DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS
22

PRIMARY BILIARY CIRRHOSIS IN ESTONIA: EPIDEMIOLOGY, CLINICAL CHARACTERIZATION AND PROGNOSTICATION OF THE COURSE OF THE DISEASE

TRIIN REMMEL

TARTU 1996

DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS
22

DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS
22

PRIMARY BILIARY CIRRHOSIS IN ESTONIA: EPIDEMIOLOGY, CLINICAL CHARACTERIZATION AND PROGNOSTICATION OF THE COURSE OF THE DISEASE

TRIIN REMMEL

TARTU UNIVERSITY
PRESS

Department of Internal Medicine, University of Tartu

Dissertation is accepted for the commencment of the degree of Doctor of Medical Science on 16th October 1996 by the Doctoral Committee of the Faculty of Medicine, University of Tartu

Opponents: Professor Heidi-Ingrid Maaroos, MD, PhD, DMSci,
University of Tartu, Estonia
Doctor Sirje Velbri, MD, PhD, DMSci, Tallinn Central Hospital, Estonia

Commencement: December 18th, 1996

Publication of this dissertation is graduated by the Estonian Science Foundation.

Tartu Ülikooli Kirjastuse trükikoda
Tiigi 78, EE 2400 Tartu
Tellimus nr. 407.

CONTENTS

LIST OF ORIGINAL PUBLICATIONS 6
ABBREVIATIONS 7
1. INTRODUCTION 8
2. REVIEW OF LITERATURE 10
2.1. Epidemiology and characteristics of patients with primary biliary cirrhosis 10
2.1.1. Prevalence and incidence 10
2.1.2. Clinical features 12
2.1.3. Laboratory data 13
2.1.4. Histology 15
2.1.5. Natural history 15
2.2. Antinuclear antibodies in primary biliary cirrhosis 16
2.3. Markers of fibrogenesis in primary biliary cirrhosis 18
3. AIMS OF THE STUDY 23
4. PART I: EPIDEMIOLOGY OF PRIMARY BILIARY CIRRHOSIS IN ESTONIA 24
4.1. Patients and methods 24
4.2. Results 25
4.3. Discussion 33
5. PART II: SIGNIFICANCE OF VARIOUS ANTINUCLEAR ANTIBODY PATTERNS IN THE COURSE OF PRIMARY BILIARY CIRRHOSIS 38
5.1. Patients and methods 38
5.2. Results 39
5.3. Discussion 43
6. PART III: AMINOTERMINAL PROPEPTIDE OF PROCOLLAGEN TYPE III AND HYALURONAN IN PRIMARY BILIARY CIRRHOSIS.
6.1. Patients and methods 47
6.2. Results 48
6.3. Discussion 55
7. CONCLUSIONS 60
8. REFERENCES 62
SUMMARY IN ESTONIAN 79
ACKNOWLEDGEMENTS 85

LIST OF ORIGINAL PUBLICATION

I. Remmel T., Remmel H., Uibo R., Salupere V. Primary biliary cirrhosis in Estonia: with special references to prevalence, incidence, clinical features and outcome. Scand. J. Gastroenterol. 1995, 4: 367–371.

II. Remmel T., Piirsoo A., Kõiveer A., Remmel H., Uibo R., Salupere V. Clinical significance of different antinuclear antibodies patterns in the course of primary biliary cirrhosis. Hepato-Gastroenterology 1996, 43: 1135–1140.

III. Remmel T., Remmel H., Salupere V. Aminoterminal propeptide of type III procollagen and hyaluronan in patients with primary biliary cirrhosis: markers of fibrosis in primary biliary cirrhosis. J. Gastroenterol. Hepatol. 1996, 11: 1016–1020.

IV. Remmel T. Primaarne biliaarne tsirroos. Eesti Arst 1994, 6: 486–490.

ABBREVIATIONS

ACA	anticentromere antibodies
AH	autoimmune hepatitis
ALD	alcoholic liver disease
AMA	antimitochondrial antibodies
ANA	antinuclear antibodies
IBS	irritable bowel syndrome
MND	multiple nuclear dots
PBC	primary biliary cirrhosis
PBS	phosphate buffered saline
PIIINP	aminoterminal propeptide of procollagen type III
SLE	systemic lupus erythematosus
UDCA	ursodeoxycholic acid

1. INTRODUCTION

Primary biliary cirrhosis (PBC) is a progressive, chronic cholestatic liver disease, mostly affecting middle-aged females and originally considered to be rare and almost invariably fatal (Ahrens *et al.* 1950).

The term PBC was coined by Ahrens in 1950 to emphasize the intrahepatic, non-suppurative nature of cholestasis (Ahrens *et al.* 1950). The development of a reproducible indirect immunofluorescence assay for antimitochondrial antibodies (AMA) has indicated that PBC is not such a rare disease (Walker *et al.* 1965). AMA is a serological hallmark of PBC, and positivity of the test is highly referred to the PBC possibility. It has been shown that 30–50% of patients may be asymptomatic at the time of the diagnosis (Christensen *et al.*1980; James *et al.* 1981; Roll *et al.* 1983; Eriksson & Lindgren 1984; Lofgren *et al.* 1985; Nyberg & Loof 1989; Danielsson *et al.* 1990; Jeffrey *et al.* 1990; Inoue *et al.* 1995).

Despite the reporting of many large series of patients, the etiology of PBC still remains obscure (Kaplan 1987). Several etiological factors have been suggested, triggering abnormal immune response (Minuk *et al.* 1987; Stemerowicz *et al.* 1988; Hopf *et al.* 1989; Morshed *et al.* 1992; O'Donohue *et al.* 1994; Vilagut *et al.* 1994; Butler *et al.* 1995), but none of them is widely accepted. Certainly, genetical factors could also be important. Different HLA associations have been found in the world, depending on the regional background (Gores *et al.* 1987; Manns *et al.* 1991; Underhill *et al.* 1993; Seki *et al.* 1993; Donaldson *et al.* 1994; Mehal *et al.* 1994; Onishi *et al.* 1994; Mella *et al.* 1995). A highly significant proportion of patients with PBC carrying HLA-DRw8 is found in several studies (Gores *et al.* 1987; Manns *et al.* 1991; Underhill *et al.* 1993; Gregory *et al.* 1993; Mehal *et al.* 1994).

There have been several systematic studies of the epidemiology of PBC. Most attempts have been made in Northern Europe (Eriksson & Lindgren 1984; Lofgren *et al.* 1985; Danielsson *et al.* 1990). Unfortunately, there are still no planned epidemiological studies of PBC in East-European countries, including Estonia. Therefore, a retrospective epidemiological study was done, comprising the period of 1973–1992 to establish the incidence and prevalence of PBC in Estonia. Study was continued prospectively until March 15th 1996. Such an investigation was necessary for the creation of a well-functioning data-base of Estonian patients with PBC, which allows to follow up the course of the disease and to predict its outcome, using immunological and fibrogenetic markers. Patients with PBC were characterized by using other clinical, laboratory and histological parameters as well.

In the second part of the study, the incidence of antinuclear antibodies (ANA) in patients with PBC and their significance to the course of the disease

was investigated. This marker was investigated, whereas about 5–10% of patients with PBC have no AMA and in a part of them ANA could be found using the immunofluorescence method. Since the course of PBC is heterogeneous, the ANA might be one of those markers, indicating an favourable or unfavourable outcome possibility or response to the treatment.

In the third part of the research, two fibrogenesis markers, i.e. aminoterminal propeptide of procollagen type III (PIIINP) and hyaluronan were studied. These markers were chosen, because there are few data of concerning their parallel detection in patients with PBC. Some authors have suggested that their determination could be valuable and should give important clinical information (Ramadori *et al.* 1991; Nyberg *et al.* 1992; Poupon *et al.* 1994; Guechot *et al.* 1994). PIIINP and hyaluronan reflect different changes in the liver tissue. It is not finally clear whether their measurement could give additional data for the evaluation of the disease process. Nevertheless, the assessment of fibrogenetic markers might diminish the liver biopsy necessity and give information in the follow-up of PBC patients, especially about their responsiveness to the treatment.

