteristic surface markers of BDLSCs and their pedigrees in the derivation of bone marrow stem cells remain obscure<sup>[7]</sup>. It is difficult to identify and sort particular cells by immunological methods, such as fluorescence-activated and magnetic-activated cell sorting<sup>[8,9]</sup>. Furthermore, the sorting of stem cells with complicated surface markers is difficult<sup>[10]</sup>. We developed a conditioning culture system to solve this problem. Within such a system, only BDLSCs could survive, while other cells could not, therefore, it was possible to purify these specific stem cells<sup>[4]</sup>.

However, the passage of BDLSCs is still a challenge, which has hindered the proliferation of the cells. No report of successful passage has been published. In our experiments, we found that the key to passage of the stem cells was to isolate purified stem cells. We were able to harvest pure BDLSC colonies with our selection system. After that, a proliferating system that contained all the nutrients required for the proliferation of liver stem cells was introduced to culture the cells<sup>[11,12]</sup>. This system contained all the known conditions required for the proliferation of oval cells<sup>[13]</sup>, with low concentrations of HGF and EGF and a mixture of FBS and cholestatic serum, so as to maintain the characteristics of the liver stem cells. At the same time, for stem cell proliferation, differentiation must be prevented. We therefore provided LIF, a factor known as a strong inhibitor of stem cell differentiation<sup>[14]</sup>. When replaced with the proliferating system on day 4, rapid proliferation occurred and the cells maintained their undifferentiated state, under the action of LIF. To harvest the differentiated cells, LIF must be discarded, and the concentration of EGF and HGF must be higher. Under the conditions that contained the differentiating factors, hepatocyte-like cells appeared from each passage of the stem cells. The morphology and phenotypic markers manifested in the differentiated cells were similar to those of liver stem cells. These cells

expressed markers of embryonic hepatocytes (AFP, albumin and CK18), biliary cells (CK19), hepatocyte functional proteins (ITR and CYP2b1), and hepatocyte nuclear factors (HNF-1 $\alpha$  and HNF-3 $\beta$ ). To confirm that the differentiated cells had functional characteristics of hepatocytes, we tested the glycogen and urea synthesis functions of the cells, and demonstrated that the cells possessed hepatocyte-like functions. These all proved that each passage of the stem cells can differentiate into hepatocyte-like cells. We also detected the stability of each passage of the stem cells with flow cytometry, and the results showed that the proliferation was stable. In addition, the increase in  $\beta 2m$  expression on the differentiated cells demonstrated their maturation because  $\beta 2m$  is only expressed on mature cells. However, we had difficulty in maintaining the proliferation after six passages. The emergence of fibroblast-like cells became inevitable. The explanation might be that the proliferation of adult stem cells, unlike tumor cells, was limited, and that new factors or a network similar to the liver tissue were required for further proliferation.

In conclusion, we demonstrated that BDLSCs could be selected from whole bone marrow cells using conditioning medium. The stem cells could also be proliferated for six passages and differentiated into hepatocyte-like cells. These methods not only provide a new and effective method for the isolation and purification of extrahepatic liver stem cells, but also provide a readily available alternate source of cells for clinical hepatocyte therapy.

## COMMENTS

### Background

Bone-marrow-derived liver stem cells (BDLSCs) were once a hot topic in the field of stem cell research because of their important therapeutic implications, but little progress has been made in recent years because of the difficulty of isolation and proliferation of this special cell population. The authors developed a culture system to isolate BDLSCs from bone marrow cells, from which they could culture pure BDLSCs *in vitro*. However, the passage of BDLSCs is still a challenge, which has hindered the proliferation of the cells.

### Research frontiers

Great interests has been aroused in the identification and isolation of liver stem cells from bone marrow cells. Several subsets of bone marrow cells have been found to have the potential to differentiate into hepatocytes, but no report of successful passage has been published.

### Innovations and breakthroughs

This study provided a new method for BDLSC isolation and proliferation. With a careful designed culture system, BDLSCs can be purified *in vitro* and be passaged, which brings new hope to the clinical use of bone-marrow-derived stem cells.

### Applications

BDLSCs can be selected directly from bone marrow cells, and pure BDLSCs can also be proliferated for six passages. The differentiated cells have hepatocyte-like phenotypes and functions. BDLSCs represent a new method to provide a readily available alternate source of cells for clinical hepatocyte therapy.

### Peer review

In this study, the authors used their original method to retrieve cells that are possibly BDLSCs. Then, they used fluorescence-activated cell sorting to determine the cells' characteristics before and after differentiation. This is an interesting and potentially important study, which suggests that bone-marrow-derived cells can be stimulated to expand and then differentiate into hepatocyte-like cells, which may possibly be used to treat liver disease.

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BRIEF ARTICLES

## B-cell clonality in the liver of hepatitis C virus-infected patients

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### Abstract

**AIM:** The association of hepatitis C virus (HCV) infection with type II mixed cryoglobulinemia is well established, but the role of HCV in B-cell lymphoma remains controversial. In patients with HCV infection, B-cell clonal expansions have been detected in peripheral blood and bone marrow, and a high prevalence of B-cell non-Hodgkin's lymphomas has been documented. Liver biopsies in chronic HCV infection frequently show portal lymphoid infiltrates with features of B follicles, whose clonality has not yet been investigated. The object of this study was to determine the frequency of liver-infiltrating monoclonal B-cells in 40 patients with HCV infection.

**METHODS:** Eight hundred and forty-eight patients were studied prospectively, including 40 HCV-positive patients and 808 patients with chronic hepatitis B virus (HBV) infection. Immunohistochemical study for B- and T-cell markers was performed on the paraffin-embedded liver tissue sections. The clonality of lymphoid B-cells was tested using a

polymerase chain reaction (PCR) approach designed to identify immunoglobulin heavy chain gene (*IgH*) rearrangements.

**RESULTS:** Liver-infiltrating monoclonal B-cells were detected in the liver for 4 (10%) of 40 HCV-positive patients but were present in only 3 (0.37%) of 808 liver biopsy specimens with chronic HBV infection. Chi-square testing showed that the monoclonal B-cells infiltration in the liver was more frequent in the HCV-infected patients ( $P = 0.000$ ). A clonal *IgH* rearrangement was detected in 5 (71.4%) of 7 liver biopsy specimens with monoclonal B-cells infiltration. In 2 of 5 patients with both a clonal B-cell expansion and monoclonal B-cells infiltration in the liver, a definite B-cell malignancy was finally diagnosed.

**CONCLUSION:** Liver-infiltrating monoclonal B-cells are detected in the liver of patients with chronic HCV and HBV infection. A high percentage of patients with monoclonal B-cells infiltration and B-cell clonality in the liver were finally diagnosed as having a definite B-cell malignancy.

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**Key words:** Hepatitis; Hepatitis C virus; B-lymphocytes; Polymerase chain reaction; Gene rearrangement; Clonality

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Fan HB, Zhu YF, Chen AS, Zhou MX, Yan FM, Ma XJ, Zhou H. B-cell clonality in the liver of hepatitis C virus-infected patients. *World J Gastroenterol* 2009; 15(13): 1636-1640 Available from: URL: <http://www.wjgnet.com/1007-9327/15/1636.asp>  
DOI: <http://dx.doi.org/10.3748/wjg.15.1636>

### INTRODUCTION

The relationship between lymphoproliferative disorders and infectious agents has been recognised and studied for many decades. A causative association between



hepatitis C virus (HCV) and non-Hodgkin's lymphoma (NHL) was postulated relatively recently and has been the subject both of intense investigation and of some debate<sup>[1-5]</sup>. On the strength of epidemiological data, emerging biological investigations and clinical observations, HCV appears to be involved in the pathogenesis of at least a proportion of patients with NHL<sup>[6-7]</sup>. This hypothesis is supported by the evidence that HCV is not only hepatotropic, but also a lymphotropic virus<sup>[8]</sup>. *In vitro*, HCV is able to replicate in human T-cell lines<sup>[9]</sup> and in normal peripheral blood mononuclear cells from healthy subjects<sup>[10]</sup>. Moreover, viral genomic sequences have been found in T- and B-cell populations, as well as in monocyte-derived cells from peripheral blood and liver tissue in patients with HCV-related chronic hepatitis<sup>[10-12]</sup>. Most studies on HCV-associated B-cell proliferations have focused on peripheral blood and bone marrow lymphocytes. During HCV infection, liver tissue is frequently characterized by prominent lymphoid aggregates in portal tracts that show histological and immunophenotypical features of B follicles<sup>[13-14]</sup>. The nature of these aggregates has not yet been investigated in detail; in particular, the clonality of B-cells within lymphoid infiltrates in the liver of HCV-infected patients has not been analyzed at the molecular level. In the present study, we analyzed the frequency of liver-infiltrating monoclonal B-cells from the paraffin-embedded liver biopsies of 40 patients with HCV infection. In 7 patients with monoclonal B-cells infiltration in the liver who were followed up, B-cell clonality was tested using a polymerase chain reaction (PCR) approach designed to identify immunoglobulin heavy chain gene (*IgH*) rearrangements.

## MATERIALS AND METHODS

### Ethics

All patients were notified of the risk of the liver puncturation and provided informed written consent.

### Selection of cases

From June 2003 to December 2005, 848 patients were enrolled in a prospective study and were followed up as outpatients at the Department of Infectious Disease at our NanFang Hospital. In 40 HCV-positive patients (anti-HCV antibody and HCV RNA positive), anti-hepatitis B virus (HBV) and anti-human immunodeficiency virus antibodies were negative. The patients had not received anti-HCV therapy for at least 6 mo. For each patient, a sample of liver biopsy was performed. A control group, consisting of 808 HBV-infected patients with other non-immune chronic liver diseases, was followed up at the same department. All the patients were diagnosed as having chronic hepatitis according to the criteria formulated by the Chinese Society of Infectious disease and Parasitology and Chinese Society of Hepatology, Chinese Medical Association.

### Immunohistochemical characterization of lymphoid aggregates

The 848 liver biopsies contained 7 monoclonal B-cell infiltrations. The composition of the 7 infiltration cell specimens was investigated by immunohistochemistry, using antibodies against B-cell (L26/CD20) markers, following the streptavidin-biotin-complex immunoperoxidase technique.

### Polymerase chain reaction of *IgH* rearrangements

The DNA from the 7 monoclonal B-cells infiltration liver specimens successfully amplified for the  $\beta$ -actin gene was then subjected to clonality assessment by PCR amplification of rearranged *IgH* genes. After heating for 10 min at 95°C, 2  $\mu$ L from each sample was added to the PCR mixture containing 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.3), 25 pmol/L of each primer, 200  $\mu$ mol/L of each dNTP, 1.25 units of *Taq* polymerase, and 4.5 mmol/L MgCl<sub>2</sub> in a final volume of 50  $\mu$ L.

We amplified the hypervariable complementary region (CDR-II and CDR-III), included between the third and joining regions (FR-III and JH) of *IgH* genes, with a 5'-primer homologous to the FR-III region, and JH as 3'-primers, using a seminested protocol. The primers were FR3, CTGTGACACGGCCGTGTATTACTG; JH, AACTGCAGAGGAGACGGTGACC. The first PCR cycle consisted of denaturation of the sample DNA at 93°C for five minutes, annealing of the primers at 55°C for one minute, then extension of the DNA at 73°C for two minutes. Each experiment was duplicated and accompanied by a negative control containing no template DNA. Ten microlitres of the PCR products were analyzed by electrophoresis on 3% agarose gels, stained by ethidium bromide, and viewed under UV light.

