Symptomatology, Prognosis and Treatment of Primary Biliary Cirrhosis and Primary Sclerosing Cholangitis

P.C.J. ter Borg

ISBN : 90-8559-040-X

Printed by Optima Grafische Communicatie, Rotterdam, The Netherlands

This study was performed at the department of Gastroenterology and Hepatology of the Erasmus MC, University Hospital Rotterdam, The Netherlands. Financial support for the thesis was kindly given by the department of Gastroenterology and Hepatology of the Erasmus MC, Zambon Nederland B.V, AstraZeneca and Roche Nederland B.V.

Symptomatology, Prognosis and Treatment of Primary Biliary Cirrhosis and Primary Sclerosing Cholangitis

Symptomatologie, prognose en behandeling van primaire biliaire cirrose en primaire scleroserende cholangitis

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de rector magnificus

Prof.dr. S.W.J. Lamberts

en volgens besluit van het College voor Promoties.
De openbare verdediging zal plaatsvinden op

woensdag 6 april 2005 om 11:45 uur

door

Pieter Cornelis Jan ter Borg

geboren te Rotterdam.

Promotiecommissie

Promotor	Prof.dr. S.W. Schalm
Overige leden:	Prof.dr. E.J. Kuipers
	Prof.dr. G.P. van Berge Henegouwen
	Prof.dr. T. Stijnen
Copromotor:	Dr. H.R. van Buuren

voor Bhartie

Contents

1

General introduction

P.C.J. ter Borg

Department of Gastroenterology and Hepatology, Erasmus MC, Rotterdam, The Netherlands

Introduction

Primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) are relatively rare chronic liver diseases characterized by cholestasis. In PBC only the small interlobular and septal bile ducts are involved, whereas in PSC both the extra- and intrahepatic bile ducts of any size may be affected (1, 2). Although for both diseases an autoimmune etiology is presumed, the cause of these diseases remains unknown.

Primary biliary cirrhosis mainly occurs in middle-aged females, and patients may present with complaints of fatigue, pruritus, painful joints and dry eyes or mouth. However, a significant proportion of patients has no symptoms at all (1). The diagnostic hallmark of the disease is the presence of antimitochondrial antibodies. These antibodies can be detected in 90-95% of patients. Further, the disease is characterized by a cholestatic liver enzyme pattern and elevated total IgM serum concentrations (1). Primary sclerosing cholangitis typically is a disease of young male patients but may occur at any age and affect both sexes. About 70-80% of patients have concurrent ulcerative colitis or, less frequently, Crohn's disease. Patients may present with cholangitis, jaundice, fatigue, pruritus or be asymptomatic. The diagnostic hallmark of the disease is the presence of bile duct abnormalities on cholangiography including strictures, focal dilatations and diverticula. The gallbladder is usually markedly enlarged and bile duct stones are frequently present. Both patients with PBC and PSC can also present with manifestations or complications of cirrhosis and portal hypertension including variceal bleeding, ascites and hepatic encephalopathy. Rarely patients present with hepatocellular or cholangiocarcinoma.

Prognosis of PBC

A number of studies have evaluated the prognosis of PBC (3, 4). However, most of these studies have been performed before the introduction of ursodeoxycholic acid (UDCA) as routine medical treatment for PBC, and data for patients treated with UDCA are scarce. The prognosis of patients with PBC can be estimated with the widely used Mayo model (3). However, this model has several disadvantages. First, it was developed before ursodeoxycholic acid was used in the treatment of PBC. Second, one of the variables of the Mayo model is edema, which is a subjective and treatment dependent factor. Third, no correction for normal values is used, and some of the laboratory test results may vary depending on the test method used.

We performed a long-term multicenter cohort study aiming to define the prognosis of patients with PBC routinely treated with UDCA. In addition, prognostic factors were evaluated and based on these prognostic factors a prognostic model for use in patients treated with UDCA was created.

The histological stage of the disease is another important prognostic factor in PBC (5). The most widely used staging system defines four histological stages, with an increasing risk of complications, liver failure and death. Stage I indicates the presence of portal hepatitis with little or no interface hepatitis, stage II portal hepatitis with interface hepatitis and ductular proliferation, in stage III fibrous septa or bridging necrosis are present and stage IV is the cirrhotic stage of the disease (6). The presence of cirrhosis (stage IV) is of particular prognostic importance, since the large majority of complications occur in cirrhotic patients. However, liver biopsy is associated with morbidity and is a procedure not appreciated by most patients. Especially because liver biopsy may not be necessary for diagnostic purposes, we attempted to create a model to predict the presence of cirrhosis based on routine laboratory tests. This model might replace liver biopsy as an instrument for staging the disease in selected, typical patients with PBC.

Many previous reports have addressed the issue of diseases associated with PBC, and it has been suggested that several diseases occur with increased frequency in patients with PBC. For example, increased prevalences of breast carcinoma, rheumatic disorders and celiac disease compared to the general population have been reported (7-11). However, all these studies focused on only one disease or a related group of diseases, and such studies may have been initiated after some cases of the associated disease had been observed. Therefore, significant bias may have occurred in these studies. This is illustrated by early findings of an increased risk of breast carcinoma, which could not be confirmed in subsequent studies (7, 8, 12-15). In order to avoid this kind of bias, and to document the prevalence of clinically recognized disorders in patients with PBC, we studied the occurrence of any comorbidity in our multicenter cohort of patients with PBC.

Fatigue and depression

Although many patients with PBC and PSC have an excellent prognosis, it has been well documented that patients with PBC frequently have decreased quality of life as a result of fatigue, which may be truly invalidating in some patients (16-18). In contrast to these studies in PBC, very few data are available for patients with PSC, and no studies have specifically

aimed to assess the prevalence and severity of fatigue. Nevertheless, it has been suggested that fatigue may be a symptom as common in PSC as in PBC (19). We performed a study to quantify fatigue and quality of life in patients with PSC as compared to patients with PBC and age and sex-matched controls.

The pathophysiological mechanism underlying the development of fatigue in these conditions remains unknown. Correlations with the severity or activity of the disease as reflected by routine laboratory tests have not been found (16-18). After the observation of a beneficial effect of antioxidant treatment on fatigue in PBC, it has been suggested that oxidative stress might be responsible for the development of fatigue, although in a recent randomized controlled trial no beneficial effects of antioxidant treatment were found (20). We aimed to measure markers of oxidative stress, and to find an association with the occurrence of fatigue in patients with PBC and PSC.

Several other mechanisms might be important in the development of fatigue. It has been previously shown that amino acid concentrations are markedly abnormal in patients with these conditions, and these alterations in amino acid metabolism might be involved in fatigue (21, 22). Finally, we evaluated the possible role of increased expression of a variant of the glucocorticoid receptor, which may be induced as a result of an inflammatory response, in the development of fatigue (23).

Finally, besides these hypothetical pathophysiological explanations for the development of fatigue, a very different explanation might exist. Two previous studies found a high prevalence of depression in patients with PBC (17, 18). Fatigue is one of the main symptoms of depressive disorders, and therefore (undetected) depression might be responsible for the occurrence of fatigue in patients with PBC and PSC. We evaluated the prevalence of depression in these patients according to a formal psychiatric interview in addition to a self-rated questionnaire. The latter instrument was used in previous studies (17, 18), but may not be adequate to reliably diagnose depression. We therefore hypothesized that previous studies may have overestimated the prevalence of depression. Since fatigue might be a symptom of depression, and treatment with antidepressants is highly effective in treating depression, we performed a randomized controlled trial evaluating the antidepressant fluvoxamine as a treatment for fatigue in patients with PBC and PSC.

References

1. Kaplan MM. Primary biliary cirrhosis. N Engl J Med 1996;335(21):1570-80.
2. Angulo P, Lindor KD. Primary sclerosing cholangitis. Hepatology 1999;30(1):325-32.
3. Dickson ER, Grambsch PM, Fleming TR, Fisher LD, Langworthy A. Prognosis in primary biliary cirrhosis: model for decision making. Hepatology 1989;10(1):1-7.
4. Prince M, Chetwynd A, Newman W, Metcalf JV, James OF. Survival and symptom progression in a geographically based cohort of patients with primary biliary cirrhosis: follow-up for up to 28 years. Gastroenterology 2002;123(4):1044-51.
5. van Hoogstraten HJF, Hansen BE, van Buuren HR, ten Kate FJW, van Berge Henegouwen GP, Schalm SW. Prognostic factors and long-term effects of ursodeoxycholic acid on liver biochemical parameters in patients with primary biliary cirrhosis. J Hepatol 1999;31:256-262.
6. Ludwig J, Dickson ER, McDonald GS. Staging of chronic nonsuppurative destructive cholangitis (syndrome of primary biliary cirrhosis). Virchows Arch A Pathol Anat Histol 1978;379(2):103-12.
7. Goudie BM, Burt AD, Boyle P, Macfarlane G, Birnie GG, Mills PR, et al. Breast cancer in women with primary biliary cirrhosis. Br Med J (Clin Res Ed) 1985;291(6509):1597-8.
8. Wolke AM, Schaffner F, Kapelman B, Sacks HS. Malignancy in primary biliary cirrhosis. High incidence of breast cancer in affected women. Am J Med 1984;76(6):1075-8.
9. Dickey W, McMillan SA, Callender ME. High prevalence of celiac sprue among patients with primary biliary cirrhosis. J Clin Gastroenterol 1997;25(1):328-9.
10. Bardella MT, Quatrini M, Zuin M, Podda M, Cesarini L, Velio P, et al. Screening patients with celiac disease for primary biliary cirrhosis and vice versa. Am J Gastroenterol 1997;92(9):1524-6.
11. Marasini B, Gagetta M, Rossi V, Ferrari P. Rheumatic disorders and primary biliary cirrhosis: an appraisal of 170 Italian patients. Ann Rheum Dis 2001;60(11):1046-9.
12. Loof L, Adami HO, Sparen P, Danielsson A, Eriksson LS, Hultcrantz R, et al. Cancer risk in primary biliary cirrhosis: a population-based study from Sweden. Hepatology 1994;20(1 Pt 1):101-4.
13. Floreani A, Baragiotta A, Baldo V, Menegon T, Farinati F, Naccarato R. Hepatic and extrahepatic malignancies in primary biliary cirrhosis. Hepatology 1999;29(5):1425-8.
14. Howel D, Metcalf JV, Gray J, Newman WL, Jones DE, James OF. Cancer risk in primary biliary cirrhosis: a study in northern england. Gut 1999;45(5):756-60.
15. Floreani A, Biagini MR, Chiaramonte M, Milani S, Surrenti C, Naccarato R. Incidence of hepatic and extra-hepatic malignancies in primary biliary cirrhosis (PBC). Ital J Gastroenterol 1993;25(9):473-6.
16. Goldblatt J, Taylor PJ, Lipman T, Prince MI, Baragiotta A, Bassendine MF, et al. The true impact of fatigue in primary biliary cirrhosis: a population study. Gastroenterology 2002;122(5):1235-41.
17. Huet PM, Deslauriers J, Tran A, Faucher C, Charbonneau J. Impact of fatigue on the quality of life of patients with primary biliary cirrhosis. Am J Gastroenterol 2000;95(3):760-7.
18. Cauch-Dudek K, Abbey S, Stewart DE, Heathcote EJ. Fatigue in primary biliary cirrhosis. Gut 1998;43 (5):705-10.
19. Wiesner RH, LaRusso NF, Ludwig J, Dickson ER. Comparison of the clinicopathologic features of primary sclerosing cholangitis and primary biliary cirrhosis. Gastroenterology 1985;88(1 Pt 1):108-14.

20. Watson JP, Jones DE, James OF, Cann PA, Bramble MG. Case report: oral antioxidant therapy for the treatment of primary biliary cirrhosis: a pilot study. J Gastroenterol Hepatol 1999;14(10):1034-40.
21. Morgan MY, Milsom JP, Sherlock S. Plasma ratio of valine, leucine and isoleucine to phenylalanine and tyrosine in liver disease. Gut 1978;19(11):1068-73.
22. Morgan MY, Marshall AW, Milsom JP, Sherlock S. Plasma amino-acid patterns in liver disease. Gut 1982;23(5):362-70.
23. Honda M, Orii F, Ayabe T, Imai S, Ashida T, Obara T, et al. Expression of glucocorticoid receptor beta in lymphocytes of patients with glucocorticoid-resistant ulcerative colitis. Gastroenterology 2000;118(5): 859-66.

2

Prognosis of ursodeoxycholic acid treated patients with primary biliary cirrhosis. Results of a 10-year cohort study involving 297 patients.

Pieter C.J. ter Borg[1], Solko W. Schalm[1], Henk R. van Buuren[1], Bettina E. Hansen[1,2] for the Dutch PBC study group

[1]Department of Gastroenterology and Hepatology, [2]Department of Epidemiology and Biostatistics, Erasmus MC, Rotterdam, The Netherlands

Submitted

Summary

The therapeutic potential of ursodeoxycholic acid (UDCA) treatment in primary biliary cirrhosis (PBC) remains controversial. In addition, relatively few data have been reported on the outcome of patients who have been treated long term. The aim of the present study was to document long-term survival of a prospectively followed large cohort of UDCA treated PBC patients in comparison to that predicted by the Mayo model and of a matched control cohort of the Dutch population.

297 Patients were included and followed during a median period of 68 (range 3-126) months until death or the end of the study. Survival free of transplantation (1-yr 99.7%, 5-yr 87% and 10 yr 71%) was significantly better than predicted by the Mayo model (p=0.01). However, for patients with abnormal serum bilirubin and/or albumin concentrations at entry, observed and predicted survival did not significantly differ. Compared with survival for a standardized cohort of the Dutch population, observed survival for the total group was significantly decreased (p=0.0003); for non-cirrhotic patients and patients with normal entry bilirubin and albumin concentrations survival was comparable. Serum bilirubin and albumin concentrations were the prognostic factors most consistently associated with survival.

In conclusion, 10-year prognosis for most UDCA treated patients with PBC is comparable to that of a matched general population. Our finding that observed survival was significantly better than predicted by the Mayo model may suggest that this model did not accurately predict prognosis in our UDCA-treated cohort. Alternatively, this finding indicates an important therapeutic effect of long-term UDCA treatment in PBC, particularly in patients with non-cirrhotic, non-advanced disease.

Introduction

Primary biliary cirrhosis (PBC) is a chronic, usually slowly progressive cholestatic liver disease (1). Ursodeoxycholic acid (UDCA) is widely accepted as the standard medical treatment, although there is controversy whether this can be considered evidence based. In particular, meta-analyses of randomized controlled trials came to different conclusions regarding a beneficial effect of UDCA on overall or transplantation-free survival (2-4). These conflicting results are largely attributable to the relatively small sample size and the short duration of (placebo-controlled) treatment in most of the trials, resulting in insufficient power to detect relatively small, but clinically important effects on end-points (5).

Since the effects of UDCA on survival remain controversial and the initiation of new trials is unlikely, information derived from long-term treated patient cohorts may be helpful to further define the therapeutic significance of UDCA. However, few such studies are available. Leuschner et al. reported maintained beneficial treatment effects up to 12 years but this study included only 22 patients (6). In a French multicenter follow-up study of 225 UDCA treated patients 10-year survival was significantly lower than survival of a matched sample of the general population, but was significantly better than predicted by the Mayo model (7, 8). This finding is compatible with a therapeutic effect of UDCA.

The ability to reliably predict prognosis in PBC is of key clinical importance. A number of prognostic models have been developed which, however, all have one or more of the following disadvantages: need to obtain liver biopsy, model created before the introduction of UDCA, insufficient data provided to actually use the model to calculate the predicted survival, no adjustment of laboratory results for different normal values and the use of subjective and treatment dependent parameters such as edema (8-14). The aim of the present study was to determine the efficacy of UDCA by comparing survival without orthotopic liver transplantation (OLT) of UDCA-treated PBC-patients with OLT-free survival as predicted by the Mayo model. A further aim was to assess overall survival, reflecting the combined effects of UDCA and OLT, in comparison with the estimated survival of a standardized control cohort of the Dutch population. Finally, we aimed to identify prognostic factors and to develop a prognostic model that can be easily used to identify low and high risk patients in clinical practice.

Patients and methods

This was a prospective multicenter cohort-study of PBC patients treated with UDCA. The results of an earlier analysis have been reported previously (15). The diagnosis of PBC was established on the basis of the criteria published by Taal et al. (16). Both untreated patients and patients already receiving UDCA were included. Inclusion started in January 1990 and follow-up data until June 2000 were analyzed. Exclusion criteria were: pregnancy, evidence of extrahepatic biliary disease, concomitant disorders limiting life expectancy and decompensated PBC, defined as Child-Pugh class B or C cirrhosis. UDCA (Ursochol, Zambon Nederland BV, Amersfoort, The Netherlands) was administered at a dose of 10 mg/kg/day. The dose was increased to 13-15 mg/kg/day in 1996 considering reported higher efficacy of the latter dose (17, 18). Follow-up data were collected at three-monthly intervals in the first year of follow-up and at six-monthly intervals thereafter. At each visit a general physical examination and measurement of total serum bilirubin, alkaline phosphatase (APh), aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, IgG, IgM and total cholesterol were performed. Liver biopsy at entry was optional. Biopsies were reviewed according to Ludwig et al. (19).

Statistical analysis

Survival analysis was performed using the Kaplan-Meier method and groups were compared using the log-rank test. Survival was analyzed as OLT-free survival (end-points: death and OLT), survival free of liver-related death or OLT (end-points: liver-related death and OLT; censored: non liver-related death) and survival including the period after OLT (end-point: death). Liver related death was defined as: death due to liver failure or hepatocellular carcinoma or death occurring within two months of an episode of variceal bleeding, spontaneous bacterial peritonitis, hepatorenal syndrome or hepatic encephalopathy.

Observed survival was compared to survival predicted by the Mayo model with a one-sample log-rank test using R version 1.5.1 (http://www.r-project.org) (8). Patients using UDCA at entry were excluded from this analysis, as the model is not based on data derived from such patients.

Overall survival was compared to survival data for a sex and age matched control cohort based on demographic data of the Dutch population. This analysis included the period after OLT, in order to avoid overestimation of death if OLT were regarded an end-point, and underestimation of death if patients were censored at the time of OLT.

Cox multivariate analysis with forward elimination was used to examine baseline prognostic variables. Analysis of the prognostic value of the presence or absence of cirrhosis was performed for patients who underwent liver biopsy within the year before entry or had established cirrhosis according to previous biopsies. Only data from patients not using UDCA at entry were included in the analyses of prognostic factors, given the effect of UDCA on APh, IgM, ALT, AST, bilirubin, gamma-glutamyltransferase and cholesterol (20).

Laboratory parameters were expressed as multiples of the upper limit of normal (bilirubin, APh, AST, ALT, IgG, IgM and cholesterol) or lower limit of normal (albumin). Logarithmic transformations (base e) were used to increase normality for all variables except albumin and the platelet count.

Patients lost to follow-up were censored at the time of their last visit. All reported p-values are two-sided and a p-value <0.05 was considered statistically significant. Statistical analysis was performed using SPSS version 9.0.0 for Windows.

Results

Patients

Patient characteristics at entry are shown in table 1. The study population comprised 297 (88% female) patients recruited in 5 university and 39 general hospitals. Median follow-up was 68 (range 3-126) months. Patients who were alive at the end of follow-up (n=231) had been followed for a median period of 77 months (range 3-126). Fifteen patients were lost to follow-up before the end of the study. Thirty-six (12%) patients were already being treated with UDCA at entry. For 12 patients serum bilirubin or albumin concentrations at entry were not available.

Survival

During follow-up 34 patients (11%) died. In 16/34 (47%) cases death was considered to be liver-related. OLT was performed in 16/297 (5%) patients. OLT-free survival was 99.7% after one year, 94% after three years, 87% after five years, 81% after seven years and 71% after ten years. Survival free of liver-related death or OLT was 99.7% after one year, 96% after three years, 90% after five years, 86% after seven years and 82% after ten years. Survival after OLT (n=16) was 81% after 1 and 3 years and 71% after 5 years. Observed overall survival was 99.7% after one year, 95% after three years, 91% after five years, 85% after seven years and

78% after ten years. OLT-free survival and survival free of liver-related death or OLT for cirrhotic patients was significantly decreased compared to non-cirrhotic patients. Five-year survival free of liver-related death or OLT was 87% for all patients, 98% for patients without cirrhosis and 60% for patients with cirrhosis ($p<0.0001$).

Prognostic factors

The following factors were included in univariate analyses of OLT-free survival and survival free of liver-related death or OLT: age, sex, weight, AMA, total serum bilirubin, serum albumin, APh, ALT, AST, AST/ALT-ratio, platelet count, serum cholesterol, IgG, IgM and the presence or absence of cirrhosis. Sex, bilirubin, albumin, APh, AST, AST/ALT-ratio, platelet count and cirrhosis were all significantly associated with OLT-free survival and survival free of liver-related death or OLT (Table 2). Age was significantly associated with OLT-free survival.

In multivariate analyses bilirubin was the most significant factor associated with survival ($p<0.0001$). Albumin was also significantly associated with survival in the models based on the total population ($p=0.006$). Age was associated ($p=0.01$) with OLT-free survival and the platelet count ($p=0.008$) was associated with survival free of liver-related death or OLT.

Since the serum bilirubin and albumin concentrations were the prognostic factors most consistently and significantly associated with survival, we attempted to use these variables to categorize patients into low and high risk groups.

Transplantation-free survival for patients with normal and increased bilirubin concentrations is shown in Figure 1a ($p<0.0001$) and for patients with normal and decreased albumin concentrations in Figure 1b ($p=0.0002$). Figure 2 shows survival for patients with normal bilirubin and albumin concentrations, with abnormal bilirubin or abnormal albumin concentrations and for patients with both abnormal bilirubin and albumin concentrations, thus identifying patients at low, medium and high risk ($p<0.0001$).

Observed versus predicted survival by the Mayo model

OLT-free survival was significantly improved compared to survival predicted by the Mayo model ($p=0.01$). For patients with normal bilirubin and albumin concentrations the expected number of events was 17, whereas only 7 events were observed (Table 3, $p=0.005$). Survival for patients with abnormal bilirubin and/or albumin concentrations was not significantly different from predicted survival ($p=0.43$ for both groups).

Comparison with Dutch population

Survival of our UDCA treated cohort was significantly decreased compared to survival of a standardized Dutch population (Figure 3, p=0.0003). Survival for patients with normal bilirubin and albumin levels (n=180) was not significantly decreased compared to survival predicted from the general population (p=0.90). However, survival for patients with abnormal bilirubin and/or albumin (one parameter abnormal in 85 patients and two in 20) was significantly decreased (Figure 4, p<0.0001). Survival for non-cirrhotic patients was comparable to survival of the Dutch population (p=0.95), while survival for cirrhotic patients was significantly lower (p<0.0001).

Discussion

Primary biliary cirrhosis is considered to be a progressive liver disease eventually leading to liver failure or necessitating transplantation in the majority of patients (1). An important finding in the present study, involving the largest reported UDCA-treated patient cohort, and other recent studies is that ten year prognosis for many patients with (UDCA-treated) PBC is only slightly decreased compared to the general population (7, 21). Our study also demonstrates that, based on the serum bilirubin and albumin concentrations, patients who have a 10-year survival rate comparable to that of the general population can be easily identified. Prognosis for patients with abnormal bilirubin and/or albumin levels, however, is markedly worse. We confirmed the finding by Poupon et al. and Koulentaki et al. that the Mayo model underestimates survival in UDCA-treated PBC patients, particularly for patients with normal bilirubin and albumin concentrations (7, 21). This is compatible with a beneficial effect of UDCA, especially in early PBC, as has been suggested previously (7, 21-23). Alternatively, the difference between observed and expected survival may suggest that the Mayo model does not accurately predict prognosis in European patients and overestimates the risk of death. In the original cross-validation study the Mayo model predicted prognosis reliably, also for low-risk patients (8). This was confirmed in other studies in the US (24, 25). A study including 770 patients from the UK, of which two thirds were never treated with UDCA, confirmed that survival was predicted well by the model, also for low-risk cases (11). In contrast, a Polish study reported that the model overestimated death risks, not only in low risk groups but also in medium- and high-risk patients (10). However, in this study approximately 50% of patients were treated with UDCA, and thus this overestimation of death risks might be related to a treatment effect.

In addition, Prince et al., as well as the landmark study by Rohl et al, suggested an increased risk of death of patients with PBC, also for cases with non-advanced disease, compared to the general population (11, 13). In contrast, the present study as well as the studies by Poupon et al. and Koulentaki et al., reported a survival of UDCA-treated patients with early PBC comparable to the general population (7, 21).

These partially conflicting data do not exclude the possibility that the Mayo model may not reliably predict prognosis in current European PBC patients. Most available data, however, clearly indicate that the Mayo model accurately predicts prognosis, also in patients with non-advanced disease.

The serum bilirubin concentration is widely used to identify high-risk patients, and the present study confirms its prognostic value in patients with PBC (8, 26). An interesting finding is the lack of additional value of including the presence or absence of cirrhosis in the model. This suggests that in patients with low bilirubin concentrations the excess risk caused by cirrhosis alone is negligible.

In univariate analysis, an increased AST/ALT-ratio was significantly associated with decreased survival. To our knowledge, the prognostic value of this ratio in PBC has not been evaluated before, but these findings support the previously reported association between cirrhosis and high AST/ALT-ratio in patients with viral hepatitis and non-alcoholic steatohepatitis (27, 28).

In conclusion, survival for our cohort of PBC patients treated with UDCA was only slightly lower than survival for a sex- and age-matched control group of the general Dutch population. Patients with a clearly increased mortality risk can easily be identified according to serum bilirubin and albumin concentrations. Survival without OLT in low-risk patients was significantly better than survival predicted by the Mayo model. This can be explained by either failure of the model to accurately predict long-term prognosis, or - more likely - by a therapeutic effect of UDCA in non-advanced PBC.

Acknowledgments

The following members of the Dutch Multicenter PBC Study group participated in the study:
C.M.J. van Nieuwkerk, C.J.J. Mulder (Amsterdam), R.J. Robijn (Apeldoorn), R.A. de Vries (Arnhem), B.J.M. Witteman, J.D. van Bergeijk (Bennekom), P.J.J. Leeuwerik, P. Stokkers (Bergen op Zoom), C.T.B.M. van Deursen (Brunssum), I.P. van Munster, A.M. Smit, Th.J.M. van Ditzhuijsen, J.W. de Bruijne, E.W. v.d. Hoek (Den Bosch), A.E.G. Luckers (Boxmeer),

M.C.M. Rijk, G.J. Ras (Breda), J. Scherpenisse (Delft), F. ter Borg (Deventer), H.H. Ponssen, R. Beukers, W. Lesterhuis, A.C.M. van Vliet (Dordrecht), A. Stronkhorst (Eindhoven), M.J. Kerbert-Dreteler, J.H. van Lijf (Enschede), K.J. Heering (Gouda), E.B. Haagsma (Groningen), S.D.J. v.d. Werf, M.H.M.G. Houben, R.M. Valentijn (Den Haag), J. Ferwerda (Haarlem), J.N. Groen (Harderwijk), T.G. Tan (Hengelo), R. Zwertbroek (Hoorn), R.W. de Koning (Nijmegen), J.C. Thijs (Hoogeveen), J.W. Kappelle, P. Spoelstra (Leeuwarden), D. van Lammeren-Venema (Lelystad), G.H. Koek (Maastricht), P.A.M. van Hees (Nieuwegein), F.J. Schuitemaker (Oosterhout), P. Biemond (Roosendaal), A.J.P. van Tilburg, F.J.G.M. Kubben, J.W. den Ouden (Rotterdam), R.N.M. Zeijen (Schiedam), B.J. Looy, L.G.J.B. Engels (Sittard), G.P. van Berge Henegouwen, J. van Hattum (Utrecht), R.P.R. Adang, V.M.C. Verstappen (Venlo), J.G.S. Breed (Weert), O.A. van Dobbenburgh (Zutphen), J. Lambert (Zwolle).

References

1. Kaplan MM. Primary biliary cirrhosis. N Engl J Med 1996;335(21):1570-80.
2. Poupon RE, Lindor KD, Cauch-Dudek K, Dickson ER, Poupon R, Heathcote EJ. Combined analysis of randomized controlled trials of ursodeoxycholic acid in primary biliary cirrhosis. Gastroenterology 1997;113(3):884-90.
3. Gluud C, Christensen E. Ursodeoxycholic acid (UDCA) in primary biliary cirrhosis (PBC) - A Cochrane hepato-biliary systematic review. J Hepatol 1999;30:83A.
4. Goulis J, Leandro G, Burroughs AK. Randomised controlled trials of ursodeoxycholic-acid therapy for primary biliary cirrhosis: a meta-analysis. Lancet 1999;354:1053-60.
5. Flather MD, Farkouh ME, Pogue JM, Yusuf S. Strengths and limitations of meta-analysis: larger studies may be more reliable. Control Clin Trials 1997;18(6):568-79; discussion 661-6.
6. Leuschner M, Dietrich CF, You T, Seidl C, Raedle J, Herrmann G, et al. Characterisation of patients with primary biliary cirrhosis responding to long term ursodeoxycholic acid treatment. Gut 2000;46(1):121-126.
7. Poupon RE, Bonnand AM, Chretien Y, Poupon R. Ten-year survival in ursodeoxycholic acid-treated patients with primary biliary cirrhosis. The UDCA-PBC Study Group. Hepatology 1999;29(6):1668-71.
8. Dickson ER, Grambsch PM, Fleming TR, Fisher LD, Langworthy A. Prognosis in primary biliary cirrhosis: model for decision making. Hepatology 1989;10(1):1-7.
9. Christensen E, Altman DG, Neuberger J, De Stavola BL, Tygstrup N, Williams R. Updating prognosis in primary biliary cirrhosis using a time-dependent Cox regression model. PBC1 and PBC2 trial groups. Gastroenterology 1993;105(6):1865-76.
10. Krzeski P, Zych W, Kraszewska E, Milewski B, Butruk E, Habior A. Is serum bilirubin concentration the only valid prognostic marker in primary biliary cirrhosis? Hepatology 1999;30(4):865-9.
11. Prince M, Chetwynd A, Newman W, Metcalf JV, James OF. Survival and symptom progression in a geographically based cohort of patients with primary biliary cirrhosis: follow-up for up to 28 years. Gastroenterology 2002;123(4):1044-51.
12. Murtaugh PA, Dickson ER, Van Dam GM, Malinchoc M, Grambsch PM, Langworthy AL, et al. Primary biliary cirrhosis: prediction of short-term survival based on repeated patient visits. Hepatology 1994;20(1 Pt 1):126-34.
13. Roll J, Boyer JL, Barry D, Klatskin G. The prognostic importance of clinical and histologic features in asymptomatic and symptomatic primary biliary cirrhosis. N Engl J Med 1983;308(1):1-7.
14. Christensen E, Neuberger J, Crowe J, Altman DG, Popper H, Portmann B, et al. Beneficial effect of azathioprine and prediction of prognosis in primary biliary cirrhosis. Final results of an international trial. Gastroenterology 1985;89(5):1084-91.
15. van Hoogstraten HJF, Hansen BE, van Buuren HR, ten Kate FJW, van Berge Henegouwen GP, Schalm SW. Prognostic factors and long-term effects of ursodeoxycholic acid on liver biochemical parameters in patients with primary biliary cirrhosis. J Hepatol 1999;31:256-262.
16. Taal BG, Schalm SW, ten Kate FW, Hermans J, Geertzen RG, Feltkamp BE. Clinical diagnosis of primary biliary cirrhosis: a classification based on major and minor criteria. Hepatogastroenterology 1983;30(5):178-82.

17. Heathcote EJ. Management of primary biliary cirrhosis. Hepatology 2000;31(4):1005-13.
18. Van Hoogstraten HJ, De Smet MB, Renooij W, Breed JG, Engels LG, Den Ouden-Muller JW, et al. A randomized trial in primary biliary cirrhosis comparing ursodeoxycholic acid in daily doses of either 10 mg/kg or 20 mg/kg. Dutch Multicentre PBC Study Group. Aliment Pharmacol Ther 1998;12(10):965-71.
19. Ludwig J, Dickson ER, McDonald GS. Staging of chronic nonsuppurative destructive cholangitis (syndrome of primary biliary cirrhosis). Virchows Arch A Pathol Anat Histol 1978;379(2):103-12.
20. Poupon RE, Balkau B, Eschwege E, Poupon R. A multicenter, controlled trial of ursodiol for the treatment of primary biliary cirrhosis. UDCA-PBC Study Group. N Engl J Med 1991;324(22):1548-54.
21. Koulentaki M, Moscandrea J, Dimoulios P, Chatzicostas C, Kouroumalis EA. Survival of anti-mitochondrial antibody-positive and -negative primary biliary cirrhosis patients on ursodeoxycholic acid treatment. Dig Dis Sci 2004;49(7-8):1190-5.
22. Corpechot C, Carrat F, Bonnand AM, Poupon RE, Poupon R. The Effect of Ursodeoxycholic Acid Therapy on Liver Fibrosis Progression in Primary Biliary Cirrhosis. Hepatology 2000;32(6):1196-1199.
23. Paumgartner G. Ursodeoxycholic acid for primary biliary cirrhosis: treat early to slow progression. J Hepatol 2003;39(1):112-4.
24. Grambsch PM, Dickson ER, Kaplan M, LeSage G, Fleming TR, Langworthy AL. Extramural cross-validation of the Mayo primary biliary cirrhosis survival model establishes its generalizability. Hepatology 1989;10(5):846-50.
25. Kim WR, Lindor KD, Locke GR, 3rd, Therneau TM, Homburger HA, Batts KP, et al. Epidemiology and natural history of primary biliary cirrhosis in a U. S. community [In Process Citation]. Gastroenterology 2000;119(6):1631-6.
26. Pugh RNH, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 1973;60:646-9.
27. Pohl A, Behling C, Oliver D, Kilani M, Monson P, Hassanein T. Serum aminotransferase levels and platelet counts as predictors of degree of fibrosis in chronic hepatitis C virus infection. Am J Gastroenterol 2001;96(11):3142-6.
28. Angulo P, Keach JC, Batts KP, Lindor KD. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. Hepatology 1999;30(6):1356-62.