2. REVIEW OF LITERATURE

2.1. Epidemiology and characteristics of patients with primary biliary cirrhosis

2.1.1. Prevalence and incidence

The first study about the epidemiology of primary biliary cirrhosis (PBC) was performed by Hamlyn and Sherlock (1974) who examined deaths due to PBC. It was estimated to account for up to 2 per cent of patients dying from liver cirrhosis (Hamlyn & Sherlock 1974). There have been several epidemiological studies conducted in the world so far (Triger 1980; Hamlyn *et al.* 1983; Eriksson & Lindgren 1984; Triger *et al.* 1984; Sasaki *et al.* 1985; Lofgren *et al.* 1985; Moreno-Sanchez *et al.* 1990; Robson *et al.* 1990; Caballero Plasencia *et al.* 1990; Witt-Sullivan *et al.* 1990; Danielsson *et al.* 1990; Myszor & James 1990) indicating that the prevalence of PBC varies between countries and between districts within the same country (Triger 1980). So far, the highest prevalence has been found in Sweden and North-East England (Lofgren *et al.* 1985; Danielsson *et al.* 1990; Myszor & James 1990), being 151 per million in North Sweden (Danielsson *et al.* 1990), and the lowest in Australia and Asia (Chan *et al.* 1990; Jeffrey *et al.* 1990; Ilan & Shouval 1992; Watson *et al.* 1995). In a study, performed in Sheffield, geographical clustering of PBC cases between different districts in the same town were found. It was suggested that different water supply could be responsible for this (Triger 1980).

The annual incidence of PBC is at the present time 5.8–15.0 per million (Eriksson & Lindgren 1984; Lofgren *et al.* 1985; Moreno Sanchez *et al.* 1990; Danielsson *et al.* 1990; Witt-Sullivan *et al.* 1990; Caballero Plasencia *et al.* 1991). PBC has previously been regarded as being very rare, but recent reports show that the prevalence and incidence of PBC may be increasing (Lofgren *et al.* 1985; Moreno-Sanchez *et al.* 1990; Myszor & James 1990; Danielsson *et al.* 1990; Witt-Sullivan *et al.* 1990; Caballero-Plasencia *et al.* 1991). It could be, either a true high occurrence of the disease due to the environmental factor or infection, or it could be a consequence of better laboratory screening (there is a shift from symptomatic patients to asymptomatic ones during the past decade), greater awareness of the disease and efficient and properly functioning medical registers (Danielsson *et al.* 1990; Myszor & James 1990).

No epidemiological studies in East-European countries have been done so far. Data on the largest epidemiological studies performed in the world, are presented in Table 1.

Table 1

Epidemiological studies of PBC in the world

Investigation area	Investigation period	Incidence /per million	Prevalence/per million	No. of patients	Main investigator
Picardy, Spain	1975–1984	2.6	13	31	Sevenet (1986)
Cities in Europe	1977–1981	4.0	23		Triger (1984)
South Granada, Spain	1976–1989	4.1	36.4		Caballero Plasencia (1991)
Navarra, Spain	1974–1987		25.15	50	Borda (1989)
Madrid, Spain	1974–1988	7.45	45.5	54	Moreno Sanchez (1990)
Sheffield, UK	1977–1979	5.8	54	34	Triger (1980)
Ontario, Canada	–1987	3.26	22.39	225	Witt-Sullivan (1990)
Malmö, Sweden	1973–1982	13.7	92	33	Eriksson (1984)
Örebro, Sweden	1976–1983	14	128	18	Lofgren (1985)
North-East England	1965–1987	19.8	128.5	471	Myszor (1990)
Newcastle-upon-Tyne, England	1972–1977	10	144 (industrial area) 37 (rural area)	117	Hamlyn (1983)
Northern Sweden	1973–1982	13.3	151	111	Danielsson (1990)

PBC is more common in women who have about ten times higher incidence than men and account for 90 per cent of cases (Triger *et al.* 1984; Jeffrey *et al.* 1990; Rydning *et al.* 1990; Inoue *et al.* 1995). The cause for PBC prevalence in women is not known. Due to the marked female preponderance of the disease, clinical studies for the establishment of the differences in the course of PBC between males and females are not numerous. Among them, in one study it was established that the course of the disease in the two sexes is similar (Lucey *et al.* 1986), but men suffer less commonly from pruritus, and more frequently PBC is diagnosed in them on routine blood tests screening (Triger *et al.* 1984; Lucey *et al.* 1986). Rubel with co-authors (1984) found that male patients have higher serum alkaline phosphatase activities and a lower frequency of the occurrence of piecemeal necrosis, as compared to women. A Japanese study concluded that male patients with PBC tend to have more favourable prognosis, comparing with female patients (Shibata *et al.* 1990).

The age of onset is between 20 and 80 years, with the peak incidence between 40 and 60 years of age (Sherlock & Scheuer 1973, Mistry & Seymour 1992). In older patients (over 65 years old) PBC could be less marked both in the number of symptoms and in the progression of serum bilirubin (Lehman *et al.* 1985; Mayama *et al.* 1990).

2.1.2. Clinical features

In approximately two-thirds of the patients, pruritus and fatigue are the initial symptoms, followed months to years later by jaundice and pigmentation which are late features of the disease (Sherlock & Scheuer 1973, Christensen *et al.* 1985; Rydning *et al.* 1990; Mistry & Seymour 1992; Laurin & Lindor 1994; Inoue *et al.* 1995). Pruritus can also develop at the same time with jaundice in 20 per cent or after jaundice in 8 per cent of patients (Sherlock & Scheuer 1973). Patients with PBC had frequently xantelasmas and xanthomas which are caused by chronic cholestasis and cholesterol accumulation in the blood. In a small part of patients the disease could present initially with symptoms of an advanced liver disease, manifesting as variceal hemorrhage, ascites and hepatic encephalopathy (Hamlyn & Sherlock 1974, Sherlock & Scheuer 1973).

Intestinal bile salt deficiency marginally impairs fat absorption in PBC patients (Ros *et al.* 1984; Lanspa *et al.* 1985), leading to the development of the malabsorption syndrome. Hepatic osteodystrophy is one of the serious late complications, leading to chronic disability, due to the osteoporotic fractures and pain syndrome (Almdal *et al.* 1989; Shiomi *et al.* 1994; Laurin & Lindor 1994). Reasons for the development of osteoporosis are several: liver disease itself as it causes a lack of fat-soluble vitamins and calcium due to chronic cholestasis (Bengoa *et al.* 1984; Wills & Savory 1984; Fonsesca *et al.* 1987; Van Berkum *et al.* 1990; Guanabens *et al.* 1990; Lindor 1993; McCaughan & Feller 1994); belonging to the female sex as women prevail among the patients with PBC; diminished osteoblasts function and treatment with such an immunosuppressive agent as prednisolone (Hodgson *et al.* 1985; Stellon *et al.* 1987; Mitchison *et al.* 1988; Guanabens *et al.* 1990; Mitchison *et al.* 1992; Crippin *et al.* 1994). Osteodystrophy in PBC is strongly correlated to the duration of the liver disease (Guanabens *et al.* 1990; Van Berkum *et al.* 1990; Hodgson *et al.* 1993).

In several studies, approximately 50 per cent of the patients were diagnosed at an early, asymptomatic stage of the disease (Eriksson & Lindgren 1984; Lofgren *et al.* 1985; Nyberg & Loof 1989; Danielsson *et al.* 1990; Jeffrey *et al.* 1990; Inoue *et al.* 1995). Those patients have no liver disease associated symptoms as pruritus, jaundice, ascites, varices, pigmentation, hepatomegaly and splenomegaly. Usually asymptomatic patients are discovered by routine medical examination, mostly due to the other autoimmune diseases. They have

AMA and could have an increased activity of alkaline phosphatase as well. The finding of AMA at a titre 1:40 or more is strongly suggestive of PBC even in the absence of symptoms and the presence of normal alkaline phosphatase (Mitchison *et al.* 1986).

Up to 80 per cent of patients may have one or more symptoms or signs of an associated autoimmune extrahepatic disorder (Murray-Lyon *et al.* 1970; Clark *et al.* 1978; Crowe *et al.* 1980; Keeffe 1987; Nyberg & Loof 1989; Jeffrey *et al.* 1990; Clark & Sack 1991; Laurin & Lindor 1994; Inoue *et al.* 1995). Autoimmune disorders, most frequently associated with PBC, are Sjögren's syndrome, rheumatoid arthritis, CREST syndrome, scleroderma, autoimmune thyreoiditis and fibrosing alveolitis, which are sometimes subclinical (Wolke *et al.* 1984; Bescwick *et al.* 1985; Wallaert *et al.* 1986; Modena *et al.* 1986; Uddenfeldt & Danielsson 1986; Wallace *et al.* 1987; Powell *et al.* 1987; Spiteri & Clarke 1989; Spiteri *et al.* 1990; Rydning *et al.* 1990; Tsianos *et al.* 1990; Krowka *et al.* 1991; Uddenfeldt *et al.* 1991; Horita *et al.* 1992; Thompson *et al.* 1994; Inoue *et al.* 1995). It should be pointed out that sometimes the joint symptoms may even dominate in the clinical picture (Uddenfeldt & Danielsson 1986; Goldenstein *et al.* 1989; Danielsson *et al.* 1990). Gallstone disease occurs in 20–30% of the patients (Lofgren *et al.* 1985; Chan *et al.* 1990).