### The histologic features of liver biopsy specimens were analyzed

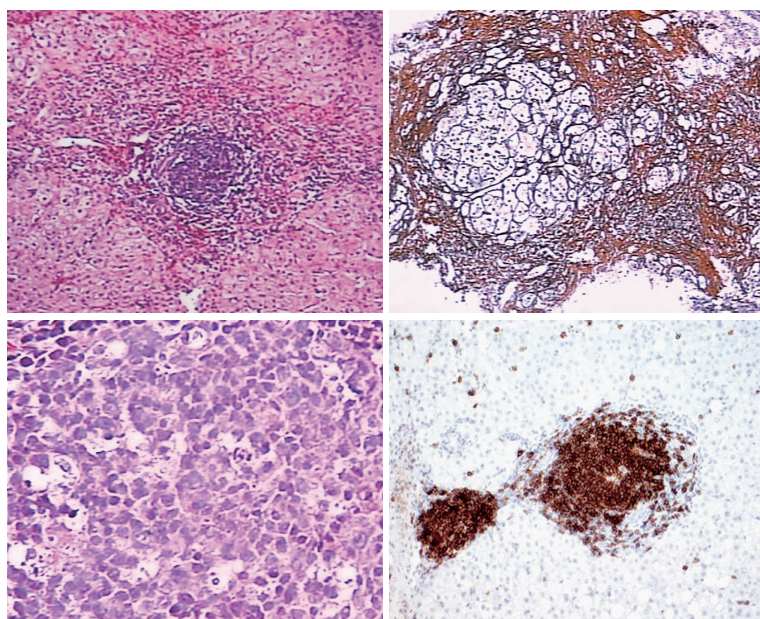
Liver biopsy specimens (> 10 mm in length) were fixed, paraffin-embedded, and stained with hematoxylin-eosin-saffron, and Warthin-Starry stained for collagen. For each liver biopsy specimen, a stage of fibrosis and a grade of activity were established according to the criteria formulated by the Chinese Society of Infectious Disease and Parasitology and Chinese Society of Hepatology, Chinese Medical Association.

### Data analysis

Categorical variables were compared by chi-square testing, and continuous variables were compared by the two-sided Student *t* test.

## RESULTS

Among the 848 patients prospectively included in this study, 40 were HCV-positive (anti-HCV antibody



**Figure 1** Histological appearances of a B-cell lymphoma, best classified as a marginal zone lymphoma involving the liver. A, B: Low power view of liver core biopsy shows chronic hepatitis and marked portal lymphoid infiltrates. C: High power view of liver core biopsy shows a monotonous portal lymphoid infiltrate composed of small lymphocytes with moderate amount of clear cytoplasm (so-called monocytoid appearance). Note that the infiltrate does not involve the biliary epithelium. D: CD20 immunostain shows that virtually all of the lymphoid cells are B-cells.

**Table 1** Baseline characteristics of patients with, and those without, chronic HCV infection

Characteristic	HCV positive (n = 40)	HCV negative (n = 808)	P
Age, (mean $\pm$ SD, yr)	50 $\pm$ 14	51 $\pm$ 15	> 0.05
Male	28/40	550/808	> 0.05
ALT, (mean $\pm$ SD, upper limit of normal value)	136 $\pm$ 7.8	132 $\pm$ 6.9	> 0.05
Liver histologic activity			
None or mild (G0-G1)	7/40	142/808	> 0.05
Moderate or severe (G2-G4)	33/40	666/808	> 0.05
Liver histologic fibrosis			
None or portal fibrosis (S0-S1)	8/40	17/808	> 0.05
Few or many septa or cirrhosis (S2-S4)	32/40	791/808	> 0.05
Liver lymphoid infiltrate			
None	0/40	0/808	> 0.05
Mild	32/40	646/808	> 0.05
Severe	8/40 (65)	162/808	> 0.05
Liver lymphoid aggregate			
None	36/40	805/808	
Yes	4/40	3/808	0.000

and HCV RNA positive), and 808 were HBV-infected patients. The main characteristics of patients with, and those without, chronic HCV infection are detailed in Table 1.

The histological appearance of a B-cell lymphoma is shown in Figure 1.

A clonal B-cell expansion was detected in 5 (71.4%) of 7 of the livers with monoclonal B-cells infiltration. In 2 of 5 patients with both a clonal B-cell expansion and monoclonal B-cells infiltration in the liver, a definite B-cell malignancy was finally diagnosed.

## DISCUSSION

In this study, we addressed the question of whether

lymphoid aggregates in the liver of patients with chronic hepatitis C are clonal B-cell proliferations.

Seven of the 848 patients have monoclonal B-cells infiltrating in the livers, including 4 of 40 chronic HCV-infected patients and 3 of 808 chronic HBV-infected patients. Five of the 7 patients with monoclonal B-cells infiltration showed a single band, suggesting that they were formed by a single B-cell clone. We were able to observe progression in patients who continued to be followed up after the end of this study. Lymphoma developed in 2 HCV-infected patients out of 5 patients who had monoclonal B-cells infiltration and B-cell proliferation in the liver.

Although epidemiological data link HCV infection and NHL, the pathobiological processes leading to clonal B-cell expansion and subsequent malignant transformation are only recently becoming better understood. CD81 has emerged as a potentially key mediator of B-cell/HCV interaction, in light of the finding that CD81 can bind to at least two sites on the HCV envelope protein, E2. CD81/E2 interaction does not apparently promote viral entry into B-cells; however, B-cells with specific anti-HCV surface immunoglobulins can simultaneously interact with viral E2 protein *via* CD81, resulting in dual activation signals leading to B-cell proliferation. Furthermore, clonal immunoglobulin gene rearrangements from HCV-positive lymphomas often share a similar restricted gene segment usage pattern, as seen in B-cells from patients with mixed cryoglobulinemia, and also show somatic hypermutation, emphasising the link between chronic viral antigenic stimulation and NHL pathogenesis<sup>[15-20]</sup>.

A multistep process has also been documented in a lymphoproliferative disorder in an HCV-infected patient, in whom the bcl-2 translocation was followed by myc translocation during the clinical progression of the disease<sup>[21]</sup>. However, the wide spectrum of lymphomas that have been described in patients with HCV infection, ranging from lymphoplasmacytoid<sup>[2]</sup>, to MALT-type<sup>[22]</sup>, to

follicle-centre cell lymphomas<sup>[23]</sup>, seem to indicate that more heterogeneous and complex processes are probably involved in the lymphomagenesis associated with HCV.

In conclusion, in patients with chronic HCV infection, the presence of a B-cell clonality and monoclonal B-cells infiltration in the liver may be useful for detecting patients at high risk for developing malignant lymphoproliferative disease. The importance of B-cell clonality analysis in the course of chronic HCV disease needs to be further evaluated, as do the indications for, and the efficacy of, antiviral treatment in patients at risk for B-cell malignancy.

## COMMENTS

### Background

The association of hepatitis C virus (HCV) infection with type II mixed cryoglobulinemia is well established, but the role of HCV in B-cell lymphoma remains controversial. The incidence of B-cell lymphoma is currently rising in line with the progression of hepatitis C though the cause of this increase is largely unknown.

### Research frontiers

Investigating the clonality of B-cells in the liver by analyzing the *IgH* gene rearrangement has been shown to correlate the development of B-cell lymphoma with HCV-infected patients. Liver biopsies in chronic hepatitis C frequently show portal lymphoid infiltrates with features of B follicles, whose clonality has not yet been investigated. In this study, the authors demonstrate that the clonality of B-cells in the liver may represent a low-grade lymphoma.

### Innovations and breakthroughs

Recent reports have highlighted the importance of antiviral treatment in the HCV-infected patient with B-cell clonality in the liver. This is the first study to analyze the association of monoclonal B-cells infiltration in the liver with the B-cell clonality. Furthermore, our follow up study showed that the lymphoma developed more frequently in the patients who had monoclonal B-cells infiltration and B-cell proliferation in liver.

### Applications

The presence of a B-cell clonality and monoclonal B-cells infiltration in the liver may be useful for detecting patients at high risk for developing malignant lymphoproliferative disease. The study results suggest a strategy for antiviral treatment in patients at risk for B-cell malignancy.

### Terminology

Polymerase chain reaction (PCR) amplification is a method currently in widespread use for detection of clonal *IgH* rearrangements. In PCR, rearranged DNA is amplified with a series of consensus primers that are complementary to sequences of variable regions; framework 1, framework 2, and framework 3 and to joining regions of the *IgH* gene.

### Peer review

The authors investigate the association of HCV infection and liver B-cell clonality in a prospective clinical trial. Liver biopsy specimens from 40 HCV-positive patients were analyzed, and specimens from hepatitis B virus (HBV)-positive patients served as a control. This is the first study to describe B-cell clonality in the liver of HCV-infected patients, and the results of their study are of interest.

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# Liver transplantation for severe hepatic trauma: Experience from a single center

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## Abstract

Liver transplantation has been reported in the literature as an extreme intervention in cases of severe and complicated hepatic trauma. The main indications for liver transplant in such cases were uncontrollable bleeding and postoperative hepatic insufficiency. We here describe four cases of orthotopic liver transplantation after penetrating or blunt liver trauma. The indications were liver failure, extended liver necrosis, liver gangrene and multiple episodes of gastrointestinal bleeding related to portal hypertension, respectively. One patient died due to postoperative cerebral edema. The other three patients recovered well and remain on immunosuppression. Liver transplantation should be considered as a saving procedure in severe hepatic trauma, when all other treatment modalities fail.

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**Key words:** Liver injury; Orthotopic liver transplantation; Severe liver trauma; Hepatic coma; Hepatic trauma

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## INTRODUCTION

The liver is the most commonly injured abdominal organ, despite its protected location under the rib cage. The therapeutic options for the management of both blunt and penetrating hepatic trauma include a range of operative and non-operative treatment modalities<sup>[1-3]</sup>. Currently available methods for the management of hepatic trauma include observation, laparotomy with direct suturing, perihepatic gauze packing, application of fibrin tissue glue, mesh hepatorrhaphy, limited debridement resection and partial lobectomy. Extensive surgical techniques, such as formal hepatectomy or total hepatectomy with liver replacement, have been documented only in selected patients<sup>[4,5]</sup>. The surgical aim is control of hemorrhage, preservation of sufficient hepatic function and prevention of secondary complications. Liver transplantation has a limited, though very important, role in specific life threatening cases, when all the above mentioned methods fail to control bleeding or when liver failure ensues. We here describe our experience over the course of 11 years (1996 through 2007) with four cases of severe hepatic trauma requiring liver transplantation.