Table 1: Baseline patient characteristics

Age (years; mean ± SD)	55.7 ± 11.3
Sex (male/female)	35 (12%) / 262 (88%)
UDCA at entry	36 (12%)
Fatigue	176 (60%)
Pruritus	113 (39%)
AMA +	283 (95%)
Alkaline phosphatase (ULN, range)*	3.4 (0.4-12.2)
AST (ULN, range)*	1.9 (0.3-7.4)
ALT (ULN, range)*	2.4 (0.3-12.1)
Bilirubin (ULN, range)*	1.0 (0.1-5.6)
Albumin (LLN, range)*	1.1 (0.5-1.5)
IgM (ULN, range)*	2.5 (0.3-17.5)
IgG (ULN, range)*	1.0 (0.1-2.9)
Histological stage**	
I	15 (12%)
II	43 (35%)
III	33 (27%)
IV	33 (27%)

* Data for patients not using UDCA at entry
** Data for patients with established cirrhosis and/or with liver biopsy within 1 year of entry
ULN: upper limit of normal range
LLN: lower limit of normal range

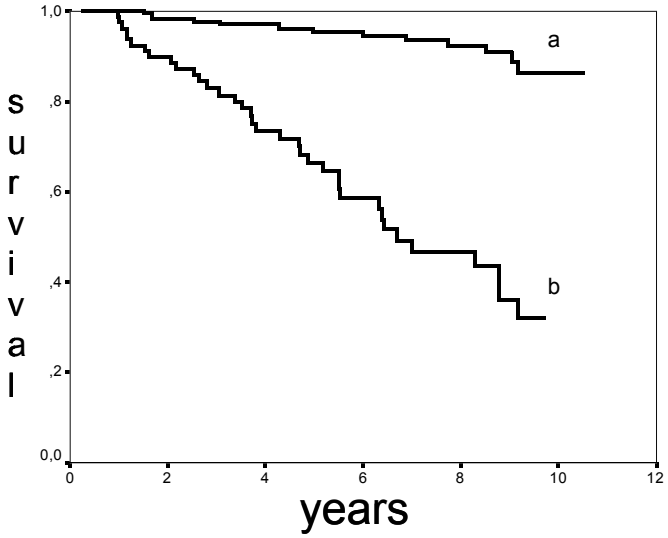
Table 2: Univariate analyses of OLT-free survival and survival free of liver-related death or OLT in UDCA treated patients

	Survival free of transplantation			Survival free of liver related death or transplantation		
	Coefficient	SD	P-value	Coefficient	SD	P-value
Age	0.03	0.014	0.03	0.01	0.017	0.51
Sex	0.74	0.36	0.039	0.6365	0.46	0.164
Weight	0.0042	0.014	0.76	0.000044	0.017	0.998
AMA	-0.48	0.72	0.50	-0.94	0.73	0.20
Ln(Bilirubin)	1.39	0.21	<0.0001	1.97	0.27	<0.0001
Albumin	-3.17	0.80	0.0001	-4.80	0.90	<0.0001
Ln(AF)	0.55	0.25	0.028	0.61	0.31	0.048
Ln(ALT)	0.19	0.23	0.41	0.45	0.29	0.12
Ln(AST)	1.01	0.28	0.0003	1.63	0.35	<0.0001
AST/ALT-ratio	0.71	0.23	0.0017	0.88	0.26	0.0006
Platelet count	-0.0042	0.0020	0.033	-0.0094	0.0025	0.0002
Ln(cholesterol)	-0.38	0.55	0.49	-1.21	0.73	0.096
Ln(IgG)	0.047	0.33	0.89	0.20	0.44	0.65
Ln(IgM)	-0.20	0.25	0.42	-0.23	0.31	0.45
Cirrhosis	-2.00	0.41	<0.0001	-3.06	0.62	<0.0001
Pruritus	0.26	0.30	0.40	0.075	0.37	0.84
Fatigue	0.12	0.30	0.70	0.038	0.38	0.92

Table 3: Observed versus expected events (death and liver transplantation)

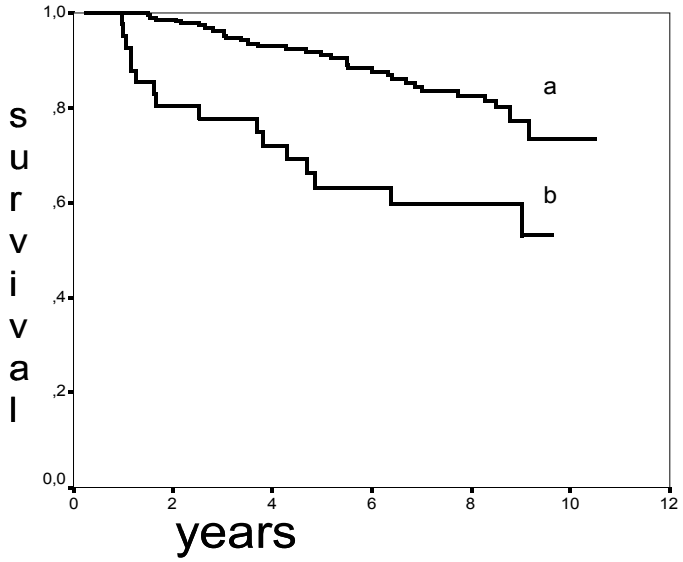
	Observed number of events	Expected number of events	P-value
All patients (n=285)	36	42	0.01
Bilirubin and albumin normal (n=180)	7	17	0.005
Bilirubin or albumin abnormal (n=85)	17	16	0.43
Bilirubin and albumin abnormal (n=20)	12	9	0.43

Figure 1a



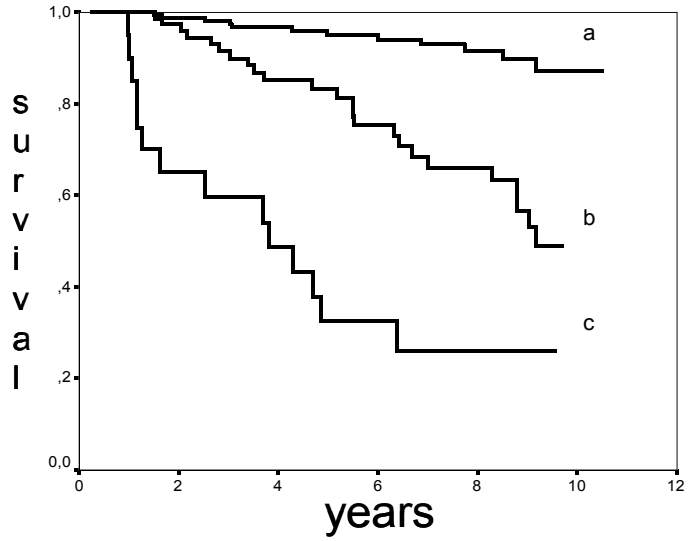
Survival free of transplantation in PBC patients with normal (a) and increased serum bilirubin concentrations (b) ($p<0.0001$, log-rank test).

Figure 1b



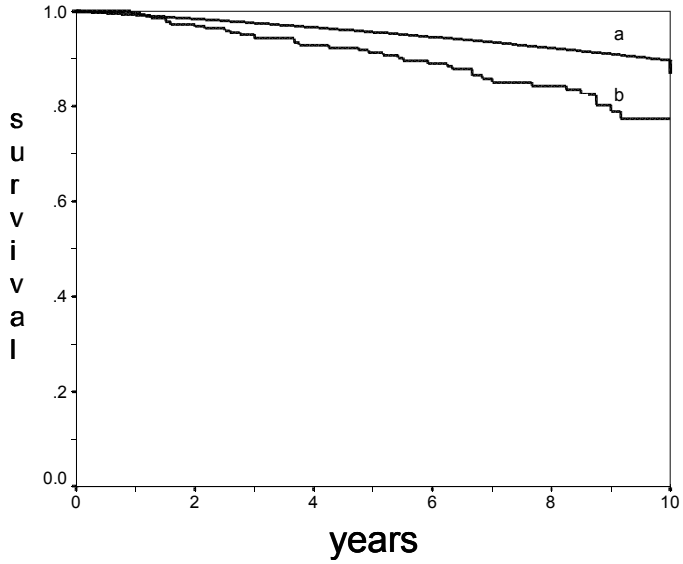
Survival free of transplantation in PBC patients with normal (a) and decreased serum albumin concentrations (b) ($p=0.0002$, log-rank test).

Figure 2



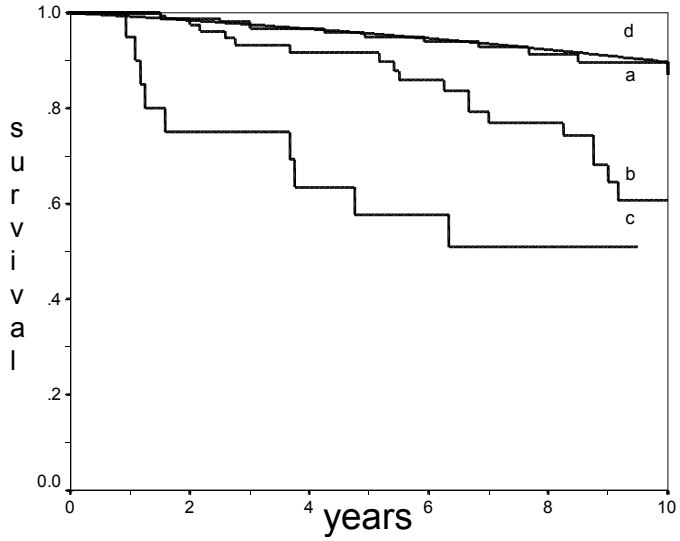
Survival free of transplantation in PBC patients with normal serum bilirubin and albumin concentrations (a), abnormal bilirubin or albumin concentrations (b) and abnormal bilirubin and albumin concentrations (c) (p=0.0002, log-rank test).

Figure 3



Observed survival in UDCA treated PBC patients (n=297, b) compared to survival of an age- and sex matched control group of the general Dutch population (a). Observed survival at 10 years was 78% compared to 90% in the control population (p=0.0003, log-rank test).

Figure 4



Comparison of observed survival in UDCA treated patients with PBC and (a) normal serum albumin and bilirubin concentrations (n=180), (b) abnormal serum bilirubin or albumin concentrations (n=85) and (c) abnormal serum bilirubin and albumin concentration (n=20) with survival of an age- and sex matched control group of the general Dutch population (d). Survival in patients with abnormal serum bilirubin and/or albumin concentrations was significantly decreased ($p<0.0001$, log-rank test).

3

A model for predicting cirrhosis in patients with primary biliary cirrhosis

P.C.J. ter Borg[1], F.J.W. ten Kate[2], H.R. van Buuren[1]

[1]Department of Gastroenterology and Hepatology, Erasmus MC, Rotterdam, [2]Department of Pathology, Academic Medical Center, Amsterdam, The Netherlands

Summary

Primary biliary cirrhosis (PBC) is a chronic cholestatic liver disease with a relatively favorable prognosis for the majority of patients. Once cirrhosis has developed the risk of complications is markedly increased. Since liver biopsy is an invasive procedure that is potentially associated with morbidity and, although rarely, mortality, a method allowing reliable prediction of cirrhosis based on routinely available data would be valuable. We reviewed all histological specimens obtained in a multicenter study and created a predictive model after assessing variables possibly predicting the presence of cirrhosis. The total serum bilirubin concentration and the platelet count predicted the presence of cirrhosis, and a model was created based on these variables. The sensitivity of this model was 70% and the specificity was 94%. Because of the relatively low sensitivity, some patients with cirrhosis would not be detected if only the model would be used to assess the stage of the disease. However, long-term survival in cirrhotic patients not detected by the prognostic model was similar to survival in patients without cirrhosis, and was significantly increased compared to patients in whom the model correctly predicted the presence of cirrhosis. Thus, patients missed by the current model appear to have a more benign course of their cirrhotic liver disease.

In conclusion, the presence of cirrhosis can be predicted using a simple model based on routinely available laboratory tests in most cirrhotic patients, whereas patients with a false-negative prediction of cirrhosis have a similar prognosis compared to truly non-cirrhotic patients.

Introduction

Primary biliary cirrhosis (PBC) is a chronic cholestatic liver disease with a relatively favorable prognosis for the majority of patients. Once cirrhosis has developed, however, risks for subsequent deterioration of liver function and complications, including hepatocellular carcinoma, variceal bleeding and ascites, are markedly increased. Therefore, histological documentation of cirrhosis has important negative prognostic significance. (1, 2). Since liver biopsy is an invasive procedure that is potentially associated with morbidity and, although rarely, mortality, a method allowing reliable prediction of cirrhosis based on routinely available data would be valuable. It could be used to assess the severity of the disease and especially the need for more intensive follow-up, including screening for hepatocellular carcinoma and the presence of esophageal varices (3). The present study aimed to define such a model using easily available, non-histological parameters.

Patients and Methods

Data collected since 1990 in the context of an ongoing multicenter follow-up study of patients with PBC in the Netherlands were used. The results of an analysis of the long-term clinical course of this cohort during treatment with ursodeoxycholic acid have been published previously (4). The diagnosis of PBC was established on the basis of previously reported criteria (5). The database of the cohort study was reviewed to identify patients with histologically documented cirrhosis, irrespective of the time interval between liver biopsy and entry into the study, and patients who underwent liver biopsy in the year prior to inclusion. Patients meeting these criteria were subsequently included in the present study. Biopsy specimens were scored (FK) according to the criteria defined by Ludwig et al. (6), without knowledge of clinical data. Laboratory values, including total serum bilirubin, albumin, alkaline phosphatase, alanine-aminotransferase (ALT), aspartate-aminotransferase (AST), IgG, IgM and platelet count were obtained when patients entered the cohort study. At that time none of the patients except one was being treated with ursodeoxycholic acid or other specific treatments including prednisone, azathioprine or colchicine.

Statistical analysis

Differences between groups of patients were compared using Student's t-test and the chi-square test. Logistic regression analyses were performed to determine predictors of cirrhosis and to construct the model. All variables found to be significant in univariate analyses were

entered in a backward elimination multivariate logistic regression model. The predictive model was constructed using all variables with significant and independent predictive value ($p<0.05$) in this multivariate analysis. Statistical analyses were performed using SPSS 9.0.0 for Windows.

Results

The total study population consists of 297 PBC patients, diagnosed and followed in 44 hospitals in the Netherlands. A liver biopsy meeting the inclusion criteria for the present study was available for 124/297 (42%) patients. An overview of characteristics of patients meeting or not meeting these entry-criteria, is shown in table 1. No significant differences were found between the two groups for any of the tested variables. In addition, there was no significant difference in transplantation-free survival between the two groups when data collected until June 2000 were analyzed (log-rank test, p=0.17).

Age, sex, weight, presence of antimitochondrial antibodies (AMA), serum alkaline phosphatase, ALT, AST, AST/ALT-ratio, total bilirubin, albumin, IgM, IgG, total cholesterol, platelet count and the presence of pruritus and fatigue (recorded as a simple 'yes' or 'no') were included in univariate analyses assessing their predictive value for the presence of cirrhosis (Table 2). Age, total bilirubin, albumin, alkaline phosphatase, AST, AST/ALT-ratio and platelet count were all significantly associated with the presence of cirrhosis. Subsequently, these variables were included in a backward elimination multivariate logistic regression analysis (Table 3). Total bilirubin and platelet count significantly and independently predicted the presence of cirrhosis, and these variables were included in the final predictive model. For any patient, the risk can be obtained using the following expression, which was derived from the model: probability of cirrhosis=1/(1+exp(-logit(p)), where logit(p)=2.97+2.54*loge(bilirubin as ULN)-0.019*platelet count (10^9/l). A ROC-curve was constructed using the probability of cirrhosis obtained with the model (Figure 1). The area under the ROC-curve is 0.92. Sensitivities, specificities, and predictive values according to various cut-off values of the calculated risk score are reported in table 4. Using a cut off probability of 0.50 resulted in a specificity of the model of 94% and a sensitivity of 70%. Thus, the model misclassified several cirrhotic patients as being non-cirrhotic, and vice versa. In order to assess the possible consequences of this misclassification, survival analysis was performed using follow-up data until June 2000. For 9/124 cases relevant data (platelet count in 8 and bilirubin in 1) were not available. The remaining 115 patients were divided into four

groups: correctly classified as cirrhotic (n=21), correctly classified as non-cirrhotic (n=80), incorrectly classified as cirrhotic (n=5) and incorrectly classified as non-cirrhotic (n=9). Transplantation-free survival according to the Kaplan-Meier technique for these four groups is shown in figure 2. Survival for correctly classified cirrhotic patients was significantly decreased compared to survival for cirrhotic patients incorrectly classified as non-cirrhotic ($p<0.001$), whereas survival for cirrhotic patients classified as non-cirrhotic was not significantly different from survival for true non-cirrhotics ($p=0.56$). During follow-up, two patients erroneously classified as non-cirrhotic died, both of liver-related causes. However, when the probability of cirrhosis was calculated at the last regular visit before death, these patients had a calculated probability of cirrhosis of 99.3% and 99.6%, respectively.

Finally, in order to compare the prognostic value of the model with the prognostic value of (histologically documented) cirrhosis, Cox regression analysis of transplantation-free survival was performed including presence of cirrhosis and the model-derived probability of cirrhosis as possible prognostic factors. In this regression model, presence of cirrhosis was significant at the $p=0.40$ level, whereas the model-based probability of cirrhosis was significant with a p-value of 0.0001, indicating superior prognostic capability of the model.

Internet version of the model

Using the scripting language PHP version 4 (http://www.php.net), an internet page was created which can be used to obtain the probability of cirrhosis using the model by simply entering serum bilirubin and platelets count (http://www.sloweb.nl/pbc/cirrmod.html).

Discussion

The present study shows that the presence of cirrhosis in patients with PBC can be predicted with acceptable reliability using two routinely available blood tests: platelets count and total serum bilirubin. Prognostic factors in PBC have been defined in a number of previous studies. Most studies, including the study resulting in the creation of the Mayo model, found that histological stage was of independent prognostic significance (1, 2, 4, 7-9). We are unaware of previous studies aiming to identify factors predictive of cirrhosis. Knowledge of such factors may be valuable in order to be able to identify in a non-invasive way those patients who may require more intense follow-up or should be considered for inclusion in surveillance protocols for detection and prevention of complications associated with cirrhosis, including esophageal varices, hepatocellular carcinoma and osteoporosis. Although the model classified 30% of

cirrhotic patients as non-cirrhotic, transplantation-free survival of these patients and true non-cirrhotic patients was comparable. This may suggest, from a clinical point of view, that failure to detect cirrhosis using the model may be of limited importance. The variables included in the final model appear to reflect two clinically important aspects of cirrhosis: impaired conjugation and excretion of bilirubin and thrombocytopenia, which is supposed to be a consequence of hypersplenism due to portal hypertension and decreased thrombopoiesis due to impaired thrombopoietin production (10). This may explain the favorable prognosis of cirrhotic patients not detected by the model, since patients without hyperbilirubinemia and thrombocytopenia may have preserved liver function without (significant) portal hypertension. This is supported by the finding in a previous study that low platelet counts were predictive of variceal hemorrhage (11). In addition, the same study identified serum bilirubin level as a predictor (although not in multivariate analysis) of variceal bleeding. Thus, patients in whom application of the model results in a low probability of cirrhosis can not only be supposed to have a good prognosis, but also a low risk of variceal bleeding.

Bias could have been introduced into the present study because availability of a recent liver biopsy was not a selection criterion for the cohort study. However, this seems unlikely since there were no statistically significant differences in any of the variables tested for their predictive value between patients with and without an available biopsy. In addition, there was no significant difference in long-term survival between the two groups.

Several previous studies identified the AST/ALT ratio as a factor independently associated with fibrosis and cirrhosis as a result of non-cholestatic liver diseases (mainly hepatitis C and non-alcoholic steatohepatitis) (12-14). We confirmed its association with cirrhosis in PBC, but in the multivariate analysis the ratio had no significant predictive value independent from the other variables, resulting in exclusion of the AST/ALT ratio from the final model.

Ursodeoxycholic acid has been shown to decrease serum levels of bilirubin although this effect may be temporary (4, 15). Further studies are clearly necessary to assess whether serum bilirubin and platelet counts remain valid in predicting cirrhosis in patients on ursodeoxycholic acid. Moreover, the present model should be validated in other patient groups before use outside a research setting can be advocated.

In conclusion, we have constructed a simple model for the prediction of cirrhosis in PBC, based on serum bilirubin and platelets count. This non-invasive approach may allow identifying patients with an increased risk for complications, who may be considered for more intensive follow-up.

References

1. Poupon RE, Bonnand AM, Chretien Y, Poupon R. Ten-year survival in ursodeoxycholic acid-treated patients with primary biliary cirrhosis. The UDCA-PBC Study Group. Hepatology 1999;29(6):1668-71.
2. Shibuya A, Tanaka K, Miyakawa H, Shibata M, Takatori M, Sekiyama K, et al. Hepatocellular carcinoma and survival in patients with primary biliary cirrhosis. Hepatology 2002;35(5):1172-8.
3. Gilmore IT, Burroughs A, Murray-Lyon IM, Williams R, Jenkins D, Hopkins A. Indications, methods, and outcomes of percutaneous liver biopsy in England and Wales: an audit by the British Society of Gastroenterology and the Royal College of Physicians of London. Gut 1995;36(3):437-41.
4. van Hoogstraten HJF, Hansen BE, van Buuren HR, ten Kate FJW, van Berge Henegouwen GP, Schalm SW. Prognostic factors and long-term effects of ursodeoxycholic acid on liver biochemical parameters in patients with primary biliary cirrhosis. J Hepatol 1999;31:256-262.
5. Taal BG, Schalm SW, ten Kate FW, Hermans J, Geertzen RG, Feltkamp BE. Clinical diagnosis of primary biliary cirrhosis: a classification based on major and minor criteria. Hepatogastroenterology 1983;30(5):178-82.
6. Ludwig J, Dickson ER, McDonald GS. Staging of chronic nonsuppurative destructive cholangitis (syndrome of primary biliary cirrhosis). Virchows Arch A Pathol Anat Histol 1978;379(2):103-12.
7. Dickson ER, Grambsch PM, Fleming TR, Fisher LD, Langworthy A. Prognosis in primary biliary cirrhosis: model for decision making. Hepatology 1989;10(1):1-7.
8. Roll J, Boyer JL, Barry D, Klatskin G. The prognostic importance of clinical and histologic features in asymptomatic and symptomatic primary biliary cirrhosis. N Engl J Med 1983;308(1):1-7.
9. Christensen E, Altman DG, Neuberger J, De Stavola BL, Tygstrup N, Williams R. Updating prognosis in primary biliary cirrhosis using a time-dependent Cox regression model. PBC1 and PBC2 trial groups. Gastroenterology 1993;105(6):1865-76.
10. Peck-Radosavljevic M, Zacherl J, Meng YG, Pidlich J, Lipinski E, Langle F, et al. Is inadequate thrombopoietin production a major cause of thrombocytopenia in cirrhosis of the liver? J Hepatol 1997;27 (1):127-31.
11. Plevris JN, Dhariwal A, Elton RA, Finlayson ND, Bouchier IA, Hayes PC. The platelet count as a predictor of variceal hemorrhage in primary biliary cirrhosis. Am J Gastroenterol 1995;90(6):959-61.
12. Pohl A, Behling C, Oliver D, Kilani M, Monson P, Hassanein T. Serum aminotransferase levels and platelet counts as predictors of degree of fibrosis in chronic hepatitis C virus infection. Am J Gastroenterol 2001;96(11):3142-6.
13. Angulo P, Keach JC, Batts KP, Lindor KD. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. Hepatology 1999;30(6):1356-62.
14. Giannini E, Botta F, Fasoli A, Ceppa P, Risso D, Lantieri PB, et al. Progressive liver functional impairment is associated with an increase in AST/ALT ratio. Dig Dis Sci 1999;44(6):1249-53.
15. Gluud C, Christensen E. Ursodeoxycholic acid for primary biliary cirrhosis. Cochrane Database Syst Rev 2002(1):CD000551.

Table 1: Patient characteristics

	Biopsy available N=124	No biopsy N=173	Significance
Age (years; mean)	62	60	NS
Sex (male/female)	16/108	19/154	NS
Weight (kg)	69	68	NS
AMA -/+	5/119	9/164	NS
Alkaline phosphatase (ULN)	3.5	3.1	NS
AST (ULN)	1.9	1.8	NS
ALT (ULN)	2.3	2.3	NS
Bilirubin (ULN)	1.0	1.0	NS
Albumin (LLN)	1.1	1.1	NS
IgM (ULN)	2.3	2.4	NS
Cholesterol (ULN)	1.1	1.1	NS
Platelets	228	246	NS
IgG (ULN)	1.1	1.0	NS
Pruritus (%)	39	39	NS
Fatigue (%)	67	56	NS

ULN = upper limit of normal; LLN = lower limit of normal

Table 2: Univariate analysis

	Coeff.	SD	P-value
Age	0.04	0.02	**0.05**
Sex	0.51	0.68	0.45
Weight	-0.02	0.02	0.29
AMA	-1.49	0.94	0.11
Log Bilirubin	2.53	0.52	**<0.0001**
Albumin	-4.12	1.31	**0.0016**
Log AF	0.73	0.37	**0.05**
Log ALT	0.19	0.35	0.60
Log AST	1.92	0.49	**0.0001**
AST/ALT	2.63	0.65	**0.0001**
Platelets	-0.02	0.004	**<0.0001**
Log cholesterol	-1.31	0.77	0.09
IgG	0.62	0.60	0.31
IgM	0.01	0.32	0.97
Pruritus	-0.36	0.42	0.39
Fatigue	-0.32	0.43	0.46

Table 3: Multivariate analysis

	Coefficient	SD	P-value
Serum bilirubin	2.54	0.69	**0.0002**
Platelets	-0.019	0.0046	**<0.0001**
Constant	2.97	0.91	**0.0011**

Table 4: Model characteristics

Cut-off value	Sensitivity	Specificity	Positive predictive value	Negative predictive value
0.10	93%	67%	50%	97%
0.30	77%	85%	64%	91%
0.40	70%	91%	72%	90%
0.45	70%	94%	81%	90%
0.50	70%	94%	81%	90%
0.55	63%	96%	86%	88%
0.60	63%	96%	86%	88%
0.70	60%	98%	90%	87%
0.90	20%	100%	100%	78%

Figure 1: ROC curve

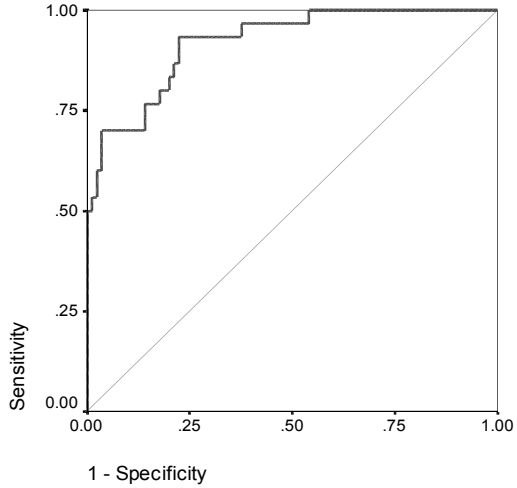
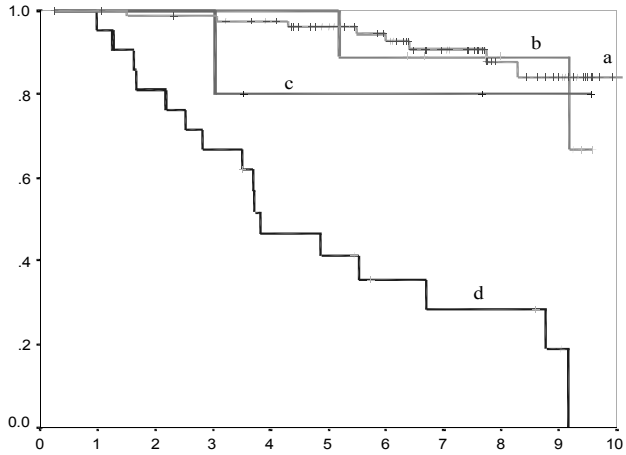


Figure 2: Patient survival



a. correctly classified as non-cirrhotic; b. incorrectly classified as non-cirrhotic; c. incorrectly classified as cirrhotic; d. correctly classified as cirrhotic

4

A survey of concurrent disorders in a multicenter cohort of primary biliary cirrhosis patients

Pieter C.J. ter Borg[1], Henk R. van Buuren[1], Karin M.J. van Nieuwkerk[2], Els B. Haagsma[3], Janny W. den Ouden[4], Martin H.M.G. Houben[5], Rudolf W. de Koning[6], Egbert W. van der Hoek[7], Rob P.R. Adang[8], Gerard P. van Berge Henegouwen[9] for the Dutch Multicenter PBC study group

[1]Department of Gastroenterology and Hepatology, Erasmus MC, Rotterdam, [2]Department of Gastroenterology and Hepatology, VU Hospital, Amsterdam, [3]Department of Internal Medicine, University Hospital, Groningen, [4]Department of Internal Medicine, St. Franciscus Hospital, Rotterdam, [5]Department of Internal Medicine and Gastroenterology, Rode Kruis Hospital, Den Haag, [6]Department of Internal Medicine, Canisius Wilhelmina Hospital, Nijmegen, [7]Department of Internal Medicine, Carolus Hospital, Den Bosch, [8]Department of Internal Medicine, VieCuri Medisch Centrum, Venlo, [9]Department of Gastroenterology and Hepatology, University Medical Center, Utrecht, The Netherlands

Contents published in Cholestatic Liver Diseases Therapeutic Options and Perspectives, 2004 Kluwer Academic Publishers and Falk Foundation e.V. ISBN 0-7923-8793-7. Pages 117-121.

Summary

PBC is a presumed autoimmune chronic liver disease. Several reports emphasized the prevalence of associated disorders in PBC. An association with the following disorders has been quantified in previous studies: thyroid dysfunctions and rheumatic disorders seem common. Additional associations have been described with SLE, systemic sclerosis, renal tubular acidosis, bacteriuria, celiac disease, ulcerative colitis and malignant diseases. Since most of these studies seemed to be initiated because of a suspected association, significant bias may have occurred. The present study aimed to quantify the presence of all previously reported associated diseases as well as other disorders in a large, prospectively followed cohort of 237 PBC patients.

A questionnaire including all previously reported associated conditions was completed for all included patients. No specific screening studies were performed for diagnosing clinically occult disorders.

Mean follow-up was 11 years. No association with celiac disease was found. There was a high prevalence of rheumatic diseases and Raynaud syndrome (total 14%) and endocrine disorders (16%): diabetes (9%), thyroid disorders (7%). The prevalence of GE-diseases was similar to the general population (gallstones 20%, peptic ulcers 6%, reflux esophagitis 4%). There was no clear-cut association with malignant diseases (breast cancer 4%, colorectal cancer 2%, skin cancer 2%), with the exception of hepatocellular carcinoma (2%).

There seem only few truly associated diseases with PBC. These are rheumatoid disorders (rheumatoid arthritis and Raynaud syndrome) and thyroid disorders (thyroiditis and hypothyroidism). We could not confirm the association with celiac disease or with malignant disease with the exception of hepatocellular carcinoma. Associated disorders in an unselected population of PBC-patients are only limited to a very few specific diseases. Most co-existing diseases have comparable frequency with the general population.

Introduction

Primary biliary cirrhosis (PBC) is a chronic cholestatic liver disease of presumed auto-immune aetiology (1). Numerous autoimmune and also non-autoimmune conditions have been reported to be (potentially) associated with PBC. The occurrence of some of these disorders, including thyroid (2) and rheumatic diseases (2-4), Sjögren's syndrome (5, 6), systemic lupus erythematosus (7), systemic sclerosis (8, 9), renal tubular acidosis (10), bacteriuria (11), celiac disease (12-21), ulcerative colitis (22) and malignant diseases (23-28) has been quantified by previous studies. Some of these studies may well have been initiated because an association was suspected after cases with concurrent disorders were seen, and this may have influenced results. This is illustrated by the finding of an increased risk of breast carcinoma in two early studies (23, 24), which could not be confirmed in a number of subsequent reports (25-29). Although a number of previous studies on associated diseases was of considerable size, including over hundred patients, (2, 4, 21, 23-28) most data are derived from relatively small and single center series of patients. In addition, a potential association with thyroid disorders, systemic lupus erythematosus, renal tubular acidosis, bacteriuria and ulcerative colitis has only been reported by single center studies, all including less than 100 patients.

The present multicenter study aimed to assess the prevalence of all diseases previously reported to be associated with PBC, as well as that of other comorbidity, in a large group of prospectively followed PBC patients.

Patients and Methods

We collected data of 237 patients with PBC who were being, or had been, followed in 42 university and non-university hospitals. These centers participated in a long-term prospective follow-up study of PBC initiated in 1990 (32). The diagnosis of PBC was based on previously defined criteria (31). Information on comorbidity was recorded until death, liver transplantation or March 2003. For the purpose of this study no specific diagnostic tests or procedures were carried out, and results are based on data as recorded in the medical files. General patient data and the presence of other diseases were recorded on a form listing the following conditions: thyroid disorders, diabetes mellitus, celiac disease, rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis, scleroderma, CREST, mixed connective tissue disease, polymyositis, dermatomyositis, Raynaud's syndrome, retroperitoneal fibrosis, Sjögren's syndrome, ankylosing spondylitis, temporal arteritis, polymyalgia rheumatica,

ulcerative colitis, pernicious anemia, eosinophilic colitis or gastroenteritis, pancreatitis, adrenal insufficiency, hepatocellular carcinoma, breast carcinoma, auto-immune hemolytic anemia, thrombocytopenic purpura, lymphoma, multiple myeloma, myelofibrosis, pericarditis, cardiomyopathy, atherosclerosis, thromboembolic events, pulmonary fibrosis, renal tubular acidosis, glomerulonephritis, recurrent urinary tract infections, lichen planus, vitiligo, herpetiform dermatitis, myelitis, neuritis, myasthenia and multiple sclerosis. In addition to these diseases, which have been previously associated with PBC, the presence of any other condition was recorded. For the diagnosis of the following diseases standardized, generally accepted and widely used diagnostic criteria were used: rheumatoid arthritis (30), Sjögren's syndrome (31), systemic lupus erythematosus (32), scleroderma (33), mixed connective tissue disease (34) and diabetes mellitus (35).

Results

General patient characteristics are shown in table 1. Celiac disease, the most extensively studied disease previously associated with PBC, was not diagnosed in any of the patients. Antibodies against endomysium were found in 1 out of the 27 patients in whom this test had been obtained, and antibodies against gliadine in 5 out of 22 tested patients. Duodenal microscopy, however, did not show evidence of celiac disease in any of the 22 patients in whom biopsies were obtained. Results with regard to other diseases are shown in tables 2-10. One or more malignancies occurred in 32 (14%) patients, whereas in 21 (70%) of cases malignancies were diagnosed after the diagnosis of PBC was made. Rheumatic disorders, not including Raynaud's phenomenon, were diagnosed in 24 (10%) patients, in 18 (78%) after PBC had been diagnosed. In all patients TSH serum levels were measured. Thyroid disorders were diagnosed in 16 (7%) patients, in 5 (33%) after PBC had been diagnosed. Genitourinary disorders were reported in 43 patients (18%), in 25 (58%) cases diagnosis preceding the diagnosis of PBC.

Table 11 shows the prevalence of a number of conditions as found by previous studies and the present study. There are noticeable differences in the prevalence of celiac disease, thyroid disorders, rheumatoid arthritis, Sjögren's syndrome, systemic sclerosis, renal tubular acidosis and bacteriuria. The number of associated diseases per patient varied from 0 to 9 (Figure).

Discussion

Although many of the autoimmune and non-autoimmune disorders formerly reported to be related with PBC were also noted in the present study, the frequency of most of these was rather low. (2, 4, 7, 9) An important feature of the present study is that in contrast to a number of previous studies, screening for concomitant diseases was not routinely performed. Only diseases that were clinically apparent were included, potentially resulting in underdiagnosis of particularly those conditions not associated with significant symptoms. Many earlier studies also included comparatively few patients and may have overestimated the prevalence of some disorders. This is illustrated by studies on cancer risks, some of the larger and more recent studies finding lower risks than initially reported. Some of the differences may also be attributable to the use of other or less stringent diagnostic criteria. The majority of our patients was followed in general hospitals and not in referral centers. Selection of different patient groups may constitute yet another feature explaining dissimilar study outcomes.

The previously reported prevalence of celiac disease in PBC varies from 0-30% (13-22), with a median of 3.4%. Based on this figure the expected number of PBC patients with this disease in our series was eight while, surprisingly, no single case was identified. We have no satisfactory explanation for this finding but a general conservative diagnostic approach seems likely to be involved. In general, diagnostic studies were only performed when celiac disease was clinically suspected. Since celiac disease can occur with minimal or absent symptoms, the present study almost certainly underestimates the true prevalence of the disease.

The single most prevalent disorder in the present study was symptomatic cholelithiasis, which occurred in 21% of patients. As most patients were females of typical cholelithiatic age this prevalence was not unexpected. The proportion is comparable to earlier reported data on cholelithiasis in patients with cirrhosis (36-38) and females over 50 years of age (39-41).

Two earlier studies reported an increased risk of breast cancer in patients with PBC (23, 24) but subsequent studies could not confirm this finding (25-27, 29). In the current study, breast cancer at any time before or during follow-up occurred in 8 (4%) of patients. In only 3 cases breast cancer occurred after the diagnosis of PBC had been made.

Previous studies, including 95 (2) and 113 (3) PBC patients, reported thyroid disease in 14% and 19% of patients resp., compared to 7% in the present study. Notable variable data have been reported for scleroderma (including CREST) with prevalences in PBC ranging from 0% up to 22% (2, 4, 7, 9), and rheumatoid arthritis, prevalences ranging from 2% to 27%. Only two cases (0.9%) of scleroderma/CREST and 10 (4%) of rheumatoid arthritis were found in

the present series according to criteria of the American Rheumatism Association. Coexisting Sjögren's syndrome was previously established in 27% of PBC patients while another study reported histological changes compatible with the disease in the same proportion of patients (5, 6). This diagnosis, based on recorded data, could be made in no more than 2% of our patients, which again is very likely to be the result of adherence to strict diagnostic criteria (33). Renal tubular acidosis was not diagnosed in any of the patients in the present study. Since this disorder has no typical clinical symptoms, it may have been underdiagnosed in the present study. However, the clinical relevance of establishing this diagnosis in patients with PBC remains unclear.

Our study does not allow estimations as to the prevalence of concurrent disorders in comparison with the general population, as no sex- and age matched control group was studied. Hence the question remains whether the frequency of some disorders is indeed higher than expected.

In conclusion, although the majority of patients with PBC has one or more additional disorder, the prevalence of most of previously reported associated diseases, especially rheumatic diseases, was relatively low and in general lower than found by others. This can be largely explained by differences in diagnostic policy, diagnostic criteria and patient selection. Our results reflect the co-occurrence of clinically obvious disorders in PBC but with respect to a number of disorders, including celiac disease, Sjögren syndrome, bacteriuria and renal tubular acidosis, our survey probably underestimates the true prevalence.