Hepatocellular carcinoma was initially thought to be a rare complication of PBC, but retrospective studies of patients revealed that a part of those patients are at risk of this complication (Melia *et al.* 1984; Nakanuma *et al.* 1990; Floreani *et al.* 1993; Farinati *et al.* 1994; Loof *et al.* 1994). Two studies have established a significant increase of the incidence of breast cancer (Wolke *et al.* 1984; Goudie *et al.* 1985), however the other studies have not confirmed it (Witt-Sullivan *et al.* 1990; Floreani *et al.* 1993; Loof *et al.* 1994).

2.1.3. Laboratory data

Based on numerous cellular and humoral immunity studies, autoimmune reactions are thought to play a central role in the development of PBC (Kaplan 1987). The most imposing evidences are the demonstration of AMA and an abnormal expression of major histocompatibility class II antigens on biliary epithelium in patients with PBC (Spengler *et al.* 1988; Baum 1989; Berg & Klein 1992; Bassendine & Yeaman 1992; Laurin & Lindor 1994). AMA have been found in about 95 per cent of PBC patients and therefore they are highly specific of PBC, especially if they are at high titre (Sherlock & Scheuer 1973; Kaplan 1987; Brenard & Geubel 1991; Berg & Klein 1992; Laurin & Lindor 1994).

Other abnormalities of immunity in PBC patients included humoral changes such as the occurrence of different non-specific antibodies, polyclonal hypergammaglobulinemia, failure of conversion of IgM to IgG, hypocomplemen-

temia, low IgE level and the presence of immune complexes (James *et al.* 1983; Bird *et al.* 1988; Minuk *et al.* 1989; Roberts-Thomson & Shepard 1990; Sundewall & Lefvert 1990; Benbassat *et al.* 1992; Menendez-Caro *et al.* 1994, Chou *et al.* 1995). Abnormalities of cellular immunity include the presence of granuloma and defective suppressor T cell function in peripherial blood as well as in the inflammatory infiltrate around the damaged bile ducts (Meuer *et al.* 1988; Van de Vater *et al.* 1989; Kram *et al.* 1990; Nouri Aria *et al.* 1991; Nakanuma *et al.* 1990; Vierling 1992; Onishi *et al.* 1993; Bjorkland *et al.* 1994; Martinez *et al.* 1995; Jones *et al.* 1995).

The liver-related laboratory tests are compatible with cholestasis, with a greater rise of membrane-bound enzymes, alkaline phosphatase and γ-glutamyl transpeptidase, as compared with the cytosolic enzymes, alanine or aspartate aminotransferase (Rydning *et al.* 1990; Mitchison *et al.* 1990; Ilan & Shouval 1992; Inoue *et al.* 1995). Due to cholestasis, serum cholesterol and circulating bile acids are also increased (Batta *et al.* 1989; Rosenson *et al.* 1990; Miller 1990; Crippin 1992).

At the time of diagnosis, bilirubin is usually normal, but as the disease progresses, it becomes elevated. With an increasing extent of liver damage, the hepatocellular synthetic function also becomes impaired, manifesting as coagulopathy and hypoalbuminemia.

2.1.4. Histology

The most frequently used histological classification was worked out by Scheuer (1967). It divides PBC into four histological stages. Stage I or periportal/cholangitic stage is characterized by patchy destruction of bile ducts which exhibit damaged epithelium surrounded by a dense infiltrate of chronic inflammatory cells, the principal components of which are T lymphocytes (Nakanuma *et al.* 1990). Non-caseating granulomas may be detected surrounding damaged bile ducts. Stage II, the periportal stage, is the stage of unfocused ductal proliferation sometimes associated with periportal hepatitis. In stage III, changes of precirrhosis and fibrosis take place and there is a characteristic marked destruction of bile ducts. Changes of established biliary cirrhosis and the disruption of liver cell architecture are seen in stage IV, when typical features of PBC are lacking, making it difficult to distinguish from other types of biliary cirrhosis on histological grounds alone (Scheuer 1967; Scheuer 1988). Histological classification of PBC allows to differentiate patients into the non-advanced (I–II stages) and advanced (III and IV stages) disease stage. This will make it easier to follow up the progression rate of the disease.

Liver damage in PBC is non-diffuse. Therefore, in needle biopsy sometimes it is not possible to receive tissue from the damaged area. Therefore, in some

cases, it is difficult to confirm the disease morphologically and to determine the histological stage of PBC (Scheuer 1988).

2.1.5. Natural history

The natural history of PBC is variable. Generally in asymptomatic patients, the disease progresses slowly and has a better prognosis (James *et al.* 1981; Roll *et al.* 1983; Lofgren *et al.* 1985; Beswick *et al.* 1985; Nyberg & Loof 1989) than in symptomatic patients. Patients with PBC but without liver-related symptoms have a good prognosis until they remain asymptomatic. However, from the time that liver disease symptoms first appear, the rate of survival is similar to that of patients with symptomatic PBC and significantly worse than the normal age- and sex-matched population (Balasubramaniam *et al.* 1990; Mitchison *et al.* 1990; Kaplan 1990; Mahl *et al.* 1994). The coexistence of autoimmune disease (Beswick *et al.* 1984), hepatomegaly, or histological grades III, IV are associated with a more rapid progression in the asymptomatic patients (Mitchison *et al.* 1990).

A over 10-year follow-up study of 279 patients with PBC demonstrated that the mean predicted survival time from the time of the diagnosis is twice as long for patients without symptoms compared to symptomatic patients (Mahl *et al.* 1994).

Patients with hepatobiliary symptoms have an average survival period from the time of the diagnosis of about 7–15 years (Roll *et al.* 1983; Christensen *et al.* 1985; Rydning *et al.* 1990; Mahl *et a.* 1994). 50% of mortality in the symptomatic group in a 10-year study were found (Eriksson & Lindgren. 1984). The prognosis is especially poor in the presence of clinical features of the advanced liver disease, such as jaundice, malnutrition, ascites, hypoalbuminemia and cirrhotic stage on biopsy (Roll *et al.* 1983). Serum bilirubin is the most important prognostic factor: when the level exceeds 34 μmol/l, the mean survival is 4 years (90 percent confidence limits, 3–6 years) and at levels >170 μmol/l, the mean survival is reduced to 1.5 years (Shapiro *et al.* 1979). In an extensive Japanese multicentre study the most frequent causes of death were hepatic failure and/or gastrointestinal bleeding, which affected 78.3% of the patients who died (Inoue *et al.* 1995).

A number of models have been derived, based on the Cox regression analysis of a range of clinical, laboratory and histological indices, in order to predict the prognosis in an individual patient (Roll *et al.* 1983; Christensen *et al.* 1985; Dickson *et al.* 1989; Christensen *et al.* 1993; Goudie *et al.* 1989; Rydning *et al.* 1990; Inoue *et al.* 1995). Most frequently the Mayo model, based on patients' age, total serum bilirubin and serum albumin concentrations, protrombin time and severity of oedemas, has been used (Dickson *et al.* 1989).

Although different variables are analysed in models, the bilirubin level remains the most important prognostic indicator (Shapiro *et al.* 1979; Christensen *et al.* 1980; Roll *et al.* 1983; Beswick *et al.* 1985; Nyberg & Loof 1989; Goudie *et al.* 1989; Rydning *et al.* 1990; Murtaugh *et al.* 1994). Such analyses have proved valuable in assessing the effects of treatment and in the optimum timing of liver transplantation (Bonsel *et al.* 1990; Neuberger *et al.* 1990).

Whereas in most earlier worked-out models, data about the patients with PBC, who did not receive treatment, were used, a requirement for new models is especially important. For instance, Poupon *et al.* (1994) have indicated that in PBC patients treated with ursodeoxycholic acid (UDCA), high bilirubin concentration loses its predictive value for prognosis. It is replaced by hyaluronan and PIIINP (Poupon *et al.* 1994). Which markers could be useful for predicting the outcome in patients, treated with such immunosuppressive drugs as prednisolone and azathioprine is not known yet.

2.2. Antinuclear antibodies in primary biliary cirrhosis

Antibodies to nuclear constituents are a common features of systemic rheumatic diseases and autoimmune liver disorders (Nakamura *et al.* 1985; Czaja *et al.* 1994). Different ANA patterns show remarkable but not absolute disease associations (Nakamura *et al.* 1985). In some diseases ANA may have a prognostic value (Kelley *et al.* 1989).