## CASE REPORT

### Case 1

A 25-year-old Caucasian male presented with hypovolemic shock to the Trauma Center due to a gunshot wound to the abdomen. The patient was severely acidotic, requiring intense fluid resuscitation. His Glasgow Coma Scale (GCS) score was 9/15 upon admission. The patient was initially managed according to the "advanced trauma life support" (ATLS) guidelines and very shortly thereafter was transferred to the operating theatre, due to signs of active bleeding. During an exploratory laparotomy, a trajectory wound affecting segments VII and VIII of the liver was documented, with active bleeding. A Pringle manoeuvre was initially used

along with repair of liver injury. The abdomen was then packed. On the first postoperative day the patient remained unstable and acidotic with further bleeding from the liver surface requiring re-exploration. Right hepatic artery ligation and packing were performed and the patient was transferred to the intensive care unit with a plan for a possible right hepatectomy. Liver and renal functions, however, deteriorated progressively, with persistent acidosis, prolonged prothrombin time, low fibrinogen level and acute renal failure. The patient was placed on the transplant list and two days later underwent an orthotopic liver transplant. A portal and systemic veno-venous bypass was utilized. During re-exploration of the abdomen, the native liver appeared necrotic; mass clamping of the hilum following by supra and infra-hepatic vena cava clamping was performed. The donor liver was implanted using a conventional method for the inferior vena cava. Postoperatively, the patient remained unstable, with progressive lactic acidosis, liver dysfunction and cerebral edema. Cerebral edema was managed with direct monitoring of intracranial pressure (ICP) and drainage of cerebrospinal fluid when decompression was necessary. Despite the above treatment and the complete support in the intensive care unit (ICU), with elevation of the patient's head by 25 degrees and maintenance of cerebral perfusion pressure by supporting systemic arterial pressure, reducing central venous pressure and avoiding agitation, the patient's condition gradually deteriorated and he died on the eleventh post-operative day.

#### Case 2

A 68-year-old white female developed a subcapsular hematoma of the right lobe of the liver due to blunt abdominal trauma. A right liver lobectomy was performed in another institution because of hematoma expansion. Liver function, however, continued to deteriorate after surgery. The patient was referred for further evaluation. GCS was 15/15 upon admission. Doppler ultrasound revealed main portal vein thrombosis. An exploratory laparotomy was performed to attempt portal vein thrombectomy through the right portal vein stump, but this was unsuccessful. The common bile duct was also found to be partially necrotic and external bile drain placement was performed. Due to postoperative liver failure, the patient was listed as a status 1 candidate for liver transplant. Transplantation was performed two days later using a veno-venous bypass, with caval reconstruction in a piggyback fashion. The patient recovered after prolonged hospitalization and remains on immunosuppression with tacrolimus and mycophenolate mophetil ten years after transplantation.

#### Case 3

A 58-year-old white female suffered a gunshot wound to the abdomen which resulted in a penetrating right lobe liver injury and a through-and-through injury of the duodenum. Suture ligation with packing and duodenal repair performed in another institution, were adequate to control initial bleeding. However, over the course of the

following two years she experienced multiple episodes of cholangitis due to biliary strictures and she required a choledoco-duodenostomy. Additionally, she went on to develop an arterio-venous fistula between the right hepatic artery and the right portal vein, which resulted in the development of significant portal hypertension. She experienced multiple episodes of gastrointestinal bleeding related to secondary biliary cirrhosis and the portal hypertension. An attempt to embolize the arterio-venous fistula failed and orthotopic liver transplantation was then considered. Her GCS score was 15/15. The native liver was cirrhotic with partial main portal vein thrombosis and a dilated hepatic artery. Under veno-venous bypass, a piggyback technique was used for the caval dissection and the recipient portal vein was thrombectomized. The spleno-portal junction was used for venous reconstruction. Due to intra-operative injury to the duodenum during the dissection, a Billroth II gastrojejunostomy was performed and a Roux-en-Y hepatico-jejunostomy was created for bile duct reconstruction. The patient recovered after an uneventful postoperative course. Explant pathology revealed cirrhotic liver with periportal abscess formation. Six months later, the patient developed cholestasis and hepatic artery thrombosis. He underwent re-transplantation and is alive and well 11 years later.

#### Case 4

A 35-year-old female was admitted to the casualty department with a gunshot injury. She presented in hypovolemic shock. Her GCS score upon admission was 9/15. After initial management according to ATLS guidelines she underwent exploratory laparotomy and segment II and III penetrating liver injuries with concomitant portal vein laceration were discovered. Longitudinal venorrhaphy of the portal vein, along with liver packing was performed without liver resection. She was then taken to angiography for embolization of the left hepatic artery. Two months later she developed liver gangrene with hepatic artery pseudo-aneurysm. Although septic, the patient was not excluded from evaluation for liver transplant due to the fact that the liver was primarily the source of infection. After removal of the native liver, the patient's hemodynamic status markedly improved. During transplant, the liver was fragile and the hilar structures were impossible to identify. The hilum was mass clamped and the structures isolated in a serial fashion after hepatectomy. The portal vein was dissected free to the confluence with the splenic vein because of the associated fibrosis and the native hepatic artery was suture ligated after removal of the pseudo-aneurysm. Transplant was performed in a piggyback fashion using a supra-celiac jump graft for the arterial inflow. The patient was discharged on postoperative trauma day 85 and is currently doing well at home nine years after transplantation.

## DISCUSSION

The overall mortality of hepatic trauma has declined

Table 1 Liver injury scale (AAST)

Grade		Description
I	Hematoma	Subcapsular, < 10% surface area
	Laceration	Capsular tear, < 1 cm parenchymal depth
II	Hematoma	Subcapsular, 10%-50% surface area: intraparenchymal, < 10 cm in diameter
	Laceration	1-3 cm parenchymal depth, < 10 cm in length
III	Hematoma	Subcapsular, > 50% surface area or expanding; ruptured subcapsular or intraparenchymal hematoma > 10 cm or expanding
	Laceration	> 3 cm parenchymal depth
IV	Laceration	Parenchymal disruption involving 25%-75% of hepatic lobe or 1-3 Couinaud's segments within a single lobe
V	Laceration	Parenchymal disruption involving > 75% of hepatic lobe or > 3 Couinaud's segments within a single lobe
	Vascular	Juxtahepatic venous injuries; i.e. retrohepatic vena cava/central major hepatic veins
VI	Vascular	Hepatic avulsion

Table 2 Type of injury, operations performed and patient outcome

Patient	Age	Injury	Primary operation	Indication for OLT	Re-transplant	Outcome
1	25	Gun shot wound right lobe	Packing, hepatic artery ligation	Acute liver failure	No	Died (cerebral edema)
2	68	Blunt trauma subcapsular hematoma right lobe	R lobectomy, failed portal vein thrombectomy	Portal thrombosis progressive liver failure	No	Discharged POD 45
3	58	Gun-shot wound right lobe, A-V fistula	Hepatorrhaphy, duodenal repair, embolization	Portal hypertension (A-V fistula), left portal vein thrombosis	Yes	Alive at 11 yr
4	35	Gun-shot wound left lateral lobe, hepatic artery pseudoaneurysm	Packing, embolization	Liver gangrene	No	Discharged POD 85

from 60% in the first half of the last century to approximately 6% today<sup>[6]</sup>. As many as 90% of patients with liver trauma are non-surgically managed with a remarkably high success rate, with only 10% requiring surgical intervention. The American Association for the Surgery of Trauma classified liver trauma degree and reported a liver injury scale (Table 1)<sup>[7]</sup>. The need for orthotopic liver transplantation (OLT) after liver trauma is clearly restricted. However, since the mortality rate of severe and complicated hepatic injuries remains significantly high, reaching 46% for grade IV and 80% for grade V hepatic injury<sup>[8,9]</sup>, OLT must be taken under consideration when all other methods to achieve hemostasis have failed or cannot be applied.

The indications for liver transplantation in the setting of severe and complicated liver trauma, reported in the literature are: (1) uncontrollable bleeding despite repeated previous surgical interventions; (2) postoperative evolution towards hepatic insufficiency (acute or progressive); (3) injuries of the portal vein that cannot be reconstructed<sup>[4,5,9-13]</sup>. In our series, the indications for OLT were the following: portal hypertension due to portal thrombosis and arterio-venous shunt; liver failure from massive injury; and portal thrombosis and liver gangrene with pseudo-aneurysm formation (Table 2). Sepsis was not an absolute contraindication in our study provided that the source of infection was limited to the liver. The above indications, such as fulminate liver failure without irreversible brain injury or extra hepatic sepsis, can also be used as criteria for prompt referral.

Esquivel<sup>[12]</sup> first reported the use of liver transplantation in two patients with progressive hepatic failure and uncontrollable bleeding. Ringe *et al.*<sup>[4]</sup> proposed a two-stage procedure (total hepatectomy and subsequent liver transplantation) in cases of severe hepatic trauma,

when all other conventional methods failed to control bleeding. In reviewing the literature between 1987 and 2005, we found 13 reported cases of patients who underwent OLT for the management of severe and life threatening hepatic trauma<sup>[4,5,10-14]</sup>. All of them had severe (grade IV or V) hepatic trauma according to the organ injury scale of the American Association for the Surgery of Trauma<sup>[7]</sup>, and were hemodynamically unstable upon admission.

Furthermore, all patients in these studies had undergone a primary or even secondary operation to control bleeding, before they were finally referred to a transplant center. All our patients had also been managed with more conservative surgical procedures to control bleeding prior to referral for OLT. In our cohort OLT was partly planned due to complications related to the initial surgical management in addition to the severity of the initial liver injury.

To our knowledge, this is the largest series from a single center reported so far. The postoperative mortality rate was 25% and involves a patient with significant hemodynamic instability. In agreement with previous reports, we feel that OLT might be contraindicated when patients do not show any signs of hemodynamic stabilization despite intensive medical support. In such cases, rapid clinical deterioration follows the transplant surgery, leading to multi organ failure and death<sup>[15]</sup>.

Although liver transplantation can be life saving in selective cases with severe liver injury, the lack of immediately available liver grafts combined with the inability to keep a patient in an anhepatic state, are the main causes of why such a few cases have been reported. Patients have to be listed as status 1 and donors with expanded criteria may also be accepted (size mismatch or steatotic livers). Reduced liver grafts have also been used

in the literature but primary non-function is possible<sup>[4]</sup>.

Preexisting sepsis and associated organ injuries are usual contraindications of liver transplantation for the management of severe hepatic trauma<sup>[16]</sup>. Bowel perforation with peritonitis, severe pancreatic trauma and loss of a large portion of the abdominal wall increase the mortality rate and preclude liver transplantation. A severe closed head injury with associated cerebral edema is also an absolute contraindication for orthotopic liver transplantation<sup>[17]</sup>. However, localized sepsis in the liver is a relative contraindication, since the septic focus can be eradicated by the transplant itself<sup>[10]</sup>.

It is worth noting that from a technical point of view: (1) veno-venous bypass is favored due to absence of portal hypertension; (2) mass clamping of the hilum is advocated in situations of difficult dissection or need for rapid liver removal and (3) a piggyback technique is facilitated by the absence of pre-existing portal hypertension. *Ex situ* liver surgery with subsequent auto-transplantation has been reported for the management of otherwise unresectable hepatobiliary malignancies, with good results<sup>[18,19]</sup>. It could be a viable alternative option for severe liver trauma, especially if a liver graft is not immediately available. In our series *ex vivo* liver repair was not performed. Patients with lethal injuries to the liver can survive only if they are referred to a transplantation center promptly as documented by our experience.

Liver transplantation is an acceptable surgical method for management of patients with severe traumatic liver injury, under the previously mentioned life-threatening conditions. Further reports are awaited, in order to support and expand the application of OLT in such devastating cases.