Appendix

The following investigators participated in the study:

R.A. de Vries, Ziekenhuis Rijnstate, Arnhem, Tonino, St. Jans Gasthuis, Weert, J.G.S. Breed, St. Jans Gasthuis, Weert, K.J. Heering, Groene Hart Ziekenhuis, Gouda, J.C. Thijs, Bethesda Ziekenhuis, Hoogeveen, R.W. de Koning, Nijmeegs Interkonfessioneel Ziekenhuis Canisius-Wilhelmina, Nijmegen, J. Scherpenisse, Reinier de Graaf Groep, Delft, J. Ferwerda, Kennemer Gasthuis, Haarlem, E.W. v.d. Hoek, Carolus Ziekenhuis, Den Bosch, J.W. de Bruijne, Jeroen Bosch Ziekenhuis, Den Bosch, J.H. van Lijf, Enschede, M.J. Kerbert-Dreteler, Medisch Spectrum Twente, Enschede, L.G.J.B. Engels, Maaslandziekenhuis, Sittard, J.W. den Ouden, Sint Franciscus Gasthuis, Rotterdam, E.B. Haagsma, Academisch Ziekenhuis Groningen, Groningen, G.H. Koek, Academisch Ziekenhuis Maastricht, Maastricht, F.J.G.M. Kubben, Medisch Centrum Rijnmond Zuid, Rotterdam, R.M. Valentijn, Rode Kruis Ziekenhuis, Den Haag, Welten, Den Haag, M.H.M.G. Houben, Rode Kruis Ziekenhuis, Den Haag, C.J.J. Mulder, Academisch Medisch Centrum, Amsterdam, Th.J.M. van Ditzhuijsen, Jeroen Bosch Ziekenhuis, Den Bosch, P. Biemond, Ziekenhuis Franciscus, Roosendaal, R.N.M. Zeijen, Vlietland Ziekenhuis, Schiedam, A.C.M. van Vliet, Albert Schweitzer Ziekenhuis, Dordrecht, D. van Lammeren-Venema, Lelystad, C.M.J. van Nieuwkerk, VU Medisch Centrum, Amsterdam, O.A. van Dobbenburgh, Gelre Ziekenhuis, Zutphen, R.J. Robijn, Gelre Ziekenhuis, Apeldoorn, T.G. Tan, Streekziekenhuis Midden Twente, Hengelo, G.J. Ras, Amphia Ziekenhuis, Breda, J.D. van Bergeijk, Ziekenhuis Gelderse Vallei, Bennekom, P. Stokkers, Lievensbergziekenhuis, Bergen op Zoom, G.P. van Berge Henegouwen, Academisch Medisch Centrum Utrecht, Utrecht, J. van Hattum, Academisch Medisch Centrum Utrecht, Utrecht, F.J. Schuitemaker, Pasteur Ziekenhuis, Oosterhout, S.D.J. v.d. Werf, Westeinde Ziekenhuis, Den Haag, W. Lesterhuis, Albert Schweitzer Ziekenhuis, Dordrecht, A.M. Smit, Den Bosch, V.M.C. Verstappen, VieCuri MC, Venlo, R.P.R. Adang, VieCuri MC, Venlo, P. Spoelstra, Medisch Centrum Leeuwarden, Leeuwarden, J.W. Kappelle, Medisch Centrum Leeuwarden, Leeuwarden, A.J.P. van Tilburg, Sint Franciscus Gasthuis, Rotterdam, E.B. Haagsma, Academisch Ziekenhuis Groningen, Groningen, B.J. Looy, Maaslandziekenhuis, Sittard, J. Lambert, Isala Klinieken, Zwolle, R. Zwertbroek, Westfries Gasthuis, Hoorn, J.N. Groen, Ziekenhuis St. Jansdal, Harderwijk, M.C.M. Rijk, Amphia Ziekenhuis, Breda, A. Stronkhorst, Catharina Ziekenhuis, Eindhoven, P.J.J. Leeuwerik, Lievensberg Ziekenhuis, Bergen op Zoom, R. Beukers, Albert Schweitzer Ziekenhuis, Dordrecht, H.H. Ponssen, Albert Schweitzer Ziekenhuis, Dordrecht, F. ter Borg, Deventer Ziekenhuis, Deventer, P.A.M. van Hees, Sint Antonius Ziekenhuis, Nieuwegein, C.T.B.M. van Deursen, Atrium Medisch Centrum, Brunssum, B.J.M. Witteman, Ziekenhuis Gelderse Vallei, Bennekom, Van Aken, Bennekom, I.P. van Munster, Jeroen Bosch Ziekenhuis, Den Bosch, A.E.G. Luckers, Maasziekenhuis, Boxmeer

References

1. Kaplan MM. Primary biliary cirrhosis. N Engl J Med 1996;335(21):1570-80.
2. Culp KS, Fleming CR, Duffy J, Baldus WP, Dickson ER. Autoimmune associations in primary biliary cirrhosis. Mayo Clin Proc 1982;57(6):365-70.
3. Uddenfeldt P, Danielsson A. Evaluation of rheumatic disorders in patients with primary biliary cirrhosis. Ann Clin Res 1986;18(3):148-53.
4. Marasini B, Gagetta M, Rossi V, Ferrari P. Rheumatic disorders and primary biliary cirrhosis: an appraisal of 170 Italian patients. Ann Rheum Dis 2001;60(11):1046-9.
5. Uddenfeldt P, Danielsson A, Forssell A, Holm M, Ostberg Y. Features of Sjogren's syndrome in patients with primary biliary cirrhosis. J Intern Med 1991;230(5):443-8.
6. Tsianos EV, Hoofnagle JH, Fox PC, Alspaugh M, Jones EA, Schafer DF, et al. Sjogren's syndrome in patients with primary biliary cirrhosis. Hepatology 1990;11(5):730-4.
7. Fonollosa V, Simeon CP, Castells L, Garcia F, Castro A, Solans R, et al. Morphologic capillary changes and manifestations of connective tissue diseases in patients with primary biliary cirrhosis. Lupus 2001;10 (9):628-31.
8. Powell FC, Schroeter AL, Dickson ER. Primary biliary cirrhosis and the CREST syndrome: a report of 22 cases. Q J Med 1987;62(237):75-82.
9. Pares A, Grande L, Bruix J, Pera C, Rodes J. Esophageal dysfunction in primary biliary cirrhosis. J Hepatol 1988;7(3):362-7.
10. Pares A, Rimola A, Bruguera M, Mas E, Rodes J. Renal tubular acidosis in primary biliary cirrhosis. Gastroenterology 1981;80(4):681-6.
11. Burroughs AK, Rosenstein IJ, Epstein O, Hamilton-Miller JM, Brumfitt W, Sherlock S. Bacteriuria and primary biliary cirrhosis. Gut 1984;25(2):133-7.
12. Bardella MT, Quatrini M, Zuin M, Podda M, Cesarini L, Velio P, et al. Screening patients with celiac disease for primary biliary cirrhosis and vice versa. Am J Gastroenterol 1997;92(9):1524-6.
13. Dickey W, McMillan SA, Callender ME. High prevalence of celiac sprue among patients with primary biliary cirrhosis. J Clin Gastroenterol 1997;25(1):328-9.
14. Kingham JG, Parker DR. The association between primary biliary cirrhosis and coeliac disease: a study of relative prevalences. Gut 1998;42(1):120-2.
15. Niveloni S, Dezi R, Pedreira S, Podesta A, Cabanne A, Vazquez H, et al. Gluten sensitivity in patients with primary biliary cirrhosis. Am J Gastroenterol 1998;93(3):404-8.
16. Sorensen HT, Thulstrup AM, Blomqvist P, Norgaard B, Fonager K, Ekbom A. Risk of primary biliary liver cirrhosis in patients with coeliac disease: Danish and Swedish cohort data. Gut 1999;44(5):736-8.
17. Gillett HR, Cauch-Dudek K, Jenny E, Heathcote EJ, Freeman HJ. Prevalence of IgA antibodies to endomysium and tissue transglutaminase in primary biliary cirrhosis. Can J Gastroenterol 2000;14(8):672-5.
18. Chatzicostas C, Roussomoustakaki M, Drygiannakis D, Niniraki M, Tzardi M, Koulentaki M, et al. Primary biliary cirrhosis and autoimmune cholangitis are not associated with coeliac disease in Crete. BMC Gastroenterol 2002;2(1):5.

19. Floreani A, Betterle C, Baragiotta A, Martini S, Venturi C, Basso D, et al. Prevalence of coeliac disease in primary biliary cirrhosis and of antimitochondrial antibodies in adult coeliac disease patients in Italy. Dig Liver Dis 2002;34(4):258-61.
20. Volta U, Rodrigo L, Granito A, Petrolini N, Muratori P, Muratori L, et al. Celiac disease in autoimmune cholestatic liver disorders. Am J Gastroenterol 2002;97(10):2609-13.
21. Habior A, Lewartowska A, Orlowska J, Zych W, Sankowska M, Bauer A, et al. Association of coeliac disease with primary biliary cirrhosis in Poland. Eur J Gastroenterol Hepatol 2003;15(2):159-64.
22. Koulentaki M, Koutroubakis IE, Petinaki E, Tzardi M, Oekonomaki H, Mouzas I, et al. Ulcerative colitis associated with primary biliary cirrhosis. Dig Dis Sci 1999;44(10):1953-6.
23. Wolke AM, Schaffner F, Kapelman B, Sacks HS. Malignancy in primary biliary cirrhosis. High incidence of breast cancer in affected women. Am J Med 1984;76(6):1075-8.
24. Goudie BM, Burt AD, Boyle P, Macfarlane G, Birnie GG, Mills PR, et al. Breast cancer in women with primary biliary cirrhosis. Br Med J (Clin Res Ed) 1985;291(6509):1597-8.
25. Loof L, Adami HO, Sparen P, Danielsson A, Eriksson LS, Hultcrantz R, et al. Cancer risk in primary biliary cirrhosis: a population-based study from Sweden. Hepatology 1994;20(1 Pt 1):101-4.
26. Floreani A, Biagini MR, Chiaramonte M, Milani S, Surrenti C, Naccarato R. Incidence of hepatic and extra-hepatic malignancies in primary biliary cirrhosis (PBC). Ital J Gastroenterol 1993;25(9):473-6.
27. Howel D, Metcalf JV, Gray J, Newman WL, Jones DE, James OF. Cancer risk in primary biliary cirrhosis: a study in northern england. Gut 1999;45(5):756-60.
28. Nijhawan PK, Therneau TM, Dickson ER, Boynton J, Lindor KD. Incidence of cancer in primary biliary cirrhosis: the Mayo experience. Hepatology 1999;29(5):1396-8.
29. Floreani A, Baragiotta A, Baldo V, Menegon T, Farinati F, Naccarato R. Hepatic and extrahepatic malignancies in primary biliary cirrhosis. Hepatology 1999;29(5):1425-8.
30. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31(3):315-24.
31. Vitali C, Bombardieri S, Moutsopoulos HM, Balestrieri G, Bencivelli W, Bernstein RM, et al. Preliminary criteria for the classification of Sjogren's syndrome. Results of a prospective concerted action supported by the European Community. Arthritis Rheum 1993;36(3):340-7.
32. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25(11):1271-7.
33. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Arthritis Rheum 1980;23(5):581-90.
34. Alarcon-Segovia D, Villareal M. Classification and diagnostic criteria for mixed connective tissue disease. In: Kasukawa R, editor. Mixed Connective Tissue Disease and Anti-nuclear Antibodies.; 1987. p. 33-40.
35. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 2003;26 Suppl 1:S5-20.
36. Conte D, Barisani D, Mandelli C, Bodini P, Borzio M, Pistoso S, et al. Cholelithiasis in cirrhosis: analysis of 500 cases. Am J Gastroenterol 1991;86(11):1629-32.

37. Elzouki AN, Nilsson S, Nilsson P, Verbaan H, Simanaitis M, Lindgren S. The prevalence of gallstones in chronic liver disease is related to degree of liver dysfunction. Hepatogastroenterology 1999;46(29):2946-50.
38. Del Olmo JA, Garcia F, Serra MA, Maldonado L, Rodrigo JM. Prevalence and incidence of gallstones in liver cirrhosis. Scand J Gastroenterol 1997;32(10):1061-5.
39. Barbara L, Sama C, Morselli Labate AM, Taroni F, Rusticali AG, Festi D, et al. A population study on the prevalence of gallstone disease: the Sirmione Study. Hepatology 1987;7(5):913-7.
40. Caroli-Bosc FX, Deveau C, Harris A, Delabre B, Peten EP, Hastier P, et al. Prevalence of cholelithiasis: results of an epidemiologic investigation in Vidauban, southeast France. General Practitioner's Group of Vidauban. Dig Dis Sci 1999;44(7):1322-9.
41. Jorgensen T. Prevalence of gallstones in a Danish population. Am J Epidemiol 1987;126(5):912-21.
42. Crowe JP, Christensen E, Butler J, Wheeler P, Doniach D, Keenan J, et al. Primary biliary cirrhosis: the prevalence of hypothyroidism and its relationship to thyroid autoantibodies and sicca syndrome. Gastroenterology 1980;78(6):1437-41.

Table 1: Patient characteristics

Total number of patients	237
Sex (male / female)	23 / 214
Age at diagnosis (years: mean, range)	51 (27-82)
Duration of PBC (years: mean, range)	12 (1-29)
Age at end of follow-up (years: mean, range)	62 (33-88)

Table 2: Disorders of the immune system, connective tissue and joints

Disorder	number (%)
Rheumatoid arthritis	10 (4%)
Raynaud's phenomenon	8 (3%)
Sjögren's syndrome	5 (2%)
Coxarthrosis	3 (1%)
Dupuytren's contracture	2 (1%)
Chondrocalcinosis	2 (1%)
Achondroplasia	1 (0.4%)
Dysplasia of the hip	1 (0.4%)
Polyarthrosis	1 (0.4%)
Systemic sclerosis / scleroderma	1 (0.4%)
CREST	1 (0.4%)
Temporal arteritis	1 (0.4%)
Systemic lupus erythematosus	1 (0.4%)
Retroperitoneal fibrosis	1 (0.4%)
Vasculitis	1 (0.4%)
Undefined connective tissue disease	1 (0.4%)

Table 3: Gastrointestinal disorders

Disorder	number (%)
Cholelithiasis	48 (20%)
Peptic ulcer disease	13 (5%)
Esophagitis	10 (4%)
Appendicitis	9 (4%)
Diverticulosis	5 (2%)
Colonic polyps	3 (1%)
Helicobacter associated gastritis	3 (1%)
Ulcerative colitis	2 (1%)
Haemorrhoids	2 (1%)
Pernicious anemia	2 (1%)
Diverticulitis	1 (0.4%)
Diverticular bleeding	1 (0.4%)
Atrophic gastritis	1 (0.4%)
Rheumatic fever	1 (0.4%)
Colonic angiodysplasia	1 (0.4%)

Table 4: Endocrine disorders

Disorder	number (%)
Diabetes mellitus	20 (8%)
Hypothyroidism	10 (4%)
Hyperthyroidism	5 (2%)
Thyroiditis	1 (0.4%)
Hyperparathyroidism	1 (0.4%)

Table 5: Malignant and hematologic disorders

Disorder	number (%)
Breast carcinoma	8 (3%)
Hepatocellular carcinoma	4 (2%)
Colonic carcinoma	4 (2%)
Basocellular carcinoma	4 (2%)
Lung carcinoma	3 (1%)
Prostatic carcinoma	3 (1%)
Monoclonal gammopathy of undetermined significance	3 (1%)
Non-Hodgkin lymphoma	2 (1%)
Idiopathic thrombocytopenic purpura	2 (1%)
Gastric carcinoma	2 (1%)
Carcinoma of Vater's papilla	1 (0.4%)
Renal cell carcinoma	1 (0.4%)
Hodgkin's disease	1 (0.4%)
Auto-immune haemolytic anaemia	1 (0.4%)
Essential thrombocytosis	1 (0.4%)
Ovarian tumour	1 (0.4%)
Von Willebrand disease	1 (0.4%)
Laryngeal carcinoma	1 (0.4%)

Table 6: Cardiovascular and pulmonary disorders

Disorder	number (%)
Essential hypertension	36 (15%)
Chronic obstructive pulmonary disease	10 (4%)
Stroke	8 (3%)
Angina pectoris	6 (3%)
Pulmonary embolism	5 (2%)
Atrial fibrillation	4 (2%)
Transient ischaemic attack	4 (2%)
Myocardial infarction	4 (2%)
Peripheral atherosclerotic disease	4 (2%)
Aneurysm of the abdominal aorta	2 (1%)
Deep venous thrombosis	2 (1%)
Supraventricular tachycardia	2 (1%)
Aortic valve stenosis	2 (1%)
Pulmonary emphysema	1 (0.4%)
AV-malformation pineal gland	1 (0.4%)
Aortic valve insufficiency	1 (0.4%)
Portal vein thrombosis	1 (0.4%)

Table 7: Disorders of kidney, urinary tract and reproductive tract

Disorder	number (%)
Recurrent urinary tract infections	8 (3%)
Urolithiasis	2 (1%)
Endometriosis	2 (1%)
Renal cysts	1 (0.4%)
Prostatic hyperplasia	1 (0.4%)
Vesico-ureteral reflux	1 (0.4%)
Salpingitis	1 (0.4%)
Recurrent spontaneous abortion	1 (0.4%)
Extra-uterine pregnancy	1 (0.4%)

Table 8: Skin disorders

Disorder	number (%)
Psoriasis	4 (2%)
Lichen planus	2 (1%)
Vitiligo	2 (1%)
Alopecia areata	2 (1%)
Annular granuloma	2 (1%)
Herpes zoster	1 (0.4%)

Table 9: Neurological disorders

Disorder	number (%)
Hernia nuclei pulposi	8 (3%)
Carpal tunnel syndrome	4 (2%)
Dementia	2 (1%)
Migraine	2 (1%)
Myasthenia gravis	1 (0.4%)
Poliomyelitis	1 (0.4%)
Subarachnoidal bleeding	1 (0.4%)
Neurogenic claudication	1 (0.4%)

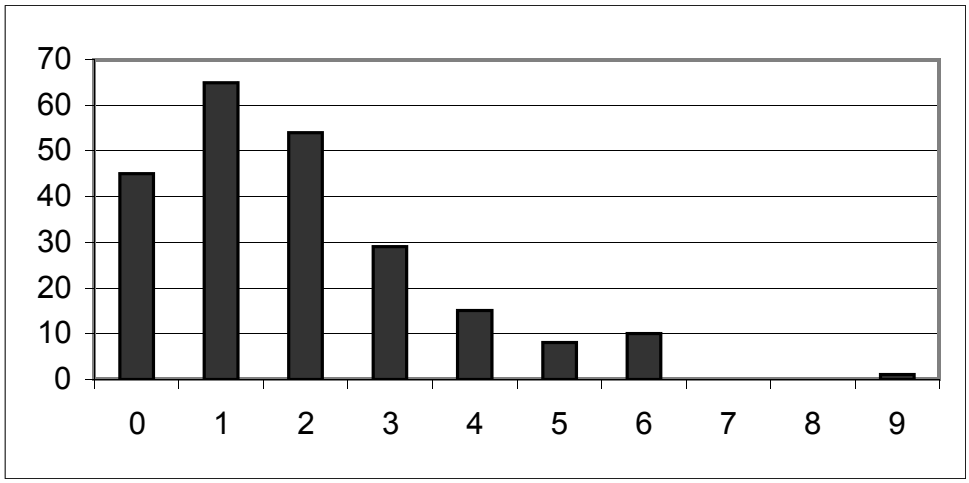
Table 10: Miscellaneous disorders and surgical procedures

Disorder	number (%)
Extirpation of the uterus	15 (6%)
Sterilisation	7 (3%)
Inguinal herniation	5 (2%)
Cataract	5 (2%)
Tonsillectomy	4 (2%)
Umbilical herniation	4 (2%)
Retinal ablation	2 (1%)
Mitochondrial myopathy	1 (0.4%)
Malaria	1 (0.4%)
Labyrintitis	1 (0.4%)
Vitrial ocular bleeding	1 (0.4%)
Tuberculosis	1 (0.4%)
Anterior uveitis	1 (0.4%)
Thoracic outlet syndrome	1 (0.4%)
Macular degeneration	1 (0.4%)
Lyme's disease	1 (0.4%)
Osteomyelitis	1 (0.4%)

Table 11: Prevalence of concurrent disorders in PBC as previously reported and according to the present study

	Present study	Previous studies, %
Celiac disease	0%	0-30% (12-21)
Thyroid disorders	6.6%	14-19% (2, 42)
Rheumatoid arthritis	4%	2-27% (2-4)
Sjögren's syndrome	2%	26-27% (5, 6)
Scleroderma/CREST	0.9%	0-22% (2, 4, 7, 9)
Renal Tubular Acidosis	0%	33% (10)
Bacteriuria	3%	35% (11)
Ulcerative colitis	1%	2% (22)

Figure: Histogram showing the number of concurrent disorders per patient



5

Does primary sclerosing cholangitis develop after colectomy for inflammatory bowel disease?

P.C.J. ter Borg[1], F.P. Vleggaar[2], H.R. van Buuren[1]

[1]Department of Gastroenterology and Hepatology, Erasmus MC, Rotterdam, [2]Department of Gastroenterology and Hepatology, University Medical Center, Utrecht, The Netherlands

Summary

Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease which is strongly associated with the presence of inflammatory bowel disease (IBD). Although the pathogenesis of PSC is unknown, it has been hypothesized that, besides a genetic predisposition, portal bacteremia or endotoxinemia may be involved. In the literature, we could not find any cases of newly developed PSC after colectomy for ulcerative colitis had been performed previously. We aimed to find such patients not in series of IBD patients, but by studying a large cohort of prospectively included PSC patients, in order to find support for the hypothesis that the colon plays a role in the development of PSC in patients with IBD. A total of 163 PSC patients were included, 96 (59%) of these patients had IBD. Twenty patients underwent colectomy, but in only 4 of these colectomy was performed before the diagnosis of PSC was made. However, in one of these only a hemicolectomy was performed. In the remaining three patients, evidence of chronic liver disease was already present at the time of colectomy, as demonstrated by an abnormal cholangiography in one and chronically elevated liver enzymes compatible with a diagnosis of PSC in two.

In conclusion, both in the literature and in the present study we could not identify any cases of newly developed PSC after colectomy for ulcerative colitis was performed. This supports a role for the inflamed colon in the pathogenesis of PSC.

Introduction

Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease which is strongly associated with the presence of inflammatory bowel disease (IBD) (1). Approximately 65% of patients with PSC have associated IBD, and 2-7.5% of patients with IBD have PSC (2-5). Although the pathogenesis of PSC is unknown, it has been hypothesized that, besides a genetic predisposition, portal bacteremia or endotoxinemia may be involved (6-9). The increased risk of developing PSC in patients with IBD might thus be explained by the presence of inflamed and damaged colonic mucosa. However, this hypothesis has been rejected since PSC can occur years after colectomy for IBD has been performed (1, 2, 9). Indeed, several papers have reported on patients in whom a diagnosis of PSC was made after colectomy for IBD (10-12). A total number of 12 patients who had a colectomy before the diagnosis of PSC was established were reported in these papers, but for none of these patients evidence showing that the liver disease was not already present at the time of the colectomy was provided. Since PSC can be a very slowly progressive and indolent disease with the possibility of large delays in diagnosis, and may initially exist without liver enzyme or cholangiographic abnormalities, the presence of early stage PSC at the time of colectomy cannot be excluded in these patients (13, 14). Only one previous study aimed to find cases of newly developed PSC after previous colectomy. Mikkola et al. studied a series of patients with IBD and normal liver biochemistry at the time of colectomy, aiming to find newly developed liver test abnormalities. They did not find any such patients in a series of 169 colectomy cases, which suggests that colectomy might indeed prevent PSC (15).

In the present study, we aimed to find such patients not in series of IBD patients, but by studying a large cohort of prospectively included PSC patients, in order to find support for the hypothesis that the colon plays a role in the development of PSC in patients with IBD.

Patients and Methods

The clinical results of this cohort study have been reported previously (16). The cohort consisted of all patients seen at the Erasmus Medical Center in the period 1980-1998 and all patients who participated in a prospective cohort study in the Netherlands in the period 1990-1998. The diagnosis of PSC was based on typical findings on cholangiography (endoscopic, percutaneous or intra-operative) or liver biopsy, in combination with serum liver tests indicating cholestasis (16). For all patients the presence of IBD or previous colectomy was recorded, in addition to clinical and laboratory characteristics.

For all patients with colectomy, we recorded the date at which it was performed, and we obtained additional data aimed at confirming the presence of liver disease before or at the the time of the colectomy in patients with colectomy prior to the diagnosis of PSC.

Results

Clinical, laboratory and histological baseline characteristics have previously been described (16). A summary of these data is shown in table 1. A total of 163 PSC patients were included. Ninety-six (59%) of these patients were known to have IBD at the time of entry, and a total number of 20 patients underwent colectomy. However, in only 4 patients, colectomy had been performed before the diagnosis of PSC had been made. Two of these patients were female and two were male. In one patient a left hemicolectomy had been performed, and thus the other hemicolon remained. In the remaining 3 patients a total colectomy was performed. In 1 of these 3 patients a previous cholangiography showed changes characteristic of PSC, although at that time the diagnosis had not been made. In 1 of the remaining 2 patients, gamma-glutamyltransferase (GGT) and alkaline phosphatase (AP) levels had been persistently elevated for a period of at least five years, although cholangiography was not performed until after the colectomy (Table 2). Finally, in the fourth patient, GGT and AP levels had been persistently elevated during a period of at least one year before the colectomy was performed.

Discussion

In the present study, we found no cases of newly developed PSC after previous colectomy was performed in a large cohort of patients with PSC. Although this does not prove that PSC does not develop in IBD patients without a colon, it supports the hypothesis that the presence of an inflamed or damaged colon facilitates its development. In addition, in the literature, including those papers referred to in order to demonstrate that the hypothesis of portal bacteremia or endotoxinemia is incorrect, we could not find a single such case with normal liver tests before the colectomy (1, 2, 9-12, 15). However, since colectomy is performed in 15-35% of patients with IBD, and not infrequently early in its course, one would have expected to find such patients (17-19). Although this may have been caused by publication bias, it suggests that the risk of PSC may indeed decrease after colectomy.

Since the incidence of PSC is low, even in patients with IBD, it is unlikely that prospective studies will be performed to study the development of PSC after colectomy, especially since for reliably excluding the presence of PSC, liver enzymes, liver biopsy and cholangiography

should be obtained. It would however be highly interesting to retrospectively study cohorts of IBD patients, in order to determine whether the risk of developing PSC decreases after colectomy. Although such a finding would not directly influence the management of patients with IBD or PSC, the results of such studies might further support the hypothesis that portal bacteremia or endotoxinemia are in part responsible for the development of PSC, and thus direct future research into the pathogenesis of this disease.

Besides the argument that PSC can occur years after colectomy for IBD, several other arguments have been used to support or reject the hypothesis. Other arguments in favor of the hypothesis have been that in a majority of patients with PSC and IBD, the diagnosis of PSC was made after the IBD had been discovered and that patients with PSC more frequently have extensive colitis compared to IBD patients without PSC, and have been reported to have an earlier diagnosis and longer duration of IBD (7, 13, 15). However, it arguments against the hypothesis that although IBD was more frequently extensive, the severity of IBD in patients with PSC is usually mild and that colectomy does not seem to influence the course of PSC (7, 13, 15). In addition, it has been reported that in patients undergoing colectomy for severe IBD portal bacteremia is rare, and that in patients with PSC portal phlebitis, which is a result of portal bacteremia, was usually absent or mild, and thus that direct evidence supporting a role for portal bacteremia is lacking (7, 20, 21). More evidence supports a role for bacterial endotoxinemia. In a rat model of colitis, it has been shown that bacterial peptides caused liver disease mimicking PSC, and in another model, intestinal bacterial overgrowth caused similar lesions (22, 23). Altogether there is substantial evidence in support, and insufficient evidence against it in order to definitely reject the hypothesis of a role for portal bacteremia or especially endotoxinemia in the development of IBD associated PSC.

In conclusion, neither in the present study nor in the literature did we find a single case of newly developed PSC after previous colectomy. Further studies are needed to clarify the role of the colon in the development of PSC.

References

1. Angulo P, Lindor KD. Primary sclerosing cholangitis. Hepatology 1999;30(1):325-32.
2. Schrumpf E, Fausa O, Elgjo K, Kolmannskog F. Hepatobiliary complications of inflammatory bowel disease. Semin Liver Dis 1988;8(3):201-9.
3. Mitchell SA, Chapman RW. Review article: the management of primary sclerosing cholangitis. Aliment Pharmacol Ther 1997;11(1):33-43.
4. Warren KW, Athanassiades S, Monge JI. Primary sclerosing cholangitis. A study of forty-two cases. Am J Surg 1966;111(1):23-38.
5. Nolan JP. Intestinal endotoxins as mediators of hepatic injury--an idea whose time has come again. Hepatology 1989;10(5):887-91.
6. Boberg KM, Lundin KE, Schrumpf E. Etiology and pathogenesis in primary sclerosing cholangitis. Scand J Gastroenterol Suppl 1994;204:47-58.
7. Narayanan Menon KV, Wiesner RH. Etiology and natural history of primary sclerosing cholangitis. J Hepatobiliary Pancreat Surg 1999;6(4):343-51.
8. LaRusso NF, Wiesner RH, Ludwig J, MacCarty RL. Current concepts. Primary sclerosing cholangitis. N Engl J Med 1984;310(14):899-903.
9. Lee YM, Kaplan MM. Primary sclerosing cholangitis. N Engl J Med 1995;332(14):924-33.
10. Wiesner RH, LaRusso NF. Clinicopathologic features of the syndrome of primary sclerosing cholangitis. Gastroenterology 1980;79(2):200-6.
11. Cangemi JR, Wiesner RH, Beaver SJ, Ludwig J, MacCarty RL, Dozois RR, et al. Effect of proctocolectomy for chronic ulcerative colitis on the natural history of primary sclerosing cholangitis. Gastroenterology 1989;96(3):790-4.
12. Shepherd HA, Selby WS, Chapman RW, Nolan D, Barbatis C, McGee JO, et al. Ulcerative colitis and persistent liver dysfunction. Q J Med 1983;52(208):503-13.
13. Olsson R, Danielsson A, Jarnerot G, Lindstrom E, Loof L, Rolny P, et al. Prevalence of primary sclerosing cholangitis in patients with ulcerative colitis. Gastroenterology 1991;100(5 Pt 1):1319-23.
14. Boberg KM, Schrumpf E, Fausa O, Elgjo K, Kolmannskog F, Haaland T, et al. Hepatobiliary disease in ulcerative colitis. An analysis of 18 patients with hepatobiliary lesions classified as small-duct primary sclerosing cholangitis. Scand J Gastroenterol 1994;29(8):744-52.
15. Mikkola K, Kiviluoto T, Riihela M, Taavitsainen M, Jarvinen HJ. Liver involvement and its course in patients operated on for ulcerative colitis. Hepatogastroenterology 1995;42(1):68-72.
16. Vleggaar FP. Advances in Classification, Prognostication and Treatment of Immunocholangitis. Rotterdam: Erasmus University; 2000.
17. Hiwatashi N, Yao T, Watanabe H, Hosoda S, Kobayashi K, Saito T, et al. Long-term follow-up study of ulcerative colitis in Japan. J Gastroenterol 1995;30 Suppl 8:13-6.
18. Farmer RG, Easley KA, Rankin GB. Clinical patterns, natural history, and progression of ulcerative colitis. A long-term follow-up of 1116 patients. Dig Dis Sci 1993;38(6):1137-46.
19. Hendriksen C, Kreiner S, Binder V. Long term prognosis in ulcerative colitis--based on results from a regional patient group from the county of Copenhagen. Gut 1985;26(2):158-63.

20. Ludwig J, Barham SS, LaRusso NF, Elveback LR, Wiesner RH, McCall JT. Morphologic features of chronic hepatitis associated with primary sclerosing cholangitis and chronic ulcerative colitis. Hepatology 1981;1(6):632-40.
21. Palmer KR, Duerden BI, Holdsworth CD. Bacteriological and endotoxin studies in cases of ulcerative colitis submitted to surgery. Gut 1980;21(10):851-4.
22. Hobson CH, Butt TJ, Ferry DM, Hunter J, Chadwick VS, Broom MF. Enterohepatic circulation of bacterial chemotactic peptide in rats with experimental colitis. Gastroenterology 1988;94(4):1006-13.
23. Lichtman SN, Sartor RB, Keku J, Schwab JH. Hepatic inflammation in rats with experimental small intestinal bacterial overgrowth. Gastroenterology 1990;98(2):414-23.

Table 1: Patient characteristics

Age (mean, range) in years	35.1 (12-72)
Male / female (n)	109 / 54
No cirrhosis / cirrhosis (n)	108 / 23
IBD (%)	
no	41.4%
ulcerative colitis	37.0%
Crohn's disease	11.7%
indeterminate colitis	9.9%
Bilirubin (mmol/L), normal <17	15.5 (4-307)
Albumin (g/L), normal >35	41.1 (21-52)
Alkaline Phosphatase	294 (35-2325)
Aspartate aminotransferase	55 (4-918)

Table 2: Characteristics of the four patients with colectomy before PSC

	Patient 1	Patient 2	Patient 3	Patient 4
Age at the time of diagnosis	33 years	30 years	23 years	43 years
Procedure	Left hemicolectomy	Subtotal colectomy	Proctocolectomy	Subtotal colectomy
Interval colectomy-PSC	3 years	3 years	4 years	3 years
Abnormalities before colectomy	None, but right hemicolon was not removed	Cholangio-graphy with typical changes before colectomy	Persistently elevated GGT and AP levels for 5 years	Persistently elevated GGT and AP levels for 1 year
Outcome	Survived, no complications	Survived, no complications	Survived, no complications	Survived, no complications

6

The impact of fatigue in primary sclerosing cholangitis

P.C.J. ter Borg, H.R. van Buuren

Department of Gastroenterology and Hepatology, Erasmus MC, Rotterdam, The Netherlands

Submitted

Summary

It has been previously reported that fatigue is a frequent symptom of PSC. However, no studies are available that have assessed fatigue using specific instruments to quantify the severity of fatigue and to determine the impact of this symptom on quality of life. We aimed to quantitatively assess severity of fatigue and quality of life in patients with PSC and compare results with data for PBC patients and healthy controls. Fatigue and quality of life were assessed in a group of consecutive, clinically stable PSC patients using the following instruments: a fatigue Visual Analogue Scale, the FISK Fatigue Severity Scale, the Multidimensional Fatigue Inventory and the SF-36 questionnaire. Control groups of patients with PBC and healthy individuals were included. Routine serum liver tests were performed in all patients. Twenty-seven patients with PSC, 47 patients with PBC and 18 healthy controls were included. Fatigue was reported by 56% of patients with PSC and 68% of patients with PBC. Fatigue was significantly increased in patients (PSC and PBC) as compared to controls, whereas no significant differences between PSC and PBC were established. A negative correlation was found between severity of fatigue and quality of life. The severity of the disease did not correlate with fatigue and quality of life.

In conclusion, fatigue is a significant problem largely influencing quality of life in patients with PSC. Fatigue seems a problem as significant in PSC as in PBC.

Introduction

Primary sclerosing cholangitis (PSC) is a chronic hepatobiliary disease characterized by inflammation, fibrosis and stricturing of the intrahepatic and/or extrahepatic bile ducts (1). Symptoms may be secondary to liver cirrhosis, portal hypertension and liver failure or to typical complications including dominant bile duct strictures, bacterial cholangitis and cholangiocarcinoma. Many patients, however, may remain free of major somatic problems during variable periods of time and experience fatigue as the most important symptom of their disease. In early studies on the natural history of PSC fatigue was not recognized as one of the major symptoms of the disease (2-4). More recent studies (5, 6), however, have reported fatigue in 14-73% of patients and nearly all found this to be the single most prevalent symptom (5, 7-9). One study comparing the clinical features of PSC and primary biliary cirrhosis (PBC) reported that the frequency of fatigue was comparable: 73% in PSC and 77% in PBC (8). In these studies no details were given as to how the presence of fatigue was scored, and probably this may have been a simple "yes" or "no" according to the patients history. In one study fatigue was considered to be present when this affected the patients lifestyle (5). In contrast to PBC, where fatigue and also quality of life have been studied quantitatively using specific instruments (10-12), no such studies are available for PSC.

The present study aimed to quantify fatigue and quality of life in patients with PSC in comparison to patients with PBC and healthy controls.

Patients and Methods

The study was approved by our institution's medical ethics committee. All consecutive patients with a diagnosis of PBC or PSC visiting the hepatology outpatient clinic of the Erasmus Medical Center between October 2001 and June 2002 were invited to participate. PBC was diagnosed on the basis of previously defined criteria (13). The diagnosis of PSC was based on typical findings on cholangiography or liver biopsy (14, 15). All patients were attending for scheduled follow-up visits, and were free of recent major events including surgery, endoscopic intervention or hospitalization. Exclusion criteria were age less than 18 years, previous liver transplantation and incomplete understanding of the Dutch language. Healthy controls were selected by asking patients to bring a healthy control person of the same age group and sex. All patients and controls provided informed consent.