AMA are not detectable in about 5–10% of patients with clinical and pathological features of PBC (Kaplan 1987, Gershwin & Mackay 1991). For these reasons, autoantibodies of other specifities could be useful as supplements to the diagnosis of PBC with ANA being a frequent finding among them. Using indirect immunofluorescence on rat liver tissue sections, ANA were found in 24–31% of patients (Doniach & Walker 1972; Christensen *et al.* 1980; Cassani *et al.* 1985; McMillan *et al.* 1987; Nickowitz *et al.* 1994). However, using cells with large nucleus (HEp-2 or HeLa cells) as a substrate for immunofluorescence, the sensitivity for the detection of ANA has been increased in PBC to 35–58% (Powell *et al.* 1984; Bernstein *et al.* 1984; Cassani *et al.* 1985; McMillan *et al.* 1987; Chou *et al.* 1995). ANA patterns include nuclear dots, fine speckled, homogeneous, nucleolar, anti-centromere and perinuclear ones (Bernstein *et al.* 1984). Among them, two distinct ANA, one giving a membrane-like pattern of positivity and the other, seen as multiple nuclear dots (MND) by immunofluorescence, have been demonstrated to be specifically associated with PBC and thus proposed as alternative markers in the AMA-negative cases (Tartakowsky & Worman 1995). The prevalence of MND-ANA is 6–27% (Powell *et al.* 1984; Bernstein *et al.* 1984; Cassini *et al.* 1985; Szostecki *et al.* 1990; Hansen *et al.* 1991) and the antibody seems to be associ-

ated with cases with lachrymal dysfunction (Bernstein *et al.* 1984; Cassini *et al.* 1985). It was shown that the pattern of nuclear dots is caused by autoantibodies directed against a soluble 100 kD protein (Sp-100 antigen) (Szostecki *et al.* 1987).

A small number of patients with rheumatic disorders but without obvious signs of PBC were also found to have anti-Sp 100 antibodies (Szostecki *et al.* 1990). As other autoimmune diseases are often associated with PBC, those patients may develop overt PBC at a later stage of rheumatic disorder (Szostecki *et al.* 1990).

A subset of ANA that stain the nucleus in a rimlike fashion on immunofluorescence microscopy recognize proteins of the nucleus envelope. The most frequently recognized nuclear envelope antigen in these patients is an integral membrane protein with molecular mass of 210 kD (Courvalin *et al.* 1990). The prevalence of anti-gp-210 in patients with PBC ranged from 10 to 52% (Ruffati *et al.* 1985; Lozano *et al.* 1988; Lassoued *et al.* 1990; Nickowitz *et al.* 1994; Tartakowsky & Worman 1995). In a large series of 150 patients with PBC, those positive for gp-210 were significantly less commonly affected by asthenia, arthralgias, associated extrahepatic diseases and the Raynaud phenomenon (Lassoued *et al.* 1990). The presence of perinuclear ANA (against gp-210 or lamin B receptor) had a sensitivity of 11% and specificity of 100% for PBC and may be useful in diagnosing PBC cases without AMA and in identifying patients with an increased incidence of associated rheumatoid arthritis (Nickowitz *et al.* 1994).

Occurrence of anticentromere antibodies in PBC patients is associated with the presence of scleroderma-like features (Raynaud's phenomenon and sclerodactyly) as found by most investigators (Bernstein *et al.* 1984; Modena *et al.* 1986; Prost *et al.* 1987; McHugh *et al.* 1990), but not at all (Chan *et al.* 1994).

Ben Ari *et al.* (1993) have first described the four cases of ANA-positive AMA-negative patients with high cholestatic indices and liver biopsy suggestive to PBC, and suggested the term autoimmune cholangiopathy in those cases. Those patients responded quite well to the prednisolone treatment (Ben-Ari *et al.* 1993). Later it was found, that the AMA-negative patients with severe cholestasis had also significantly higher smooth muscle antibody titres and lower serum IgM and aspartate aminotransferase activities than the AMA-positive controls (Michiletti *et al.* 1994). Taylor *et al.* (1994) found at the same time that AMA-negative ANA-positive patients did not respond to prednisolone and azathioprine treatment. The AMA-negative group patients were slightly younger on the average (50 vs. 55 years) than AMA-positives (Goodman *et al.* 1995). Since the only consistently distinguishing feature among these patients are the autoantibody (AMA and ANA) profile, and they otherwise have virtually identical clinical and histopathologic features, autoimmune cholangiopathy can be considered to be the same as AMA-negative PBC (Goodman *et al.* 1995,

Lacerda *et al.* 1995). Whether those patients respond better to the prednisolone/azathioprine treatment or UDCA treatment is not exactly known yet.

So far, there have been no studies, dealing with the outcome peculiarities in the ANA-negative and ANA-positive groups of patients with PBC. In addition, no studies have been done, dealing with ANA dynamical determination in PBC patients.

2.3. Markers of fibrogenesis in primary biliary cirrhosis

Most chronic liver diseases are accompanied by biochemical reactions and morphological changes, termed fibrosis. In general, this may be defined as the deposition of connective tissue to a higher than normal extent (Chojiker & Brenner 1988; Johnson 1996). It is clearly established that hepatic fibrogenesis is an active process in which connective tissue synthesis is stimulated in both mesenchymal and parenchymal liver cells (Burt 1992; Friedman 1993; Gressner & Bachem 1994; Gressner 1996).

In the normal liver, the de novo production of extracellular matrix is minimal (Friedman *et al.* 1985; Bissell *et al.* 1990; Johnson 1996). When the extracellular matrix is replaced by an abnormal matrix, as in hepatic fibrosis and cirrhosis, diminished liver function occurs (Bissell *et al.* 1990; Schuppan 1990; Gressner 1991; Johnson 1996).

Fibrotic changes can be determined by the morphological examination of the liver, but this approach cannot be used to assess accurately the activity of collagen synthesis at any given point of time. Thus, the development of biochemical markers of hepatic fibrosis might allow a promising diagnostic approach for the identification and quantitation of this process (Gressner 1987; Goldberg & Brown 1987; Wu & Danielsson 1995). Among them, serum hyaluronan and aminoterminal propeptide of procollagen type III (PIIINP) as useful prognostic markers have been recommended (Eriksson & Zettervall 1986; Niemela *et al.* 1988; Neuberger 1989; Nyberg *et al.* 1992; Teare *et al.* 1993). They are valuable whereas it is possible to measure them in serum. In some liver diseases it has been shown that their dynamic determination could indicate responsiveness to the treatment (Weigand *et al.* 1984; Ballardini *et al.* 1984; McCullough *et al.* 1987; Risteli *et al.* 1988; Schuppan 1991; Trinchet *et al.* 1992; Capra *et al.* 1993; Camps *et al.* 1993; Guechot *et al.* 1994; Teran *et al.* 1994; Ueno *et al.* 1995).

Procollagens are synthesized intracellularly and have extension peptides at both amino and carboxy ends of the molecule (Burgeson & Nimni 1991). The aminoterminal peptides are precursor-specific segments, which are cleaved off by specific proteases and released into the blood circulation during the conversion of procollagen into collagen (Schuppan 1990; Burgeson & Nimni 1991).

Since procollagen peptides are liberated in stoichiometric amounts during the conversion of procollagen into collagen and persist for some time in the body, it has been suggested that the procollagen level reflects the intensity of the connective tissue formation in the liver (Timpl & Glanville 1981). Type III is the predominant collagen type in hepatic fibrosis and only in late cirrhosis is type I predominant (Rojkind & Martinez-Paloma 1976; Seyer *et al.* 1977; Rojkind *et al.* 1979; Murata *et al.* 1984; Aycock & Seyer 1989).

Bile and urine seem to be the major excretion routes of PIIINP and its degradation products. The biliary excretion of propeptide seems to be quantitatively more important than its renal excretion (Raedsch *et al.* 1983; Bentsen *et al.* 1990). In large quantities it is a physiological function of the scavenger receptor in liver sinusoidal endothelial cells (Smedsrod 1988; Bentsen *et al.* 1988; Melkko *et al.* 1994). Consequently, increased serum levels may be, at least in part, attributed to the compromised function in advanced liver fibrosis or in acute hepatic decompensation as a result of a reduced liver PIIINP extraction rate (Wu & Danielsson 1995).

The original radioimmunological assay for PIIINP determination developed by Rohde *et al.* (1979) employs a heterogeneous antigen existing in two immunoreactive molecular sizes. One antigen resembles the intact triple-stranded aminopeptide in size (Col_{1-3}; Mr=45000), the other resembles the globular domain of peptide (Col_1; Mr=10000) believed to be a proteolytic degradation product of the intact molecule. In an assay worked out by Niemela *et al.* (1988) only intact procollagens was used, making this test system more specific to determine the level of fibrogenesis.