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## Guillain-Barré syndrome following hepatitis E

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### INTRODUCTION

Guillain-Barré syndrome (GBS) is an acute polyradiculoneuropathy presenting, in its classical form, as a rapidly evolving symmetric and ascending motor paralysis with hypotonia and areflexia accompanied by an leukocytic cerebrospinal fluid with elevated protein level. In over two-third of cases, an infection precedes the onset of neuropathy by 1 to 3 wk. Cytomegalovirus and Epstein-Barr virus account for a large proportion of virus-triggered cases. There are also many reports linking acute hepatitis A, B, and C, with GBS. Hepatitis E is a frequent cause of acute hepatitis in Asia, the Middle East, North Africa and South or Central America. Locally acquired hepatitis E in individuals who have not travelled to endemic areas is, however, becoming an emerging problem in European countries<sup>[1,2]</sup>. We report a case of Guillain-Barré syndrome in a patient sporadically contaminated in a Western country. This is the third report of GBS in a patient with hepatitis E<sup>[3,4]</sup>, and the first occurring in a patient sporadically contaminated in a Western country. This is the first time, to our knowledge, that ganglioside molecular mimicry is suggested in the pathogenesis of GBS-associated with a hepatotropic virus.

### CASE REPORT

A 66-year-old male general practitioner who worked in an urban area consulted due to an acute elevation of liver function tests (AST: 1062 IU/L, ALT: 1813 IU/L,  $\gamma$ -GT: 90 IU/L). Serum bilirubin and alkaline phosphatases were normal. The liver tests had been carried out during a routine check-up and the patient was completely asymptomatic at presentation. Three months prior to consultation his blood tests were normal. The patient had not recently received any hepatotoxic or neurotoxic drugs or vaccinations, and had not travelled abroad during the last year.

### Abstract

Guillain-Barré syndrome (GBS) is often triggered by a preceding bacterial or viral infection. Occasionally, it has been observed in association with acute hepatitis A, B and C, and three cases have been previously described in India in which GBS was associated with acute hepatitis E. A molecular mimicry mechanism is supposed to be involved in the pathogenesis of GBS triggered by infectious agents, although the nature of the shared epitopes has not been characterized in most instances, including that in the case of hepatotropic viruses. We report a case of GBS following acute hepatitis E in a European individual. The presence of antiganglioside GM2 antibodies in this patient suggested molecular mimicry involving ganglioside GM2 in the pathogenesis of GBS associated with hepatitis E.

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**Key words:** Gangliosides; Guillain-Barré syndrome; Hepatitis E; Molecular mimicry; Viral hepatitis

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A few days later, the patient developed neurological symptoms, beginning with progressive loss of strength in both legs and paraesthesia of the lower limbs, mainly in the evening. Ataxia and neuropathic pain appeared a few days later. These symptoms led the patient to be hospitalized in the neurology department of our institution.

On physical examination, the patient was afebrile. Blood pressure was normal. Examination of heart, lungs and abdomen was unremarkable. There were no features of chronic liver disease and no signs of encephalopathy. The neurological examination showed a stance and gait ataxia with Romberg's sign and distal hypopallesthesia. Symmetric hyporeflexia in the upper limbs and areflexia in the lower limbs were associated with a severe proximal weakness prominent in the lower limbs.

Routine blood examination showed an AST of 68 IU/L, ALT of 443 IU/L and  $\gamma$ -GT of 94 IU/L. Renal function, electrolytes, glucose, and haematologic values were normal.

Cerebrospinal fluid analysis showed a major increase in protein concentration at 1722 mg/L, associated with a high level of immunoglobulin G, without an increased number of leucocytes. Electrophysiological examinations of the lower limbs demonstrated an acute demyelinating polyradiculoneuropathy.

These findings were consistent with a diagnosis of GBS. Of note, serum antiganglioside antibodies GM2 IgM were positive (Dotzen ganglio profile Ab IgG and IgM by Zentech®). Other antiganglioside antibodies were negative (GM1, GM3, GD1A and GD1B, GD3, GQ1B, GT1A and GT1B). Antibodies to Purkinje cells, to neurons and to myelin were negative. Sulfatide antibodies were negative.

A serological study showed IgM antibodies to hepatitis E (two assays were used: HEV IgM ELISA by Genelabs, with a sensitivity of 93% and a specificity of 99%; and Recomblot HEV IgM by Mikrogen, with a sensitivity of 85.7% and a specificity of 100% in non endemic regions<sup>[5]</sup>).

Hepatitis B surface antigen, antibodies to hepatitis C, IgM anti-HAV were absent. The following serological tests were also negative: antibodies to HIV, Varicella-Zoster virus and cytomegalovirus. Serology for campylobacter was negative. IgG were positive, with negative IgM, for Epstein-Barré virus, adenovirus and herpesvirus.

A diagnosis of GBS associated with acute hepatitis E was made. Intravenous immunoglobulins were given at a dose of 0.4 g/kg per day for five days. This treatment significantly improved the patient's neurological condition with progressive recovery of walking perimeter and a reduction in neuropathic pain. Liver enzymes completely normalized. Four months later, a near-complete neurological recovery was noted.

## DISCUSSION

Hepatitis E has become an emerging cause of acute hepatitis in western countries<sup>[1,2]</sup>. In most cases, acute

hepatitis E in these regions is of autochthonous origin<sup>[6]</sup>. The most frequent risk factors for hepatitis E, reported in a French survey, were water consumption from a personal water supply, uncooked shellfish consumption, and the recent acquisition of a pet pig<sup>[7]</sup>. None of these risk factors were present in our patient. It is possible that the contamination was related to the patient's profession as a general practitioner. It has been shown that the clinical evolution of hepatitis E can be different in patients infected sporadically compared with patients infected in endemic areas. In autochthonous cases, the mean age is higher and the prognosis is more severe with a higher rate of fulminant liver failure<sup>[8]</sup>.

Guillain-Barré syndrome is clinically defined as an acute inflammatory demyelinating polyradiculoneuropathy causing limb weakness<sup>[9]</sup>. Paralysis of muscles develops acutely over a period of days, but can take up to 4-6 wk. In most patients, after a brief plateau, improvement begins with a gradual resolution of paralysis which lasts from weeks to months. The syndrome is considered to be an autoimmune disease triggered by a preceding bacterial or viral infection. The most commonly identified triggering agents are *Campylobacter jejuni*, followed by cytomegalovirus, Epstein-Barr virus, and mycoplasma pneumonia. HIV, shigella, clostridium, haemophilus influenza, as well as hepatitis A, B and C were also identified as triggering agents<sup>[10]</sup>. In our case, the temporal association between acute hepatitis E and GBS strongly suggested a relation between both disorders.

The mechanism by which infection can trigger GBS is not completely understood. It is thought that the immune system mistakenly attacks myelin or axons by a molecular mimicry mechanism (in which the host generates an immune response against an infectious organism that shares epitopes with the host's peripheral nerves)<sup>[11]</sup>. The nature of the epitope, although still uncertain, is likely to be a glycolipid. The most attractive candidate targets are gangliosides, which are present in nodal and internodal membranes of nerve fibres<sup>[12]</sup>. Ganglioside antibodies may perturb nerve conduction and, in a complement-dependant fashion, disrupt the molecular topography of nodal and paranodal proteins and induce motor axonal degeneration<sup>[13]</sup>. It is postulated that infected cells can produce ganglioside-like epitopes that trigger the immune response. This mechanism of molecular mimicry has been observed for *Campylobacter jejuni* with the implication of several gangliosides (GM1, GD1B, and GQ1B)<sup>[14]</sup>. The implication of antiganglioside GM2 antibodies in the pathogenesis of GBS related to CMV has been described<sup>[15]</sup>. It has been demonstrated that CMV-infected fibroblasts express ganglioside-like epitopes that specifically recognize anti-GM2 antibodies<sup>[16]</sup>. These results suggest that, in CMV-infected GBS patients, infected cells with CMV can express epitopes inducing an immune response against gangliosides.

For GBS related to hepatitis A, B, C, or E, no homologous epitopes to a component of the peripheral nerves have been described to date. We report the first

description of the presence of antiganglioside GM2 antibodies in GBS associated with a hepatotropic virus, suggesting possible molecular mimicry involving gangliosides. This possible relationship should be further documented in the very rare cases of GBS associated with viral hepatitis to confirm the mechanism.

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## CASE REPORT

# Schistosomal appendicitis: Incidence in Japan and a case report

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## Abstract

Schistosomal appendicitis is very rare in developed countries like the USA, Europe, and Japan. The author reviewed 311 pathologic archival specimens of vermiform appendix over the past 10 years. One case of schistosomal appendicitis was recognized. Therefore, the incidence of this disease was 0.32% in all appendices surgically resected in our hospital. The patient was a 41-year-old woman presenting with lower abdominal pain. She was a sailor traveling to many countries including endemic areas. Physical examination, laboratory data, and imaging modalities suggested an acute appendicitis, and appendectomy was performed under the diagnosis of ordinary appendicitis. Histologically, numerous schistosomal eggs were present in the vasculatures throughout the appendiceal walls. Some of the eggs were calcified. Stromal foreign body reaction was also recognized. The appendicitis was phlegmonous consisting of severe infiltrations of neutrophils and eosinophils. Acute serositis was also noted. Examination of feces revealed numerous eggs of *Schistosoma mansoni*. Clinicians should be aware of schistosomal appendicitis.

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**Key words:** Appendix; Histopathology; Pathologic archival specimens; Schistosomiasis; Acute appendicitis

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Terada T. Schistosomal appendicitis: Incidence in Japan and a case report. *World J Gastroenterol* 2009; 15(13): 1648-1649 Available from: URL: <http://www.wjgnet.com>

## INTRODUCTION

Schistosomiasis is caused by *Schistosoma mansoni* or *Schistosoma japonica*, and is a disease of waterborne trematode infestation. Schistosomal appendicitis is very rare in developed countries like the USA, Europe, and Japan<sup>[1-5]</sup>. However, it is prevalent in endemic areas such as Africa and South Asia<sup>[1-5]</sup>. In Japan, Yamanashi and Fukuoka prefectures are endemic areas. However, the incidence and pathological features of schistosomal appendicitis are not known. Therefore, the author herein reports the incidence and pathological features of this disease in Japan.

## CASE REPORT

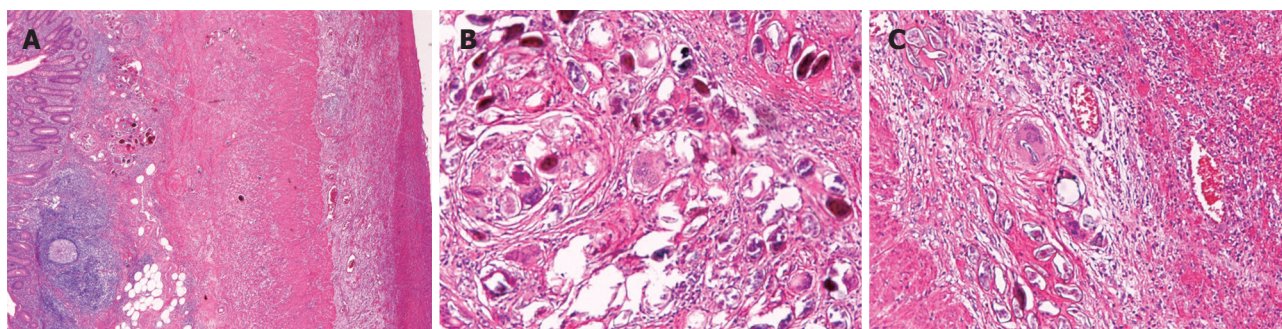
The author re-examined 311 pathologic archival specimens of vermiform appendix over the past 10 years. Of these specimens, one case of schistosomal appendicitis was recognized. Therefore, the incidence of this disease was 0.32% of all appendices surgically resected in our hospital. The specimen was from a 41-year-old woman, who was a sailor traveling to many countries including endemic areas, who had complained of lower abdominal pain. Physical examination was suggestive of acute appendicitis. Blood laboratory data showed leukocytosis. Imaging modalities including CT indicated an appendiceal swelling. An appendectomy was performed under the clinical diagnosis of ordinary appendicitis.