Assessment of fatigue and quality of life

Fatigue was measured using a visual analogue scale and two self-report instruments, the Fisk Fatigue Severity Scale (FFSS) and the Multidimensional Fatigue Inventory (MFI) (16, 17). The visual analogue scale consisted of a 10 cm line, indicating on one side the presence of no fatigue at all, and at the other side the worst possible fatigue. Fatigue was graded by distance with higher scores relating to more severe fatigue. Subjects were asked to rate the severity of their fatigue over the previous month. The 40 question FFSS assesses the impact of fatigue on aspects of daily life over the previous month. The score per individual item ranges from zero to four, higher scores indicating greater impact of fatigue. The FFSS consists of three domains measuring the impact of fatigue on psycho-social, cognitive and physical activity. The maximum scores for these domains are 80, 40 and 40, respectively. The FFSS is a validated instrument in PBC (18) that has been used to evaluate the extent and impact of fatigue (10, 12) and the effect of therapeutic intervention (19).

The 20 question MFI quantifies general fatigue, physical fatigue, reduction in motivation, reduction in activity and cognitive fatigue. For each question, the patient is asked to respond on a five-item scale, with a question being true on the one side to not true on the other side. Using pre-defined criteria, the score for each of the domains was calculated (17). The maximum score for each of the domains is 20.

The impact of the disease on the quality of life was assessed using the SF-36 (20). The SF-36 has been widely used in the evaluation of decreased quality of life as a result of disease. In addition, it has been previously used to quantify quality of life in patients with cholestatic liver disease (21, 22). It consists of 36 questions regarding different aspects of quality of life: physical functioning, role functioning physical, bodily pain, general health, vitality, social functioning, role emotional functioning, mental health and reported health transition. The score for each of the domains was calculated as previously described (20).

Increasing scores on the visual analogue scale, the FFSS and the MFI correspond with increasing severity of fatigue. Increasing SF-36 scores indicate better quality of life.

Other investigations

The following routine laboratory tests were obtained in all patients: serum total bilirubin, alkaline phosphatase, ALT, AST, albumin, prothrombin time, total immunoglobulin M, hemoglobin and platelet count. The following clinical variables were recorded: age, sex, UDCA dose, presence of fatigue and pruritus, date of diagnosis, weight and length. Finally,

patients and controls completed a visual analogue scale for pruritus and a pruritus sleep interruption score. The visual analogue scale for pruritus consisted of a 10 cm line with 0 corresponding to no pruritus at all and 10 corresponding to the worst possible pruritus, as experienced during the previous month. The sleep interruption score asked patients how frequently sleep was interrupted by pruritus: never, very occasionally, less than 1 night per week, more than 1 but less than 4 nights per week, more than 4 nights per week but not every night or every night (18).

Statistical analysis

Comparisons between groups were performed using χ^2 and the Mann-Whitney tests. Non-parametric tests were chosen because a normal distribution of the questionnaire results could not be expected. Correlations were tested using Pearson's correlation method. Multivariate regression analyses were performed using a backward elimination model. P-values of less than 0.05 were considered to be statistically significant. All statistical analyses were performed using SPSS version 10.

Results

Patient characteristics

A total number of 27 patients with PSC, 47 patients with PBC and 18 healthy controls were included. Two patients refused to be included in the study. All study subjects adequately completed the questionnaires and other self-report instruments. Patient characteristics are shown in table 1. There were significant differences between patients with PBC and PSC in male/female-ratio, age, UDCA dose, length and hemoglobin and immunoglobulin M levels. Laboratory tests of disease activity and severity showed no significant differences between the two groups. The median age of healthy controls was 42.5 years (range 31-74); five were male and 13 female. Ten patients (21%) with PBC and 10 patients (37%) with PSC had serum bilirubin concentrations above the upper limit of normal (p=0.14). Two patients with PSC had documented cirrhotic disease, whereas none had previous variceal bleeding or ascites. Twelve (44%) of patients with PSC were previously treated because of a dominant biliary stricture, and 16 (59%) had associated inflammatory bowel disease (13 ulcerative colitis, 2 Crohn's disease and 1 indeterminate colitis). No patients had chronic anemia or thyroid disease.

Fatigue

The prevalence of fatigue, defined as the answer to a ‘’yes/no’’ question was 56% in patients with PSC, 68% in patients with PBC and 17% in controls. This results in a p-value of 0.01 for the patients with PSC vs. controls, a p-value of 0.28 for patients with PSC vs. PSC and a p-value of <0.001 for patients with PBC vs. controls.

In patients with PSC, FFSS and MFI scores in all domains, except for mental fatigue, were higher than in the control subjects, but differences were only significant for MFI scores. Also, fatigue measured by VAS was more severe: the median fatigue score was 4.5 for PSC patients and 1.4 for controls (p=0.01).

There were no significant differences in fatigue scores between patients with PSC and PBC, although in all domains of both the FFSS and MFI patients with PBC had higher scores, thus showing a trend towards increased fatigue in these patients (Table 2).

In patients with PBC, FFSS and MFI scores in all domains were significantly higher than in control subjects, except for the mental fatigue MFI scores.

VAS scores for fatigue indicated that fatigue was significantly more severe in patients with PBC than in controls (p<0.001). No significant difference was found between PSC and PBC patients. Correlations between VAS, FFSS and MFI scores are shown in figures 1 and 2.

Twenty-nine patients with PBC (62%), 11 with PSC (41%) and 2 controls (12%) had visual analogue scores more than 50% of the maximum (10) score (PBC vs. PSC p=0.10, PBC vs. controls p<0.001 and PSC vs. controls p=0.05). Ten patients with PBC (21%), 7 with PSC (26%) but no controls had a total FFSS score more than 50% of the maximum (160) score (PBC vs. PSC p=0.78, PBC vs. controls p=0.05 and PSC vs. controls p=0.03). Thirty-eight patients with PBC (81%), 19 with PSC (70%) and 4 controls (22%) had a total MFI score more than 50% of the maximum (100) score (PBC vs. PSC p=0.30, PBC vs. controls p<0.001 and PSC vs. controls p=0.02).

There were no significant differences in fatigue scores between patients with PSC and associated inflammatory bowel disease and patients without inflammatory bowel disease (p=0.73 for the VAS, p=0.84 for the FFSS and p=0.96 for the MFI).

No significant correlations were found between total fatigue VAS, FFSS and MFI scores and serum bilirubin, alkaline phosphatase, AST, ALT, and albumin in either group of patients.

The correlation coefficient for the FFSS and the fatigue VAS was 0.71 (p<0.001), for the MFI and the fatigue VAS 0.76 (p<0.001) and for the FFSS and the MFI was 0.81 (p<0.001).

Quality of life (SF-36)

There were significant differences in SF-36 scores between PSC patients and controls in the role functioning physical, general health and vitality domains, whereas between PBC patients and controls significant differences in role functioning physical, bodily pain, general health, vitality, social functioning and reported health transition were found (Table 3). The only significant difference between PSC and PBC was found in the physical functioning domain, where patients with PBC performed worse. This domain measures the extent to which physical activities are limited by health.

There were no significant differences in SF-36 scores between patients with PSC and associated inflammatory bowel disease and patients without inflammatory bowel disease ($p=0.56$ for the overall SF-36 score).

Impact of fatigue on quality of life

In order to assess the impact of fatigue on the quality of life, we used the VAS score and the total FFSS and MFI scores. Overall quality of life was quantified using the sum of the individual SF-36 domain scores. Correlations between quality of life and fatigue scores are shown in table 4. The relation between fatigue and quality of life is illustrated in figure 3. Correlation testing showed that, depending on the fatigue questionnaire used, 74-79% and 67-74% of the variation in SF-36 scores could be explained by variation in fatigue in patients with PSC and PBC, respectively.

Pruritus and sleep quality

The mean visual analogue scores for pruritus were 2.4, 2.3 and 1.0 in patients with PSC, PBC and controls, respectively. There was a significant difference between PSC and controls ($p=0.02$), and a trend towards increased pruritus in patients with PBC vs. controls ($p=0.06$). The results of the sleep interruption scale are shown in table 5. The differences between any of the three groups were not significant ($p=0.99$ for PBC vs. PSC, $p=0.37$ for PBC vs. controls and $p=0.13$ for PSC vs. controls).

Baseline characteristics and questionnaire results

There was a significant difference in the SF-36 physical functioning domain scores between patients with PSC and PBC. In addition, there was an overall trend towards more severe fatigue and worse quality of life in patients with PBC. Since patients were not equally distributed with respect to baseline characteristics, univariate and multivariate analyses were performed to assess the relation between baseline variables and fatigue or quality of life. In these analyses, the following variables were included: diagnosis, age, sex and hemoglobin level. Both univariate and the multivariate analyses did not result in any factor significantly associated with fatigue questionnaire scores. With respect to quality of life, univariate analysis showed that hemoglobin level (p=0.02), sex (p=0.001), age (p=0.03) and diagnosis (p=0.001) were associated with the physical functioning domain scores. Multivariate analysis demonstrated that sex (p=0.001) was the only independently associated variable. Tests for the other SF-36 domains did not result in any significant associations.

Discussion

This is the first study that quantified fatigue and its impact on quality of life in patients with PSC, showing, compared to healthy controls, increased fatigue and decreased quality of life. Although patients with PBC had worse overall fatigue scores compared to PSC, differences were not statistically significant. We could not find any correlation with routine laboratory tests of disease activity and severity or other baseline characteristics in patients with PSC or PBC. This confirms previous findings in PBC, whereas in PSC such a relation had not been studied previously (11, 12, 18).

Compared to previous studies on fatigue in patients with PBC by Huet et al. and Prince et al., PBC patients in our study demonstrated less severe fatigue as measured by the FFSS. The median total FFSS score for PBC patients in the present study was 41, compared to 78 in the study by Prince et al, whereas the mean total FFSS score in the present study was 44, compared to 60 in the study by Huet et al. (12, 18). However, a more recent and larger study on fatigue in PBC, with data from a geographically based cohort, reported a median total FFSS score of 40 (10). The results of the latter study are therefore highly comparable with the current findings in patients with PBC.

The differences in fatigue between patients with cholestatic liver disease and controls became more apparent when the frequency of more intense fatigue was studied. None of the controls had a FFSS score exceeding 50% of the maximum score, whereas this was the case in 26% of

patients with PSC and 21% of patients with PBC. Analysis of VAS and MFI scores showed similar differences. Thus, many patients with these diseases have relatively mild fatigue, whereas approximately 25% of patients have fatigue to an extent not observed in healthy controls.

The results of the MFI showed that in both PSC and PBC all measurements of physical fatigue and general fatigue, but not mental fatigue, were decreased compared to controls. Although the FFSS did not result in significant differences between PSC and controls, a similar trend could be observed. The predominant occurrence of physical fatigue is compatible with the finding by Goldblatt et al. that fatigue correlates with grip strength decrease in patients with PBC, and suggests the presence of an as yet unidentified factor causing physical fatigue (23).

The SF-36 resulted in significant differences in role functioning physical (the extent to which physical problems limit activities), general health, vitality and social functioning between patients with PSC and controls. In PBC, in addition to those domains identified in PSC, physical function and pain were different from controls. These results suggest that patients with PSC and PBC are limited in physical activities and social functioning mainly because of physical fatigue, but not because of mental fatigue or emotional problems. We found that approximately 75% of variation of SF-36 scores can be explained by variation in fatigue, suggesting that indeed fatigue is the most important factor limiting quality of life in these patients. Regression analysis further showed that the difference in physical functioning between PBC and PSC was explained by the marked difference in sex distribution between the two groups, and was not caused by true differences between the two diseases.

Most patients had no or modest pruritus, and only in a minority pruritus resulted in sleep disturbances.

Although we carefully aimed to include all eligible patients the sample of the present study was relatively small. Therefore, additional studies are clearly needed to confirm the present findings. Further, many patients with PSC have concurrent IBD, a disease that may also be associated with fatigue and decreased quality of life (24). This markedly impairs the ability to distinguish the role of either PSC or inflammatory bowel disease in relation to symptoms as fatigue and quality of life changes. However, our preliminary data indicate that severity of fatigue and quality of life did not differ for patients with and without inflammatory bowel disease.

Currently, no effective drug or other treatment for fatigue is available. Although liver transplantation has been suggested as a possible treatment of invalidating fatigue associated

with cholestatic liver disease, the reported effect of liver transplantation on fatigue is not consistent (10, 25). Moreover, studies evaluating liver transplantation for the treatment of fatigue in the absence of other accepted indications have not been performed. This suggests that fatigue as the sole indication for transplantation should be considered critically.

In conclusion, this study shows that in PSC fatigue is a problem as significant as in PBC that influences quality of life in a major way.

References

1. Angulo P, Lindor KD. Primary sclerosing cholangitis. Hepatology 1999;30(1):325-32.
2. Schrumpf E, Elgjo K, Fausa O, Gjone E, Kolmannskog F, Ritland S. Sclerosing cholangitis in ulcerative colitis. Scand J Gastroenterol 1980;15(6):689-97.
3. Wiesner RH, LaRusso NF. Clinicopathologic features of the syndrome of primary sclerosing cholangitis. Gastroenterology 1980;79(2):200-6.
4. Chapman RW, Arborgh BA, Rhodes JM, Summerfield JA, Dick R, Scheuer PJ, et al. Primary sclerosing cholangitis: a review of its clinical features, cholangiography, and hepatic histology. Gut 1980;21(10): 870-7.
5. Farrant JM, Hayllar KM, Wilkinson ML, Karani J, Portmann BC, Westaby D, et al. Natural history and prognostic variables in primary sclerosing cholangitis. Gastroenterology 1991;100(6):1710-7.
6. Floreani A, Zancan L, Melis A, Baragiotta A, Chiaramonte M. Primary sclerosing cholangitis (PSC): clinical, laboratory and survival analysis in children and adults. Liver 1999;19(3):228-33.
7. Helzberg JH, Petersen JM, Boyer JL. Improved survival with primary sclerosing cholangitis. A review of clinicopathologic features and comparison of symptomatic and asymptomatic patients. Gastroenterology 1987;92(6):1869-75.
8. Wiesner RH, LaRusso NF, Ludwig J, Dickson ER. Comparison of the clinicopathologic features of primary sclerosing cholangitis and primary biliary cirrhosis. Gastroenterology 1985;88(1 Pt 1):108-14.
9. Okolicsanyi L, Fabris L, Viaggi S, Carulli N, Podda M, Ricci G. Primary sclerosing cholangitis: clinical presentation, natural history and prognostic variables: an Italian multicentre study. The Italian PSC Study Group. Eur J Gastroenterol Hepatol 1996;8(7):685-91.
10. Goldblatt J, Taylor PJ, Lipman T, Prince MI, Baragiotta A, Bassendine MF, et al. The true impact of fatigue in primary biliary cirrhosis: a population study. Gastroenterology 2002;122(5):1235-41.
11. Cauch-Dudek K, Abbey S, Stewart DE, Heathcote EJ. Fatigue in primary biliary cirrhosis. Gut 1998;43 (5):705-10.
12. Huet PM, Deslauriers J, Tran A, Faucher C, Charbonneau J. Impact of fatigue on the quality of life of patients with primary biliary cirrhosis. Am J Gastroenterol 2000;95(3):760-7.
13. van Hoogstraten HJF, Hansen BE, van Buuren HR, ten Kate FJW, van Berge Henegouwen GP, Schalm SW. Prognostic factors and long-term effects of ursodeoxycholic acid on liver biochemical parameters in patients with primary biliary cirrhosis. J Hepatol 1999;31:256-262.
14. MacCarty RL, LaRusso NF, Wiesner RH, Ludwig J. Primary sclerosing cholangitis: findings on cholangiography and pancreatography. Radiology 1983;149(1):39-44.
15. Ludwig J, Barham SS, LaRusso NF, Elveback LR, Wiesner RH, McCall JT. Morphologic features of chronic hepatitis associated with primary sclerosing cholangitis and chronic ulcerative colitis. Hepatology 1981;1(6):632-40.
16. Fisk JD, Ritvo PG, Ross L, Haase DA, Marrie TJ, Schlech WF. Measuring the functional impact of fatigue: initial validation of the fatigue impact scale. Clin Infect Dis 1994;18 Suppl 1:S79-83.
17. Smets EM, Visser MR, Willems-Groot AF, Garssen B, Oldenburger F, van Tienhoven G, et al. Fatigue and radiotherapy: (A) experience in patients undergoing treatment. Br J Cancer 1998;78(7):899-906.

18. Prince MI, James OF, Holland NP, Jones DE. Validation of a fatigue impact score in primary biliary cirrhosis: towards a standard for clinical and trial use. J Hepatol 2000;32(3):368-73.
19. Watson JP, Jones DE, James OF, Cann PA, Bramble MG. Case report: oral antioxidant therapy for the treatment of primary biliary cirrhosis: a pilot study. J Gastroenterol Hepatol 1999;14(10):1034-40.
20. Ware JE, Jr., Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care 1992;30(6):473-83.
21. Younossi ZM, Kiwi ML, Boparai N, Price LL, Guyatt G. Cholestatic liver diseases and health-related quality of life. Am J Gastroenterol 2000;95(2):497-502.
22. Kim WR, Lindor KD, Malinchoc M, Petz JL, Jorgensen R, Dickson ER. Reliability and validity of the NIDDK-QA instrument in the assessment of quality of life in ambulatory patients with cholestatic liver disease. Hepatology 2000;32(5):924-9.
23. Goldblatt J, James OF, Jones DE. Grip strength and subjective fatigue in patients with primary biliary cirrhosis. Jama 2001;285(17):2196-7.
24. Minderhoud IM, Oldenburg B, van Dam PS, van Berge Henegouwen GP. High prevalence of fatigue in quiescent inflammatory bowel disease is not related to adrenocortical insufficiency. Am J Gastroenterol 2003;98(5):1088-93.
25. Gross CR, Malinchoc M, Kim WR, Evans RW, Wiesner RH, Petz JL, et al. Quality of life before and after liver transplantation for cholestatic liver disease. Hepatology 1999;29(2):356-64.

Table 1: Patient characteristics

	PSC (n=27)	PBC (n=47)	p-value
Male / Female	19/8	4/43	<0.001
Age (median, range)	44 (23-68)	60 (34-78)	<0.001
UDCA (median, range in mg/day)	750 (0-1500)	900 (0-1200)	0.005
Pruritus (yes / no)	11/16	16/31	0.56
Fatigue (yes / no)	15/12	32/15	0.28
Weight (median, range in kg)	78 (62-103)	72 (51-103)	0.09
Length (median, range in cm)	179 (168-192)	169 (143-186)	0.001
Body Mass Index (median, range)	24 (20-32)	25 (19-31)	0.34
Albumin (median, range in gram/l)	42 (33-47)	41 (31-48)	0.19
Bilirubin (median, range in mmol/l)	14.5 (8-33)	10 (4-98)	0.89
ALT (median, range in U/l)	34 (10-662)	43 (17-484)	0.44
AST (median, range in U/l)	31 (18-338)	37 (21-241)	0.66
Alkaline phosphatase (median, range in U/l)	146 (53-1141)	154 (68-441)	0.21
Immunoglobulin M (median, range in g/l)	1.39 (0.59-3.1)	2.62 (1.1-10.9)	<0.001
Haemoglobin (median, range in mmol/l)	9.0 (6.90-10.5)	8.4 (6.7-9.4)	0.003
Platelet count (median, range, 10^9/l)	245 (109-316)	212 (59-466)	0.26

Normal values: albumin >35 gram/l, bilirubin <17 mmol/l, ALT < 31U/l, AST < 31U/l, Alkaline phosphatase < 120 U/l, Immunoglobulin M < 2.4 g/l, haemoglobin > 7.6 mmol/l, platelet count 120 – 320 x 10^9/l

Table 2: Total and individual domain scores of fatigue in PSC, PBC and controls

	median scores (range)		
	PSC	PBC	Controls
VAS Fatigue (0–10)	4.5 (0-9.6)*	6.4 (0-10)**	1.4 (0-5.1)
FFSS			
Physical domain (max. 40)	13 (0-32)	16 (0-29)**	4.5 (0-25)
Cognitive domain (max. 40)	7 (0-30)	7 (0-27)	5.5 (0-20)
Social domain (max. 80)	10 (0-58)	20 (0-55)*	9.5 (0-37)
Total score (max. 160)	28 (0-118)	41 (0-105)*	20 (0-71)
MFI			
General Fatigue (max. 20)	16 (4-20)*	15 (4-20)**	6 (4-18)
Physical Fatigue (max. 20)	12 (4-20)*	13 (4-20)**	6 (4-17)
Reduction in Activity (max. 20)	12 (4-20)	12 (4-20)**	4.5 (4-20)
Reduction in Motivation (max. 20)	10 (4-19)	11 (4-20)**	5.5 (4-18)
Mental Fatigue (max. 20)	11 (8-16)	12 (7-20)	11.5 (8-16)
Total score (max. 100)	58 (24-95)*	63 (24-100)**	36 (24-87)

* $p<0.05$ compared with controls, ** $p<0.001$ compared with controls
No significant differences were found between scores for PBC and PSC patients.

Table 3: Comparison of total and individual domain SF-36 scores for PBC and PSC patients and controls

	median scores (range)		
	PSC (n=27)	PBC (n=47)	Controls (n=18)
Physical Functioning (max. 100)	87.5 (55-100) †	72.5 (20-100)**†	92.5 (45-100)
Role Functioning Physical (max. 100)	50 (0-100)*	50 (0-100)*	100 (0-100)
Bodily Pain (max. 100)	67 (41-100)	72 (0-100)*	92 (41-100)
General Health (max. 100)	37.5 (0-82)**	45 (5-82)**	73.5 (40-100)
Vitality (max. 100)	55 (20-100)*	50 (5-85)**	75 (40-100)
Social Functioning (max. 100)	87.5 (25-100)	62.5 (12.5-100)*	100 (62.5-100)
Role Emotional Functioning (max. 100)	100 (0-100)	100 (0-100)	100 (0-100)
Mental Health (max. 100)	80 (32-100)	72 (0-100)	78 (48-100)
Reported Health Transition (max. 5)	3.5 (1-5)	3 (1-5)*	3 (1-4)
Total score (max. 800) (excl. Reported Health Transition)	527 (208-778)*	510 (143-747)**	681 (319-800)

* $p<0.05$ as compared with controls, ** $p<0.001$ as compared with controls, † PBC vs. PSC $p=0.001$

Table 4: Correlation between fatigue and quality of life scores

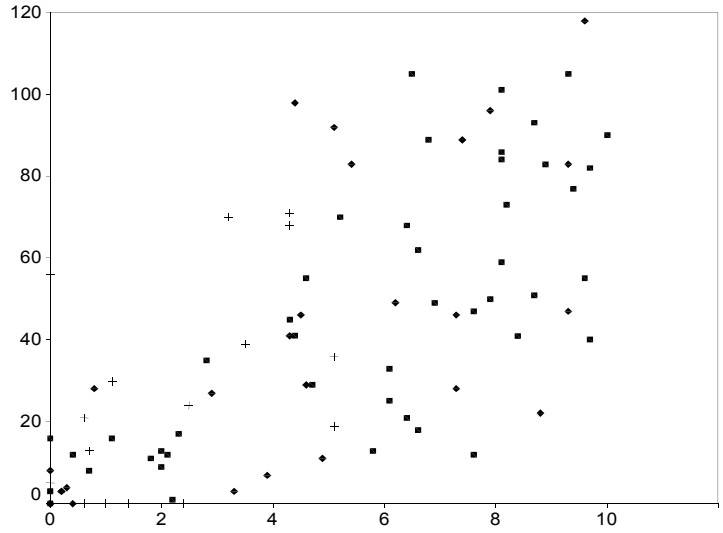
	SF-36 PBC	SF-36 PSC	SF-36 controls
VAS	-0.77**	-0.66**	-0.62*
FFSS	-0.86**	-0.86**	-0.55*
MFI	-0.82**	-0.89**	-0.63*

* $p < 0.05$, ** $p< 0.001$

Table 5: Sleep interruption score

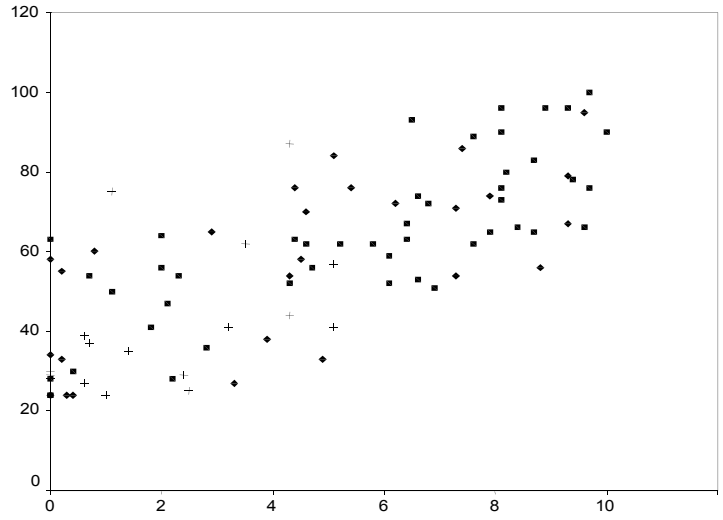
	Number of PSC patients (%)	Number of PBC patients (%)	Number of controls (%)
Never	16 (59%)	30 (64%)	17 (94%)
Very occasionally	6 (22%)	10 (21%)	1 (6%)
Less than 1 night per week	3 (11%)	4 (8.5%)	0
More than 1, less than 4 nights per week	1 (4%)	1 (2%)	0
More than 4 nights per week, not every night	1 (4%)	1 (2%)	0
Every night	0	1 (2%)	0

Figure 1



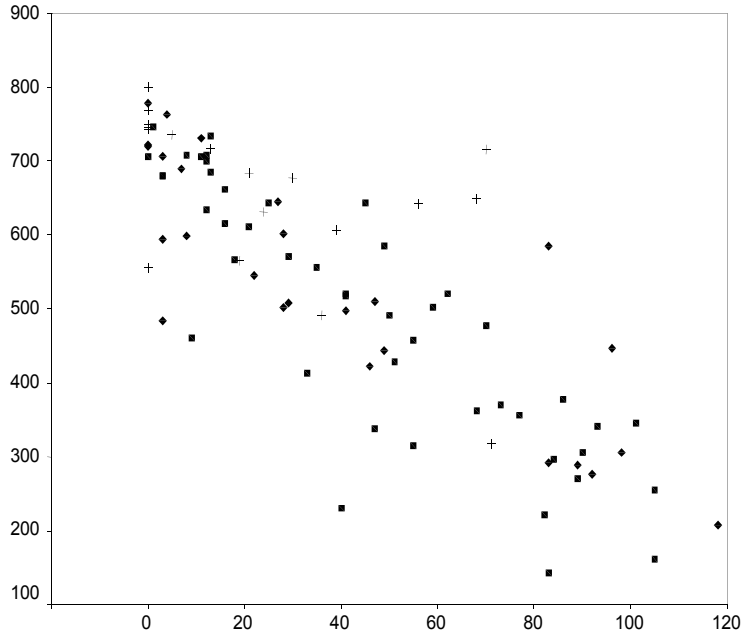
Relation between the visual analogue scale for fatigue (X-axis) and the FFSS score (Y-axis). Diamonds indicate PSC patients, squares indicate PBC patients, + signs indicate healthy controls.

Figure 2



Relation between the visual analogue scale for fatigue (X-axis) and the MFI score (Y-axis). Diamonds indicate PSC patients, squares indicate PBC patients, + signs indicate healthy controls.

Figure 3



Relation between the FFSS score (X-axis) and the SF-36 score (Y-axis). Diamonds indicate PSC patients, squares indicate PBC patients, + signs indicate healthy controls.

7

Depression in patients with primary biliary cirrhosis and primary sclerosing cholangitis

Erik van Os[1], Walter W. van den Broek[1], Paul G. H. Mulder[3] , Pieter. C. J. ter Borg[2], Henk R. van Buuren[2], Jan A. Bruijn[1]

[1] Department of Psychiatry, [2] Department of Gastroenterology and Hepatology, [3] Department of Epidemiology & Biostatistics, Erasmus MC, Rotterdam, The Netherlands

Conditionally accepted, Gut

Summary

Former studies reported a very high prevalence of depression in patients with primary biliary cirrhosis. The aim of the present study was to study the prevalence of depression in a Dutch population with PBC and PSC. Patients with PBC and PSC completed a self report questionnaire measuring the severity of a depression. Patients with scores higher than the cutoff score were interviewed with a structured psychiatric interview. Seventy-three patients were included. 41.3% had depressive symptoms according to the self-report questionnaire. Only 4.9% had a depressive syndrome according to the structured psychiatric interview.

In conclusion, the prevalence of depressive syndrome in the patients with PBC and PSC is not increased compared to the general population.

Introduction

Multiple large community studies utilizing structured psychiatric interviews have demonstrated a prevalence of major depression in the general population between the 2 and 4% (1-3). Compared to the general population, the prevalence of depression in patients with a chronic medical illness is increased (4). Patients with one or more chronic medical illnesses had a 41% increase in the relative risk of having any recent psychiatric illness (5). Primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) are chronic cholestatic liver diseases with a relatively favorable prognosis for most patients (6,7). Former studies in populations with PBC found a high prevalence of depression between 20% and 45% (8,9). However both studies did not perform a structured psychiatric interview to confirm the diagnosis of depression. The first study (8) used the Hamilton Rating Scale for Depression (HRSD) (10) and the Center for Epidemiologic Studies Depression Rating Scale (CES-D) (11) for the screening of depression. For the HRSD they used a cutoff score of 17 and for the CES-D a score of 16. The second study (9) used the Beck Depression Inventory (BDI) (12) to screen for depression with a frequently used cutoff score of 10.

In the present study we determined the prevalence of depression in the population of patients with PBC and PSC of the liver unit of the Erasmus University Medical Center. Because the BDI had been developed as an instrument to measure the severity of the depression and not the presence of a depression, we also performed a structured psychiatric interview: Schedule for Affective Disorders and Schizophrenia (SADS) (13), to verify whether the groups of patients with a BDI score ≥ 10 included those patients with a depressive disorder (DSM-IV). We expected to find a lower prevalence, because the former studies did not perform a structured psychiatric interview. Probably these patients have some symptoms of a depressive disorder, but lack all criteria necessary for the diagnosis. We will try to specify the symptoms these patients have and describe the syndrome.

Methods

Subjects

Data for the study were obtained from a study by the Erasmus MC: Department of Gastroenterology, section Hepatology: Fluvoxamine treatment in depression related to primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC); a double-blind placebo-controlled trial.

In this trial 73 patients were included. All subjects were outpatients with PBC or PSC. Inclusion criteria were a diagnosis of PBC or PSC, age > 18 years and all patients were asked an informed consent. Exclusion criteria were patients using ursodeoxycholic acid (UDCA) or antipruritic treatment, initiated or changed less than less than three months prior to entry, severe psychiatric disorder (other than depression), incomplete understanding of the Dutch language, pregnancy or lack of adequate contraception in women with childbearing potential, lactation, Child-Pugh score ≥ 6, other study medication in the preceding three months, use of benzodiazepines which could not be decreased to less than 3 mg lorazepam or equivalent per day.

Before the visit the patients were asked to self-assess the Beck Depression Inventory (BDI). This was sent to them before their visit to the out-patient clinic. The research physician of the Department of Gastroenterology counted the Beck score and consulted us for the structured psychiatric interview. If the Beck score was ≥ 10, the patient was interviewed with a structured psychiatric interview (SADS). The group of patients with a BDI < 10 were randomized and 50 % were interviewed with a structured psychiatric interview as well. Raters were blind with respect to the BDI score of the patients.

Next to the SADS the HRSD: Hamilton Rating Scale for Depression (10) was assessed.

Reference standard

The reference standard in this trial is the depression-section of the Schedule for Affective Disorders and Schizophrenia: SADS (13). This structured psychiatric interview was administered in the screening period, before the start of medication to obtain Research Diagnostic Criteria (RDC) (14) and to confirm the DSM-IV diagnosis.

Test methods

The structured psychiatric interview was performed by a trained psychiatrist or a trained resident from the department of psychiatry. The first 15 interviews were performed by both as inter-rater sessions.

The cutoff score for the Beck was 10, which is considered a good indication for depression (12). The BDI (15) is a 21-item self-report depression scale. Scoring is completed by summing the severity of individual symptoms rated from 0 to 3; overall scores ranges from 0 to 63. The BDI focuses on the following dimensions: (a) depressive mood, anhedonia, and inhibition of activities, (b) negative self-concept, and (c) somatic/ vegetative features (16).

Statistical Analysis

Data were analyzed with SPSS 9.0 software. Differences between groups were tested with $\chi 2$ tests. Statistical tests were two-tailed. P-values of 0.05 or less were considered statistically significant. The estimation of the prevalence and its asymptotic standard error is based on a weighted sum of the observed prevalences in the interviewed patients of either BDI group, with the sizes of the two BDI groups as weights.

Results

A total of 73 patients were included (See Table 1: Baseline characteristics). Of these patients, 43 had a BDI score < 10 and 30 a BDI score ≥ 10. In the group patients with a BDI ≥ 10, there were 5 who refused a psychiatric interview. The other 25 were all interviewed with the SADS. There was a depression present according to the SADS in 3 of the 25 with a BDI ≥ 10, resulting in a prevalence of depression of 4.9% with a standard error of 5.2 percent points. Of the 43 patients with a BDI score < 10, only 9 were interviewed with the SADS and were all negative for a depressive disorder. The other patients of the random half of the 43 refused a psychiatric interview.

We tested the 9 different DSM-IV criteria for depression in the two groups BDI < and ≥ 10 (See Table 2: DSM-IV criteria in the different groups). We found that only the fatigue criterion showed a statistically significant difference in the two groups. This means that the main symptom of the patients with a BDI score ≥ 10 is fatigue in comparison to patients with a BDI score < 10.

Next to this we tested the three questions of the HRSD concerning the sleeping disorders (question 4, 5, and 6) in the two groups BDI < and ≥ 10 (See Table 3: Sleeping Disorders). There was no statistically significant difference in the two groups.

Discussion

In our study we found a much lower prevalence of depression compared to the two previously reported studies. The difference in prevalence can be explained by the use of different screening instruments. The former studies used the BDI, CES-D and the HRSD to screen for depression. These questionnaires were developed as instruments to determine the severity of a depression, but not to determine the presence of a depression. We used a structured psychiatric interview (SADS) to diagnose a depression (DSM-IV).

We found that 31 patients had a BDI ≥ 10. According to the BDI score, this would mean a prevalence of [30/73] 41.3% and resembles the prevalence found in the former studies (8,9).
It is possible that patients with a chronic somatic illness have some features of a depressive disorder, but lack all features necessary for the DSM-IV diagnosis. These patients suffer from a 'subsyndromal depression' (17). This includes patients who suffer from a cluster of depressive symptoms, in which the number, duration, or quality of symptoms is insufficient to meet the DSM criteria necessary for a diagnosis of major depression. Therefore this includes patients with minor depression (less than 5 symptoms), recurrent brief depression (less than 2 weeks), as well as patients who fail to satisfy criterion A of the major depression even though they may have more than 5 symptoms for more than 2 weeks.
Cauch-Dudek et al. 1998 (8) mention that the fatigue in this population was not related to the severity of the liver disease. Because we could not diagnose depression in most patients with the BDI ≥ 10, we conclude that depression did not cause the fatigue as well. Another explanation for the fatigue in this population could be a high prevalence of sleeping disorders. We tested the three questions of the HRSD concerning the sleeping disorders in the two groups BDI < and ≥ 10. There was no statistically significant difference in the presence of sleeping disorders in the two groups (BDI < and ≥ 10), which might indicate that fatigue is not caused by a sleeping disorder.
We conclude that in our study the prevalence of depression in patient with PBC and PSC is not as high as was reported in former studies. We found a prevalence of 4.9%. The high prevalence in other studies was likely caused by the screening instruments used, and by not performing a structured psychiatric interview. Probably a subsyndromal depression is present in a large proportion of patients with these chronic somatic diseases, which resembles a depression, but does not satisfy all the DSM-IV features necessary to diagnose a major depression.