PIIINP concentration was significantly elevated in most patients with untreated chronic active hepatitis and cirrhosis (Niemela *et al.* 1983; Frei *et al.* 1984; Weigand *et al.* 1984; Surrenti *et al.* 1987; van Zanten *et al.* 1988; Hayasaka *et al.* 1990; Gonzalez-Reimers *et al.*1990; Misaki *et al.*1990; Ramadori *et al.* 1991) and also in PBC (Eriksson & Zettervall 1986; Weigand *et al.* 1984; Nyberg *et al.* 1988; Babbs *et al.* 1988; Niemela *et al.* 1988; van Zanten *et al.* 1988; Mutimer *et al.* 1989; Nyberg *et al.* 1992), but not in chronic persistent hepatitis patients (Frei *et al.* 1984; Weigand *et al.* 1984; Surrenti *et al.* 1987; Bell *et al.* 1989; Hayasaka *et al.* 1990; Ramadori *et al.* 1991).

A good correlation between PIIINP and the extent of liver fibrosis as determined by biopsy was found by various authors (Frei *et al.* 1984; Torres-Salinas *et al.* 1986; *et al.* Gabrielli *et al.* 1989; Diodati *et al.* 1990; Trinchet *et al.* 1991; Muller *et al.* 1991; Trinchet *et al.* 1992; Gonzalez-Reimers *et al.* 1992; Murawaki *et al.* 1994). As a matter of fact, most authors regard PIIINP as the most reliable serum marker for the ongoing fibrogenesis in the liver, rather than as an indicator of the fibrotic extent, since it is usually in normal range in inactive cirrhosis (McCullough *et al.* 1987; Hayasaka *et al.* 1990). Several authors have not found any significant difference in the serum levels of PIIINP between chronic active hepatitis and biopsy-proven cirrhosis (McCullough *et*

al. 1987; Hayasaka *et al.* 1990; Gonzalez-Reimers *et al.* 1990), but PIIINP levels were found to correlate with the clinical stage of the disease (McCullough *et al.* 1987). In PBC a significant correlation was found between serum PIIINP and the histological stage, the highest levels being found in patients with bridging fibrosis and cirrhosis (Eriksson & Zettervall 1986; Babbs *et al.* 1988; Mutimer *et al.* 1989; Niemela *et al.* 1988; Nyberg *et al.* 1992; Teare *et al.* 1993). On the contrary, the serial determination of PIIINP in 18 patients, treated by cyclosporine or cyclosporine\prednisolone combination (followed up for 13 years) indicated that PIIINP is not reliable for predicting the histological progression (Beukers *et al.* 1988).

Some authors described the clinical usefulness of the serial determination of PIIINP in the follow-up of patients with different chronic liver diseases, particularly in chronic active hepatitis (Weigand *et al.* 1984; Annoni *et al.* 1986; McCullough *et al.* 1987; Diodati *et al.* 1990; Wu & Danielsson 1995). It has been found that PIIINP has correlations with several clinical and biochemical characteristics of chronic liver diseases (e.g. in PBC patients). In different studies the correlated parameters are unlike (Eriksson & Zettervall 1986; Mutimer *et al.* 1989; Bell *et al.* 1989; Gabrielli *et al.* 1989; Gonzalez-Reimers *et al.* 1990; Trinchet *et al.* 1991; Nyberg *et al.* 1992; Gonzalez-Reimers *et al.* 1992; Diaz *et al.* 1993) and in some studies the prognostic significance of PIIINP in chronic liver diseases has been established (Weigand *et al.* 1984; Gonzalez-Reimers *et al.*1990; Bayerdorffer *et al.* 1991). By the Cox multivariate analysis, it has been discovered that PIIINP has prognostic value for patients with PBC (Eriksson & Zettervall 1986; Babbs *et al.* 1988; Poupon *et al.* 1994). At the same time Mutimer with co-authors found that PIIINP did not give prognostic information and it had no benefit over the existing conventional measurements in routine management of this disease (1989).

Immunosuppressive therapy (with prednisolone alone or in combination with azathioprine) significantly decreases serum PIIINP levels in both cirrhotic and noncirrhotic patients (Ballardini *et al.* 1984; Weigand *et al.* 1984; McCullough *et al.* 1987; Hayasaka *et al.* 1993).

Hyaluronan is a high molecular weight polysaccharide which is widely distributed in the extracellular matrix, especially of soft connective tissues (Gressner & Haarmann 1988; Laurent 1987; Laurent & Fraser 1992; Johnson 1996). It is synthesized in the plasma membrane of fibroblasts and other cells by addition of sugars to the reducing end of the polymer, whereas the nonreducing end protrudes into the pericellular space (Fraser & Laurent 1989; Laurent & Fraser 1992; Gressner 1994). Polysaccharide is catabolized locally or carried by lymph to lymph nodes or into general blood circulation, from where it is cleared by the endothelial cells of the liver sinusoids (Fraser & Laurent 1989; Laurent & Fraser 1992; Ueno *et al.* 1993). In hepatic sinusoidal capillarization, sinusoidal endothelial cells morphologically change and also seem to decrease hyaluronate degradation (Babbs *et al.* 1990; Ueno *et al.* 1993). Hyaluronan is

taken up and metabolized in liver endothelial cells by means of a receptor (Laurent *et al.* 1986; Fraser *et al.* 1986; Forsberg & Gustafson 1991; McGary *et al.* 1993). Patients with high serum hyaluronan levels, 200 ng/ml or more, have liver cirrhosis with typical hepatic sinusoidal capillarization (Ueno *et al.* 1993).

The pathophysiological mechanisms of hyaluronan increase in serum are not completely understood. The most probable hypothesis is that hyaluronan levels in serum reflect either an increased production and/or outflow from the tissues or a decreased elimination of polysaccharide by the liver endothelial cells or by other parts of the reticuloendothelial system or through the kidneys (Frebourg *et al.* 1986; Engström-Laurent 1989; Laurent & Fraser 1992). In cirrhosis, both hepatic secretion by sinusoidal endothelial cells and renal elimination are reduced, and the half-life of hyaluronan is prolonged in liver failure (Gressner 1991).

There are notably fewer clinical studies with hyaluronan than with PIIINP. Significantly increased hyaluronan was found in patients with chronic active hepatitis or cirrhosis (Engström-Laurent *et al.* 1985; Frebourg *et al.* 1986; Ramadori *et al.* 1991; Gibson *et al.* 1992; Ueno *et al.* 1993; Wu & Danielsson 1995). Increased hyaluronan levels might be a sensitive indicator for the prediction of cirrhosis and the progression of PBC (Nyberg *et al.* 1988; Nyberg *et al.* 1992). In alcoholic cirrhosis serum hyaluronan levels differed for the various Pugh grades and were significantly higher in Pugh grade C (Gibson *et al.* 1992). Indices of hepatocyte synthetic function, sinusoidal blood flow and degree of intrahepatic shunting are independent predictors of serum hyaluronan in alcoholic liver disease. (Gibson *et al.* 1992).

Statistical analysis of the data has revealed positive correlations between serum hyaluronan concentrations and several liver functional tests as galactose tolerance test, indocyanine green clearance, indocyanine green extraction, serum prothrombin time, serum albumin, serum bilirubin, ASAT (Frebourg *et al.* 1986; Nyberg *et al.* 1988; Ramadori *et al.* 1991; Nyberg *et al.* 1992; Gibson *et al.* 1992; Gibson *et al.* 1993). The serum hyaluronan appears to be associated more with liver function and portal hypertension than with the degree of the fibrotic process (Gressner 1991; Wu & Danielsson 1995).

Serum hyaluronan increases in PBC merit special emphasis as there is a close relation between these levels and the histopathological changes in the liver (Nyberg *et al.* 1988; Nyberg *et al.* 1992; Floreani *et al.* 1994; Guechot *et al.* 1994), the clinical course of PBC (Nyberg *et al.* 1988; Nyberg *et al.* 1992) and other serum variables reflecting liver functions (Fraser *et al.* 1986; Nyberg *et al.* 1988; Nyberg *et al.* 1992; Guechot *et al.* 1994). Plebani with co-authors (1990) has found that hyaluronan concentration is useful to separate early PBC (stages I–II) from the advanced disease complicated by fibrosis or cirrhosis. In advanced cases, serum hyaluronan displays negative correlation with the survival time (Nyberg *et al.* 1992). Circulating hyaluronan levels in patients with PBC and chronic viral C hepatitis correlated well with the severity of liver fi-

brosis (Guechot *et al.* 1994; Guechot *et al.* 1995). All these findings suggest that serum hyaluronan could be helpful in detecting and monitoring hepatic fibrosis, especially in PBC patients when it is measured dynamically (Nyberg *et al.* 1988). Hyaluronan seems to be a predictive factor for determining whether patients with PBC, treated with UDCA, enter the terminal phase of the disease (Poupon *et al.* 1994).