Histologically, numerous schistosomal eggs were present in the vasculatures throughout the appendiceal walls (Figure 1A). This finding was unexpected. Some of the eggs were calcified (Figure 1B). Stromal foreign body reaction was also recognized (Figure 1B). Severe infiltrations of neutrophils and eosinophils were recognized (Figure 1C). Acute serositis was also noted (Figure 1A). Examination of feces revealed numerous eggs of *Schistosoma mansoni*. The involvement of other organs was unclear.

## DISCUSSION

Schistosomiasis is a waterborne parasitic disease caused by *Schistosoma mansoni* and *Schistosoma japonica*. This





**Figure 1** Histologic findings of schistosomal appendicitis. A: Low power view of the appendiceal walls in schistosomal appendicitis. Acute inflammation is recognized throughout the walls. Schistosomal eggs are seen in the mucosa and muscular layer. The serosa shows acute serositis (right). HE,  $\times 40$ ; B: Schistosomal eggs in the mucosa. Foreign body granulomatous reaction is recognized. Some eggs show calcification. HE,  $\times 200$ ; C: Schistosomal eggs in the subserosa. The eggs were located within vasculatures. Severe infiltrations of neutrophils and hemorrhage are recognized. HE,  $\times 200$ .

disease is endemic and particularly prevalent in Africa and South Asia<sup>[1-5]</sup>. Our hospital is located in a non-endemic area. Schistosomiasis is a disease of intestine and liver, where the parasite resides and produces eggs in the vasculatures.

The incidence of schistosomal appendicitis is unclear in Japan. The present study revealed that the incidence was 0.32% of all vermiform appendices resected.

The present case is pathologically typical of schistosomal appendicitis, and feces examination strongly supported the diagnosis. Clinically, schistosomal appendicitis was not considered, and clinicians were first informed after pathologic examination. The present patient was a sailor traveling to many countries including endemic areas. Thus, clinicians should be aware of schistosomal appendicitis.

It is uncertain whether schistosomiasis of the vermiform appendix induces acute appendicitis<sup>[1]</sup>. However, it is now thought that appendiceal schistosomiasis may cause acute appendicitis. This may be due to ischemic changes caused by egg emboli. This situation may diminish mucosal immunity, thus leading to bacterial infection.

In endemic areas like Nigeria, Badmos *et al*<sup>[4]</sup> reported that appendices with schistosomiasis were present in 35/843 (4.2%) of surgically resected cases. Of these 35 positive cases, 23 (65.7%) were associated with acute appendicitis, while the remaining 12 cases (34.3%) were not associated with inflammation. Thus,

the presence of the parasite does not always give rise to acute appendicitis. In developed countries like the USA, Nandipati *et al*<sup>[5]</sup> reported that schistosomal appendicitis was found in 3/1690 (0.2%) of surgically resected cases. All three cases were African Americans<sup>[5]</sup>. Thus, in developed countries, schistosomal appendicitis is preferentially found in travelers or in an endemic area population.

In summary, the incidence of schistosomal appendicitis is 0.34% in Japan. The present patient with this disease was a sailor traveling to many countries. Thus, clinicians should be aware of schistosomal appendicitis.

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CASE REPORT

## A combination treatment of entecavir and early-phase corticosteroid in severe exacerbation of chronic hepatitis B

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Matsumoto K, Miyake Y, Miyatake H, Takahara M, Imada T, Yagi S, Toyokawa T, Nakatsu M, Ando M, Hirohata M. A combination treatment of entecavir and early-phase corticosteroid in severe exacerbation of chronic hepatitis B. *World J Gastroenterol* 2009; 15(13): 1650-1652 Available from: URL: <http://www.wjgnet.com/1007-9327/15/1650.asp> DOI: <http://dx.doi.org/10.3748/wjg.15.1650>

### Abstract

Of patients with severe exacerbation of chronic hepatitis B accompanied by jaundice and coagulopathy, 20%-30% have a fatal outcome. In this report, we describe 2 cases of severe exacerbation of chronic hepatitis B with jaundice and coagulopathy who were successfully treated with a combination of entecavir and corticosteroid. In both cases, rapid reductions in serum hepatitis B virus (HBV)-DNA levels were observed, and corticosteroid was stopped after serum HBV-DNA levels became undetectable. Entecavir treatment was continued. Generally, entecavir treatment reduced serum HBV-DNA levels rapidly, although the improvement in liver function was delayed by a few weeks. During this time lag, liver cell injury continued and the disease progressed. Corticosteroid suppressed the excessive host immune response and was useful for stopping progressive deterioration. A combination of entecavir and early-phase corticosteroid may be a useful treatment in severe exacerbation of chronic hepatitis B.

### INTRODUCTION

An estimated 400 million people worldwide have chronic hepatitis B virus (HBV) infection, and more than 500 000 people die every year from complications of HBV-related chronic liver disease<sup>[1]</sup>. In patients chronically infected with HBV, acute exacerbations are clinically important because they can have severe or even fatal consequences<sup>[2]</sup>. An estimated 10%-30% of hepatitis B carriers experience acute exacerbation each year<sup>[3]</sup>. Hepatitis B e antigen (HBeAg) seroconversion and mortality occur in 2.7% and 0.7% of patients with acute exacerbation, respectively<sup>[4]</sup>. On the other hand, mortality occurs in 20%-30% of cases with severe exacerbation accompanied by jaundice and coagulopathy<sup>[5,6]</sup>.

In the past decade, lamivudine (LMV) has revolutionized the treatment of chronic hepatitis B. Treatment with LMV significantly decreases the rate of hepatic decompensation and prevents the development of hepatocellular carcinoma<sup>[7]</sup>. On the other hand, LMV monotherapy confers no significant protection against rapid progression to hepatic failure in severe exacerbation of chronic hepatitis B, although LMV results in long-term benefits<sup>[5]</sup>. Furthermore, viral resistance, which is usually followed by a loss of clinical response, a rise in aminotransferase levels and worsening of hepatic histology, is related to mutations in the tyrosine-methionine-aspartate-aspartate (YMDD) motif which

occurs in 70%-80% of patients treated continuously for 4-5 years<sup>[8]</sup>. Recently, the early introduction of high-dose corticosteroid was reported to improve the short-term prognosis of patients with severe exacerbation of chronic hepatitis B<sup>[9]</sup>.

In this report, we describe 2 cases with severe exacerbation of chronic hepatitis B successfully treated with a combination of entecavir (ETV) and corticosteroid.

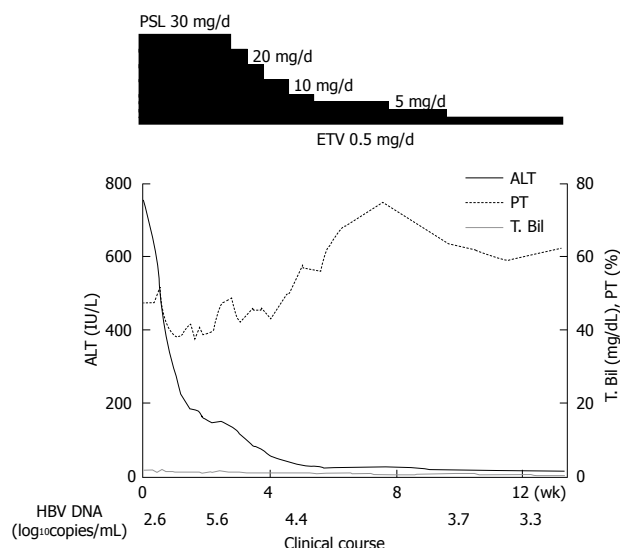
## CASE REPORT

### Case 1

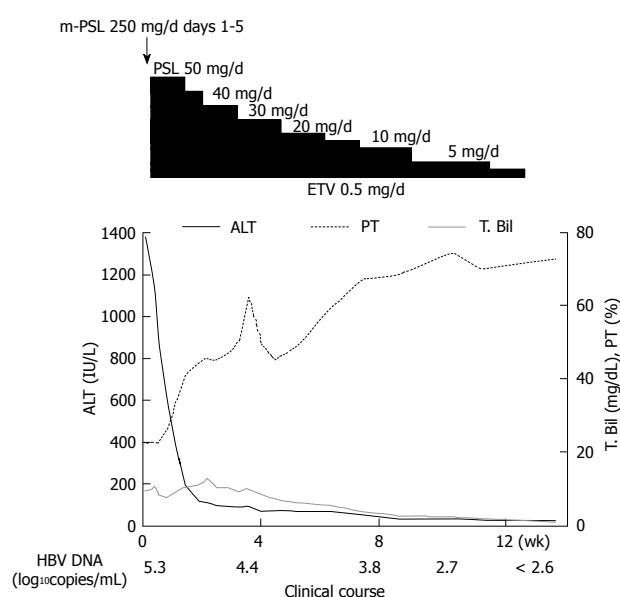
A 33-year-old Japanese man was admitted to our hospital with general fatigue. He had been diagnosed as a carrier of HBV but had never received treatment. His family history included chronic hepatitis B in his late mother, who had died of severe exacerbation of the disease. On physical examination at admission, he was conscious and the bulbar conjunctiva was not icteric. Neither ascites nor pretibial edema was noted. Laboratory data on admission were as follows: bilirubin, 19 mg/L; aspartate aminotransferase, 698 IU/L; alanine aminotransferase, 756 IU/L; albumin, 33 g/L; and prothrombin activity, 47.7%. He was positive for both hepatitis B surface antigen (HBsAg) and HBeAg, and his serum HBV-DNA level measured by real-time quantitative polymerase chain reaction (TaqMan PCR, Roche Diagnostics) was 2.6 LGE/mL. He was diagnosed with severe exacerbation of chronic hepatitis B, and oral ETV treatment (0.5 mg/d) was initiated. On his fifth day of hospitalization, prednisolone (30 mg/d) was added. At 6 wk, his serum transaminase level was normal, after which the dose of prednisolone was tapered. At 12 wk, his serum HBV-DNA level was 3.3 LGE/mL and prednisolone was stopped. Since then, his transaminase level has remained normal (Figure 1).