References

1. Weissman MM, Myers JK, Thompson WD. Depression and its treatment in a US urban community 1975-1976. Arch. Gen. Psychiatry 1981;38:417-21.
2. Robins LN, Helzer JE, Weissman MM, et al. Lifetime prevalence of specific psychiatric disorders in three sites. Arch Gen Psychiatry 1984;41:949-58.
3. Offord DR, Boyle MH, Campbell D, et al. One-year prevalence of psychiatric disorder in Ontarians 15 to 64 years of age. Can. J. Psychiatry 1996;41:559-63.
4. Katon W, Sullivan MD. Depression and chronic medical illness. J. Clin. Psychiatry 1990;51:suppl 3-11.
5. Wells KB, Golding JM, Burnham MA. Psychiatric in a sample of the general population with and without chronic medical conditions. Am. J. Psychiatry 1988;145:976-81.
6. Van Hoogstraten HJF, Hansen BE, van Buuren HR, et al. Prognostic factors and long-term effects of ursodeoxycholic acid on liver biochemical parameters in patients with primary biliary cirrhosis. J. Hepatology 1999;31:256-62.
7. Broome U, Olsson R, Loof L, et al. Natural history and prognostic factors in 305 Swedish patients with primary sclerosing cholangitis. Gut 1996;38:610-5.
8. Cauch-Dudek K, Abbey S, Stewart DE, et al. Fatigue in primary biliary cirrhosis. Gut 1998;43:705-10.
9. Huet P, Deslauriers J, Tran A, et al. Impact of fatigue on the quality of life in patients with primary biliary cirrhosis. American Journal of Gastroenterology 2000;95:760-7.
10. Hamilton M. A rating scale for depression (HRS). J of Neurology, Neurosurgery and Psychiatry 1960;23:56-61.
11. Radlo LS. CES-D scale: a self-report depression scale research in the general population. Psychol. Meas. 1977;1:385-401
12. Beck AT, Ward CH, Mendelson M. An inventory for measuring depression. Arch. Gen. Psychiatry 1961;6:561-71.
13. Spitzer RL, Endicott J. Schedule for Affective Disorders and Schizophrenia (SADS), 3rd edition New York, Biometric Research 1981, New York State Psychiatric Institute.
14. Spitzer RL, Endicott J, Robins E. Research Diagnostic Criteria (RDC): Rationale and reliability. Arch. Gen. Psychiatry 1978;35:773-82
15. Beck AT, Rush AJ, Shaw BF, et al. Cognitive therapy of depression. 1979 New York: Guilford.
16. Steer RA, Beck AT, Brown G, et al. Self-reported depressive symptoms that differentiate recurrent-episode major depression from dysthymic disorders. J. Clin. Psychology 1987;43:246-50.
17. Sadek N, Bona J. Subsyndromal symptomatic depression: a new concept. Depression and Anxiety 2000;12:30-9.

Table 1: Baseline characteristics

Male/Female	25/48
Age median (range)	54 (23 - 79)
Age mean	53.80
PBC/PSC	46/27
Duration illness mean yrs (range)	10.0 (0-25.45)

Table 2: DSM-IV criteria in the different groups

	Depressed mood	Loss of interest	Loss of weight	Insomnia	Agitation or retardation	Fatigue	Worthlessness	Loss of concentration	Suicidal ideation
BDI ≥ 10 N = 25	7	3	8	11	1	18	3	7	3
BDI < 10 N = 9	0	0	0	1	0	2	0	0	0
P-value	0.151	0.549	0.077	0.113	1.0	**0.017**	0.549	0.151	0.549

Table 3: Sleeping disorders (Hamilton Rating Scale for Depression question 4,5 and 6)

	HRSD question 4	HRSD question 5	HRSD question 6
BDI ≥ 10	9	7	6
BDI < 10	1	0	0
P-value	0.23	0.15	0.16

8

Beck Depression Inventory in patients with primary biliary cirrhosis and primary sclerosing cholangitis

Erik van Os[1], Walter W. van den Broek[1], Paul G. H. Mulder[3] , Pieter. C. J. ter Borg[2], Henk R. van Buuren[2], Jan A. Bruijn[1]

[1] Department of Psychiatry, [2] Department of Gastroenterology and Hepatology, [3] Department of Epidemiology & Biostatistics, Erasmus MC, Rotterdam, The Netherlands

Submitted

Summary

The Beck Depression Inventory (BDI) is the most widely used screening instrument for depression. The aim is to study the validity of the BDI as a screening tool for depression in patients with a chronic illness. We studied a population of the Department of Gastroenterology. These patients had primary biliary cirrhosis (PBC) or primary sclerosing cholangitis (PSC). Patients with PBC and PSC completed the BDI. Patients with a score higher than the cutoff score were interviewed with a structured psychiatric interview. Of the patients with a score lower than the cutoff score 50% were randomized and were interviewed with a structured psychiatric interview as well. Seventy-three patients were included. 41.3% had depressive symptoms according to the BDI (cutoff score > 10). Only 4.9% had a depression according to the structured psychiatric interview.

In conclusion, the BDI is not an effective screening tool for depression in this population. The prevalence of depression using the BDI is ten times higher compared to the prevalence using a structured psychiatric interview.

Introduction

The Beck Depression Inventory (BDI) is the most widely used self-rating scale for depression. Although it was developed to measure the intensity of depression, it is now also used as a screening instrument to detect depression in clinical practice and research projects (1). It is generally considered to be one of the best screening instruments for assessing depression (2).

The BDI (3) is a 21-item self-report depression scale. Scoring is completed by summing the severity of individual symptoms rated from 0 to 3; overall scores ranges from 0 to 63. The BDI focuses on the following dimensions: (a) depressive mood, anhedonia, and inhibition of activities, (b) negative self-concept, and (c) somatic/ vegetative features (4).

Beck et al. (1) claim that the BDI was derived from clinical observations. The items were chosen to assess the intensity of depression on the basis of the main symptoms of the depression. Moran and Lambert (5) discussed this issue. They mentioned that the BDI only reflects 6 of the 9 DSM-III symptoms. Two are only partially addressed (sleep disturbances and eating behavior) and one is not included (agitation).

If we wish to adopt this questionnaire as a screening instrument for research use, we should try to verify its criterion validity using an appropriate methodological design. Therefore it is necessary to compare the diagnostic results using the BDI with the DSM-IV diagnoses obtained by performing a reliable structured psychiatric interview for clinical evaluation. Former studies already tried to validate the BDI. Richter et al. (6) conclude in their review on the validity of the BDI, that the BDI measures the intensity of a depression by means of the main symptoms of the depression, but that the BDI is not reliable to determine the existence of a depressive nosological disorder nor to objectify it.

Schotte et al. (7) also conclude in their study on the validity of the Beck in a depressive population, that the BDI score is not a valid categorical diagnostic indicator for clinical depression. They further emphasize that the appropriateness of the various BDI cutoff score ranges depends on the nature of the sample.

We studied a population with a chronic cholestatic liver disease (primary biliary cirrhosis or primary sclerosing cholangitis). Former studies in patients with PBC found a prevalence of depression of 20-45% (8, 9). However both studies did not perform a structured psychiatric interview to diagnose a depression. The first (8) used the Hamilton Rating Scale for Depression (HRSD) (10) and the Center for Epidemiologic Studies Depression Rating Scale (CES-D) (11) to screen for depression. They used a cutoff score of 18 for the HRSD and a

cutoff score of 16 for the CES-D. The second (9) used the Beck Depression Inventory (BDI) (12) to screen for depression. They used a cutoff score of 10.

The purpose of the present study is to validate the BDI as a screening instrument for depression in patients with PBC or PSC.

Methods

Subjects

Data for the study were obtained from a study by the department of Gastroenterology and Hepatology of the Erasmus MC: Fluvoxamine treatment in depression related to primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC); a double-blind placebo-controlled trial. The rationale for this study was the suggested higher incidence of depression in patients with PBC and PSC compared to the prevalence in the normal population.

A total number of 73 patients (25 men and 48 women) were included in the study. All subjects were outpatients with a diagnosis of PBC or PSC. Inclusion criteria were a diagnosis of PBC or PSC, age > 18 years and informed consent. Exclusion criteria were patients with ursodeoxycholic acid (UDCA) or antipruritic treatment, initiated or changed less than three months prior to entry, severe psychiatric disorder (other than depression), incomplete understanding of the Dutch language, pregnancy or lack of adequate contraception in women with childbearing potential, lactation, Child-Pugh score ≥ 6, other study medication in the preceding three months, use of benzodiazepines which could not be decreased to less than 3 mg lorazepam or equivalent per day.

In advance of the visit the patients were asked to self-assess the Beck questionnaire (BDI). This was sent to them before their visit to the out-patient clinic. The research physician of the Department of Gastroenterology counted the Beck score and consulted WWB or EO for the structured psychiatric interview. If the BDI score was higher than 10, the patient was interviewed with a structured psychiatric interview (SADS). The group of patients with a BDI lower than 10 were randomized and 50% were interviewed with a structured psychiatric interview as well. Raters were blind with respect to the BDI score of the patients.

In addition to the SADS the Hamilton Rating Scale for Depression (HRSD) (10) was assessed.

Reference standard

The reference standard in this trial is the section which relates to depression of the Schedule for Affective Disorders and Schizophrenia (SADS) (13). This structured psychiatric interview was administered in the screening period, before the start of medication to obtain Research Diagnostic Criteria (14) and to confirm the DSM-IV diagnosis.

Test methods

The structured psychiatric interview was performed by a trained psychiatrist and/or a trained resident from the department of psychiatry. The first 15 interviews were performed by both as inter-rater sessions.

The cutoff score for the Beck was 10, which is generally considered as a good indication for depression (12).

Results

A total of 73 patients were included. Baseline characteristics are shown in table 1. 43 patients had a BDI score lower than the cutoff score (< 10) and 30 a BDI score higher than the cutoff score (≥ 10). On the basis of a randomization scheme 9 patients of the 43 patients with a BDI < 10 were selected to be interviewed with the SADS. The other patients of the random half of the 43 refused a psychiatric interview. In the group of patients with a BDI score lower than 10 no SADS interview resulted in the diagnosis depressive disorder according to the DSM-IV criteria. In the group of patients with a BDI higher than 10, 5 of the 30 patients refused a psychiatric interview. The other 25 were all interviewed using the SADS. In 3 of the 25 patients the SADS interview resulted in the diagnosis depressive disorder according to the DSM-IV criteria. (See Table 2) No depression was present in all 9 subjects interviewed in the BDI < 10 group.

According to these results the sensitivity of the BDI is 100% (95% CI: 29-100%) and the specificity is 61% (95% CI: 44-79%). These percentages are adjusted by correcting towards the BDI score distribution as observed in the total group of 73.

Discussion

Although the BDI was not developed to be a screening tool for depression, it has frequently been used for this purpose. Especially in the patients with a chronic medical illness, it has

often been used to confirm the suggested higher prevalence of depression. We aimed to validate the BDI for the screening for depression in patients with a chronic cholestatic liver disease: primary biliary cirrhosis or primary sclerosing cholangitis. We found that the BDI has a very high number of false-positives. We found that 31 patients had a BDI higher than 10. According to the BDI score, this would mean a prevalence of depression of 41.3%. This resembles the prevalence found in the former studies (8, 9). We conclude that in our study the prevalence of depression in patients with PBC and PSC (using the SADS interview and diagnosing the depressive disorder according to the DSM-IV criteria) was 4.9%. The limitation of the study is the high number of patients with a BDI lower than 10, that refused a structured psychiatric interview. We planned to interview 50% of the patients with a BDI lower than 10, but we only interviewed 21%.

We conclude that the BDI is not an adequate screening tool for depression. According to the literature (6,7) the BDI is only reliable to measure the intensity of an otherwise diagnosed depressive disorder. We think some symptoms seem to be part of a depression but actually are part of the chronic medical illness.

References

1. Beck, A. T., Steer, R. A., Garbin, M. G., 1988. Psychometric properties of the Beck Depression Inventory: 25 years of evaluation. Clin. Psychol. Review 8, 77-100.
2. McDowell, I., Newell, C., 1996. Measuring helath: a guide to rating scales and questionnaires. Oxford University Press, New York.
3. Beck, A. T., Rush, A. J., Shaw, B. F., et al. 1979. Cognitive therapy of depression. New York: Guilford.
4. Steer, R. A., Beck, A. T., Brown, G., et al. 1987. Self-reported depressive symptoms that differentiate recurrent-episode major depression from dysthymic disorders. J. Clin. Psychology 43, 246-50.
5. Moran, P. W., Lambert, M. J., 1983. A review of current assessment tools for monitoring changes in depression; in Lambert, M. S., Christensen, E. R., DeJullio, S. S. (eds): The Assessment of Psychotherapy Outcome. New York, Wiley, pp. 263-303.
6. Richter, P., Werner, J., Heerlein, A., et al. 1998. On the validity of the Beck Depression Inventory, a review. Psychopathology 31, 160-8.
7. Schotte, C. K. W., Maes, M., Cluydts, R., et al. 1997. Construct validity of the Beck Depression Inventory in a depressive population. J Affect. Disorders 46, 115-25.
8. Cauch-Dudek, K., Abbey, S., Stewart, D. E., et al. 1998. Fatigue in primary biliary cirrhosis. Gut 43, 705-10.
9. Huet, P., Deslauriers, J., Tran, A., et al. 2000. Impact of fatigue on the quality of life in patients with primary biliary cirrhosis. American Journal of Gastroenterology 95, 760-7.
10. Hamilton, M., 1960. A rating scale for depression (HRS). J of Neurology, Neurosurgery and Psychiatry 23, 56-61.
11. Radlo, L. S., 1977. CES-D scale: a self-report depression scale research in the general population. Psychol. Meas. 1, 385-401
12. Beck, A.T., Ward, C. H., Mendelson, M., 1961. An inventory for measuring depression. Arch. Gen. Psychiatry 6, 561-71.
13. Spitzer, R.L., Endicott, J., 1981. Schedule for Affective Disorders and Schizophrenia (SADS), 3rd edition New York, Biometric Research, New York State Psychiatric Institute.
14. Spitzer, R. L., Endicott, J., Robins, E., 1978. Research Diagnostic Criteria (RDC): Rationale and reliability. Arch. Gen. Psychiatry 35, 773-82.

Table 1: Baseline characteristics

Male/Female	25/48
Age median (range)	54 (23 - 79)
Age mean	53.80
PBC/PSC	46/27
Duration mean yrs (range)	10.0 (0-25)

Table 2: Results

	SADS DSM-IV depression	SADS DSM-IV no depression	Total
Beck ≥ 10	3	22	25 (30)
Beck < 10	0	9	9 (43)
Total	3	31	34 (73)

9

Oxidative stress in primary biliary cirrhosis and primary sclerosing cholangitis is not associated with fatigue or quality of life

Pieter C.J. ter Borg[1], Kees Schoonderwoerd[2], Willem Sluiter[2], Durk Fekkes[3], Henk R. van Buuren[1]

[1]Department of Gastroenterology and Hepatology, [2]Department of Biochemistry, [3]Department of Psychiatry, Erasmus MC, The Netherlands

Submitted

Summary

In primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) fatigue is a major clinical problem. Oxidative stress has been implicated in the development of fatigue in several non-hepatological conditions but for PBC and PSC no data are available. This study aimed to investigate the association between oxidative stress, fatigue and quality of life in patients with these diseases.

Plasma concentrations of glutathione, neopterin, vitamin A and vitamin E were determined in plasma of patients with PBC (n=45), PSC (n=27) and healthy controls. Fatigue and quality of life were quantified using the Fisk Fatigue Severity Scale, a visual analogue scale and the SF-36.

There was a significant increase in neopterin concentrations, and a decrease in vitamin A, vitamin E and glutathione concentrations in patients with PBC and PSC compared to controls. No significant correlations between oxidative stress and disease activity or severity, fatigue or quality of life scores were found.

In patients with PBC and PSC, oxidative stress is increased compared to controls. However, we did not find any associations between oxidative stress, fatigue and quality of life. Therefore it seems unlikely that oxidative stress is implicated in the pathophysiology of fatigue in these disorders.

Introduction

Primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) are chronic cholestatic liver diseases characterized by a usually slowly progressive course (1, 2). It is now recognized that in many patients fatigue is a major and chronic symptom that may adversely affect quality of life (3-5). Fatigue has not been found to correlate with the severity of the disease, and the pathophysiology is unknown (3, 4, 6). One of the mechanisms that might be responsible for the development of fatigue is oxidative stress. Several previous studies have demonstrated that oxidative stress is increased in animal models of cholestasis as well as in human cholestasis, whereas in other conditions oxidative stress has been associated with the occurrence of fatigue (7-12).

These data led us to hypothesize that oxidative stress in patients with chronic cholestatic liver diseases might be involved in the development of fatigue. Thus, we aimed to study the relation between oxidative stress and fatigue and quality of life in patients with PBC and PSC.

Patients and Methods

The study was approved by our institution's medical ethics committee and written informed consent was obtained from each patient. All consecutive patients with a diagnosis of PBC or PSC visiting the hepatology outpatient clinic of the Erasmus Medical Center between October 2001 and June 2002 were invited to participate. Exclusion criteria were an age of less than 18 years and failure to obtain written informed consent. Patients were asked to invite an age and sex matched healthy control to participate in the study. Fatigue was quantified using a visual analogue scale (VAS) and the Fisk Fatigue Severity Scale (FFSS). The VAS consisted of a ten cm line with on one end no fatigue at all and at the other end the worst possible fatigue, on which the patient was asked to indicate the severity of fatigue. The FFSS has been previously validated for use in patients with PBC, and quantifies the physical, social and cognitive impact of fatigue (6, 13). Quality of life was quantified using the SF-36 questionnaire (14). The total serum bilirubin concentration and the serum activities of alkaline phosphatase (AP) and aspartate aminotransferase (AST) were measured as markers of disease activity and severity. The presence of cirrhosis was determined on the basis of histological and, if not available, clinical criteria.

Laboratory techniques

The following markers of oxidative stress were measured: total glutathione, neopterin, vitamin A and vitamin E. Glutathione can be oxidized as a result of oxidative stress, resulting in decreased concentrations (15). Vitamin A and vitamin E are fat-soluble vitamins with anti-oxidant properties (16, 17). Neopterin is a pteridine known to be released from macrophages and monocytes at increased rates in cellular immune reactions. Particularly, increased amounts of neopterin are produced by human monocytes/macrophages upon stimulation by the cytokine interferon-gamma (18). High neopterin production is also associated with increased production of reactive oxygen species and with low serum concentrations of antioxidants. Therefore, neopterin can also be regarded as a marker for oxidative stress due to activation of the cellular immune system (19).

Total neopterin in plasma was measured - after acid oxidation of the reduced forms of both pteridines - by high-performance liquid chromatography employing fluorescence detection as described earlier (20). Glutathione was measured according to Tietze (21) and modified by Adams et al. (22). Blood plasma was immediately acidified by the addition of sulphosalicylic acid and kept frozen at –80ºC. After neutralization total glutathione was determined with 5,5'-dithio-bis-2-nitrobenzoic acid. Concentrations of vitamin A and vitamin E (n=200) and neopterin (n=51) determined in healthy persons used as a laboratory reference group were used as controls. Within this group, no relation with age or sex was found. Since no validated reference values were available for glutathione, concentrations determined in a group of 18 sex and age-matched controls were used as controls.

Statistics

Differences in baseline characteristics between groups were tested using Student's t-test and the χ^2 test. Correlations were tested using Pearson's correlation method. The normality of distributions was assessed visually using histograms, and non-parametric tests would be used when appropriate. The relations between oxidative stress and fatigue scores were tested by calculating correlation coefficients for VAS and FFSS domain scores and the markers of oxidative stress. A two-sided p-value <0.05 was considered statistically significant. Statistical analyses were performed using SPSS (Version 9.0, SPSS Inc, Chicago, IL, U.S.A).

Results

Patient characteristics

Demographic data and serum markers of disease activity and severity of the patients with PBC and PSC are shown in table 1. As was expected because of the unbalanced sex distribution characteristic of these diseases, the majority of patients with PBC were female and the majority of patients with PSC were male. All patients were treated with ursodeoxycholic acid in a dose of 13-15 mg. per kg and none were using antioxidant drugs. There were no significant differences between patients with PBC and PSC with respect to the total serum bilirubin concentrations and the serum activities of alkaline phosphatase, AST and ALT. The frequency of documented cirrhosis was higher in patients with PBC compared to PSC ($p=0.05$). The median age of healthy controls was 42.5 years (range 31-74); five were male and 13 female.

Markers of oxidative stress in patients and controls

Table 2 shows the plasma concentrations of the markers of oxidative stress for patients with PBC, PSC and controls. Differences between patients with PBC and PSC for reduced glutathione, vitamin A and vitamin E concentrations were not statistically significant. Neopterin concentrations were significantly higher in patients with PBC than in patients with PSC ($p<0.001$). Compared to healthy controls, vitamin A, vitamin E and glutathione concentrations were significantly decreased in patients with PBC as well as in patients with PSC, whereas the neopterin concentrations were significantly increased, all reflecting increased oxidative stress in patients versus controls.

Oxidative stress and markers of disease activity and severity

There were no significant correlations between the total serum bilirubin concentration, the serum activities of alkaline phosphatase and aspartate aminotransferase on the one hand and the markers of oxidative stress on the other hand, both for the entire patient series and for the subgroups of patients with PBC and PSC.

Oxidative stress, fatigue and quality of life

There were no significant associations between the markers of oxidative stress measured in this study (glutathione, the vitamins A, vitamin E and neopterin) and fatigue as measured by both the visual analogue score for fatigue and the total and 3 domain FISS scores. The absence of any relation between the marker of oxidative stress neopterin and the visual analogue score for fatigue is illustrated in the figure. In addition to the fatigue scores, the SF-36 scores were not significantly associated with oxidative stress.

Discussion

In the present study we confirmed previous findings that oxidative stress is significantly increased in patients with PBC as well as in patients with PSC. The increased plasma levels of neopterin suggest that this may be secondary to chronic immune activation. However, we were unable to demonstrate a relation between oxidative stress and fatigue or quality of life. The most likely explanation seems a true absence of such a relation. This is consistent with the lack of an effect of antioxidant treatment on fatigue in PBC in a recent randomized controlled trial (23).

Fatigue is a significant and occasionally invalidating problem in patients with PBC and PSC that has been studied with increasing frequency in recent years (3, 4, 6, 24). Up till now, no specific pathogenic factors have been identified, although a small recent study suggested a relation with brain alterations, possibly as a result of a disturbed manganese homeostasis in patients with PBC (25). Especially, no relation has been found with laboratory parameters of disease activity or severity, or histological stage (3, 4, 6). In addition, no effective medical treatment for fatigue associated with PBC and PSC is available. Numerous studies evaluating medical treatments, including ursodeoxycholic acid, corticosteroids, immunosuppressants and anti-inflammatory agents, have failed to show beneficial effects on fatigue.

Oxidative stress can be defined as the occurrence of damage caused by an increased production of reactive oxygen species or other free radicals, or by a decrease in antioxidant capacity (26). Increased oxidative stress may be secondary to chronic inflammatory responses, as occurs in PBC and PSC, and a role of oxidative stress has been shown in various human diseases including acute pancreatitis (27), alcoholic liver disease (28), non-alcoholic steatohepatitis (29), Parkinson's disease (15), chronic obstructive pulmonary disease (30) and depression (31). Several previous studies have demonstrated a status of increased oxidative stress in patients with cholestatic liver diseases. Plasma levels of various antioxidants were

found to be markedly decreased in patients with PBC and PSC (7) and increased levels of serum and hepatic manganese superoxide dismutase, an enzyme protecting against oxidative stress, have been reported in PBC (32). Recently it was reported that in patients with PBC, plasma glutathione, vitamin A and total antioxidant concentrations were significantly decreased, which was confirmed in the present study (10). Finally, it has been shown that oxidative DNA damage occurs in these diseases, which might be responsible for the increased risk of hepatocellular and bile duct carcinomas (11, 33). In animal models of chronic cholestasis, oxidative stress has been more extensively studied. In bile duct ligated rats, oxidative stress not only occurred in the liver, but also in plasma, kidney, brain and heart, indicating that oxidative stress is a systemic phenomenon (34). In similar rat models, antioxidant treatment reduced hepatic damage and improved behavioral changes induced by cholestasis (35-37).

Our results must be considered in light of several caveats. To our knowledge no previous attempts have been made to find a relation between oxidative stress and fatigue. Therefore, the power of the present study may not have been sufficient to detect a small but possibly relevant association, resulting in a type I statistical error.

Further, there is no universal marker of oxidative stress and although we studied several different markers of oxidative stress the possibility of a relation between other markers of oxidative stress and fatigue cannot be excluded. In this context it should also be noted that we measured plasma markers of oxidative stress, whereas fatigue in these diseases might be the result of central (central nervous system) or peripheral (muscles) mechanisms (24).

In conclusion, we confirmed and extended previous findings on the occurrence of increased oxidative stress in patients with PBC and PSC, but we found no evidence for a role of oxidative stress in relation to fatigue or quality of life.

References

1. Angulo P, Lindor KD. Primary sclerosing cholangitis. Hepatology 1999;30(1):325-32.
2. Neuberger J. Primary biliary cirrhosis. Lancet 1997;350(9081):875-9.
3. Huet PM, Deslauriers J, Tran A, Faucher C, Charbonneau J. Impact of fatigue on the quality of life of patients with primary biliary cirrhosis. Am J Gastroenterol 2000;95(3):760-7.
4. Cauch-Dudek K, Abbey S, Stewart DE, Heathcote EJ. Fatigue in primary biliary cirrhosis. Gut 1998;43:705-10.
5. Wiesner RH, LaRusso NF, Ludwig J, Dickson ER. Comparison of the clinicopathologic features of primary sclerosing cholangitis and primary biliary cirrhosis. Gastroenterology 1985;88(1 Pt 1):108-14.
6. Prince MI, James OF, Holland NP, Jones DE. Validation of a fatigue impact score in primary biliary cirrhosis: towards a standard for clinical and trial use. J Hepatol 2000;32(3):368-73.
7. Floreani A, Baragiotta A, Martines D, Naccarato R, D'Odorico A. Plasma antioxidant levels in chronic cholestatic liver diseases. Aliment Pharmacol Ther 2000;14(3):353-8.
8. Logan AC, Wong C. Chronic fatigue syndrome: oxidative stress and dietary modifications. Altern Med Rev 2001;6(5):450-9.
9. Vecchiet J, Cipollone F, Falasca K, Mezzetti A, Pizzigallo E, Bucciarelli T, et al. Relationship between musculoskeletal symptoms and blood markers of oxidative stress in patients with chronic fatigue syndrome. Neurosci Lett 2003;335(3):151-4.
10. Aboutwerat A, Pemberton PW, Smith A, Burrows PC, McMahon RF, Jain SK, et al. Oxidant stress is a significant feature of primary biliary cirrhosis. Biochim Biophys Acta 2003;1637(2):142-50.
11. Kitada T, Seki S, Iwai S, Yamada T, Sakaguchi H, Wakasa K. In situ detection of oxidative DNA damage, 8-hydroxydeoxyguanosine, in chronic human liver disease. J Hepatol 2001;35(5):613-8.
12. Engin A, Altan N. Effects of obstructive jaundice on the antioxidative capacity of human red blood cells. Haematologia (Budap) 2000;30(2):91-6.
13. Fisk JD, Ritvo PG, Ross L, Haase DA, Marrie TJ, Schlech WF. Measuring the functional impact of fatigue: initial validation of the fatigue impact scale. Clin Infect Dis 1994;18 Suppl 1:S79-83.
14. Ware JE, Jr., Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care 1992;30(6):473-83.
15. Mytilineou C, Kramer BC, Yabut JA. Glutathione depletion and oxidative stress. Parkinsonism Relat Disord 2002;8(6):385-7.
16. Meydani M, Meisler JG. A closer look at vitamin E. Can this antioxidant prevent chronic diseases? Postgrad Med 1997;102(2):199-201, 206-7.
17. Palace VP, Khaper N, Qin Q, Singal PK. Antioxidant potentials of vitamin A and carotenoids and their relevance to heart disease. Free Radic Biol Med 1999;26(5-6):746-61.
18. Murr C, Widner B, Wirleitner B, Fuchs D. Neopterin as a marker for immune system activation. Curr Drug Metab 2002;3(2):175-87.
19. Murr C, Fuith LC, Widner B, Wirleitner B, Baier-Bitterlich G, Fuchs D. Increased neopterin concentrations in patients with cancer: indicator of oxidative stress? Anticancer Res 1999;19(3A):1721-8.

20. Hoekstra R, van den Broek WW, Fekkes D, Bruijn JA, Mulder PG, Pepplinkhuizen L. Effect of electroconvulsive therapy on biopterin and large neutral amino acids in severe, medication-resistant depression. Psychiatry Res 2001;103(2-3):115-23.
21. Tietze F. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. Anal Biochem 1969;27(3):502-22.
22. Adams JD, Jr., Lauterburg BH, Mitchell JR. Plasma glutathione and glutathione disulfide in the rat: regulation and response to oxidative stress. J Pharmacol Exp Ther 1983;227(3):749-54.
23. Prince MI, Mitchison HC, Ashley D, Burke DA, Edwards N, Bramble MG, et al. Oral antioxidant supplementation for fatigue associated with primary biliary cirrhosis: results of a multicentre, randomized, placebo-controlled, cross-over trial. Aliment Pharmacol Ther 2003;17(1):137-43.
24. Goldblatt J, James OF, Jones DE. Grip strength and subjective fatigue in patients with primary biliary cirrhosis. Jama 2001;285(17):2196-7.
25. Forton DM, Patel N, Prince M, Oatridge A, Hamilton G, Goldblatt J, et al. Fatigue and primary biliary cirrhosis: association of globus pallidus magnetisation transfer ratio measurements with fatigue severity and blood manganese levels. Gut 2004;53(4):587-92.
26. Parola M, Robino G. Oxidative stress-related molecules and liver fibrosis. J Hepatol 2001;35:297-306.
27. Tsai K, Wang SS, Chen TS, Kong CW, Chang FY, Lee SD, et al. Oxidative stress: an important phenomenon with pathogenetic significance in the progression of acute pancreatitis. Gut 1998;42(6):850-5.
28. Bray TM. Dietary antioxidants and assessment of oxidative stress. Nutrition 2000;16(7-8):578-81.
29. Sanyal AJ, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, et al. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. Gastroenterology 2001;120(5):1183-92.
30. Mador MJ, Bozkanat E. Skeletal muscle dysfunction in chronic obstructive pulmonary disease. Respir Res 2001;2(4):216-24.
31. Maes M, De Vos N, Pioli R, Demedts P, Wauters A, Neels H, et al. Lower serum vitamin E concentrations in major depression. Another marker of lowered antioxidant defenses in that illness. J Affect Disord 2000;58(3):241-6.
32. Ono M, Sekiya C, Ohhira M, Namiki M, Endo Y, Suzuki K, et al. Elevated level of serum Mn-superoxide dismutase in patients with primary biliary cirrhosis: possible involvement of free radicals in the pathogenesis in primary biliary cirrhosis. J Lab Clin Med 1991;118(5):476-83.
33. Jaiswal M, LaRusso NF, Shapiro RA, Billiar TR, Gores GJ. Nitric oxide-mediated inhibition of DNA repair potentiates oxidative DNA damage in cholangiocytes. Gastroenterology 2001;120(1):190-9.
34. Ljubuncic P, Tanne Z, Bomzon A. Evidence of a systemic phenomenon for oxidative stress in cholestatic liver disease. Gut 2000;47(5):710-716.
35. Swain MG, Le T. Chronic cholestasis in rats induces anhedonia and a loss of social interest. Hepatology 1998;28(1):6-10.

36. Pastor A, Collado PS, Almar M, Gonzalez-Gallego J. Antioxidant enzyme status in biliary obstructed rats: effects of N-acetylcysteine. J Hepatol 1997;27(2):363-70.

37. Sokol RJ, McKim JM, Jr., Goff MC, Ruyle SZ, Devereaux MW, Han D, et al. Vitamin E reduces oxidant injury to mitochondria and the hepatotoxicity of taurochenodeoxycholic acid in the rat. Gastroenterology 1998;114(1):164-74.

Table 1: Patient characteristics

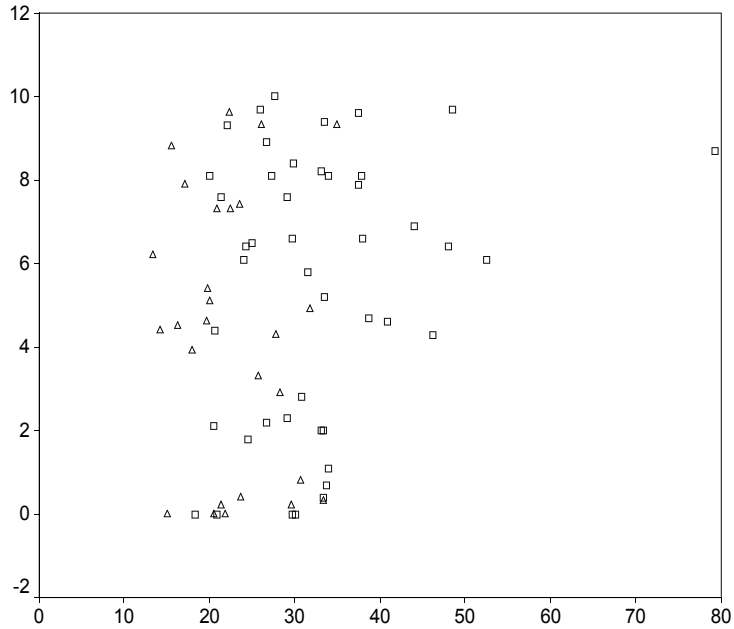
	PBC (n=45)	PSC (n=27)
Age (mean, range in years)	58 (34-78)	45 (23-68)
Sex (male / female)	4/45	19/8
Fatigue (yes / no)	32/15	15/12
Cirrhosis (yes / no)	12 / 33	2 / 25
Total serum bilirubin (mean, range in μmol/l)	17 (4-98)	16 (6-37)
Serum ALT activity (mean, range in U/l)	58 (17-484)	77 (10-662)
Serum AST activity (mean, range in U/l)	50 (21-241)	56 (18-338)
Serum Alkaline Phosphatase (mean, range in U/l)	187 (68-441)	238 (53-1141)

Normal: bilirubin <17 μmol/l, A.Ph. < 120 U/l, ALT <41 U/l, AST <37 U/l.

Table 2: Plasma markers of oxidative stress in PBC, PSC and control subjects

Plasma marker	PBC (n=45,SD)	p vs. PSC	p vs. controls	PSC (n=27, SD)	p vs. controls	controls
Glutathione (nmol/l)	213 (311)	0.14	0.0001	410 (659)	0.03	692 (435)
Vitamin A (μmol/l)	1.61 (0.62)	0.33	<0.001	1.47 (0.45)	<0.001	2.13 (0.44)
Vitamin E (μmol/l)	25.6 (5.9)	0.50	0.04	24.6 (5.7)	0.008	27.5 (4.75)
Neopterin (nmol/l)	32.6 (10.8)	<0.001	<0.0001	22.7 (6.0)	0.003	19.3 (4.7)

Figure



Relation between the plasma concentrations of neopterin (x-axis, nmol/L) and fatigue scores (visual analogue scale, y-axis) in patients with PBC (squares) and PSC (triangles). No significant relation was found (p=0.11).

10

Fluvoxamine for fatigue in primary biliary cirrhosis and primary sclerosing cholangitis: a randomized controlled trial [ISRCTN88246634]

Pieter C.J. ter Borg[1], Erik van Os[2], Walter W. van den Broek[2], Bettina E. Hansen[1,3], Henk R. van Buuren[1]

[1]Department of Gastroenterology and Hepatology, [2]Department of Psychiatry, [3]Department of Epidemiology and Biostatistics, Erasmus MC, Rotterdam, The Netherlands

BMC Gastroenterology 2004; 4: 13.

Summary

Fatigue is a major clinical problem in many patients with primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC). An effective treatment has not been defined. Recently, a large proportion of patients with these diseases was found to have symptoms of depression. Because fatigue is a frequent symptom of depression and there is some evidence that treatment with an antidepressant improves fatigue in patients with fibromyalgia, we hypothesized that the antidepressant fluvoxamine might improve fatigue related to PBC and PSC.

Fatigued patients were randomized to receive fluvoxamine (75 mg BID) or placebo for a six-week period. Fatigue and quality of life were quantified using a visual analogue scale, the Fisk Fatigue Severity Scale, the Multidimensional Fatigue Inventory and the SF-36.

Seventeen and 16 patients were allocated to fluvoxamine and placebo, respectively. There was no statistically significant beneficial effect of fluvoxamine on fatigue or quality of life. The median VAS scores in the fluvoxamine and placebo groups were 7.40 and 7.45 at day 0, 6.9 and 7.15 at day 14, 7.45 and 7.65 at day 42 and 7.8 and 8.0 four weeks after treatment discontinuation.

We found no evidence for a beneficial effect of fluvoxamine on fatigue in these patients with cholestatic liver disease and severe chronic fatigue.

Introduction

Primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) are chronic cholestatic liver diseases with a relatively favorable prognosis for most patients (1, 2). Fatigue is now recognized as a major clinical problem in PBC, with a reported prevalence of up to 85% (3). Although fatigue has not been studied extensively in PSC, it is a problem in many of these patients as well (4). There have been no reports of drugs or other treatment modalities with a beneficial effect on fatigue associated with these diseases, and although the widely used drug ursodeoxycholic acid improves the biochemical abnormalities in these diseases, an effect on fatigue has not been shown (5, 6).

Recent studies suggest that 45-72% of patients may have a depressive disorder (3, 5). Fatigue is a frequent symptom of depression. We hypothesized that common mechanisms may be involved in the aetiology of both fatigue and depression or that fatigue could be a symptom of a depressive disorder in PBC and PSC. If this would be true, antidepressive drugs could have a beneficial effect on fatigue and potentially also on overall quality of life. Thus, the present study aimed to evaluate the effects of an antidepressant on fatigue and quality of life in PBC and PSC. The antidepressant fluvoxamine (a selective serotonin re-uptake inhibitor, SSRI) was chosen because of several reasons. First, fluvoxamine is one of the oldest SSRI's, and the risk of unexpected side-effects was therefore low. Second, there was extensive local experience with this particular drug. Third, there is a documented correlation between serum concentrations and effects for fluvoxamine, which could be used in future studies using this drug.