The results of studies, performed in PBC patients are contradictory, and frequently small amounts of patients have been studied. In addition, only few studies are dealing with a simultaneous PIIINP and hyaluronan measurement and the evaluation of their significance to the course of the disease (Ramadori *et al.* 1991; Nyberg *et al.* 1992; Poupon *et al.* 1994; Guechot *et al.* 1994).

3. AIMS OF THE STUDY

1. Establish the incidence and prevalence of PBC in Estonia, peculiarities in distribution between the ethnic groups and districts
2. Characterize PBC patients in Estonia
3. Follow the long-time course of the disease and determine the markers which could be useful for predicting the outcome
4. Verify the occurrence of ANA and their immunofluorescence patterns in patients with PBC, using tissue sections and cultured cells as antigen substrates and ascertain whether there are some ANA patterns which could be important for diagnosing PBC, especially in AMA-negative cases
5. Identify clinical, laboratory and prognostic differences between ANA-negative and ANA-positive PBC patients
6. Find out PIIINP and hyaluronan correlations with clinical, laboratory and histological features of PBC and their connections with short and long-term outcome.

4. PART I: EPIDEMIOLOGY OF PRIMARY BILIARY CIRRHOSIS IN ESTONIA (PAPERS I, IV)

4.1. Patients and methods

The investigation area was the Republic of Estonia. The population is fairly stable and 1,526,177 inhabitants lived in Estonia on 31st December 1992 (end of the retrospective epidemiological study). 62.6 percent of the inhabitants were of the Finno-Ugric origin, 35.2 percent of Slavic origin and 2.2 percent of other origin according to the data of National Census in 1989.

In the course of the epidemiological study an attempt was made to recognize all patients in whom PBC had been suspected in years 1973–1992. Firstly, in 1986 informative circulars were sent by Division of Gastroenterology, Department of Internal Medicine, University of Tartu to all district hospitals with a request to send all patients with probable PBC to Tartu for further investigations. Additionally, at the same time all gastroenterologists were asked to send information about all patients with PBC registered since 1973. Secondly, case histories of all patients with positive AMA results (with titre more than 1:40) during the period of 1978–1992 were reexamined at the Laboratory of Immunology, University of Tartu (Walker *et al.* 1965). During this period more than 9.000 sera were analysed and 83 were AMA-positive. Sixty-four of them met the conventional criteria for PBC diagnosing. (Sherlock & Scheuer 1973; Kaplan 1987). The diagnosis of PBC was based on the presence of the established clinical, biochemical, serological criteria and diagnostic or compatible liver biopsy findings (Sherlock & Scheuer 1973; Kaplan 1987). Also three patients with positive ANA reactions (AMA-negative), but with clinical, laboratory and histological data highly suggestive of PBC were included in the present study. The AMA test was not performed in two cases because there were no opportunities to detect autoantibodies in Estonia before 1978. In those cases clinical, laboratory (alkaline phosphatase more than two times above the upper reference value) and histological data of PBC were required.

After completing the epidemiological study in 1992, the collection of data on new PBC patients continued. Earlier registered patients with PBC were also under observation. Two sources of information were used, i.e. Department of Internal Medicine, University of Tartu and Division of Gastroenterology, Tallinn Central Hospital. Since 1994 AMA tests have been performed in the Tallinn Central Hospital too. During the period of December 31, 1992 until March 15, 1996, 28 new cases of PBC were diagnosed in Estonia. Altogether, 97 cases of PBC have been diagnosed in Estonia since 1973. Data of all those patients (n=97) were included in the patients analysis.

All the patients were evaluated for symptoms and signs. Several biochemical studies, including bilirubin, alkaline phosphatase, transaminases, cholesterol, total protein, albumin and globulin were done and immunoglobulins were measured. AMA were positive in 87 (91.6%) of 95 studied patients. Five out of eight AMA-negative patients had a high titre of ANA (antigen substrate — tissue sections), and clinical, laboratory and histological features highly confirmed the PBC diagnosis.

Patients were classified asymptomatic if no clinical symptoms or signs attributed to liver disease (pruritus, jaundice, pigmentation, hepatomegaly, splenomegaly, ascites and variceal bleeding) were found. Extrahepatic diseases of biliary tree were excluded by endoscopic retrograde cholangiopancreatography and/or ultrasonography. Upper gastrointestinal tract endoscopy was done in all patients for the evaluation of oesophageal varices. Both procedures were done at the time of the diagnosis and were repeated in case of need.

Liver biopsy specimens were available in 93 patients (95.9%) at the time of diagnosis. All liver specimens were reevaluated and the diagnosis confirmed by using the established histological criteria (Scheuer 1988). Liver biopsy was not available in four patients. If liver biopsy specimen was not available, a positive titre of AMA (>1:40) with an increased activity of alkaline phosphatase (more than two times above the upper reference value) was required for the PBC diagnosis.

Patients survival data and the causes of death were obtained from regional physicians or hospitals when the patients died. All patients were treated, most of the time, with prednisolone or with a combination of prednisolone and azathioprine. During the last two years, UDCA treatment was started in 27 patients.

The significance of differences was evaluated with the chi-square test. The Spearman rank correlation coefficient was used for correlation analysis. The Kaplan—Meier estimated survival curve was used for the survival analysis (Kaplan & Meier 1958). Only the survival data of the patients who had died due to the liver disease-related reasons (liver coma, variceal haemorrhages, liver cancer) were included in the final survival analysis. Stepwise regression analysis was used to estimate the factors influencing the survival. $P<0.05$ was considered statistically significant.

4.2. Results

During the period of 1973–1992, 69 cases of PBC were diagnosed in Estonia. The mean annual incidence was 2.27±1.4 per million, being 3.93 in the last year of the investigation (Fig. 1). On 31st December 1992, 41 patients were alive, giving the point prevalence of 26.9 per million (Fig. 2). Significant dif-

ferences among the various regions of Estonia were found (Fig 3). The highest prevalence was in the Viljandi County, being 123 per million on 31st December 1992 (8 cases of PBC among 64,885 inhabitants of this district, $p < 0.0001$).

As the study was continued until 15th March 1996, data on the 97 patients were used in the following analysis. Of the 97 PBC cases diagnosed so far, 76 patients were of the Finno-Ugric origin and 21 of Slavic origin ($p>0.05$). 92 were female and 5 male, yielding a male/female ratio 1:18.4. Mean age at the moment of diagnosis was 55.6±10.4 years (range 32–84 years) (Fig. 4). 61 out of 92 female patients (66.3%) were postmenopausal, the others being premenopausal.

The symptomatic period preceding the diagnosing of PBC was 2.1±1.95 years (range 0.1–10 years). Table 2 summarizes the clinical signs and symptoms at the time of the diagnosis. Most frequent symptoms and signs were pruritus, hepatomegaly, jaundice and pigmentation. Signs of portal hypertension were rare.

Table 2

Clinical characteristics of primary biliary cirrhosis (n=97)

Symptom or sign	No. of patients	Percentage
Pruritus	77	79.4
Hepatomegaly	58	59.8
Jaundice	51	52.5
Pigmentation	47	48.4
Fatigue	46	47.2
Abdominal pain	42	43.4
Xanthomas	33	34.0
Arthralgias	31	31.9
Weight loss	26	26.8
Palmar erythema	24	24.7
Teleangiectasia	20	20.6
Back pain	13	13.4
Oesophageal varices	13	13.4
Oedema	12	12.4
Splenomegaly	8	8.2
Ascites	8	8.2

In 61 out of 82 symptomatic patients (74.4%) itching was the first symptom while 9 (11%) patients were jaundiced. Itching and jaundice developed at the same time in 12 (14.6%) patients. In 40 out of 53 patients who were followed up at least for 5 years jaundice developed as follows: in 30.2% during the 1st year, in 60.4% during the 3rd year and in 75.5% during the 5th year.

Musculosceletal and joints systems complaints were found in 32 (33%) and 36 (37%) patients during the course of the disease, respectively. 13 PBC pa-

tients had back pain at the time of the diagnosis. During the course of the disease it developed in another 18 patients. On the contrary, arthralgias were early symptoms in patients with PBC, preceding the development of the liver disease associated symptoms up to 10 years in some cases.

Associated autoimmune conditions were reported in 34% of PBC patients (Table 3). Among them most frequent ones were keratoconjunctivitis sicca and Raynaud's signs.