### Case 2

A 44-year-old Japanese woman was admitted to our hospital with general fatigue and anorexia. She had been diagnosed as a carrier of HBV but had never received treatment. Her family history included a father who was a carrier of HBV. On physical examination at admission, she was conscious and her bulbar conjunctiva was icteric. Neither ascites nor pretibial edema was noted. Laboratory data on admission were as follows: bilirubin, 96 mg/L; aspartate aminotransferase, 1389 IU/L; alanine aminotransferase, 573 IU/L; albumin, 31 g/L; and prothrombin activity, 22.7%. She was positive for both HBsAg and HBeAg, and her serum HBV-DNA level measured by transcription-mediated amplification assay (Roche Diagnostics) was 5.3 LGE/mL. She was diagnosed with severe exacerbation of chronic hepatitis B. A combination treatment of oral ETV (0.5 mg/d) and intravenous methylprednisolone (250 mg/d) was immediately started. On her sixth day of hospitalization, intravenous methylprednisolone was changed to oral prednisolone (50 mg/d). At 8 wk, her serum transaminase level was normal and her serum bilirubin levels and prothrombin activity were improved to



**Figure 1 Patient clinical course.** ALT: Alanine aminotransferase; PT: Prothrombin activity; T. Bil: Total bilirubin; HBV DNA: Hepatitis B virus DNA. At 6 wk, his serum transaminase level was normalized. At 12 wk, his serum HBV-DNA level became 3.3 LGE/mL and prednisolone was stopped.



**Figure 2 Patient clinical course.** ALT: Alanine aminotransferase; PT: Prothrombin activity; T. Bil: Total bilirubin; HBV DNA: Hepatitis B virus DNA. At 8 wk, her serum transaminase level was normalized. At 15 wk, her serum HBV-DNA level became undetectable and the prednisolone was stopped.

68.8% and 29 mg/L, respectively. Subsequently, the dose of prednisolone was tapered. At 15 wk, her serum HBV-DNA level became undetectable and prednisolone was stopped. One year later, her ETV treatment has continued, and her serum HBV-DNA level has continued to be undetectable (Figure 2).

## DISCUSSION

ETV suppresses HBV replication significantly better than LMV. The mean reduction in serum HBV-DNA levels from baseline to week 48 is reported to be 6.9 log in HBeAg-positive patients and 5.0 log in HBeAg-



negative patients<sup>[10,11]</sup>. Furthermore, ETV shows a lower frequency of virologic rebound (2% in the first year of drug administration) compared with LMV. Thus, ETV is considered a first-choice therapy for patients with chronic hepatitis B not previously treated with a nucleoside analogue. However, for patients with severe exacerbation of chronic hepatitis B, treatment during the first 2 wk determines their prognosis<sup>[9]</sup>. The liver cell injury caused by HBV infection is mediated mainly by the response of CD8+ cytotoxic T lymphocytes to small epitopes of HBV proteins, especially the hepatitis B core antigen, present on the surface of liver cells<sup>[12]</sup>. ETV treatment reduces serum HBV-DNA levels rapidly, although the improvement in liver function is delayed by a few weeks. During this time lag, liver cell injury continues and the disease progresses. Corticosteroid suppresses the excessive host immune response and is useful for stopping progressive deterioration. On the other hand, patients not treated with any antiviral drugs show a subsequent rebound increase in serum transaminase levels 4 to 10 wk after withdrawal of corticosteroid<sup>[13]</sup>. Thus, we consider that a combination of ETV and early-phase corticosteroid may be reasonable for improving prognosis in severe exacerbation of chronic hepatitis B.

Corticosteroid has been reported to directly stimulate HBV replication through specific glucocorticoid receptors in the HBV genome in cultured human hepatoma cells<sup>[14]</sup>. Clinically, immunosuppressive treatment has been indicated to have a potentiating effect on HBV replication in patients with chronic active hepatitis B<sup>[15]</sup>. Furthermore, corticosteroid has been reported to delay the normalization of serum transaminase levels<sup>[16]</sup>. However, in this study, both our patients showed rapid reductions in serum HBV-DNA levels despite corticosteroid treatment. We consider that this may be attributable to the antiviral effect of ETV, which may be strong enough to overcome HBV replication activated by corticosteroid treatment.

In this study, 0.5 mg/kg or more of prednisolone was administered daily. When the patient showed a trend toward remission in serum transaminase levels, the dose of prednisolone was tapered. After serum HBV-DNA level became undetectable, prednisolone was stopped. In Japan, ETV is administered at a dose of 0.5 mg daily in order to treat chronic HBV infection in accordance with Japanese national health insurance rules. However, further study is required in order to confirm adequate doses of prednisolone and ETV in severe exacerbation of chronic hepatitis B.

In conclusion, the combination treatment of ETV and corticosteroid may improve the prognosis in severe exacerbation of chronic hepatitis B. However, this is a case report; prospective studies of large study populations are needed in order to confirm the effectiveness of this combination treatment.

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## Needle track seeding: A real hazard after percutaneous radiofrequency ablation for colorectal liver metastasis

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### Abstract

Neoplastic needle track seeding following percutaneous radiofrequency ablation (RFA) of secondary liver tumors is exceedingly rare. Reports on cutaneous tumor seeding after percutaneous RFA for colorectal liver metastasis are even rarer in the literature. Here we report a case of a 46-year-old female who developed an ulcerating skin lesion along the needle track of a previous percutaneous RFA site around 6 mo after the procedure. The previous RFA was performed by the LeVeen® needle for a secondary liver tumor from a primary rectal cancer. The diagnosis of secondary skin metastasis was confirmed by fine needle aspiration cytology. The lesion was successfully treated with wide local excision. We believe that tumor seeding after percutaneous RFA in our patient was possibly related to its unfavorable subcapsular location and the use of an expansion-type needle. Hence, prophylactic ablation of the needle track should be performed whenever possible. Otherwise, alternative routes of tumor ablation such as laparoscopic or open RFA should be considered.

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**Key words:** Radiofrequency catheter ablation; Needles; Neoplasm seeding; Liver neoplasms; Skin neoplasms; Neoplasm metastasis

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### INTRODUCTION

Radiofrequency ablation (RFA) is a well established local ablative treatment for primary or secondary hepatic neoplasms. Despite a relatively low complication rate, the percutaneous application of RFA carries a potential risk of needle track seeding. As reported by a recent systematic review<sup>[1]</sup>, the seeding risk after percutaneous RFA for hepatocellular carcinoma (HCC) is around 0.6%. However, such a seeding risk in secondary liver tumors is less well defined. Reports on tumor seeding after percutaneous RFA for colorectal liver metastasis are rare. The following report illustrates a patient with cutaneous tumor seeding after percutaneous RFA for a small colorectal liver metastasis.

### CASE REPORT

A 46-year-old female, who had no other major medical illness, was referred to our unit for stage IV rectal cancer with liver metastasis after receiving laparoscopic anterior resection of the primary rectal tumor in the private sector. Preoperative staging computed tomography (CT) of the abdomen showed no distant metastasis. The operation for the primary rectal tumor was uneventful. However, a suspicious liver lesion was incidentally found on the surface of the liver intra-operatively and was biopsied. Histopathology revealed a T3N2 well-differentiated adenocarcinoma of the rectum and a metastatic adenocarcinoma of the liver. A postoperative re-staging CT scan identified a 1.7 cm solitary subcapsular liver metastasis at segment 4 of the liver (Figure 1). A

Table 1 Literature review on neoplastic seeding after RFA for colorectal liver metastasis

Author (yr)	Age; gender	Size of liver metastasis (cm)	Subcapsular location of liver lesion	Number of RFA sessions	Size of seeding nodule (cm)	Time lag after RFA
Bonatti <i>et al</i> <sup>[4]</sup> (2003)	56; male	Not stated	Not stated	2	2	6 wk
Charalampopoulos <i>et al</i> <sup>[5]</sup> (2007)	64; male	3	Yes	2	Not stated	10 mo
Present case-2008	46; female	1.7	Yes	1	2	6 mo

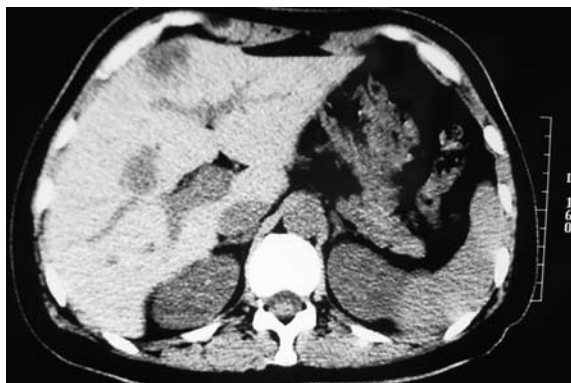


Figure 1 Subcapsular liver metastasis at segment 4 of liver.



Figure 2 Percutaneous radiofrequency ablation by LeVeen® needle.

subsequent positron emission tomography scan excluded additional distant metastasis. Unfortunately, the patient refused curative hepatic resection. Hence, a single session of CT-guided percutaneous RFA of the liver lesion using a 3 cm 17-gauge LeVeen® needle electrode (Super-slim, Boston Scientific, United States) with a single puncture was performed (Figure 2). Thermal ablation of the needle tract was not done in this case. She was subsequently put on a complete course of oxaliplatin-based chemotherapy.

Six months after RFA, she presented with a 2 cm ulcerating skin nodule at the previous RFA puncture site (Figure 3). Fine needle aspiration cytology of the nodule confirmed a metastatic adenocarcinoma of primary colorectal origin. At the same time, a CT scan revealed multiple simultaneous recurrences at the left liver. Left hepatectomy for the liver lesions and wide local excision of the cutaneous tumor were performed. Multiple suspicious peritoneal deposits were incidentally identified intra-operatively and they were all resected. Final histopathology revealed metastatic disease in the

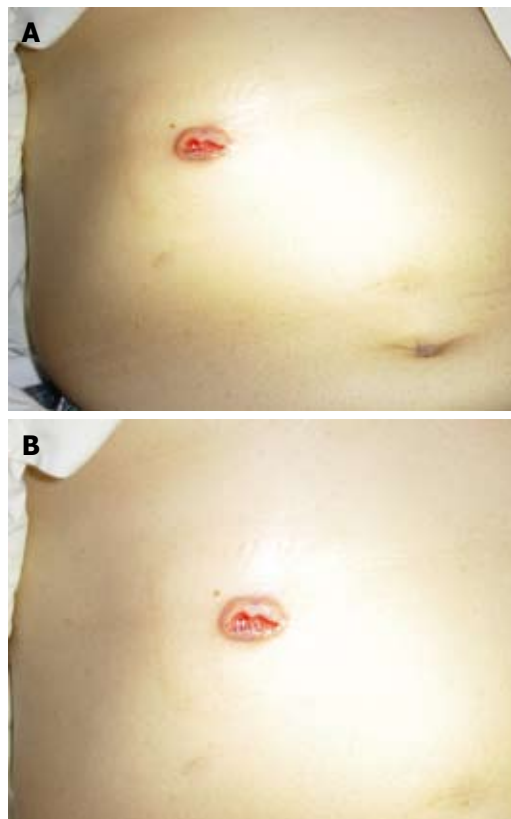


Figure 3 Ulcerating skin nodule. A: Cutaneous neoplastic seeding 6 mo after RFA. B: A closer view.

liver, skin and peritoneum. She then received further courses of palliative chemotherapy in view of likely recurrence. In the subsequent follow-up period, no cutaneous recurrence was identified at the RFA needle track. However, she developed progressive disease with lung metastasis and carcinomatosis. The patient finally died around 5 mo after the second operation.