Methods

Participants

All patients visiting the outpatient clinic of our hospital with a diagnosis of PBC or PSC and self-reported significant fatigue for which the patient was willing to receive treatment were invited to participate. Patients with a diagnosis of depression or other psychiatric disorder, any change in medication in the past three months, incomplete understanding of the Dutch language, pregnancy, current lactation or lack of adequate contraception, Child-Pugh score of >5 or receiving other study medication in the preceding 6 months were excluded. The Beck Depression Inventory was used to screen for the presence of depression, and in patients with a score > 10 a psychiatric evaluation using the SADS DSM-IV was performed (7). Written

informed consent was obtained in all patients. The institutional review board approved the study.

Treatment

Patients were treated with identical capsules containing 75 mg of fluvoxamine or placebo. They were instructed to take a capsule in the evening before sleeping on the first two days, and to add one capsule in the morning starting on day three and to continue this treatment regimen of 75 mg BID for a total period of six weeks. This dose is a widely used dose for the treatment of depression. Since previous data on the effects on fatigue were lacking, this dose was used in the present study. Patients were instructed to contact the trial coordinators in case of side effects.

Objectives

The aim of the study was to assess the efficacy of fluvoxamine in the treatment of fatigue associated with PBC or PSC.

Outcomes

The primary outcome was an improvement in fatigue, as measured by a visual analogue scale (VAS), the Fisk Fatigue Severity Scale (FFSS) (8) and the Multidimensional Fatigue Inventory (MFI) (9). The FFSS has been previously validated for assessing fatigue in PBC (10). The VAS consisted of a 10-cm line with on one end ‘no fatigue at all during the last month’ and on the other end ‘extreme fatigue during the last month’. Patients were asked to indicate the severity of fatigue by placing a marking on the line. A higher score represents increased fatigue in these three outcome measures. Secondary outcomes were changes in quality of life, assessed by the SF-36 questionnaire (11), changes in pruritus using a VAS and changes in laboratory tests. The VAS for pruritus consisted of a 10-cm line with on one end ‘no pruritus at all during the last month’ and on the other end ‘severe pruritus during the last month’. At entry and after 42 days the following tests were performed: FFSS, MFI, SF-36, VAS for fatigue and pruritus, total serum bilirubin, albumin, gamma-GT, alkaline phosphatase (APh), alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol, immunoglobulin G (IgG), immunoglobulin M (IgM), prothrombin time (PT), antithrombin-III (AT-III), hemoglobin, leukocyte and platelet count. At 14 days, the FFSS,

MFI and serum bilirubin, ALT and APh were repeated. At day 70, the MFI, FFSS and the VAS for fatigue and pruritus were obtained.

Sample Size

A formal power calculation was precluded by absence of data on the efficacy of fluvoxamine in the treatment of fatigue associated with PBC or PSC. In order to detect an effect similar to the expected therapeutic effect of fluvoxamine in depression (25% improvement in the placebo-group and 75% in the fluvoxamine-group), with a power of 80% and assuming a drop-out rate of 5%, it was calculated that 40 patients needed to be recruited (Stata 5.0, Stata Corporation, College Station, Texas, USA).

Randomization

Patients were allocated to a serial number (by authors PB or HB) corresponding to a previously prepared package of capsules. The capsules containing fluvoxamine or placebo were prepared by the hospital pharmacy. The packages were numbered according to a computer generated randomization list prepared by the trial statistician (BH). Thus, both patients and those assessing the outcomes were blinded to group assignment. Patients were stratified for disease (PBC or PSC). Opaque, closed envelopes containing information on the allocated treatment for each patient number were prepared for medical emergencies.

Statistical Methods

Data were analyzed according to both the intention-to-treat and the per-protocol principle. Differences between groups were planned to be tested with the $\chi 2$ and Student's t-test. However, in part because of the lower than expected patient recruitment and the higher than expected drop-out rate, a normal data distribution was not present for most variables. Therefore, it was decided that the Mann-Whitney test would be used for testing differences between groups. Statistical tests were two-tailed. P-values of 0.05 or less were considered statistically significant. In the analyses where the multiple time points were compared, a p-value of <0.05 divided by 3 ($p<0.017$) was considered statistically significant.

Subgroup analyses including only patients with PBC and PSC were performed using the same statistical methods. Statistical analyses were performed using SPSS (Version 9.0, SPSS Inc, Chicago, IL, U.S.A).

Results

Recruitment, allocation and participants flow

Patients were included between October 2001 and June 2002. Five patients (29%) in the fluvoxamine group and 2 patients (13%) in the placebo group discontinued treatment because of side effects ($p=0.40$). All randomized patients were included in the intention-to-treat analysis, and all patients completing the six weeks treatment period were included in the per-protocol analysis (Figure 1).

Baseline characteristics

Of the 82 patients screened for inclusion, 33 met all inclusion and exclusion criteria and gave their informed consent to participate in the trial. Although 18/33 (55%) patients had a Beck Depression Inventory score > 10 points, suggesting the presence of depression, additional psychiatric evaluation (EO and WB) according to the DSM-IV found no evidence for depression in any of these cases. In three patients not suitable for inclusion in the present trial a diagnosis of depression was made according to the DSM-IV criteria. One patient had been diagnosed with depression thirteen years before entry; however psychiatric evaluation found no evidence for depression at the time of entry. Of the 33 patients, 16 were allocated to the placebo group and 17 to the fluvoxamine group.

General baseline data and questionnaire results are shown in tables 1 and 2, respectively. All patients had been fatigued during at least two years; none had renal failure, chronic anemia or serum electrolyte abnormalities. One patient (fluvoxamine group) was receiving treatment for hypothyroidism and was already fatigued before thyroid dysfunction developed and thyroid function was documented to be normal. In all other patients thyroid function was normal. Five patients (4 in the fluvoxamine group) were using β-blockers; in all cases chronic fatigue had been present before initiation of this therapy. Patients in the fluvoxamine group had significantly higher scores in the cognitive and social domains of the FFSS ($p=0.04$ for both domains). Differences for all other baseline variables were non-significant.

Outcomes

Analysis on intention-to-treat basis (17 and 16 patients in the fluvoxamine and placebo group, respectively) yielded no significant differences in response between the fluvoxamine and the placebo group with respect to the primary outcome measures, the fatigue visual analogue score, MFI and FFSS (Table 3). Per-protocol analysis (12 patients and 14 patients in the fluvoxamine and placebo group, respectively) of the same outcomes showed no significant differences except for a borderline significant difference in the general fatigue domain of the MFI in favor of the placebo group ($p=0.04$). The results of the intention-to-treat analysis with respect to the fatigue VAS scores are shown in figure 2. Analysis (intention-to-treat) of changes in SF-36 scores revealed no differences between treatment groups. No significant difference in pruritus was found according to VAS scores at entry and at 6 weeks in either group. The only (borderline) significant difference in the laboratory test results was for serum alkaline phosphatase at day 42 and day 0, attributable to a significant decrease at 42 days in the placebo group ($p=0.03$). Subgroup analysis of only patients with PBC or PSC showed no significant differences between the fluvoxamine and placebo groups (Tables 4 and 5). In addition, no significant differences in response were found between men and women.

One patient treated with fluvoxamine who suffered from fatigue for many years prior to inclusion in the present trial reported a substantial improvement in fatigue during treatment, and a subsequent deterioration after treatment discontinuation. It was decided to continue fluvoxamine treatment in this patient and she has currently been free of fatigue for two years. Her VAS scores for fatigue were 8.4 prior to treatment, 6.9 after 14 days and 2.5 after 6 weeks. Four weeks after treatment discontinuation, her VAS score for fatigue was 7.6.

Adverse events

Treatment was discontinued due to side effects in 5 patients in the fluvoxamine group (after 2, 5, 5, 6 and 7 days) and 2 patients (after 7 and 28 days in the placebo group. These side effects were headache, nausea (2 patients), insomnia and dizziness (3 patients) in the fluvoxamine group, and diarrhea (n=2) in the placebo group. All side effects resolved rapidly after discontinuation of the treatment and none required specific therapy or hospitalization.

Discussion

In the present study no beneficial effect of the antidepressant drug fluvoxamine in the treatment of fatigue associated with PBC and PSC could be demonstrated. Further, given the results of quality of life assessment, no evidence was found for an effect on overall well being. There are several potential explanations for the negative outcome of this trial. The most logical would be that fatigue is neither a symptom of a depressive disorder commonly present in PBC and PSC nor that the aetiology of fatigue and depression are interrelated, sharing a common pathway that could be modified by anti-depressive medications. We believe this is the most likely explanation. Although a high prevalence of depression has been reported in PBC (3, 12), we doubt whether this also applies to our patients as preliminary results of an ongoing study indicate that depression in our patients with PBC and PSC is infrequent (study in progress). On the other hand, the trial may have failed to detect a true therapeutic effect of fluvoxamine. The trial was designed to detect a large treatment effect while the number of patients that was enrolled in the study was lower than expected. In addition, the number of patients discontinuing treatment was higher than expected. Therefore, a (small) beneficial effect of fluvoxamine may well have been missed, and larger trials using fluvoxamine or another SSRI may be needed to define the efficacy of these drugs in the treatment of fatigue. However, the initiation of large trials in cholestatic liver disease is particularly difficult since these diseases are relatively rare. As a result international collaboration is usually needed but acquiring sufficient funding for such collaboration is a recurrent problem.

Although on the whole patient groups were nicely balanced, the baseline scores in the cognitive and social domains of the FFSS were higher for the fluvoxamine group, suggesting fatigue might have been more severe in this group. However, since all other measurements of fatigue showed no significant differences, it appears unlikely that bias due to differences in base-line characteristics of the treatment groups has significantly influenced the results. Finally, the duration of treatment may have been too short, given the chronic character of

fatigue associated with cholestatic liver diseases. Although therapeutic effects of fluvoxamine when used for treating depression are in general apparent within 2-4 weeks, the possibility that therapeutic effects may only become apparent after more prolonged treatment cannot be excluded.

Side effects leading to discontinuation of the drug occurred in one third of patients receiving fluvoxamine treatment. This was much more frequent than expected and contrasting with available data on the tolerability of the drug. We have no clear suggestion as to a possible explanation. The 150 mg dose we used was comparable to those used in other studies and within the 100 – 200 mg dose range frequently used for treating depression. This experience raises the possibility that patients with cholestatic disorders are more susceptible for developing side effects. Unfortunately, we did not monitor plasma fluvoxamine concentrations and therefore cannot exclude the possibility that side effects were related to higher fluvoxamine blood concentrations as compared to those in normal individuals. In this context it should be noted that all patients were ambulant and had relatively mild liver dysfunction. None of the side effects required intervention or persisted after discontinuation of the drug, and there were no obvious deteriorations in liver tests in any of the patients. There have been no previous studies of fluvoxamine or other antidepressants in the treatment of fatigue associated with cholestatic liver diseases, but several studies have addressed the efficacy of these drugs in the treatment of chronic fatigue syndrome and fibromyalgia. According to a recent meta-analysis, antidepressants are effective in the treatment of many of the symptoms of fibromyalgia, including fatigue (13). Two randomized controlled trials, however, did not find an improvement in patients with the chronic fatigue syndrome (14, 15).

Conclusions

In conclusion, although the present study lacked power to detect small but possibly relevant treatment effects, no evidence for a therapeutic effect of fluvoxamine in the treatment of fatigue associated with PBC or PSC was found. The results may indirectly suggest that fatigue is not a symptom of an underlying depressive disorder in these patients with cholestatic liver disease.

Acknowledgments

A grant was provided by the Gastrostart foundation.

References

1. HJF van Hoogstraten, BE Hansen, HR van Buuren, FJW ten Kate, GP van Berge Henegouwen, SW Schalm: Prognostic factors and long-term effects of ursodeoxycholic acid on liver biochemical parameters in patients with primary biliary cirrhosis. J Hepatol 1999, 31:256-262.
2. U Broome, R Olsson, L Loof, G Bodemar, R Hultcrantz, A Danielsson, H Prytz, H Sandberg-Gertzen, S Wallerstedt, G Lindberg: Natural history and prognostic factors in 305 Swedish patients with primary sclerosing cholangitis. Gut 1996, 38:610-5.
3. PM Huet, J Deslauriers, A Tran, C Faucher, J Charbonneau: Impact of fatigue on the quality of life of patients with primary biliary cirrhosis. Am J Gastroenterol 2000, 95:760-7.
4. RH Wiesner, NF LaRusso, J Ludwig, ER Dickson: Comparison of the clinicopathologic features of primary sclerosing cholangitis and primary biliary cirrhosis. Gastroenterology 1985, 88:108-14.
5. C Gluud, E Christensen: Ursodeoxycholic acid for primary biliary cirrhosis. Cochrane Database Syst Rev 2002:CD000551.
6. HJF van Hoogstraten, FHJ Wolfhagen, PC van de Meeberg, H Kuiper, GAJJ Nix, MCJM Becx, AC Hoek, DPF van Houte, MCM Rijk, JMJI Salemans, et al: Ursodeoxycholic acid therapy for primary sclerosing cholangitis: results of a 2-year randomized controlled trial to evaluate single versus multiple daily doses. J Hepatol 1998, 29:417-423.
7. AT Beck, CH Ward, M Mendelson: An inventory for measuring depression. Arch Gen Psychiatry 1961, 6:561-71.
8. JD Fisk, PG Ritvo, L Ross, DA Haase, TJ Marrie, WF Schlech: Measuring the functional impact of fatigue: initial validation of the fatigue impact scale. Clin Infect Dis 1994, 18 Suppl 1:S79-83.
9. EM Smets, MR Visser, AF Willems-Groot, B Garssen, F Oldenburger, G van Tienhoven, JC de Haes: Fatigue and radiotherapy: (A) experience in patients undergoing treatment. Br J Cancer 1998, 78:899-906.
10. MI Prince, OF James, NP Holland, DE Jones: Validation of a fatigue impact score in primary biliary cirrhosis: towards a standard for clinical and trial use. J Hepatol 2000, 32:368-73.
11. JE Ware, Jr., CD Sherbourne: The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care 1992, 30:473-83.
12. K Cauch-Dudek, S Abbey, DE Stewart, EJ Heathcote: Fatigue in primary biliary cirrhosis. Gut 1998, 43:705-10.
13. PG O'Malley, E Balden, G Tomkins, J Santoro, K Kroenke, JL Jackson: Treatment of fibromyalgia with antidepressants: a meta-analysis. J Gen Intern Med 2000, 15:659-66.
14. JH Vercoulen, CM Swanink, FG Zitman, SG Vreden, MP Hoofs, JF Fennis, JM Galama, JW van der Meer, G Bleijenberg: Randomised, double-blind, placebo-controlled study of fluoxetine in chronic fatigue syndrome. Lancet 1996, 347:858-61.
15. AJ Wearden, RK Morriss, R Mullis, PL Strickland, DJ Pearson, L Appleby, IT Campbell, JA Morris: Randomised, double-blind, placebo-controlled treatment trial of fluoxetine and graded exercise for chronic fatigue syndrome. Br J Psychiatry 1998, 172:485-90.

Table 1: Baseline characteristics (general)

	Fluvoxamine N = 17	Placebo N = 16	P-value
Male/Female	4/13	6/10	0.38
PBC/PSC	12/5	10/6	0.57
Age (median)	51	54	0.38
Pruritus (yes/no)	9/8	8/8	0.87
UDCA dose (median, mg/day)	750	900	0.14
Previous depressive disorder (yes/no)	(0/17)	(1/15)	0.30
Weight (median, kg)	67	71	0.95
Length (median, cm)	173	171	0.67
Body Mass Index (median)	24.2	24.6	0.61
Haemoglobin (mmol/l, median)	8.3	8.8	0.21
Bilirubin (mmol/l, median)	13	11.5	0.83
Alkaline Phosphatase (U/l, median)	137	192	0.36
ALT (U/l, median)	42	43	0.55
Albumin (U/l, median)	42	41.5	0.81

Laboratory reference values: haemoglobin 7.5-9.5 mmol/l, bilirubin 0-16 μmol/l, alkaline phosphatase 0-119 U/l, ALT 0-30 U/l, albumin 35-50 g/l.

Table 2: Baseline values of outcome measures

	Fluvoxamine (n = 17)	Placebo (n = 16)	P-value
VAS Fatigue	7.40	7.45	0.91
VAS Pruritus	3.50	1.15	0.33
Beck Depression Inventory	11.0	10.5	0.45
FFSS			
Physical Domain	24.0	16.5	0.25
Cognitive Domain	15.0	9.0	0.04
Social Domain	37.0	22.5	0.04
MFI			
General Fatigue	18.0	17.5	0.78
Physical Fatigue	16.0	14.0	0.22
Reduction in Activity	15.0	13.0	0.30
Reduction in Motivation	13.0	10.0	0.17
Mental Fatigue	13.0	13.0	0.90
SF-36			
Physical Functioning	65.0	70.0	0.47
Role Functioning Physical	25.0	25.0	0.35
Bodily Pain	62.0	62.0	0.55
General Health	40.0	35.0	0.63
Vitality	45.0	40.0	0.64
Social Functioning	62.5	62.5	0.34
Role Emotional Functioning	100.0	66.7	0.46
Mental Health	56.0	60.0	0.28
Reported Health Transition	4.0	4.0	1.00

All results are expressed as median scores.

Table 3: Primary outcomes, change from baseline, intention-to-treat

	Placebo (n = 16)			Fluvoxamine (n = 17)			P-value		
	Day 14	Day 42	Day 70	Day 14	Day 42	Day 70	Day 14	Day 42	Day 70
VAS Fatigue	-1.3	-0.6	-0.55	-0.3	-0.4	-0.35	0.31	0.51	0.51
FFSS									
Physical Domain	-2.0	-3.5	-4.0	0	-2.5	0	0.31	0.40	0.12
Cognitive Domain	-1.5	1.0	0	-1.0	-1.0	0	0.69	0.85	0.60
Social Domain	-3.0	-3.5	-2.0	-3.0	-4.0	-1.5	0.66	0.93	0.93
MFI									
General Fatigue	-2.0	0	0	0	0	-1.0	0.10	0.62	0.87
Physical Fatigue	0	0	0	0	1.0	1.0	0.92	0.73	0.44
Reduction Activity	0.5	-1.0	0	1.0	-0.5	1.0	0.20	0.56	0.13
Reduction Motivation	-1.0	0	-1.0	1.0	-0.5	1.0	0.08	0.94	0.71
Mental Fatigue	-1.0	-0.5	0	0	0	0	0.06	0.35	0.89

Results are expressed as mean differences in scores compared to baseline.

Table 4: Primary outcomes for patients with PBC

	Placebo (n = 10)				Fluvoxamine (n = 12)			
	Day 0	Day 14	Day 42	Day 70	Day 0	Day 14	Day 42	Day 70
VAS Fatigue	7.6	6.9	7.9	7.5	6.8	6.9	8.8	7.6
FFSS								
Physical Domain	20	18	18	15	16	16	21	21
Cognitive Domain	9	6	14	8	11	8	10	9
Social Domain	25	26	28	19	26	20	22	15
MFI								
General Fatigue	17	15	17	16	16	16	17	16
Physical Fatigue	14	14	15	16	14	13	14	14
Reduction Activity	13	13	13	11	12	14	14	15
Reduction Motivation	9	9	12	11	11	13	11	12
Mental Fatigue	14	12	12	14	13	14	14	12

Results are expressed as median scores.

Table 5: Primary outcomes for patients with PSC

	Placebo (n =6)				Fluvoxamine (n = 5)			
	Day 0	Day 14	Day 42	Day 70	Day 0	Day 14	Day 42	Day 70
VAS Fatigue	7.3	7.4	7.5	8.3	6.4	6.8	7.5	7.9
FFSS								
Physical Domain	14	13	9	9	28	27	26	28
Cognitive Domain	8	11	8	7	20	20	19	18
Social Domain	17	12	11	13	48	49	47	48
MFI								
General Fatigue	18	18	17	17	19	19	19	20
Physical Fatigue	13	15	14	13	18	19	19	19
Reduction Activity	12	12	11	12	18	18	17	20
Reduction Motivation	10	10	8	8	18	17	18	16
Mental Fatigue	12	10	10	11	14	14	13	13

Results are expressed as median scores.

Figure 1: Trial profile

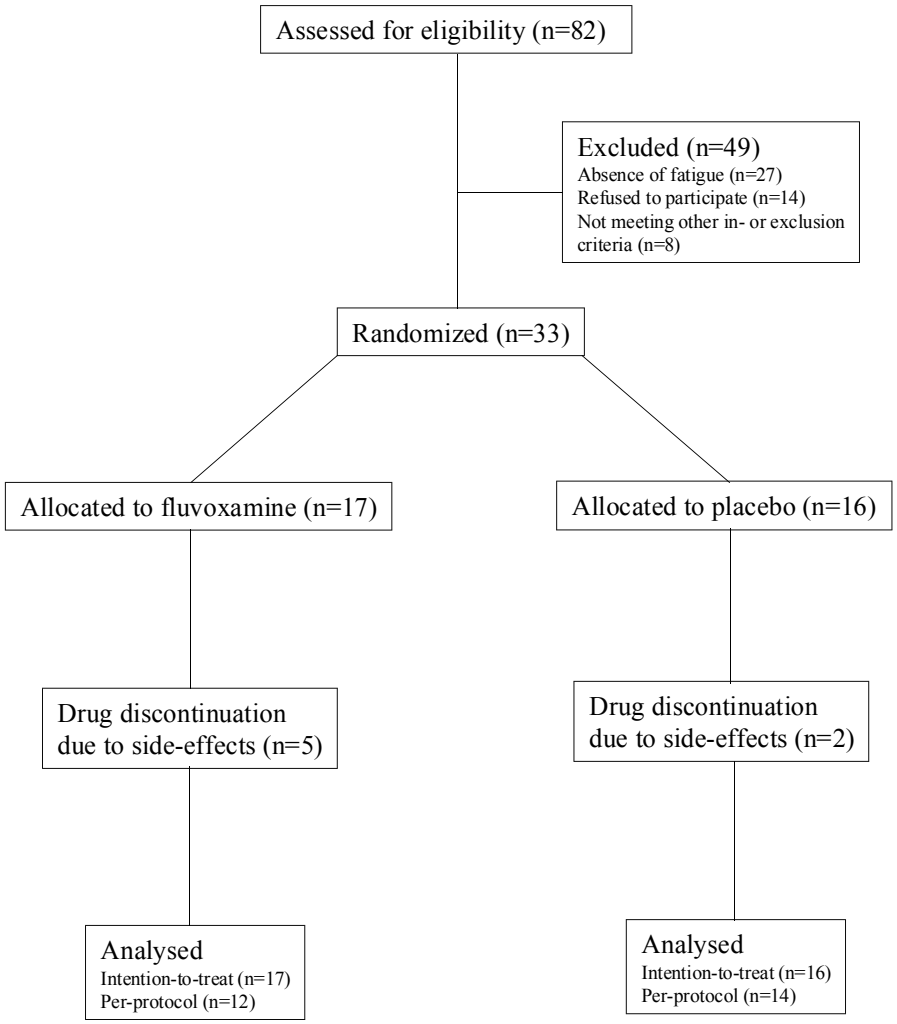
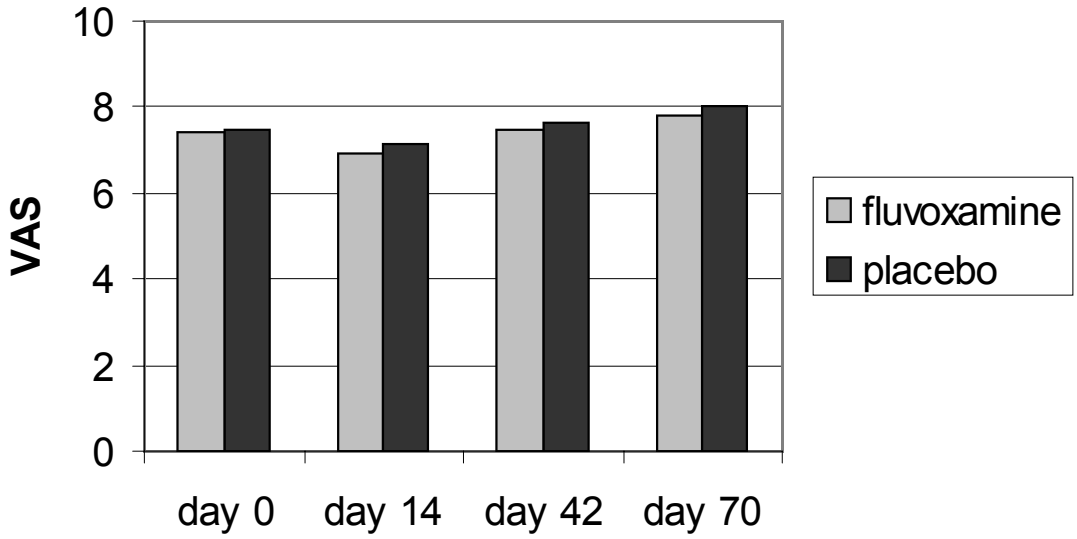


Figure 2: Mean Visual Analogue Scores for fatigue



No significant differences were found within and between groups at entry, during treatment (day 14 and day 42) and 4 weeks after stopping the trial medication.

11

The relation between plasma tyrosine concentration and fatigue in primary biliary cirrhosis and primary sclerosing cholangitis

Pieter C.J. ter Borg[1], Durk Fekkes[2], Jan Maarten Vrolijk[1], Henk R. van Buuren[1]

[1]Department of Gastroenterology and Hepatology, [2]Department of Psychiatry, Erasmus MC, Rotterdam, The Netherlands

Submitted

Summary

In primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) fatigue is a major clinical problem. Abnormal amino acid (AA) patterns have been implicated in the development of fatigue in several non-hepatological conditions but for PBC and PSC no data are available. This study aimed to identify abnormalities in AA patterns and to define their relation with fatigue.

Plasma concentrations of tyrosine, tryptophan, phenylalanine, valine, leucine and isoleucine were determined in plasma of patients with PBC (n=45), PSC (n=27), chronic hepatitis C (n=22) and healthy controls (n=73). Fatigue and quality of life were quantified using the Fisk fatigue severity scale, a visual analogue scale and the SF-36.

Valine, isoleucine, leucine were significantly decreased in PBC and PSC. Tyrosine and phenylalanine were increased ($p<0.0002$) and tryptophan decreased ($p<0.0001$) in PBC. In PBC, but not in PSC, a significant inverse relation between tyrosine concentrations and fatigue and quality of life was found. Patients without fatigue and with good quality of life had increased tyrosine concentrations compared to fatigued patients. Multivariate analysis indicated that this relation was independent from disease activity or severity or presence of cirrhosis.

In conclusion, in patients with PBC and PSC marked abnormalities in plasma AA patterns occur. Discrepant low tyrosine concentrations may be associated with fatigue and diminished quality of life.

Introduction

Primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) are chronic cholestatic liver diseases characterized by a usually slowly progressive course (1, 2). Many patients remain in good clinical condition for many years but may suffer from fatigue interfering with normal activities and general quality of life during a significant part of their life (3-5). Fatigue is not related to the severity or activity of the liver disease, and its pathophysiology remains unknown (3, 4, 6). In several non-hepatological conditions amino acids, in particular tryptophan and tyrosine, have been reported to be involved in the pathophysiology of fatigue (7, 8). Plasma amino acid abnormalities have been studied extensively in patients with liver failure and hepatic encephalopathy (9). In patients with less advanced liver disease of various etiologies, significant differences with respect to plasma amino acid concentrations and tyrosine metabolism have been reported in comparison with control individuals. These studies were performed more than two decades ago, at a time when fatigue had not been identified as a significant problem in cholestatic liver disease. Thus far, the potential role of abnormalities in amino acid metabolism in fatigue associated with cholestatic liver disease has not been evaluated and relevant data in PSC are completely lacking.

The present study aimed to identify abnormalities in plasma concentrations of several amino acids and their relation to fatigue and quality of life in patients with PBC and PSC.

Patients and Methods

The study was approved by our institution's medical ethics committee and informed consent was obtained from each patient. Patients with a diagnosis of PBC (45) or PSC (27) visiting the hepatology outpatient clinic of the Erasmus Medical Center between October 2001 and June 2002 were invited to participate. Exclusion criteria were an age of less than 18 years and incomplete understanding of the Dutch language. As controls, a group of 22 patients with untreated chronic hepatitis C virus infection (HCV) and a group of 73 healthy individuals were included. Fatigue in patients with PBC and PSC was quantified using a visual analogue scale (VAS) and the Fisk fatigue severity scale (FFSS). The FFSS has been validated for use in PBC, and quantifies fatigue in a physical, social and cognitive domain (6, 10). Quality of life was quantified using the SF-36, a widely used quality of life questionnaire (11). These questionnaires were also obtained from a separate group of 18 age and sex-matched controls. Total serum bilirubin, serum albumin, prothrombin time and serum activities of alkaline

phosphatase (AP) and aspartate aminotransferase (AST) were obtained as markers of disease activity and severity. The presence of cirrhosis was determined on the basis of histological and, if not available, clinical criteria (ultrasound findings compatible with cirrhosis if supported by the presence of thrombocytopenia or esophageal varices).

Amino acid measurement

Immediately after the venapuncture plasma was prepared by a 20 min centrifugation step at 2650 g and stored at -80 °C. The amino acids phenylalanine, tyrosine, tryptophan, isoleucine, leucine and valine were measured by means of high performance liquid chromatography as described elsewhere (12). The tryptophan ratio, which is the ratio of tryptophan to the summed concentrations of phenylalanine, tyrosine, isoleucine, leucine and valine, was determined as a measure for central availability of tryptophan for serotonin synthesis. The tyrosine ratio was determined as a measure for central availability of tyrosine for dopamine and norepinephrine synthesis and was calculated as the concentration of tyrosine divided by the sum of the concentrations of phenylalanine, tryptophan, isoleucine, leucine and valine.

Statistics

Testing for differences between groups was performed using Student's t-test and the □2 test. Correlations were tested using Pearson's correlation method. The normality of amino acid distributions was assessed visually using histograms, and non-parametric tests were used where appropriate. The relations between amino acid concentrations and fatigue scores were tested by calculating correlation coefficients for VAS and FFSS domain scores and plasma amino acid concentrations. In these tests, a p-value <0.01 was considered to be statistically significant. In order to quantify the impact of the differences in amino acid on fatigue, for those amino acids which significantly correlated with fatigue, patients were divided into groups with amino acid concentrations within the 95% confidence interval for healthy controls and patients with concentrations outside this range. Testing for differences in fatigue, quality of life and laboratory parameters between these two groups was performed using Student's t-test. Multivariate regression analysis including the biochemical tests of disease activity and severity and the presence of histological or clinical cirrhosis was performed in order to assess the independent association of amino acid abnormalities and fatigue. In all tests other than the correlation tests, a two-sided p-value <0.05 was considered statistically significant. Statistical analyses were performed using SPSS (Version 9.0, SPSS Inc, Chicago, IL, U.S.A).

Results

Patient characteristics

Patient characteristics for patients with PBC and PSC are shown in table 1. As was expected because of the unbalanced sex distribution in these diseases, the majority of patients with PBC were female and the majority of patients with PSC were male. The frequency of cirrhosis, serum bilirubin and albumin and serum activities of alkaline phosphatase, AST and ALT did not significantly differ for patients with PSC or PBC.

Amino acids in patients and controls

Table 2 shows the plasma concentrations of amino acids and the tryptophan and tyrosine ratio's for patients with PBC, PSC, HCV and healthy controls. Plasma concentrations of the aromatic amino acids tyrosine and phenylalanine were increased in patients with PBC, whereas in HCV only tyrosine concentration was increased compared to controls.

In PSC, neither of the aromatic amino acids was increased. Tryptophan concentration was decreased in patients with PBC and HCV. Plasma concentrations of the branched chain amino acids valine, isoleucine and leucine were significantly lower in both patients with PBC and PSC. The tryptophan ratio was significantly decreased in patients with PBC and HCV. The tyrosine ratio was significantly increased in all three patient groups.

Within the group of healthy controls, no differences in amino acid concentrations were found for different age groups or sex.

Amino acids and markers of disease activity and severity

In patients with PBC, significant inverse correlations were present between the branched chain amino acids valine ($p=0.002$), isoleucine ($p=0.006$) and leucine ($p=0.007$) and total serum bilirubin concentrations. Plasma concentrations of the aromatic amino acids tyrosine ($p<0.001$) and phenylalanine ($p=0.003$) correlated inversely with serum albumin concentrations. There was a significant inverse correlation between plasma valine and the serum activity of AST ($p=0.005$). Patients with cirrhosis had significantly increased tyrosine ($p=0.004$) and phenylalanine ($p=0.03$) concentrations and an increased tyrosine ratio ($p=0.004$) compared to non-cirrhotics.

However, all differences in amino acid concentrations retained their significance when only patients without cirrhosis and with normal bilirubin and albumin were compared to healthy controls.

In patients with PSC, no significant correlations were found between any of the markers of disease activity or severity and fatigue or quality of life.

Patients with PSC and inflammatory bowel disease had significantly decreased concentrations of valine, isoleucine and leucine compared to patients with PSC alone ($p=0.02$). The concentrations of tyrosine, phenylalanine and tryptophan were not significantly different.

Amino acids, fatigue and quality of life

In patients with PBC a significant negative correlation was found between tyrosine concentrations and all fatigue tests. In addition, in these patients a significant negative correlation between tryptophan concentrations and the cognitive domain of the FFSS was found, whereas trends towards significant correlations were found for the other FFSS domains. For the other amino acids, no correlations with fatigue were found (Table 3). In patients with PSC, no significant correlations between amino acids and fatigue were found.

Comparing PBC patients with normal tyrosine concentrations with patients with increased concentrations resulted in significant differences in VAS ($p=0.03$), all domains of the FFSS ($p=0.03$, $p<0.001$ and $p=0.01$ for the physical, cognitive and social domains, respectively) and the role functioning physical (the extent to which physical health interferes with work or other daily activities) ($p=0.001$), bodily pain ($p=0.001$), general health ($p=0.03$), vitality ($p=0.004$), social functioning ($p=0.005$), role functioning emotional (the extent to which emotional problems interfere with work or other daily activities) ($p=0.008$) and mental health ($p<0.001$) domains of the SF-36 (Figures 1 and 2). In order to assess confounding by disease severity or activity, we performed multivariate analyses for the measurements of fatigue in PBC including plasma tyrosine concentrations and those laboratory tests which correlated with the amino acid, as well as the presence of cirrhosis, although these laboratory tests and the presence of cirrhosis themselves did not correlate with fatigue or quality of life. These analyses showed that only the plasma tyrosine concentration, and not the laboratory tests or the presence of cirrhosis was significantly and independently associated with fatigue.

Comparing patients with normal tyrosine concentrations with healthy controls resulted in the following significant differences: VAS ($p<0.001$), the physical ($p<0.001$) and social ($p=0.004$) domains of the FFSS and the physical functioning ($p<0.001$), role functioning physical

(p<0.001), bodily pain (p=0.004), general health (p<0.001), vitality (p<0.001), social functioning (p=0.001), role emotional functioning (p=0.05) and mental health (p=0.04) domains of the SF-36. There was no significant difference in the cognitive domain of the FFSS. Comparing patients with increased tyrosine concentrations with healthy controls showed no significant differences in any of the tests except for worse scores in the general health (p=0.03) and better scores in the mental health (p=0.02) domains of the SF-36 for patients with high tyrosine concentrations.

The mean VAS scores were 6.1 and 3.3 for patients with normal and increased tyrosine concentrations, respectively (p=0.01). Patients with a VAS score > 5 had a mean tyrosine concentration of 68 μMol/l, whereas patients with a score < 5 had a mean concentration of 86 (p=0.02).

Tests for differences in fatigue for patients with normal or decreased tryptophan concentrations did not show significant differences between the two groups.

Discussion

The present study confirms previous findings that significant differences in plasma amino acid concentrations between patients with PBC and healthy controls do exist (13, 14). We found increased concentrations of the aromatic amino acids tyrosine and phenylalanine and decreased concentrations of tryptophan and the branched chain amino acids valine, isoleucine and leucine. Tyrosine concentration correlated with all measurements of fatigue, whereas tryptophan concentrations correlated only with the cognitive FFSS domain. PBC patients with increased tyrosine concentrations reported less fatigue and better quality of life compared to patients with (sub)normal concentrations. For PSC, no previous studies on amino acid patterns are available for comparison. We found significant decreases in the plasma concentrations of the branched chain amino acids, and trends towards decreased tryptophan and increased tyrosine and phenylalanine concentrations. However, in contrast to PBC, no relationship with fatigue was found. In addition, we found that valine, isoleucine and leucine concentrations were even lower in patients with PSC and inflammatory bowel diseases than in patients with PSC alone. To our knowledge, no previous data on amino acid concentrations in inflammatory bowel disease are available for comparison.