Table 3

Associated autoimmune and other disorders (n=97)

Disease or disorder	No. of patients	Percentage
Autoimmune disorders	33	34.0
Keratoconjuctivitis sicca	11	11.3
Raynaud's signs	7	7.2
Lichen planus	6	6.1
Rheumatoid arthritis	4	4.1
Autoimmune thyroid disease	4	4.1
Systemic sclerosis	4	4.1
Systemic lupus erythematosus	2	2.1
Fibrosing alveolitis	1	1.0
Hemolytic anemia	1	1.0
Psoriasis	1	1.0
Dermatomyositis	1	1.0
Ankylosing spondylitis	1	1.0
Other disorders	7	7.2
Breast cancer	4	4.1
Hypernephroma	1	1.0
Ovarial cancer	1	1.0
Colon cancer	1	1.0
Stomach cancer	1	1.0

Altogether, seven patients (7.2%) gave a history of extrahepatic cancer: breast cancer in three, stomach cancer in one, colon cancer in one, ovarial cancer in one and breast cancer together with hypernephroma in one patient. All breast cancers had been operated on before PBC was diagnosed. Besides, six patients had benign breast tumors.

In 26 out of 97 patients (26.8%) gallstones in the gallbladder at some time of the course of the disease were diagnosed. As a rule, those patients had no history of biliary colics (silent stones). In 12 out of 26 patients the gallbladder had been removed before PBC was diagnosed whereas abnormal liver functional tests were connected with the presence of gallstones.

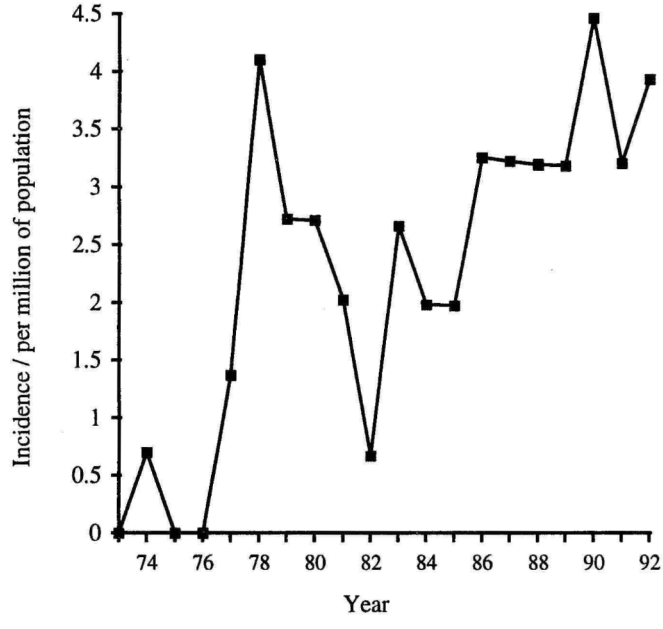

Fig. 1. Incidence of primary biliary cirrhosis in Estonia (1973–1992).

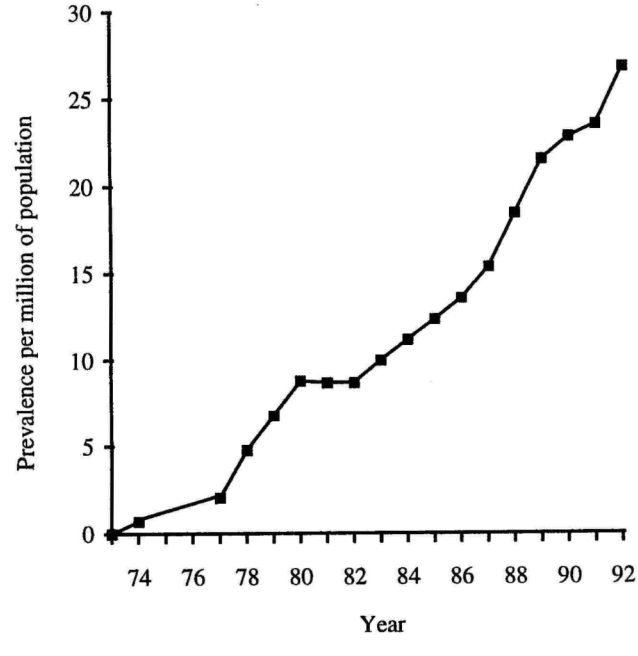

Fig. 2. Prevalence of primary biliary cirrhosis in Estonia (1973–1992).

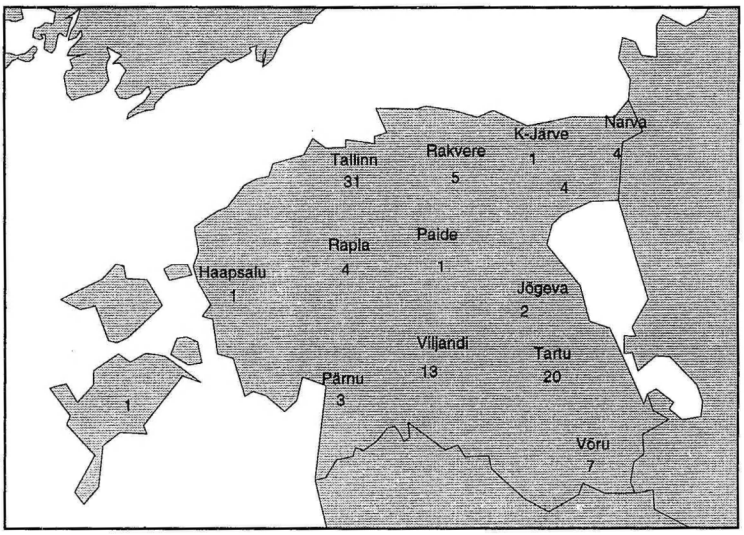

Fig. 3. Distribution of primary biliary cirrhosis in Estonia (n=97).

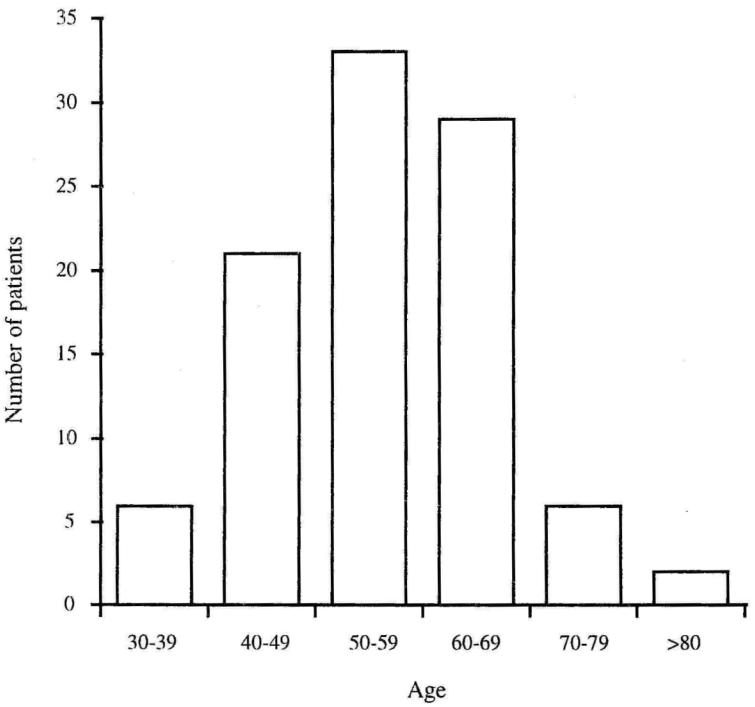

Fig. 4. Distribution of primary biliary cirrhosis by age (n=97).

Fifteen patients (15.5%) were classified as asymptomatic at the time of the PBC diagnosis. Laboratory data on symptomatic and asymptomatic patients are shown in table 4. Only bilirubin and alkaline phosphatase were different in those patients. During the mean 5.2±3.9 years of the follow-up in 7 out of 15 asymptomatic patients (47%) symptoms developed and 3 died (all deaths were liver disease associated).