## DISCUSSION

Neoplastic seeding is an uncommon but well-recognized complication following percutaneous diagnostic and therapeutic procedures for primary liver cancer. For diagnostic percutaneous biopsy, the risk of neoplastic seeding was approximately 2.2%<sup>[1]</sup>. As for therapeutic RFA of HCC, initial results from a small-scale Spanish study suggested an alarmingly high risk of 12.5%<sup>[2]</sup>. A recent large-scale multicenter study by Livraghi *et al*<sup>[3]</sup> in contrast identified a substantially lower risk of only 0.9%. As highlighted by a recent systematic review<sup>[1]</sup>, such a seeding risk for HCC was definitely lower with

an overall median risk of only 0.6%. For secondary liver tumors, objective evidence on the seeding risk following percutaneous RFA is lacking. In the English literature, there were only two related case reports identified<sup>[4,5]</sup> (Table 1).

Several associated risk factors have been identified for neoplastic seeding following RFA for HCC, notably subcapsular tumor location<sup>[2,6]</sup>, poor tumor differentiation grade<sup>[2]</sup>, multiple RFA sessions<sup>[6]</sup>, multiple electrode placements<sup>[6]</sup> and history of previous biopsy<sup>[6]</sup>. In an Italian study by Latteri *et al*<sup>[7]</sup>, the risk of neoplastic seeding after open RFA was virtually zero but the risk was as high as 1.4% after percutaneous RFA. Remarkably, most of these identified factors were based on seeding risk for HCC. As for our patient, tumor seeding in the RFA needle track was possibly related to its unfavorable subcapsular location and the use of an expansion-type electrode. The previous use of laparoscopic biopsy in such a subcapsular tumor was probably a major detrimental factor related to its peritoneal dissemination. Nevertheless, potential risk factors of neoplastic seeding solely for secondary liver tumors were still undefined. To evaluate the seeding risk, a larger-scale prospective cohort study for secondary liver tumors is required. However, these sorts of studies are practically difficult to conduct because of the rare occurrence.

To prevent neoplastic seeding, some investigators advocated the application of thermocoagulative ablation along the needle track while withdrawing the RFA needle<sup>[8]</sup>. However, this ablative technique may not be technically feasible in cases of subcapsular lesions as in our case. With regard to treatment, surgical excision with a wide margin seems to be the most justifiable option. Alternatively, different novel techniques had been described. Shibata *et al*<sup>[9]</sup> successfully treated a chest wall neoplastic seeding from HCC by transarterial embolization of the feeding vessel. Espinoza *et al*<sup>[10]</sup> suggested the use of RFA again for ablating the metastatic seeding tract as treatment.

To conclude, despite its rare occurrence, needle track seeding is a real hazard following percutaneous RFA for secondary liver tumors. Prophylactic ablation of the

needle track should be performed whenever possible for high risk patients. Otherwise, alternative routes of tumor ablation like laparoscopic or open RFA should be considered.

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## CASE REPORT

# A large congenital and solitary intrahepatic arterioportal fistula in an old woman

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## Abstract

Arterioportal fistula (APF) is a rare cause of portal hypertension and may lead to death. APF can be congenital, post-traumatic, iatrogenic (transhepatic intervention or biopsy) or related to ruptured hepatic artery aneurysms. Congenital APF is a rare condition even in children. In this case report, we describe a 73-year-old woman diagnosed as APF by ultrasonography, computed tomography, and hepatic artery selective arteriography. The fistula was embolized twice but failed, and she still suffered from alimentary tract hemorrhage. Then, selective arteriography of the hepatic artery was performed again and venae coronariae ventriculi and short gastric vein were embolized. During the 2-year follow-up, the patient remained asymptomatic. We therefore argue that embolization of venae coronariae ventriculi and short gastric vein may be an effective treatment modality for intrahepatic APF with severe upper gastrointestinal bleeding.

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**Key words:** Congenital intrahepatic arterioportal fistula; Liver; Embolization; Portal hypertension; Angiography

**Peer reviewers:** Dr. Paolo Del Poggio, Hepatology Unit, Department of Internal Medicine, Treviglio Hospital, Piazza

## INTRODUCTION

Arterioportal fistula (APF) is a rare cause of portal hypertension and may lead to death. APF can be congenital, post-traumatic, iatrogenic (transhepatic intervention or biopsy) or related to ruptured hepatic artery aneurysms. Congenital APF is a rare condition in children. To date, only 18 cases of congenital intrahepatic APF have been reported<sup>[1]</sup>. Most of them were found in their infancy, in which the oldest one was a 13-year-old boy<sup>[1]</sup>. We report here the incidental findings of a large and solitary congenital APF in a 73-year-old woman. Digital subtraction angiography revealed a stubby fistular vessel between the left hepatic artery and portal vein of the patient. Transcatheter closure of APF was performed three times using multiple coils.

## CASE REPORT

A 73-year-old woman was admitted to our hospital with complaints of ascites, splenomegaly, abdominal distension and pain. She had been asymptomatic before and denied any medication history as well as history of cirrhosis and hepatic neoplasms, blunt or penetrating trauma, percutaneous liver biopsy, transhepatic cholangiography, gastrectomy and biliary surgery. Her mother and aunt died of ascites and gastrointestinal bleeding in their thirties, about 60 years ago. There was no history of chronic hepatic disease in her family.

A recent physical examination revealed ascites, splenomegaly, and a subcutaneous varicose vein in the abdominal wall. Her laboratory results are listed in Table 1.

Values for other biochemical tests were within the normal ranges. All viral markers for hepatitis including



Table 1 Laboratory results

Item	Results
Hemoglobin	97 g/L
Hematocrit	40.30%
MCV	89.8 fL
Blood cell count	$4.0 \times 10^9/L$
Platelet count	$131 \times 10^9/L$
Prothrombin time	14 s (normal value: 9.8-15.0 s)
Serum ALT	37 IU/L (normal value: 3-40 IU/L)
Serum AST	62 IU/L (normal value: 3-40 IU/L)
Serum GGT	102 IU/L (normal value: 0-54 IU/L)
Serum ALP	141 IU/L (normal value: 30-115 IU/L)
Total bilirubin	10 $\mu\text{mol/L}$ (normal value < 22 $\mu\text{mol/L}$ )
Direct bilirubin	4 $\mu\text{mol/L}$ (normal value < 7 $\mu\text{mol/L}$ )
Albumin	39.7 g/L

hepatitis A-E viruses, autoantibodies (antinuclear, anti-mitochondrial, anti-smooth-muscle, anti-liver-kidney microsomal enzymes, anti-soluble liver antigen, and anti-mitochondrial antibody) were also negative. Serum alpha-fetoprotein level was normal. Upper gastrointestinal endoscopy revealed non-bleeding esophageal and fundus varices, but mild portal hypertensive gastropathy.

Real-time sonography of the liver demonstrated an anechoic lesion in continuity with a normal width of the left portal vein (LPV) branch, which was assumed to be an intrahepatic APF. Reverse flow in the left portal vein was observed on color Doppler sonography (Figure 1), and high-velocity flow at 1.31 m/s (Figure 2) was observed on spectral Doppler sonography. The distal portion of the LPV showed no enlargement but turbulent flow. The left hepatic artery branch was dilated, with a diameter of 5 mm. Color speckling (mosaic pattern), present in hepatic tissues adjacent to the aneurysmal lesion, was considered a typical sign of arteriovenous fistula.

Contrast-enhanced computed tomography (CT) also confirmed the diagnosis of intrahepatic APF. A high density of the LPV was observed with a CT value of 220.6 Hounsfield units (HU) (Figure 3A) at the arterial phase, and a CT value of 159.7 HU (Figure 3B) at the portal venous phase, close to the CT values of 226.2 HU (Figure 3A) and 168.0 HU (Figure 3B), respectively, for the abdominal aorta at the same phase.

After a comprehensive analysis, a diagnosis of portal hypertension secondary to hepaticoportal fistula was established.

Selective digital subtraction angiography of the hepatic artery was performed, which revealed a large left hepatic artery-left portal vein fistula (Figure 4A). The aneurysmal lesion was embolized, and angiography CT and sonography showed no shunt after operation (Figure 4B).

Three months after operation, the patients had severe upper gastrointestinal bleeding from esophageal and fundus varices. She received another transcatheter closure, with no recurrence of bleeding. She underwent somatostatin infusion and conservative treatment but hemorrhage recurred 1 mo later. She refused any surgical intervention. Selective arteriography of the hepatic



Figure 1 Color Doppler sonography of the portal vein aneurysm showing increased blood flow in the LPV and turbulence (arrow) at the distal portion of the aneurysmal sac.

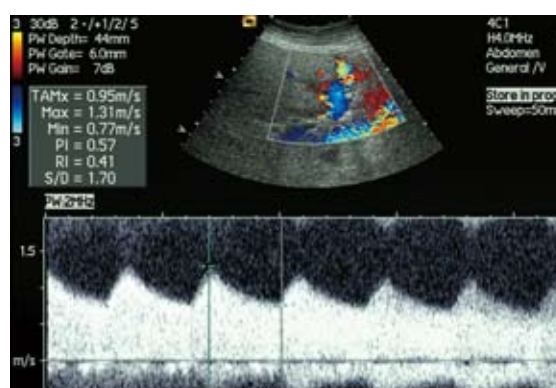
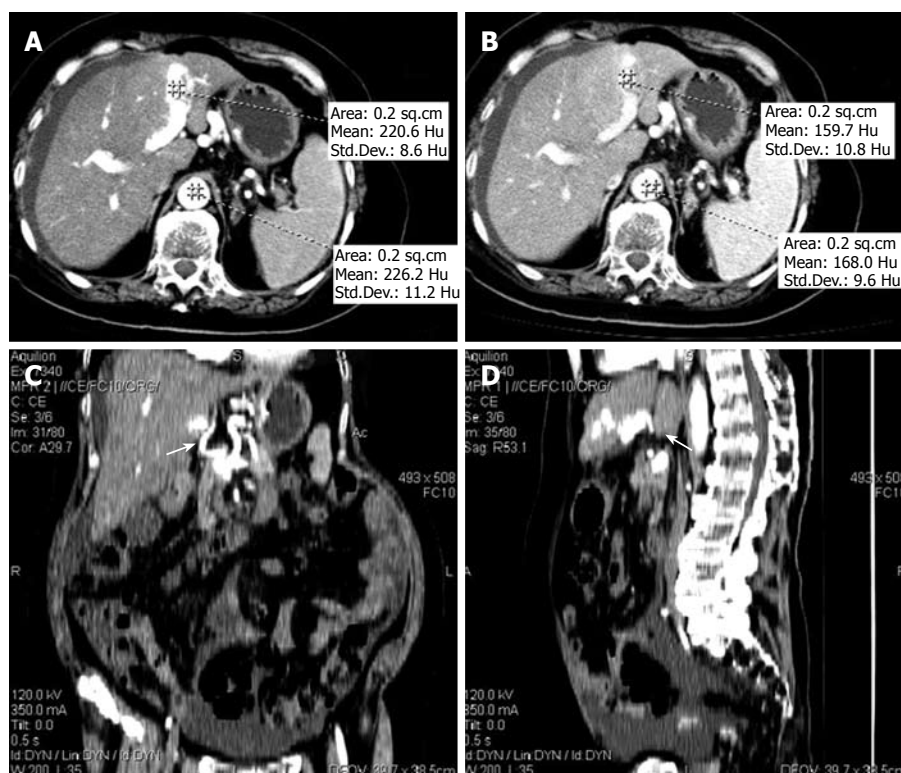


Figure 2 PW Doppler sonography of the portal vein aneurysm showing a high speed blood flow at 1.31 m/s in the aneurysm.