In several previous studies, mostly on hepatic encephalopathy in patients with advanced cirrhosis, plasma concentrations of amino acids have been studied (9, 15). However, we could identify only two studies including patients with non-cirrhotic PBC. Given the supposedly

normal liver function in these patients, these studies somewhat surprisingly found marked differences between patients and controls comparable to those observed in the present study (13, 14). In addition, although the differences appeared to be somewhat smaller, comparable results were obtained in patients with PSC. It remains unclear which mechanisms are responsible for these differences. Although correlations with the markers of disease severity were found, these do not adequately explain the differences in amino acid concentrations, since only a small proportion of the variation in amino acid concentrations could be explained by differences in these markers, and significant differences existed in the majority of patients without cirrhosis and with normal albumin and bilirubin concentrations. Therefore, we suggest other mechanisms, rather than inflammation of the liver or an overall decreased liver function, may be responsible for the noted abnormalities. The nature of these mechanisms, however, remains unknown.

Tyrosine and phenylalanine are mainly metabolized in the liver, suggesting that decreased liver function might result in increased plasma levels. The decreased tryptophan concentrations found in our study might be explained by increased use of tryptophan as a result of immune activation (15, 16). We did not analyze dietary factors that supposedly could influence amino acid concentrations. Previous studies found no evidence to suggest that this is a factor of importance (13, 14). Nearly all patients in the present study were being treated with ursodeoxycholic acid while previous studies reporting comparable plasma amino acid patterns in PBC were performed in the pre-UDCA era (13, 14). Therefore, a role for UDCA in causing these altered patterns seems unlikely.

Fatigue is a significant problem in many patients with PBC and PSC, and has been studied extensively in recent years (3, 4, 6, 17). However, so far, no specific etiological or pathogenic factors have been identified. Especially, no relation has been found with laboratory parameters for the activity or severity of the disease or histological stage.

An effective medical treatment for fatigue associated with PBC and PSC is not available. Two recent studies specifically addressing PBC-associated fatigue, indicate that treatment with antioxidants is ineffective (18, 19).

The present study suggests an association between fatigue and low tyrosine concentrations in PBC. Concentrations above the 95% confidence interval for healthy controls corresponded with statistically significantly less fatigue and better quality of life scores. Although this suggests that increased tyrosine concentrations may ‘protect’ against fatigue and low concentrations may ‘cause’ fatigue, it may well be that that tyrosine plasma concentration

alterations are an epiphenomenon and that both these and fatigue are caused by a so far unknown confounding factor or mechanism. Tyrosine is a precursor in the synthesis of dopa, dopamine, epinephrine and norepinephrine, all of which are important neurotransmitters that might play a role in fatigue. Experimental catecholamine depletion has been reported to worsen fatigue, suggesting that a (relative) lack of tyrosine might be associated with fatigue (20, 21). Further, beneficial effects of tyrosine administration in the prevention of exhaustion and fatigue after physical activity in both animals and humans have been reported (22-24). Since tyrosine concentrations, and not the tyrosine-ratio was significantly associated with fatigue, a peripheral instead of a central role for tyrosine in the development of fatigue is suggested, which is supported by previous findings supporting peripheral mechanisms in the development of PBC associated fatigue (17). In addition, other mechanisms, which we did not study, such as abnormalities in the hypothalamo-pituitary-adrenal axis, for example abnormal CRH-release, or manganese homeostasis, might be involved in the development of fatigue in these diseases (25). In addition, cytokine release as a result of an inflammatory response might also play a role, although studies supporting this hypothesis are lacking. Studies into these mechanisms might therefore be of interest.

Further studies are required to confirm the present findings and to evaluate the effect of tyrosine suppletion in PBC patients with fatigue.

In conclusion, we showed that in patients with PBC and PSC, marked abnormalities in plasma amino acids occur and that low tyrosine concentrations in patients with PBC are associated with fatigue and diminished quality of life. This association was independent from the activity and severity of the disease.

References

1. Angulo P, Lindor KD. Primary sclerosing cholangitis. Hepatology 1999;30(1):325-32.
2. Neuberger J. Primary biliary cirrhosis. Lancet 1997;350(9081):875-9.
3. Huet PM, Deslauriers J, Tran A, Faucher C, Charbonneau J. Impact of fatigue on the quality of life of patients with primary biliary cirrhosis. Am J Gastroenterol 2000;95(3):760-7.
4. Cauch-Dudek K, Abbey S, Stewart DE, Heathcote EJ. Fatigue in primary biliary cirrhosis. Gut 1998;43 (5):705-10.
5. Wiesner RH, LaRusso NF, Ludwig J, Dickson ER. Comparison of the clinicopathologic features of primary sclerosing cholangitis and primary biliary cirrhosis. Gastroenterology 1985;88(1 Pt 1):108-14.
6. Prince MI, James OF, Holland NP, Jones DE. Validation of a fatigue impact score in primary biliary cirrhosis: towards a standard for clinical and trial use. J Hepatol 2000;32(3):368-73.
7. McGuire J, Ross GL, Price H, Mortensen N, Evans J, Castell LM. Biochemical markers for post-operative fatigue after major surgery. Brain Res Bull 2003;60(1-2):125-30.
8. Georgiades E, Behan WM, Kilduff LP, Hadjicharalambous M, Mackie EE, Wilson J, et al. Chronic fatigue syndrome: new evidence for a central fatigue disorder. Clin Sci (Lond) 2003;105(2):213-8.
9. Butterworth RF. Neuroactive amino acids in hepatic encephalopathy. Metab Brain Dis 1996;11(2):165-73.
10. Fisk JD, Ritvo PG, Ross L, Haase DA, Marrie TJ, Schlech WF. Measuring the functional impact of fatigue: initial validation of the fatigue impact scale. Clin Infect Dis 1994;18 Suppl 1:S79-83.
11. Ware JE, Jr., Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care 1992;30(6):473-83.
12. Fekkes D, van Dalen A, Edelman M, Voskuilen A. Validation of the determination of amino acids in plasma by high-performance liquid chromatography using automated pre-column derivatization with o-phthaldialdehyde. J Chromatogr B Biomed Appl 1995;669(2):177-86.
13. Morgan MY, Marshall AW, Milsom JP, Sherlock S. Plasma amino-acid patterns in liver disease. Gut 1982;23(5):362-70.
14. Morgan MY, Milsom JP, Sherlock S. Plasma ratio of valine, leucine and isoleucine to phenylalanine and tyrosine in liver disease. Gut 1978;19(11):1068-73.
15. Smith AR, Rossi-Fanelli F, Ziparo V, James JH, Perelle BA, Fischer JE. Alterations in plasma and CSF amino acids, amines and metabolites in hepatic coma. Ann Surg 1978;187(3):343-50.
16. Mellor AL, Munn D, Chandler P, Keskin D, Johnson T, Marshall B, et al. Tryptophan catabolism and T cell responses. Adv Exp Med Biol 2003;527:27-35.
17. Goldblatt J, James OF, Jones DE. Grip strength and subjective fatigue in patients with primary biliary cirrhosis. Jama 2001;285(17):2196-7.
18. Watson JP, Jones DE, James OF, Cann PA, Bramble MG. Case report: oral antioxidant therapy for the treatment of primary biliary cirrhosis: a pilot study. J Gastroenterol Hepatol 1999;14(10):1034-40.
19. Prince MI, Mitchison HC, Ashley D, Burke DA, Edwards N, Bramble MG, et al. Oral antioxidant supplementation for fatigue associated with primary biliary cirrhosis: results of a multicentre, randomized, placebo-controlled, cross-over trial. Aliment Pharmacol Ther 2003;17(1):137-43.

20. Verhoeff NP, Kapur S, Hussey D, Lee M, Christensen B, Psych C, et al. A simple method to measure baseline occupancy of neostriatal dopamine D2 receptors by dopamine in vivo in healthy subjects. Neuropsychopharmacology 2001;25(2):213-23.
21. Verhoeff NP, Christensen BK, Hussey D, Lee M, Papatheodorou G, Kopala L, et al. Effects of catecholamine depletion on D(2) receptor binding, mood, and attentiveness in humans: a replication study. Pharmacol Biochem Behav 2003;74(2):425-32.
22. Avraham Y, Hao S, Mendelson S, Berry EM. Tyrosine improves appetite, cognition, and exercise tolerance in activity anorexia. Med Sci Sports Exerc 2001;33(12):2104-10.
23. Owasoyo JO, Neri DF, Lamberth JG. Tyrosine and its potential use as a countermeasure to performance decrement in military sustained operations. Aviat Space Environ Med 1992;63(5):364-9.
24. Deijen JB, Wientjes CJ, Vullinghs HF, Cloin PA, Langefeld JJ. Tyrosine improves cognitive performance and reduces blood pressure in cadets after one week of a combat training course. Brain Res Bull 1999;48 (2):203-9.
25. Forton DM, Patel N, Prince M, Oatridge A, Hamilton G, Goldblatt J, et al. Fatigue and primary biliary cirrhosis: association of globus pallidus magnetisation transfer ratio measurements with fatigue severity and blood manganese levels. Gut 2004;53(4):587-92.

Table 1: Patient characteristics

	PBC (n=45)	PSC (n=27)
Age (mean, range in years)	58 (34-78)	45 (23-68)
Sex (male / female)	4 / 45	19 / 8
Fatigue (yes / no)	32 / 15	15 / 12
Cirrhosis (yes / no)	12 / 33	2 / 25
Inflammatory bowel disease (yes / no)	0 / 45	16 / 11
Total serum bilirubin (median, range in mmol/l)	10 (4-98)	15 (6-37)
Serum albumin (median, range in g/l)	42 (31-48)	42 (33-47)
Prothrombin time (median, range in sec.)	12 (10-18)	13 (11-16)
Serum ALT activity (median, range in U/l)	43 (17-484)	35 (10-662)
Serum AST activity (median, range in U/l)	37 (21-241)	31 (18-338)
Serum Alkaline Phosphatase (median, range in U/l)	154 (68-441)	147 (53-1141)

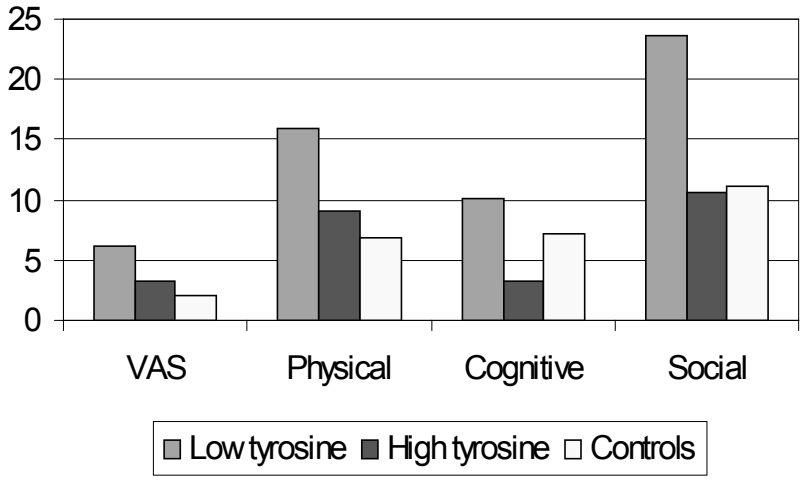
Table 2: Amino acid measurements (µMol/l)

		PBC (n=45)	PSC (n=27)	HCV (n=22)	controls
Aromatic amino acids	Tyrosine (mean ± SD)	76 ± 26 p=0.0002	69 ± 24 p=0.07	73 ± 25 p=0.01	62 ± 14.4
	Phenylalanine (mean ± SD)	65 ± 15 p<0.0001	59 ± 11 p=0.11	54 ± 8 p=0.26	56 ± 6.9
	Tryptophan (mean ± SD)	39 ± 8 p<0.0001	43 ± 11 p=0.085	40 ± 8 p=0.0003	46 ± 6.1
Branched chain amino acids	Valine (mean ± SD)	211 ± 47 p=0.002	208 ± 42 p=0.002	232 ± 45 p=0.56	238 ± 41.7
	Isoleucine (mean ± SD)	55 ± 16 p<0.0001	53 ± 13 p<0.0001	67 ± 19 p=0.51	70 ± 18.8
	Leucine (mean ± SD)	107 ± 30 p<0.0001	101 ± 25 p<0.0001	119 ± 28 p=0.053	132 ± 27.1
Calculated ratio's	Tryptophan-ratio (mean ± SD)	7.7 ± 1.5 p=0.02	8.9 ± 1.8 p=0.051	7.5 ± 1.0 p=0.004	8.3 ± 1.14
	Tyrosine-ratio (mean ± SD)	16.3 ± 6.5 p<0.0001	15.1 ± 5.0 p<0.0001	14.0 ± 3.5 p<0.0001	11.4 ± 2.17

Table 3: Correlation between amino acids and fatigue in PBC

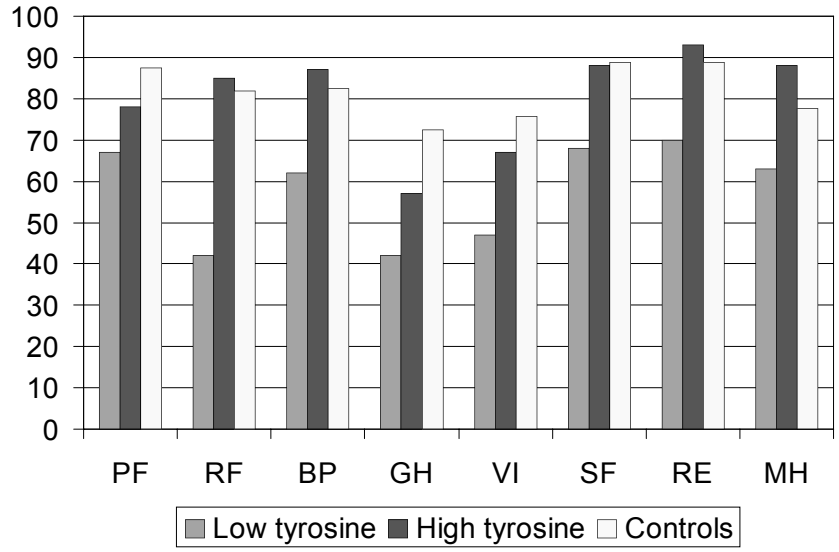
		VAS	FFSS, physical domain	FFSS, cognitive domain	FFSS, social domain
Aromatic amino acids	Tyrosine	-0.38 **p=0.01**	-0.37 **p=0.01**	-0.40 **p=0.006**	-0.37 **p=0.01**
	Phenylalanine	-0.27 p=0.08	-0.29 p=0.05	-0.25 p=0.10	-0.31 p=0.04
	Tryptophan	-0.25 p=0.10	-0.36 p=0.02	-0.42 **p=0.004**	-0.33 p=0.03
Branched chain amino acids	Valine	-0.10 p=0.50	-0.21 p=0.16	-0.06 p=0.70	-0.04 p=0.79
	Isoleucine	-0.04 p=0.76	-0.09 p=0.54	0.06 p=0.70	0.09 p=0.58
	Leucine	-0.09 p=0.57	-0.16 p=0.29	0.01 p=0.98	0.02 p=0.89
Calculated ratio's	Tryptophan-ratio	-0.02 p=0.87	-0.07 p=0.66	-0.26 p=0.09	-0.19 p=0.22
	Tyrosine-ratio	-0.30 p=0.048	-0.21 p=0.15	-0.34 p=0.02	-0.32 p=0.03

Figure 1



Mean VAS scores and scores of the physical, cognitive and social domains of the FFSS for PBC patients with normal or low tyrosine concentrations, high tyrosine concentrations and controls.

Figure 2



Mean SF-36 scores for patients with normal/low tyrosine concentrations, high tyrosine concentrations and controls. PF = Physical Functioning, RF = Role Functioning Physical, BP = Bodily Pain, GH = General Health, VI = Vitality, SF = Social Functioning, RE = Role Emotional Functioning, MH = Mental Health.

12

A pilot study exploring the role of glucocorticoid receptor variants in primary biliary cirrhosis and primary sclerosing cholangitis

Pieter C.J. ter Borg[1], Antje Hagendorf[2], Henk R. van Buuren[1], Jan W. Koper[2], Steven W.J. Lamberts[2]

[1]Department of Gastroenterology and Hepatology, [2]Department of Internal Medicine, Erasmus MC, The Netherlands

Neth. J. Med. 2004; 62: 326-31.

Summary

In primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) significant therapeutic effects of glucocorticoids have not been documented. The most important clinical problem in patients with these diseases is fatigue, which is occasionally invalidating. Abnormalities in the hypothalamo-pituitary-adrenal axis have been suggested as a cause of fatigue. Most effects of glucocorticoids are mediated by the glucocorticoid receptor (hGR α). Recently a causative role for a splicing variant of the glucocorticoid receptor (hGR β) has been proposed in glucocorticoid resistance in asthma and ulcerative colitis, whereas another splicing variant (hGR P) might be associated with glucocorticoid resistant haematological malignancies. The aims of the present pilot-study were to assess abnormalities in glucocorticoid receptor expression and to relate these abnormalities to the development of fatigue and to disease activity and severity in autoimmune cholestatic liver disease. Five fatigued and five non-fatigued patients with PBC or PSC were included, and the results were compared to healthy controls. The expression of hGR P was not different from controls, but hGR β mRNA was significantly increased ($p=0.02$) and hGR α mRNA decreased ($p=0.015$). There were no significant differences between fatigued and non-fatigued patients. A significant negative correlation between the serum activity of alkaline phosphatase and hGR α and hGR P mRNA was found. In conclusion, although there was no relation with fatigue, abnormalities in hGR expression appear to occur in patients with these diseases, and may play a role in its pathophysiology and the poor response to glucocorticoid treatment.

Introduction

Primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) are chronic cholestatic liver diseases with a relatively favorable prognosis for most patients (1, 2). Clinically, the most frequent and occasionally invalidating symptom is fatigue. In PBC, the prevalence of fatigue of any severity is around 85%. Although fatigue has not been studied as extensively in PSC, it appears to occur with comparable frequency in patients with this disease (3-5). There is no correlation with the biochemical or histological severity of the disease (6). Although several studies have attempted to elucidate the pathophysiological mechanisms causing fatigue in cholestatic liver diseases, these have so far remained unknown (7). In addition, there have been no reports of drugs or other treatment modalities with a beneficial effect on fatigue. Although the widely used drug ursodeoxycholic acid improves the biochemical abnormalities in these diseases, it usually has no effect on fatigue (6, 8).

Since fatigue is the most important and still an untreatable problem in many patients with these diseases, attempting to elucidate the mechanisms leading to fatigue may be an important step in finding an effective treatment. One of the possible mechanisms is dysfunction of the hypothalamo-pituitary-adrenal axis, a role of which has been implicated in the pathophysiology of chronic fatigue (9). Since the actions of glucocorticoids are mediated by the intracellular glucocorticoid receptor, this receptor has been studied for defects associated with abnormalities in glucocorticoid function (10). Previously, three splicing variants of the glucocorticoid receptor have been described. The hGR α is the active form of the hGR, while hGR β and hGR P are derived by alternative splicing of the original transcript (11, 12). The hGR P has been reported to increase the activity of hGR α in several cell lines, and it has been suggested that it may be related to glucocorticoid resistance in hematological malignancies (12, 13). Increased expression of the β-variant of the glucocorticoid receptor (hGR), which is formed by alternative splicing of the hGR gene-transcript and is present in normal human tissues, was associated with glucocorticoid resistance in asthma and ulcerative colitis (14-19). Although the mechanism causing the increased expression of hGR β is partially unclear, it has been repeatedly found that induction by pro-inflammatory cytokines may be involved (20-22). This finding led to the hypothesis that glucocorticoid resistance might be the result of an abnormal inflammatory response (23). Since an increased production of inflammatory cytokines has also been observed in cholestatic liver diseases, the expression of hGR β might be increased in these diseases (24-27). In addition, glucocorticoid treatment is not recommended in these diseases since studies assessing the efficacy of glucocorticoids found

only modest effects, suggesting that relative glucocorticoid resistance might exist (28, 29). No studies attempting to find a relation between expression of hGR β in chronic inflammatory diseases and fatigue have been reported. We hypothesized that increased expression of hGR β might not only be present in these diseases, but that it might also be associated with fatigue.
The present study was performed to determine whether levels of the variants of the hGR in peripheral blood mononuclear leukocytes are different from controls in these cholestatic liver diseases, as well as to assess the relation between hGR expression and fatigue.

Patients and Methods

In the present pilot study five patients with a diagnosis of PBC or PSC without fatigue and five patients with chronic and significant fatigue were included. Sex, age and dose of ursodeoxycholic acid were recorded. Serum activity of aspartate aminotransferase, alkaline phosphatase, total serum bilirubin and total immunoglobulin M were measured as markers of disease severity and activity. Fatigue severity was quantified using a visual analogue scale (VAS) and the Fisk Fatigue Severity Scale (FFSS) (30). The FFSS includes social, cognitive and physical domains, in which these aspects of fatigue are quantified. It has been validated for use in primary biliary cirrhosis (31). A visual analogue scale was used in order to quantify pruritus. Informed consent was obtained from each patient and the study was approved by the institutional review committee.

Laboratory techniques

Blood samples were obtained from a group of 12 healthy controls and the 10 patients. To isolate peripheral blood mononuclear leukocytes, the samples were diluted twofold with saline and layered over Ficoll-Hypaque (Pharmacia, Uppsala, Sweden).
Density gradient centrifugation was performed at 1410 rpm for 30 min. at room temperature. The peripheral blood mononuclear leukocytes enriched interphase was isolated and washed twice with saline and the final pellet was suspended with saline. RNA was immediately isolated using a high-resolution RNA isolation kit (Roche Diagnostics GmbH, Mannheim, Germany). After RNA elution in 55 µl elution buffer the concentration of isolated RNA was measured using a Ribo-Green RNA Quantitation Reagent and Kit (Brunschwig chemie, Amsterdam, The Netherlands).
800 ng of total RNA were used for reverse transcription reaction of 50 µl using 5 µM Random Hexamers and 200 nM Oligo-dt-primers in a first strand cDNA synthesis kit (Applied

Biosystems, Foster City, U.S.A.). Reactions lacking reverse transcriptase were also run to generate controls for assessment of genomic DNA contamination. For the different hGR splice variants 2 μl of the resulting cDNA were amplified in real-time PCR assays on the ABI Prism 7700 (ABS, Nieuwerkerk a/d IJssel, The Netherlands) in a total volume of 25 μl containing 300 pmol of each primer and 200 pmol probe in a qPCR-core kit (Eurogentec, Liege, Belgium). After an initial denaturation at 95 °C for 10 minutes, PCR was performed for 42 cycles of denaturation for 15 seconds and annealing for 1 minute at 60 °C.

To detect the expression of the hGR splice variants we used the same upstream primer: 5'-TGT TTT GCT CCT GAT CTG A-3', encoding part of exon 6, as well as the same taqman probe: 5'-FAM-TGA CTC TAC CCT GCA TGT ACG AC-TAMRA-3', encoding part of exon 7, for all isoforms. To discriminate hGR α, β and P from each other we used specific downstream primers. The sequences of these reverse primers are as follows: rev-α: 5'-TCG GGG AAT TCA ATA CTC A-3', encoding part of exon 9α, rev-β: 5'TGA GCG CCA AGA TTG T-3', encoding part of exon 9β, and rev-P: 5'-GTT TCT GCC ATA CCT ATT TG-3', encoding part of intron 7. The expression levels were determined relatively by using the expression of the HPRT housekeeping gene (hyoxantine phosphoribosyltransferase with the forward primer (500 pmol): 5'CAC TGG CAA AAC AAT GCA GAC T-3', the reverse primer (500 pmol): 5'-GTC TGG CTT ATA TCC AAC ACT TCG T-3', and the probe (200 pmol): 5'FAM-CAA GCT TGC GAC CTT GAC CAT CTT TGG A-TAMRA-3'.

Because of the supposed interactions between the hGR β and hGR P variants with the active hGR α variant, we calculated the hGR α/hGR β and the hGR α/hGR P ratio's.

Statistical analysis

Differences in the expression of variants of hGR mRNA between patients and controls, and differences between fatigued and non-fatigued patients were tested using Mann-Whitney's non-parametric test for independent samples. Correlations between the severity of fatigue and laboratory values and the hGR expression were tested using Pearson's correlation method. Logarithmic transformations of laboratory values were used. All statistical tests were performed using SPSS version 9.0.

Results

Ten patients with cholestatic liver disease were included in the study, five of whom complained about fatigue. Seven patients had a diagnosis of PBC and three had been diagnosed with PSC. A summary of patient characteristics is shown in table 1.

In order to assess differences in hGR mRNA levels related to the presence of the disease, we compared the levels of the three variants of the hGR, as well as the hGR α/hGR P-ratio and the hGR α/hGR β-ratio with the levels in a group of healthy controls. These tests resulted in non-significant p-values for hGR P and p-values of 0.015 for the hGR α variant and 0.02 for the hGR β variant, with decreased numbers of hGR α and increased increased numbers of hGR β mRNA in patients versus controls. In addition, the hGR α/hGR β-ratio was significantly decreased in patients (Table 2).

Tests for differences between fatigued and non-fatigued patients were performed, and resulted in p-values of 0.99 for hGR α, 0.65 for hGR β and 0.28 for hGR P. No significant correlations were found between GR mRNA levels and quantified fatigue (Table 3).

Finally, correlation testing was performed in order to find associations between the GR variants and the markers of disease severity and activity. A significant, negative correlation was found between the serum alkaline phosphatase activity and hGR α and hGR P, as well as total hGR mRNA. For the levels of aspartate aminotransferase, bilirubin and immunoglobulin M no significant correlations were found (Table 4). The figure illustrates this relation between alkaline phosphatase and total hGR mRNA.

Discussion

In the present study we found increased levels of hGR β mRNA and decreased levels of hGR α mRNA in patients with cholestatic liver disease compared to healthy controls. As a result, the hGR α/hGR β-ratio was significantly decreased. In addition, there was a significant inverse relation between the hGR α and hGR P variants and the serum activity of alkaline phosphatase, a routinely used marker of disease activity in PBC and PSC. A correlation between the receptor variants and fatigue was not found.

An association between increased levels of hGR β mRNA and glucocorticoid resistance in asthma and ulcerative colitis has been reported previously (14-17, 20, 21). In addition, increased expression of this variant has been observed in patients with hormone resistant nephrotic syndrome, chronic lymphatic leukaemia and nasal polyps (32-35). Several in vitro studies have shown that expression of hGR β can be induced by the inflammatory cytokines Il-2, Il-4, Il-7, Il-8 and TNF-α (20-22). The increase of hGR β as a result of cytokine exposure correlated with a decrease of glucocorticoid sensitivity in one of these studies (22). Further, the frequency of a polymorphism associated with increased stability of hGR β mRNA was increased in patients with rheumatoid arthritis. The authors suggested that this could be a cause of glucocorticoid resistance, which is a common problem in this condition (36). However, despite a significant number of studies reporting it, controversy regarding the negative effects of this variant does still exist, since several other in vivo and in vitro studies found no effects of the hGR β variant, and the mechanisms responsible for the dominant negative effect are largely unknown (13, 19, 37-39). In the present study, levels of hGR β mRNA were much lower than those of the other variants. This does not exclude a role for this variant in causing glucocorticoid resistance, since similar results have been obtained in previous studies reporting quantitative hGR mRNA levels, and it might have several explanations (14, 35). First, mRNA does not necessarily correspond with protein levels, and hGR β protein levels could better reflect the mechanism leading to glucocorticoid resistance, although a previous paper reported very low or undetectable hGR β protein levels in the presence of similar mRNA levels as in the present study (19). Second, in the present study blood samples were studied, whereas the disease occurs primarily in the liver. Thus, studying blood samples may have diluted the hypothetically higher intrahepatic hGR β levels. Third, significantly lower levels of hGR β compared to total hGR levels might be needed in order to

induce glucocorticoid resistance, although the mechanism responsible for this presumed dominant negative effect of the hGR β variant is unclear (23).

Thus, the increased levels of hGR β mRNA in patients in the present study compared to healthy controls may have been caused by the inflammatory nature of these liver diseases, and it can be hypothesized that the modest efficacy of glucocorticoid treatment in these diseases could be caused by an increased expression of hGR β (28, 29). Another explanation for the increased hGR β mRNA levels, in parallel to the hypothesis by Derijk et al. in rheumatoid arthritis, is that patients with increased hGR β expression are at increased risk of developing auto-immune diseases due to resistance to endogenous glucocorticoids (36).

An inverse correlation between the levels of hGR α and hGR P, and therefore total hGR mRNA, and the serum activity of alkaline phosphatase was found, whereas we found no correlation with the other markers of disease activity or severity. Such a relation with disease activity has been reported previously in patients with systemic lupus erythematosus, where glucocorticoid sensitivity correlated with total hGR levels (40). In patients with rheumatoid arthritis, hGR levels were decreased in patients compared to controls (41, 42). These studies suggest that, in addition to hGR β expression, hGR α and hGR P levels might also play a role in determining disease activity and glucocorticoid resistance.

The most important limitation of the present study is its small sample size, and therefore confirmation of the results of the present study in a subsequent larger study would be valuable. In addition, the present study design does not allow conclusions with regard to the cause of abnormalities in hGR expression.

In conclusion, we found increased expression of hGR β mRNA in patients with cholestatic liver diseases as compared to controls and an inverse relation between the hGR α and hGR P mRNA and the serum activity of alkaline phosphatase. This suggests that the glucocorticoid receptor might be involved in the pathogenesis of these diseases as well as in their relative glucocorticoid resistance. Since we found no correlation with fatigue, it seems unlikely that differential expression of hGR variants plays a major role in the etiology of this distressing symptom of PBC and PSC.

References

1. van Hoogstraten HJF, Hansen BE, van Buuren HR, ten Kate FJW, van Berge Henegouwen GP, Schalm SW. Prognostic factors and long-term effects of ursodeoxycholic acid on liver biochemical parameters in patients with primary biliary cirrhosis. J Hepatol 1999;31:256-262.
2. Broome U, Olsson R, Loof L, Bodemar G, Hultcrantz R, Danielsson A, et al. Natural history and prognostic factors in 305 Swedish patients with primary sclerosing cholangitis. Gut 1996;38(4):610-5.
3. Huet PM, Deslauriers J, Tran A, Faucher C, Charbonneau J. Impact of fatigue on the quality of life of patients with primary biliary cirrhosis. Am J Gastroenterol 2000;95(3):760-7.
4. Wiesner RH, Grambsch PM, Dickson ER, Ludwig J, MacCarty RL, Hunter EB, et al. Primary sclerosing cholangitis: natural history, prognostic factors and survival analysis. Hepatology 1989;10(4):430-6.
5. Wiesner RH, LaRusso NF, Ludwig J, Dickson ER. Comparison of the clinicopathologic features of primary sclerosing cholangitis and primary biliary cirrhosis. Gastroenterology 1985;88(1 Pt 1):108-14.
6. Cauch-Dudek K, Abbey S, Stewart DE, Heathcote EJ. Fatigue in primary biliary cirrhosis. Gut 1998;43 (5):705-10.
7. Bergasa NV, Mehlman JK, Jones EA. Pruritus and fatigue in primary biliary cirrhosis. Baillieres Best Pract Res Clin Gastroenterol 2000;14(4):643-55.
8. Gluud C, Christensen E. Ursodeoxycholic acid for primary biliary cirrhosis. Cochrane Database Syst Rev 2002(1):CD000551.
9. Kavelaars A, Kuis W, Knook L, Sinnema G, Heijnen CJ. Disturbed neuroendocrine-immune interactions in chronic fatigue syndrome. J Clin Endocrinol Metab 2000;85(2):692-6.
10. Beato M, Truss M, Chavez S. Control of transcription by steroid hormones. Ann N Y Acad Sci 1996;784:93-123.
11. Hollenberg SM, Weinberger C, Ong ES, Cerelli G, Oro A, Lebo R, et al. Primary structure and expression of a functional human glucocorticoid receptor cDNA. Nature 1985;318(6047):635-41.
12. Krett NL, Pillay S, Moalli PA, Greipp PR, Rosen ST. A variant glucocorticoid receptor messenger RNA is expressed in multiple myeloma patients. Cancer Res 1995;55(13):2727-9.
13. de Lange P, Segeren CM, Koper JW, Wiemer E, Sonneveld P, Brinkmann AO, et al. Expression in hematological malignancies of a glucocorticoid receptor splice variant that augments glucocorticoid receptor-mediated effects in transfected cells. Cancer Res 2001;61(10):3937-41.
14. Honda M, Orii F, Ayabe T, Imai S, Ashida T, Obara T, et al. Expression of glucocorticoid receptor beta in lymphocytes of patients with glucocorticoid-resistant ulcerative colitis. Gastroenterology 2000;118(5): 859-66.
15. Christodoulopoulos P, Leung DY, Elliott MW, Hogg JC, Muro S, Toda M, et al. Increased number of glucocorticoid receptor-beta-expressing cells in the airways in fatal asthma [In Process Citation]. J Allergy Clin Immunol 2000;106(3):479-84.
16. Hamid QA, Wenzel SE, Hauk PJ, Tsicopoulos A, Wallaert B, Lafitte JJ, et al. Increased glucocorticoid receptor beta in airway cells of glucocorticoid-insensitive asthma. Am J Respir Crit Care Med 1999;159(5 Pt 1):1600-4.

17. Sousa AR, Lane SJ, Cidlowski JA, Staynov DZ, Lee TH. Glucocorticoid resistance in asthma is associated with elevated in vivo expression of the glucocorticoid receptor beta-isoform. J Allergy Clin Immunol 2000;105(5):943-50.
18. Oakley RH, Webster JC, Sar M, Parker CR, Jr., Cidlowski JA. Expression and subcellular distribution of the beta-isoform of the human glucocorticoid receptor. Endocrinology 1997;138(11):5028-38.
19. Pujols L, Mullol J, Roca-Ferrer J, Torrego A, Xaubet A, Cidlowski JA, et al. Expression of glucocorticoid receptor alpha- and beta-isoforms in human cells and tissues. Am J Physiol Cell Physiol 2002;283(4): C1324-31.
20. Leung DYM, Hamid Q, Vottero A, Szefler SJ, Surs W, Minshall E, et al. Association of glucocorticoid insensitivity with increased expression of glucocorticoid receptor beta. J Exp Med 1997;186(9):1567-74.
21. Orii F, Ashida T, Nomura M, Maemoto A, Fujiki T, Ayabe T, et al. Quantitative analysis for human glucocorticoid receptor alpha/beta mRNA in IBD. Biochem Biophys Res Commun 2002;296(5):1286-94.
22. Webster JC, Oakley RH, Jewell CM, Cidlowski JA. Proinflammatory cytokines regulate human glucocorticoid receptor gene expression and lead to the accumulation of the dominant negative beta isoform: a mechanism for the generation of glucocorticoid resistance. Proc Natl Acad Sci U S A 2001;98 (12):6865-70.
23. Vottero A, Chrousos GP. Glucocorticoid Receptor beta: View I. Trends Endocrinol Metab 1999;10(8): 333-338.
24. Harada K, Van de Water J, Leung PS, Coppel RL, Ansari A, Nakanuma Y, et al. In situ nucleic acid hybridization of cytokines in primary biliary cirrhosis: predominance of the Th1 subset. Hepatology 1997;25(4):791-6.
25. Yamashiki M, Kosaka Y, Nishimura A, Watanabe S, Nomoto M, Ichida F. Analysis of serum cytokine levels in primary biliary cirrhosis patients and healthy adults. J Clin Lab Anal 1998;12(2):77-82.
26. Lohr HF, Schlaak JF, Gerken G, Fleischer B, Dienes HP, Meyer zum Buschenfelde KH. Phenotypical analysis and cytokine release of liver-infiltrating and peripheral blood T lymphocytes from patients with chronic hepatitis of different etiology. Liver 1994;14(3):161-6.
27. Miller LC, Kaplan MM. Serum interleukin-2 and tumor necrosis factor-alpha in primary biliary cirrhosis: decrease by colchicine and relationship to HLA-DR4. Am J Gastroenterol 1992;87(4):465-70.
28. Leuschner M, Maier KP, Schlichting J, Strahl S, Herrmann G, Dahm HH, et al. Oral budesonide and ursodeoxycholic acid for treatment of primary biliary cirrhosis: results of a prospective double-blind trial. Gastroenterology 1999;117(4):918-25.
29. van Hoogstraten HJ, Vleggaar FP, Boland GJ, van Steenbergen W, Griffioen P, Hop WC, et al. Budesonide or prednisone in combination with ursodeoxycholic acid in primary sclerosing cholangitis: a randomized double-blind pilot study. Belgian-Dutch PSC Study Group [see comments]. Am J Gastroenterol 2000;95(8):2015-22.
30. Fisk JD, Ritvo PG, Ross L, Haase DA, Marrie TJ, Schlech WF. Measuring the functional impact of fatigue: initial validation of the fatigue impact scale. Clin Infect Dis 1994;18 Suppl 1:S79-83.
31. Prince MI, James OF, Holland NP, Jones DE. Validation of a fatigue impact score in primary biliary cirrhosis: towards a standard for clinical and trial use. J Hepatol 2000;32(3):368-73.

32. Liu Y, Song L, Li B. [The expression of glucocorticoid receptor beta messenger RNA in peripheral white blood cells of hormone-resistant nephrotic syndrome patients]. Zhonghua Nei Ke Za Zhi 2001;40(11): 725-8.
33. Shahidi H, Vottero A, Stratakis CA, Taymans SE, Karl M, Longui CA, et al. Imbalanced expression of the glucocorticoid receptor isoforms in cultured lymphocytes from a patient with systemic glucocorticoid resistance and chronic lymphocytic leukemia. Biochem Biophys Res Commun 1999;254(3):559-65.
34. Hamilos DL, Leung DY, Muro S, Kahn AM, Hamilos SS, Thawley SE, et al. GRbeta expression in nasal polyp inflammatory cells and its relationship to the anti-inflammatory effects of intranasal fluticasone. J Allergy Clin Immunol 2001;108(1):59-68.
35. Pujols L, Mullol J, Benitez P, Torrego A, Xaubet A, de Haro J, et al. Expression of the glucocorticoid receptor alpha and beta isoforms in human nasal mucosa and polyp epithelial cells. Respir Med 2003;97 (1):90-6.
36. Derijk RH, Schaaf MJ, Turner G, Datson NA, Vreugdenhil E, Cidlowski J, et al. A human glucocorticoid receptor gene variant that increases the stability of the glucocorticoid receptor beta-isoform mRNA is associated with rheumatoid arthritis. J Rheumatol 2001;28(11):2383-8.
37. de Lange P, Koper JW, Brinkmann AO, de Jong FH, Lamberts SW. Natural variants of the beta isoform of the human glucocorticoid receptor do not alter sensitivity to glucocorticoids. Mol Cell Endocrinol 1999;153(1-2):163-8.
38. Hecht K, Carlstedt-Duke J, Stierna P, Gustafsson J, Bronnegard M, Wikstrom AC. Evidence that the beta-isoform of the human glucocorticoid receptor does not act as a physiologically significant repressor. J Biol Chem 1997;272(42):26659-64.
39. Carlstedt-Duke J. Glucocorticoid Receptor beta: View II. Trends Endocrinol Metab 1999;10(8):339-342.
40. Tanaka H, Akama H, Ichikawa Y, Makino I, Homma M. Glucocorticoid receptor in patients with lupus nephritis: relationship between receptor levels in mononuclear leukocytes and effect of glucocorticoid therapy. J Rheumatol 1992;19(6):878-83.
41. Schlaghecke R, Kornely E, Wollenhaupt J, Specker C. Glucocorticoid receptors in rheumatoid arthritis. Arthritis Rheum 1992;35(7):740-4.
42. Huisman AM, Van Everdingen AA, Wenting MJ, Siewertsz Van Reesema DR, Lafeber FP, Jacobs JW, et al. Glucocorticoid receptor downregulation in early diagnosed rheumatoid arthritis. Ann N Y Acad Sci 2002;966:64-7.

Table 1: Patient characteristics

Median (range)	All patients	Fatigue +	Fatigue -
Male / Female	4 / 6	3 / 2	1 / 4
PSC / PBC	3 / 7	3 / 2	0 / 5
Age	58 (40-76)	53 (40-62)	64 (55-76)
UDCA dose (mg/day)	900 (0-1200)	900 (0-1200)	900 (600-1200)
Bilirubin (μmol/l)	15.5 (7-89)	15 (10-89)	16 (7-43)
Alkaline phosphatase (U/l)	171 (72-441)	188 (122-441)	154 (72-427)
Aspartate aminotransferase (U/l)	40 (27-241)	39 (27-241)	40 (30-65)
Immunoglobulin M (g/l)	1.8 (1.1-3.9)	2.8 (1.2-3.9)	1.6 (1.1-2.4)
Visual analogue score for pruritus (cm)	0.6 (0-3.3)	0.9 (0-2.5)	0 (0-3.3)
Visual analogue score for fatigue (cm)	3.15 (0-9.3)	6.2 (4.3-9.3)	0 (0-2.0)
FFSS Physical Domain	9 (1-32)	24 (10-32)	1 (1-8)
FFSS Cognitive Domain	4.5 (0-21)	7 (4-21)	2 (0-5)
FFSS Social Domain	7.5 (0-42)	31 (9-42)	3 (0-6)

Table 2: Glucocorticoid receptor mRNA levels, number of copies in 2 μl cDNA obtained from a 50 μl reverse transcriptase reaction of 800 ng RNA

Relative number of copies	Mean (patients)	Mean (controls)	P-value
hGR α	41887	56025	0.02
hGR β	69	42	0.02
hGR P	8889	11922	0.09
hGR α/ hGR P	4.7	5.1	0.19
hGR α/ hGR β	679	1523	0.001
Total	50844	67752	0.02

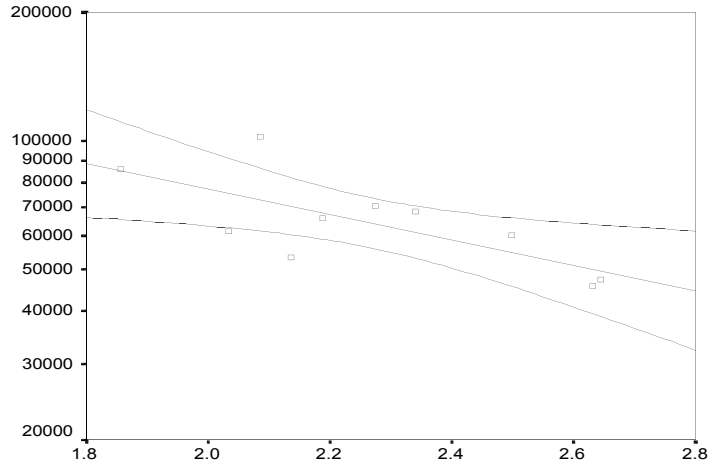
Table 3: Correlation between hGR mRNA and fatigue

	hGR α		hGR β		hGR P		Total	
	coeff.	p	coeff.	p	coeff.	p	coeff.	p
VAS	-0.089	0.81	-0.29	0.43	0.12	0.75	-0.02	0.96
FFSS Physical Domain	-0.26	0.47	-0.30	0.40	-0.07	0.86	-0.20	0.58
FFSS Cognitive Domain	-0.93	0.80	0.21	0.55	0.14	0.71	-0.01	0.97
FFSS Social Domain	-0.26	0.47	-0.10	0.79	0.15	0.69	-0.12	0.74

Table 4: Correlation between hGR mRNA and biochemical markers of disease activity and severity

	hGR α		hGR β		hGR P		Total	
	coeff.	p	coeff.	p	coeff.	p	coeff.	p
Total serum bilirubin	-0.38	0.28	0.11	0.76	-0.063	0.86	-0.28	0.43
Alkaline phosphatase	-0.65	0.041	-0.28	0.44	-0.64	0.049	-0.68	0.03
Aspartate aminotransferase	-0.39	0.27	-0.20	0.58	-0.61	0.063	-0.48	0.16
Immunoglobulin M	-0.10	0.78	0.11	0.76	-0.31	0.39	-0.18	0.62

Figure



Relation between total GR mRNA and serum activity of alkaline phosphatase. ^{10}Log(alkaline phosphatase) is shown on the x-axis and total hGR mRNA is shown on the y-axis.

13

Summary

Samenvatting (Summary in Dutch)

Dankwoord (Acknowledgments)

Curriculum vitae

Bibliograpy

Summary

Primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) are chronic, usually slowly progressive cholestatic liver diseases that may eventually lead to liver cirrhosis and liver failure. The natural history of PBC has been extensively documented, with reported median 7-year survival rates of approximately 25%. In the 1980's ursodeoxycholic acid (UDCA) was introduced in the treatment of PBC. Although several randomized controlled trials have been performed, the therapeutic effect of UDCA remains controversial, mainly because individual trials failed to show significant improvement in survival and meta-analyses came to different conclusions with respect to this end-point (chapter 1). Currently, given the facts that UDCA is widely used in the treatment of PBC and the disease is relatively rare, it is unlikely that new, decisive randomized controlled trials will be initiated. Since 1990, we prospectively studied the long-term course of patients with PBC treated with UDCA. In chapter 2 we compared patient survival in our cohort of 300 patients to the prediction by the Mayo model. This prognostic model was developed and validated before the introduction of UDCA and predicts survival based on clinical and laboratory data. In addition, we compared survival of PBC patients to that of an age- and sex-matched sample from the Dutch population. Survival of PBC patients from our cohort was significantly increased compared to the Mayo model prediction. Survival for the large majority of patients with early PBC was comparable to that of the general population. However, a minority of patients, who can be identified by increased serum bilirubin and decreased albumin concentrations, has a prognosis that was significantly worse than age- and sex-matched controls.

The study documents that the long-term outcome of PBC patients on UDCA is significantly better than the natural history of PBC and therefore adds support to the efficacy of UDCA therapy in PBC. Further studies should be considered to assess whether the efficacy of UDCA is also reflected by decreasing numbers of PBC patients undergoing liver transplantation.

The diagnosis of PBC can usually be made on the basis of clinical and laboratory data, and in most cases liver biopsy may not be needed for establishing the diagnosis. However, at the time of diagnosis liver biopsy may be useful for staging the disease. In chapter 3 we aimed to develop a non-invasive method to stage the disease, based on routinely available, simple laboratory tests. We found that cirrhosis in patients with PBC can be predicted by using the serum bilirubin concentration and the platelet count. In addition, we found that prognosis for patients with a false-negative test result (i.e. cirrhotic patients not identified by the predictive model), is favorable and comparable to prognosis for non-cirrhotic patients.

Our findings suggest that liver biopsy in patients with PBC should not remain routine practice. However, when there is any doubt about the correct diagnosis, e.g. in cases with features of an overlap-syndrome with autoimmune hepatitis, and also from a scientific point of view, liver biopsy remains an important and valuable diagnostic tool. .

PBC, as other autoimmune disorders, has been associated with many other diseases. However, most of these associations have been described in case reports or case series, and significant bias may have occurred in these studies. There are no previous studies documenting the occurrence of all concurrent disorders. In chapter 4, we report the prevalence of other diseases in a large group of patients with PBC. We found that most patients had one or more concurrent disorders, however the prevalence of most disease entities was low, and lower than reported in the case studies.

PSC is a disease which is highly associated with ulcerative colitis and Crohn's disease: approximately 75% of patients with PSC also have inflammatory bowel disease. It has previously been hypothesized that the presence of an inflamed bowel facilitates the development of PSC. However, although several cases of PSC developing after a colectomy performed for inflammatory bowel disease have been described, none of the reports convincingly show that liver tests were normal at the time of colectomy. In our large cohort of PSC patients, we were unable to identify any patient who developed PSC after previous colectomy (chapter 5).

This finding supports the hypothesis that the presence of an inflamed bowel may indeed be important in the pathogenesis of PSC. If so, new therapeutic approaches may be considered addressing factors including gut permeability and portal bacteremia and endotoxinemia. We suggest that the generally accepted view that de novo PSC may well develop after colectomy requires further study.

Recently, the occurrence of fatigue as a dominant symptom in cholestatic liver disease, especially in PBC, has become an area of interest. Although it is well recognized that patients with PSC also frequently suffer from fatigue, little information is available on the prevalence of fatigue in PSC and no previous studies have quantified the impact of fatigue in patients with this disease. We therefore studied the prevalence and severity of fatigue in patients with PSC (chapter 6). The severity and impact of fatigue were comparable to that in PBC, and were significantly increased when compared to healthy controls. In both diseases fatigue was not related to any marker of disease activity or liver function.

Clinicians should be well aware that fatigue is an important and occasionally invalidating symptom in both PBC and PSC, also in non-advanced or early disease.

In addition to fatigue, it has been reported previously that the prevalence of depressive disorders is markedly increased in patients with PBC. We studied the prevalence of depression in patients with PBC and PSC (chapter 7) and found that the prevalence of depression was comparable to that reported for the general population. Our discrepant outcome is likely the result of other diagnostic criteria: in all previous studies questionnaires were used to establish the diagnosis, whereas we diagnosed depression based on DSM-IV criteria after a formal, extensive psychiatric evaluation.

These conflicting findings of our and previous studies underline the importance of questionnaire validation. We therefore aimed to validate the Beck Depression Inventory (BDI) as a screening tool for depression in patients with PBC and PSC (chapter 8). Although the BDI correctly identified the small number of patients with a depressive syndrome, the specificity of the test was low.

Thus, the previously reported high prevalence of depression in PBC indeed appears to be the result of using screening instruments, such as the BDI, for diagnosis. In our opinion, for correct diagnosis of depression a structured psychiatric evaluation is mandatory.

Because the prevalence of depression in fatigued patients with PBC and PSC is low, fatigue is not a symptom likely to be part of a depressive disorder. Previous studies have failed to elucidate the pathophysiology of fatigue in PBC and PSC and this remains unknown. We hypothesized that oxidative stress as a result of immune activation might be involved in the development of fatigue. In chapter 9 we describe a study confirming previous findings of increased oxidative stress in patients with cholestatic liver disease when compared to controls, and suggest that oxidative stress may indeed be related to immune activation. However, we could not find any relation between oxidative stress and severity of fatigue or quality of life scores.

Unfortunately, given the high prevalence of the symptom in PBC and PSC, no effective treatment for fatigue has been defined. In particular, while UDCA improves liver tests and overall prognosis in PBC, previous studies have failed to demonstrate a clear beneficial effect on symptoms including fatigue. Fatigue is a common symptom of depression and depression usually responds to antidepressant drugs. We considered the possibility that fatigue and depression might share a common pathophysiological pathway and consequently that antidepressants could ameliorate fatigue. To test this hypothesis a randomized controlled trial

in patients with PBC and PSC was performed, comparing the antidepressant fluvoxamine to placebo with respect to the effect on fatigue (chapter 10). No serious adverse events of fluvoxamine were observed but we were also unable to document a significant beneficial therapeutic effect.

This finding does therefore not support the hypothesis that the pathophysiological mechanisms leading to fatigue and depression are related. Treatment of fatigue with an antidepressant in patients with PBC or PSC cannot be recommended.

Previous studies in the early 1980's showed that marked abnormalities in serum amino acid concentrations occur in patients with PBC. However, these studies only included patients with advanced PBC. We initiated a study to confirm these abnormalities in patients with early PBC and PSC (chapter 11). We found that abnormalities in serum amino acid concentrations occur, largely irrespective of disease severity or activity. Thus, the previously reported abnormalities in amino acid concentrations in patients with PBC were confirmed in patients with early disease, while data in patients with PSC were not previously available. In addition to these abnormalities in amino acid concentrations, we found a relation between the serum tyrosine concentration and fatigue in PBC, suggesting that low tyrosine concentrations may be associated with fatigue and diminished quality of life. Further studies into the role of tyrosine in the development of fatigue in patients with cholestatic liver disease should be considered.

Recently, a variant of the glucocorticoid receptor has been described, which may be associated with glucocorticoid resistance in ulcerative colitis and asthma. The problem of glucocorticoid resistance in PBC and PSC, both disorders of presumed autoimmune etiology, is relevant given the poor response to corticosteroid treatment. Further, abnormalities in the hypothalamo-pituitary-adrenal axis have been considered as a potential factor involved in fatigue, a common symptom in these disorders. No previous studies on glucocorticoid receptor variants in patients with liver diseases have been performed. Chapter 12 reports a pilot study exploring abnormalities in glucocorticoid receptor expression in patients with PBC and PSC. The expression of the beta variant of this receptor was significantly increased compared to controls, whereas the expression of the biologically active variant of the receptor was decreased. These preliminary findings may contribute to understanding the limited efficacy of corticosteroids in PBC and PSC.

Samenvatting

Primaire biliaire cirrose (PBC) en primaire scleroserende cholangitis (PSC) zijn chronische, meestal langzaam progressieve cholestatische leverziekten, die uiteindelijk kunnen leiden tot levercirrose en leverfalen. Het natuurlijk beloop van PBC is uitvoerig vastgelegd met een gerapporteerde mediane overleving na 7 jaar van ongeveer 25%. In de jaren '80 is ursodeoxycholzuur (UDCA) geïntroduceerd in de behandeling van PBC. Hoewel verscheidene gerandomiseerde studies zijn verricht, blijft het therapeutische effect van UDCA controversieel. Dit komt met name doordat de individuele studies geen significante verbetering lieten zien in overleving en meta-analyses tot verschillende conclusies kwamen. Aangezien UDCA algemeen wordt gebruikt in de behandeling van PBC en PBC hiernaast relatief zeldzaam is, is het onwaarschijnlijk dat grote doorslaggevende gerandomiseerde studies zullen worden gestart. Vanaf 1990 is het lange termijnbeloop van patiënten met PBC die behandeld werden met UDCA prospectief vastgelegd. In hoofdstuk 2 wordt de overleving van de patiënten in ons cohort van 300 patiënten vergeleken met de voorspelling van het Mayo model. Dit prognostische model is ontworpen en gevalideerd voor de introductie van UDCA en voorspelt de overleving aan de hand van klinische en laboratoriumgegevens. Hiernaast vergeleken we de overleving van de patiënten met PBC met de voor leeftijd en geslacht gecorrigeerde overleving van de Nederlandse bevolking. De overleving van de patiënten in ons cohort was significant toegenomen in vergelijking met de voorspelling door het Mayo model. De overleving van de grote meerderheid van patiënten met PBC is zelfs vergelijkbaar met die van de Nederlandse bevolking. Een klein deel van de de patiënten, te herkennen aan een gestegen serum bilirubine en een gedaald albumine, heeft echter een prognose die significant slechter is dan die van de algehele bevolking.

Deze studie toont dat de lange termijnoverleving van patiënten met PBC die behandeld worden met UDCA significant beter is dan het natuurlijk beloop van PBC en onderbouwt dus de effectiviteit van deze behandeling bij patiënten met PBC. Het verrichten van aanvullende studies om te beoordelen of behandeling met UDCA ook leidt tot minder levertransplantaties wegens PBC verdient aanbeveling.

De diagnose PBC kan meestal worden gesteld op basis van klinische en laboratorium-gegevens, en in de meeste gevallen is een leverbiopt hiervoor niet nodig. Op het moment dat de diagnose wordt gesteld kan een leverbiopt van nut zijn voor het stageren van de ziekte. In hoofdstuk 3 beschrijven we een niet invasieve methode om de ziekte te stageren op basis van routinematig beschikbare eenvoudige laboratoriumbepalingen. De aanwezigheid van cirrose

kan worden voorspeld aan de hand van de serum bilirubineconcentratie en het thrombocytenaantal. De prognose van de patiënten met een foutnegatieve uitkomst (d.w.z. patiënten met cirrose die niet door het model werden herkend) is gunstig en vergelijkbaar met de prognose van patiënten zonder cirrose.

Deze bevindingen suggereren dat een leverbiopsie bij patiënten met PBC niet routinematig verricht hoeft te worden. Bij twijfel aan de diagnose, bijvoorbeeld bij patiënten met kenmerken van een overlapsyndroom met auto-immuun hepatitis, en ook vanwege wetenschappelijke redenen, blijft het leverbiopt een belangrijk en waardevol hulpmiddel.

Het voorkomen van PBC, zoals van de meeste auto-immuunziekten, is geassocieerd met vele andere ziekten. De meeste van deze associaties zijn echter beschreven in case reports of case series, waarbij belangrijke bias kan zijn opgetreden. Er zijn geen eerdere studies die de aanwezigheid van alle andere aandoeningen probeerden vast te leggen. In hoofdstuk 4 beschrijven we de prevalentie van andere aandoeningen in een grote groep patiënten met PBC. De meeste patiënten hadden een of meer andere aandoeningen, maar de prevalentie van de meeste van deze aandoeningen was laag, en in het algemeen lager dan in de eerdere case studies.

Het voorkomen van PSC is sterk geassocieerd met colitis ulcerosa en de ziekte van Crohn: ongeveer 75% van de patiënten met PSC heeft ook inflammatory bowel disease (IBD). Eerder bestond al de hypothese dat de aanwezigheid van een ontstoken darm de kans op het ontwikkelen van PSC vergroot. Hoewel verscheidene patiënten die PSC kregen nadat een colectomie wegens IBD was verricht zijn beschreven, is bij geen van deze patiënten overtuigend getoond dat de levertests normaal waren ten tijde van de colectomie. In ons grote cohort van patiënten met PSC konden we geen enkele patiënt vinden die na een eerdere colectomie nog PSC ontwikkelde (hoofdstuk 5).

Deze bevinding ondersteunt de hypothese dat de aanwezigheid van een ontstoken darm van belang kan zijn in de pathogenese van PSC. Als dit inderdaad zo is, kunnen andere, nieuwe therapeutische mogelijkheden worden overwogen die relatie hebben met de permeabiliteit van de darm en portale bacteriëmie en endotoxinemie. De algemeen aanvaarde mening dat PSC zich kan ontwikkelen ruime tijd na een colectomie verdient al met al nader onderzoek.

De laatste jaren is belangstelling ontstaan voor het voorkomen van vermoeidheid als belangrijk symptoom bij cholestatische leverziekten, met name bij PBC. Hoewel het bekend is dat patiënten met PSC ook kunnen lijden aan vermoeidheid, zijn weinig gegevens bekend over de prevalentie van vermoeidheid bij PSC en zijn geen eerdere studies gedaan naar de

gevolgen van vermoeidheid bij patiënten met deze ziekte. We bestudeerden derhalve de prevalentie en de ernst van vermoeidheid bij patiënten met PSC (hoofdstuk 6). De ernst en gevolgen van vermoeidheid waren vergelijkbaar met die bij patiënten met PBC, en significant toegenomen vergeleken met gezonde controles. Bij beide aandoeningen was vermoeidheid niet gecorreleerd met enige maat voor de activiteit van de ziekte of de leverfunctie.

Clinici moeten zich derhalve bewust zijn dat vermoeidheid een belangrijk en soms invaliderend symptoom is bij patiënten met zowel PBC als PSC, zelfs bij patiënten met een vroeg stadium van de ziekte.

Naast vermoeidheid is eerder gevonden dat de prevalentie van depressie bij patiënten met PBC sterk is toegenomen. We bestudeerden het voorkomen van depressie bij patiënten met PBC en PSC (hoofdstuk 7) en vonden dat de prevalentie vergelijkbaar was met die in de algemene bevolking. Dit verschil met eerder onderzoek is waarschijnlijk het gevolg van het gebruik van andere diagnostische criteria: in de eerdere studies werden vragenlijsten gebruikt om de diagnose te stellen, terwijl in onze studie de diagnose was gebaseerd op de DSM-IV criteria na een psychiatrisch onderzoek.

Deze conflicterende bevindingen onderstrepen het belang van het valideren van vragenlijsten. We probeerden derhalve de Beck Depression Inventory (BDI) als screeningsinstrument voor het vaststellen van depressie bij patiënten met PBC en PSC te valideren (hoofdstuk 8). Hoewel de BDI het kleine aantal patiënten met een depressie inderdaad herkende, was de specificiteit laag.

De eerder gerapporteerde hoge prevalentie van depressie bij patiënten met PBC lijkt dus inderdaad het gevolg van het gebruik van vragenlijsten als de BDI voor het stellen van de diagnose. Wij zijn van mening dat voor het stellen van de diagnose depressie een gestructureerd psychiatrisch onderzoek noodzakelijk is.

Gezien de lage prevalentie van depressie bij patiënten met PBC en PSC, is het onwaarschijnlijk dat vermoeidheid bij deze patiënten een onderdeel is van depressie. Eerdere studies konden de pathofysiologie van vermoeidheid bij PBC en PSC niet ophelderen en deze is dus onbekend. Het is mogelijk dat oxidatieve stress als gevolg van immuunactivatie een rol speelt bij het ontwikkelen van vermoeidheid. In hoofdstuk 9 beschrijven we een studie die eerdere bevindingen dat oxidatieve stress is toegenomen bij patiënten met cholestatische leverziekte bevestigt. Ook suggereren de uitkomsten dat oxidatieve stress inderdaad gerelateerd kan zijn aan immuunactivatie. We konden echter geen relatie vinden tussen oxidatieve stress en de ernst van vermoeidheid of de kwaliteit van leven.

Helaas, zeker gezien de hoge prevalentie bij patiënten met PBC en PSC, is geen effectieve behandeling voor vermoeidheid beschikbaar. Hoewel UDCA de levertests en prognose bij patiënten met PBC verbetert, is een duidelijk effect op vermoeidheid nooit gevonden. Aangezien vermoeidheid een belangrijk symptoom is bij patiënten met depressie, en depressie doorgaans goed reageert op behandeling met antidepressiva, overwogen we de mogelijkheid dat vermoeidheid een soortgelijke ontstaanswijze heeft als depressie. We verrichtten daarom een gerandomiseerde trial bij patiënten met PBC en PSC waarin de effectiviteit van het antidepressivum fluvoxamine op vermoeidheid werd onderzocht (hoofdstuk 10). Hoewel geen ernstige bijwerkingen van fluvoxamine voorkwamen, werd ook geen significant therapeutisch effect gevonden.

Deze bevinding ondersteunt derhalve de hypothese dat de pathofysiologie van vermoeidheid gerelateerd is aan die van depressie niet. Behandeling van vermoeidheid bij patiënten met PBC en PSC met een antidepressivum kan dan ook niet worden geadviseerd.

Eerdere studies in het begin van de jaren '80 lieten duidelijke afwijkingen zien in serum aminozuurconcentraties bij patiënten met PBC. Deze studies werden echter verricht bij patiënten met een gevorderd stadium van de ziekte. Wij verrichtten een studie met als doel het bevestigen van deze resultaten bij patiënten met een vroeger stadium van PBC en PSC (hoofdstuk 11). We vonden significante afwijkingen in aminozuurconcentraties, grotendeels onafhankelijk van de ernst van de ziekte. De eerdere bevindingen bij patiënten met PBC werden dus bevestigd, terwijl voor patiënten met PSC tot nu toe geen eerdere gegevens beschikbaar waren. Hiernaast vonden we een relatie tussen de serum tyrosineconcentratie en vermoeidheid bij patiënten met PBC, die suggereert dat een lage tyrosineconcentratie geassocieerd kan zijn met vermoeidheid en verminderde kwaliteit van leven.

Aanvullende studies naar de rol van tyrosine bij het ontwikkelen van vermoeidheid bij patiënten met cholestatische leverziekte kunnen worden overwogen.

Recent is een variant van de glucocorticoïdreceptor beschreven, die geassocieerd lijkt te zijn met glucocorticoïdresistentie bij patiënten met colitis ulcerosa en astma. Gezien de slechte reactie van PBC en PSC, die beide worden beschouwd als auto-immuunziekte, op behandeling met corticosteroïden speelt glucocorticoïdresistentie mogelijk ook een rol bij deze ziekten. Hiernaast zijn afwijkingen in de hypothalamus-hypofyse-bijnieras overwogen als factor in het ontstaan van vermoeidheid. Eerdere studies naar varianten van de glucocorticoïdreceptor bij patiënten met leverziekten zijn niet verricht. Hoofdstuk 12 beschrijft een pilot studie naar afwijkingen in de expressie van de glucocorticoïdreceptor bij

patiënten met PBC en PSC. De expressie van de beta-variant van de receptor was significant toegenomen ten opzichte van controles, terwijl de expressie van de biologisch actieve variant was afgenomen. Deze voorlopige bevindingen kunnen mogelijk bijdragen aan het begrip van de beperkte effectiviteit van glucocorticosteroïden bij PBC en PSC.

Dankwoord

Vele mensen hebben bijgedragen aan het voltooien van dit proefschrift. Zonder de medewerking van honderden patiënten uit heel Nederland was dit onderzoek, evenals welke vorm van klinisch onderzoek dan ook, niet mogelijk geweest. Mijn dank gaat dan ook allereerst naar hen uit.

Professor Solko Schalm dank ik voor het continu in mij gestelde vertrouwen en het op de juiste momenten bijsturen van mijn onderzoek. Je onnavolgbare enthousiasme en inzet zullen me altijd blijven verbazen.

Beste Henk, allereerst bedankt voor het delen van je kamer, de onmeetbare hoeveelheden koffie, Oldtimer drop en andere versnaperingen. Nog belangrijker was natuurlijk je bijdrage in het opzetten en uitvoeren van het onderzoek, maar natuurlijk ook het onderwijs in de Maag-darm-leverziekten. Zonder jouw jarenlange voorbereiding op het totstandkomen van dit proefschrift (vanaf 1990 is immers onder jouw aanvoering gewerkt aan het instandhouden en uitbreiden van de cohorten patiënten) was het nooit zover gekomen.

De collega's van andere afdelingen van het Erasmus MC die betrokken waren bij de verschillende onderzoeken: Durk Fekkes, Kees Schoonderwoerd, Willem Sluiter, Walter van den Broek, Erik van Os, Antje Hagendorf, Jan Willem Koper en prof. dr. S.W.J. Lamberts, allen van harte bedankt voor jullie hulp en bijdragen.

Mijn voorgangers Frank Wolfhagen, Bart van Hoogstraten en Frank Vleggaar: bedankt voor het samen met Henk leggen van de basis van dit voorlopig vierde proefschrift op rij!

Ook zonder de hulp van de vele MDL-artsen en internisten in heel Nederland die betrokken zijn bij de cohorten is onderzoek als dit onmogelijk. In het bijzonder wil ik professor Gerard van Berge Henegouwen bedanken voor zijn belangrijke inbreng bij verschillende onderdelen van dit proefschrift.

Secretaresses van de sectie Hepatologie: Mieke, Sylvia, Margriet en natuurlijk alle medewerkers van het trialbureau bedankt voor jullie hulp bij het verzamelen en verwerken

van de data voor dit proefschrift. Marion, bedankt voor alle begeleiding in de afgelopen maanden!

Collega-onderzoekers Leonieke, Annick, Jan Maarten, Thjon, Annemiek, Monika, Dave, Bart, Marjolein, Rachel, Sarwa: hartelijk dank voor de leuke jaren in onze fraaie dakappartementen op de 4e verdieping.

Begeleiders en kamergenoten gedurende mijn afstudeeronderzoek, bedankt voor het leggen van de basis voor mijn interesse in onderzoek op deze afdeling: Michael Groeneweg, Frank Vleggaar, Tekla van Rossum en ook toen al Leonieke en Annick. Mirjam Hollemans, ook jij natuurlijk bedankt voor de enthousiaste samenwerking in deze periode!

Mijn opleiders in de Maag-darm-leverziekten, Professor Ernst Kuipers en Rob de Man, bedankt voor de samenwerking en vooral jullie vertrouwen in mijn opleiding tot MDL-arts. Ernst, zoals je weet zal onze eerste kennismaking me altijd bijblijven: tijdens de vooropleiding interne ben ik de eerdere periode zonder pieper steeds meer gaan waarderen!

Mijn huidige opleider Interne Geneeskunde, Adrie van Vliet en alle andere collega's in het Albert Schweitzer Ziekenhuis, bedankt voor het bieden van een leuke nieuwe werkomgeving en voor jullie belangstelling voor mijn onderzoek.

Zonder de dames van de prikkamer van de polikliniek was er van een groot deel van dit proefschrift niets terechtgekomen. Bedankt voor jullie vaak spontane en flexibele hulp!

Natuurlijk alle andere collega's van de MDL-afdeling bedankt voor de samenwerking en leuke tijd: MDL-artsen, research-verpleegkundigen, polikliniek-assistentes (Karin, Esther en alle anderen) en secretaresses.

Mijn ouders, Martijn, Marjolein, mijn schoonfamilie, Ron, Robbert en Laura: bedankt voor jullie aanwezigheid en steun in deze jaren!

Bhartie, jij bent deze jaren natuurlijk het meest bij me geweest en hebt alle dalen en hoogtepunten met me gedeeld. Zonder jou was het me niet gelukt!

Curriculum vitae

De auteur van dit proefschrift werd geboren op 20 juli 1976 te Rotterdam. Na het behalen van het V.W.O. diploma in 1994 studeerde hij Geneeskunde aan de Erasmus Universiteit te Rotterdam en behaalde in 2000 het artsdiploma. Tijdens deze studie verrichte hij afstudeeronderzoek op de afdeling Maag-, darm en leverziekten van het Erasmus MC. Na het afleggen van het artsexamen werkte hij van 2000 tot 2003 als arts-onderzoeker op dezelfde afdeling (hoofd ad interim prof. dr. S.W. Schalm, vanaf augustus 2000 hoofd prof. dr. E.J. Kuipers). Vanaf 2003 is hij in opleiding tot Maag-darm-leverarts (opleider prof. dr. E.J. Kuipers). De vooropleiding Interne Geneeskunde heeft plaats in het Albert Schweitzer ziekenhuis te Dordrecht (opleider dr. A.C.M. van Vliet). Hij is gehuwd met Bhartie.

Bibliography

P.C.J. ter Borg, M. van Donselaar, H.R. van Buuren. Clinical events after TIPS: correlation with hemodynamic findings. Gastroenterology 1998;115:1607.

M. Malinchoc, P.S. Kamath, F.D. Gordon, C.J. Peine, J. Rank, P.C.J. ter Borg. A Model to Predict Poor Survival in Patients Undergoing Transjugular Intrahepatic Portosystemic Shunts. Hepatology 2000; 31: 864-71.

P.C.J. ter Borg, H.R. van Buuren, A.C.T.M. Depla. Bacterial cholangitis causing secondary sclerosing cholangitis: A case report. BMC Gastroenterology 2002;2:14.

H.R. van Buuren, P.C.J. ter Borg. Transjugular intrahepatic portosystemic shunt (TIPS): indications and long-term patency. Scand J Gastroenterol Suppl. 2003; 239:100-104.

P.C.J. ter Borg, H.R. van Buuren, W. Visser. Zwangerschapscholestase. Ned Tijdschr Geneeskd. 2003;147:1056-1059.

P.C.J. ter Borg, H.R. van Buuren. Appendicectomy and ulcerative colitis. Gut. 2003;52:768.

P.C.J. ter Borg, M. van Donselaar, J.S. Lameris, F.P. Vleggaar, M. Groeneweg, W.C.J. Hop, H.R. van Buuren. Transjugular intrahepatic portosystemic shunts: long-term patency and clinical results in a patient cohort observed for 3-9 years. Radiology 2004; 231: 537-45.

P.C.J. ter Borg, E. van Os, W.W. van den Broek, B.E. Hansen, H.R. van Buuren. Fluvoxamine for fatigue in primary biliary cirrhosis and primary sclerosing cholangitis: a randomised controlled trial [ISRCTN88246634]. BMC Gastroenterol. 2004; 4: 13.

P.C.J. ter Borg, A. Hagendorf, H.R. van Buuren, J.W. Koper, S.W.J. Lamberts. A pilot study exploring the role of glucocorticoid receptor variants in primary biliary cirrhosis and primary sclerosing cholangitis. Neth. J. Med. 2004; 62: 326-31.

P.C.J. ter Borg, H.R. van Buuren, K.M.J. van Nieuwkerk, E.B. Haagsma, J.W. den Ouden, M.H.M.G. Houben, R.W. de Koning, E.W. van der Hoek, R. Adang, G.P. van Berge Henegouwen. Diseases associated with primary biliary cirrhosis. In: Cholestatic Liver Diseases Therapeutic Options and Perspectives, 2004 Kluwer Academic Publishers and Falk Foundation e.V. ISBN 0-7923-8793-7. Pages 117- 121.

L.E. Bakker-Jonges, P.C.J. ter Borg, H.R. van Buuren, H.H. Hooijkaas. De rol van 'anti-Soluble Liver Antigen' en andere autoantistoffen bij de diagnostiek van autoimmuun hepatitis. Submitted.

P.C.J. ter Borg, S.W. Schalm, H.R. van Buuren, B.E. Hansen for the Dutch PBC study group.Prognosis of UDCA treated patients with primary biliary cirrhosis. Results of a 10-year cohort study involving 297 patients. Submitted.

P.C.J. ter Borg, H.R. van Buuren. The Impact of Fatigue in Primary Sclerosing Cholangitis. Submitted.

E. van Os, W.W. van den Broek, P.G.H. Mulder, P.C. J. ter Borg, H.R. van Buuren, J.A. Bruijn. Depression in patients with primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC). Submitted.

E. van Os, W.W. van den Broek, P.G.H. Mulder, P.C. J. ter Borg, H.R. van Buuren, J.A. Bruijn. Beck Depression Inventory (BDI) in patients with primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC). Submitted.

P.C.J. ter Borg, K. Schoonderwoerd, W. Sluiter, D. Fekkes, H.R. van Buuren. Oxidative stress in primary biliary cirrhosis and primary sclerosing cholangitis is not associated with fatigue or quality of life. Submitted.

P.C.J. ter Borg, D. Fekkes, J.M. Vrolijk, H.R. van Buuren. The relation between plasma tyrosine concentration and fatigue in primary biliary cirrhosis and primary sclerosing cholangitis. Submitted.