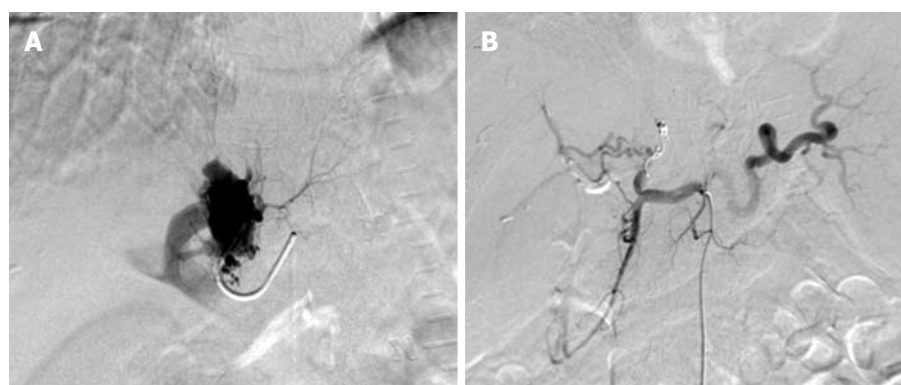
artery was performed again with her venae coronariae ventriculi and short gastric vein embolized (Figure 5A and B). During the 2-year follow-up, the patient remained asymptomatic.

## DISCUSSION

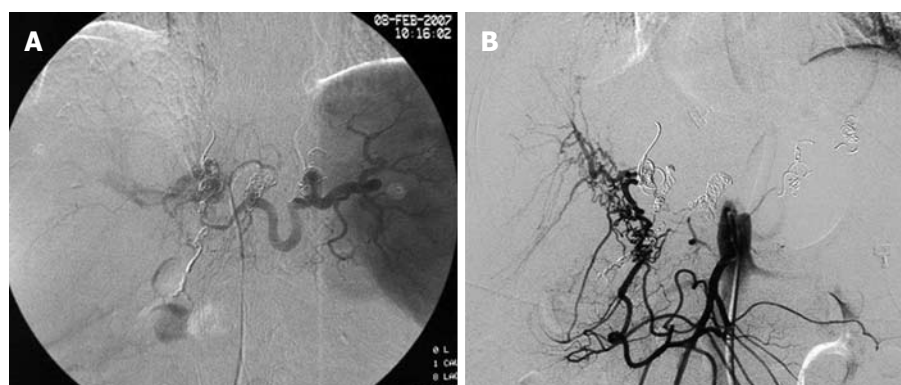
Congenital APF is a rare cause of severe portal hypertension, with challenging diagnostic and therapeutic implications<sup>[2,3]</sup>. Upon physical examination, a characteristic bruit can be heard over the right upper quadrant. Liver function tests are usually normal. Radiologic evaluation of APF is usually performed with color Doppler ultrasonography (US), helical CT, and magnetic resonance imaging. Arterial and direct portography as well as splenoportography may also be used. Doppler ultrasound is the best way in making the decisive diagnosis and helpful in the subsequent evaluation of these patients<sup>[4,5]</sup>. Angiography is the most useful test because it can not only identify multiple APFs but also be therapeutic. These APFs are mostly intrahepatic and represent 12% of the cases developmental intrahepatic shunts<sup>[3]</sup>. The presence of a patent ductus venosus is protective in the initial postnatal period. Symptoms usually develop after 1 mo of age, and these children are often investigated for generalized abdominal findings and are later noted to



**Figure 3** Contrast-enhanced CT showing a LPV density of 220.6 Hu and an abdominal aorta density of 226.2 Hu at artery phase (A), a LPV density of 159.7 Hu and an abdominal aorta density of 168.0 Hu at portal venous phase (B), and CT of coronal section (C) and sagittal section (D) showing the direct shunt of APF.



**Figure 4** Selective digital subtraction angiography of the hepatic artery. A: A large left hepatic artery-left portal vein fistula; B: No more shunt after embolism.



**Figure 5** Angiography showing collateral circulation in fistula after embolism (A) and no more shunt after embolism (B).

develop symptoms related to portal hypertension<sup>[2,3]</sup>. APF often presents in conjunction with major gastrointestinal tract bleeding and should be differentiated between small peripheral intrahepatic APF (type 1) and large central APF (type 2), whereas diffuse congenital intrahepatic APF that is difficult to manage is defined as typed 3<sup>[6]</sup>. A few cases of congenital fistula from the hepatic artery to the

portal vein have been reported<sup>[4]</sup>, but this abnormality is not a common cause of portal hypertension. Increased blood flow in the portal system is considered to be the cause of hyperkinetic portal hypertension in patients with hepatoportal arteriovenous fistula. Arterioportal venous fistula can be treated with percutaneous arterial occlusion<sup>[7]</sup>. Ligation of the hepatic artery has been proved

to be successful in reported cases<sup>[8]</sup>. Recently, transcatheter arterial embolization has been attempted as the first choice, because of its low invasiveness and success in some cases. Recanalization of the embolized artery in some cases has also been reported<sup>[9,10]</sup>. Therefore, some of the cases can undergo hepatic resection, including fistula.

To date, in the literature, the oldest congenital APF case was a 13-year-old child<sup>[1]</sup>. We report here a 73-year-old woman. This patient had no history of cirrhosis and hepatic neoplasms, blunt or penetrating trauma, percutaneous liver biopsy, transhepatic cholangiography, gastrectomy, and biliary surgery, and was finally diagnosed having a congenital APF. Initially, the APF was demonstrated by contrast-enhanced CT and the fistula was subsequently identified by color Doppler imaging and angiography. The case was classified as type 3.

No more shunt was found after coil occlusion. Notably, esophageal varices and ascites disappeared after embolization. However, color Doppler sonography still displayed the shunt 3 mo after operation.

The aneurysmal lesion was embolized twice, but transcatheter closure did not work well. After undergoing embolization twice, she suffered alimentary tract hemorrhage again, suggesting that transcatheter closure is not so effective against a large APF. The reason why embolization failed was because of too much collateral circulation. The purpose of embolizing the venae coronariae ventriculi and short gastric vein is to cut off the collateral circulation, because it can effectively control hemostasis. Since it cannot cure the disease, the treatment modality is radical surgery.

In conclusion, this case suggests that interventional radiology plays an important role in the treatment of congenital APF with severe upper gastrointestinal bleeding caused by esophageal and fundus varices.

Liver function test, abdomen ultrasonography and gastroscopy should be performed regularly during the follow-up.

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Westin San Diego Hotel, San Diego, CA  
Advances in Prostate Cancer Research

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Carefree Resort and Villas, Carefree, AZ (Greater Phoenix Area)  
Second AACR Conference  
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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  
Hong Kong Convention and Exhibition Centre, Hong Kong, China  
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](http://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](mailto:bsg@mailbox.ulcc.ac.uk)

April 8-9, 2009  
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  
the 44th Annual Meeting of the European Association for the Study of the Liver (EASL)  
<http://www.easl.ch/>

May 17-20, 2009  
Denver, Colorado, USA  
Digestive Disease Week 2009

May 29-June 2, 2009  
Orange County Convention Center  
Orlando, Florida  
45th ASCO Annual Meeting  
[www.asco.org/annualmeeting](http://www.asco.org/annualmeeting)

May 30, 2009  
Chicago, Illinois  
Endpoints Workshop: NASH

May 30-June 4, 2009  
McCormick Place, Chicago, IL  
DDW 2009  
<http://www.ddw.org>

June 17-19, 2009  
North Bethesda, MD  
Accelerating Anticancer Agent Development

June 20-26, 2009  
Flims, Switzerland  
Methods in Clinical Cancer Research (Europe)

June 24-27, 2009  
Barcelona, Spain  
ESMO Conference: 11th World Congress on Gastrointestinal Cancer  
[www.worldgicancer.com](http://www.worldgicancer.com)

June 25-28, 2009  
Beijing International Convention Center (BICC), Beijing, China  
World Conference on Interventional Oncology  
<http://www.chinamed.com.cn/wcio2009/>

July 5-12, 2009  
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

August 1-7, 2009  
Vail Marriott Mountain Resort, Vail, CO, United States  
Methods in Clinical Cancer Research

August 14-16, 2009  
Bell Harbor Conference Center, Seattle, Washington, United States  
Practical Solutions for Successful Management  
<http://www.asge.org/index.aspx?id=5040>

September 23-26, 2009  
Beijing International Convention Center (BICC), Beijing, China  
19th World Congress of the International Association of Surgeons, Gastroenterologists and Oncologists (IASGO)  
<http://iasgo2009.org/en/index.shtml>

September 27-30, 2009  
Taipei, China  
Asian Pacific Digestive Week  
<http://www.apdwcongress.org/2009/index.shtml>

October 7-11, 2009  
Boston Park Plaza Hotel and Towers, Boston, MA, United States  
Frontiers in Basic Cancer Research

October 13-16, 2009  
Hyatt Regency Mission Bay Spa and Marina, San Diego, CA, United States  
Advances in Breast Cancer Research: Genetics, Biology, and Clinical Applications

October 20-24, 2009  
Versailles, France  
Fifth International Conference on Tumor Microenvironment: Progression, Therapy, and Prevention

October 30-November 3, 2009  
Boston, MA, United States  
The Liver Meeting

November 15-19, 2009  
John B. Hynes Veterans Memorial Convention Center, Boston, MA, United States  
AACR-NCI-EORTC Molecular Targets and Cancer Therapeutics

November 21-25, 2009  
London, UK  
Gastro 2009 UEGW/World Congress of Gastroenterology  
[www.gastro2009.org](http://www.gastro2009.org)



### Global Collaboration for Gastroenterology

For the first time in the history of gastroenterology, an international conference will take place which joins together the forces of four pre-eminent organisations: Gastro 2009, UEGW/WCOG London. The United European Gastroenterology Federation (UEGF) and the World Gastroenterology Organisation (WGO), together with the World Organisation of Digestive Endoscopy (OMED) and the British Society of Gastroenterology (BSG), are jointly organising a landmark meeting in London from November 21-25, 2009. This collaboration will ensure the perfect balance of basic science and clinical practice, will cover all disciplines in gastroenterology (endoscopy, digestive oncology, nutrition, digestive surgery, hepatology, gastroenterology) and ensure a truly global context; all presented in the exciting setting of the city of London. Attendance is expected to reach record heights as participants are provided with a compact "all-in-one" programme merging the best of several GI meetings. Faculty and participants from all corners of the earth will merge to provide a truly global environment conducive to the exchange of ideas and the forming of friendships and collaborations.



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*Chinese journal article (list all authors and include the PMID where applicable)*

- 2 Lin GZ, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

*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 PMID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

- 5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ, Alf-One Study Group. Sexual dysfunction in 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]

No author given

- 6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

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

Issue with no volume

- 8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; **(401)**: 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

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

## Books

Personal author(s)

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

Chapter in a book (list all authors)

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

Author(s) and editor(s)

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

Conference proceedings

- 13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

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

Electronic journal (list all authors)

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

Patent (list all authors)

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

## Statistical data

Write as mean  $\pm$  SD or mean  $\pm$  SE.

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Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as  $\chi^2$  (in Greek), related coefficient as *r* (in italics), degree of freedom as  $\nu$  (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

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