Table 4

Laboratory data on symptomatic and asymptomatic PBC patients

Parameters	Asymptomatic patients (n=15) Mean±SD	Symptomatic patients (n=82) Mean±SD	p
Age (years)	51.6±9.1	56.4±10.5	>0.05
Bilirubin (μmol/l)	15.7±7.6	64.8±58.3	<0.00001
Alkaline phosphatase (U/l)	808±494	1610±994	<0.05
Cholesterol (mmol/l)	7.6±2.1	9.0±3.8	>0.05
ASAT (U/l)	69±37	87±48	>0.05
ALAT (U/l)	69±41	77±76	>0.05
Total protein (g/l)	89.0±9.4	86.4±9.3	>0.05
Albumin (g/l)	44.8±5.8	41.0±7.7	>0.05
Globulin (g/l)	45.6±6.9	44.9±8.4	>0.05
Sedimentation rate (mm/h)	37±15	40±6	>0.05
IgM (g/l)	8.1±5.0	5.3±4.6	>0.05
IgG (g/l)	17.9±6.3	16.7±7.0	>0.05
IgA (g/l)	3.0±0.9	3.0±1.7	>0.05
Follow-up period (years)	5.2±3.9	4.3±3.9	>0.05

Eighty-seven out of 95 patients (91.6%) were AMA-positive at a titre 1:40 or more. The activity of alkaline phosphatase was elevated in 94 of the 97 patients (97%) at the time of the diagnosis. Increased values of alanine and aspartate aminotransferase were found in 86 (87%) patients. Bilirubin was higher than 34 μmol/l (2 mg/dl) in 55 (56.7%) and more than 69 μmol/l (4 mg/dl) in 27 (27.8%) patients at the time of the diagnosis. Low albumin level (<35 g/l) was detected in 12 (12.4%) patients. Immunoglobulins were determined in 71 patients. IgM was higher than normal in 50 (70.4%) patients, IgG in 29 (40.8%) and IgA in 17 (23.9%) patients.

Hepatitis B surface antigen (HBsAg) was detectable in two patients and HCV antibodies were found in four cases during the course of the disease.

In 78 out of 93 cases (83.4%) liver biopsy finding was diagnostic for PBC. There were 6 cases of PBC in the first stage (7.7%), while 21 (26.9%) were found in the second stage, 27 (34.6%) in the third stage and 24 (30.8%) patients in the fourth stage. In 15 cases it was impossible to classify with the histological stage due to small specimens.

The clinical presentation of male patients was similar to that of female patients, except that they complained of less fatigue than females at the time of

diagnosis ($p<0.05$). Premenopausal and postmenopausal female patients with PBC differed in several aspects. Postmenopausal patients had at the time of the diagnosis more weight loss ($p<0.05$), splenomegaly ($p<0.05$), oesophageal varices ($p<0.05$), more advanced histological stages ($p<0.01$) than the premenopausal patients. At the same time premenopausal patients had higher alanine aminotransferase activity than the postmenopausal patients ($p<0.05$). Symptomatic period before the diagnosis of PBC was significantly longer in postmenopausal patients than premenopausal ones (2.3 ± 2.1 and 0.8 ± 1.2 years, respectively, $p<0.001$). Postmenopausal PBC patients' survival was significantly lower than in premenopausal patients since the time of the diagnosis (mean 4.2 ± 3.4 and 8.1 ± 5.3 years, respectively, $p<0.05$).

All patients were followed up from 0.1 to 20.4 years (mean 2.85 years). 38 patients died during the follow-up period: 22 of liver coma, 7 of bleeding of esophageal varices, 2 of liver carcinoma and 4 of non-liver associated disease (gastric cancer, car accident, stroke, arrhythmia). It was not possible to establish the cause of death in three cases. The mean survival time from the diagnosis of PBC was 5.5 ± 4.5 years (range 0.1–20.4 years) and from the development of the first symptom associated with the liver disease 7.8 ± 4.65 years (range 1.4–22.3 years). The overall estimated five-year survival was 78.1%. The estimated survival of patients with PBC is indicated in figure 5. Statistically significant rank correlations between the survival time and different clinical and biochemical markers are demonstrated in table 5. Patients with low survival had higher age, higher bilirubin concentration and γ-globulin relative concentration, but lower albumin values. They also had a more advanced histological stage, more ascites, oesophageal varices, oedema and insignificant or no hepatomegaly at the time of the diagnosis. None of the patients died in the first histological stage. The patients in the fourth histological stage had shortest survival time, being on the average 3.1 ± 2.8 years. Survival, depending on the different histological stages, has been shown in table 6.

Using the stepwise regression analysis, old age ($p<0.05$) and high bilirubin concentration ($p<0.05$) were established to be associated with poor outcome.

Table 5

Spearman's rank correlations between the survival (liver-disease-associated deaths) and different parameters.

Clinical or laboratory parameter	Rank correlation coefficient	p
Age	–0.4148	<0.05
Bilirubin	–0.4287	<0.05
Albumin	+0.4374	<0.05
γ-globulin (relative%)	–0.395	<0.05
Histological stage	–0.4128	<0.05
Hepatomegaly	–0.5540	<0.01
Ascites	–0.5639	<0.01
Oesophageal varices	–0.5230	<0.01
Oedema	–0.3606	<0.05

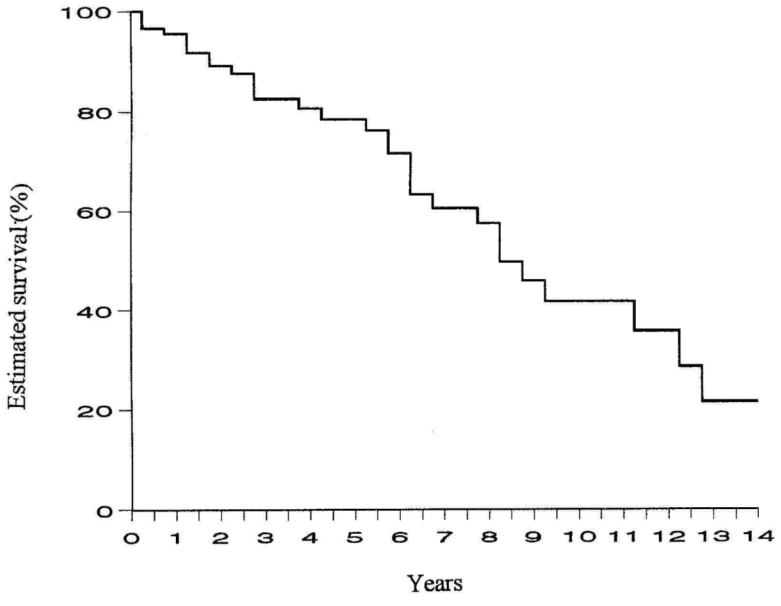

Fig. 5. Kaplan-Meier estimated survival curve for primary biliary cirrhosis patients (n=97).

Table 6

Survival of PBC patients belonging to the different histological stages (only deaths due to the liver disease included)

Histological stage	No. of patients	Deaths	Mean (years)	Range (years)
I stage	6	0 (0%)		
II stage	21	9 (42.9%)	8.0±5.9	1.2–20.4
III stage	27	6 (22.2%)	6.5±5.0	1.2–12.8
IV stage	24	11 (45.8%)	3.1±2.8	0.1–7.9
Not classified	15	4 (26.7%)	5.6±1.1	4.0–6.7

4.3. Discussion

The present study is dealing with the incidence, prevalence and clinicopathological features of PBC in Estonia. As PBC is a liver disease in which the treatment with liver transplantation is especially effective, the collection of PBC patients into the database is extremely necessary.

So far, the real prevalence of PBC is not known in the former East-European socialist countries. Only a few data about the epidemiology of PBC concerning Finno-Ugric peoples (including Finns) are published (Triger *et al.* 1984, Salupere *et al.* 1987).

The prevalence and incidence of PBC is low in Estonian population as compared with other European countries (Eriksson & Lindgren 1984; Löfgren *et al.* 1985; Myszor & James 1990; Danielsson *et al.* 1990). The point prevalence was 26.9 per million. Nevertheless, two peaks of incidence were found: first in 1978, when AMA testing was first applied in Estonia. The second elevation was in 1986. It could be explained by the distribution of an informative circular to all district hospitals. Since that year the PBC incidence has remained at a relatively stable level, being 5–7 new PBC cases per year. The highest number of PBC cases was diagnosed in 1993. It could be explained by an especially intensive education program in the field of PBC in Estonia (due to the continuation of a prospective epidemiological study).

In spite of a thorough search for new patients, PBC seems to be a rare disease in Estonia. One of the reasons for such a low prevalence of PBC in Estonia could be an insufficient use of routine screening methods (AMA, activity of alkaline phosphatase) at district hospitals. This may have led to an underdiagnosing of asymptomatic cases of PBC. For instance, during the past 10 years the percentage of asymptomatic PBC cases in studies carried out in Western Europe has increased to 70% (Eriksson & Lindgren 1984; Lofgren *et al.* 1985; Nyberg & Loof 1989; Danielsson *et al.* 1990; Jeffrey *et al.* 1990; Inoue *et al.* 1995) if compared to 15.5 per cent in Estonia. A greater use of screening methods rather large differences in the distribution of PBC in Estonia. The highest

prevalence was 123.3 per million in the Viljandi County in southern Estonia. The region is rural and its population is mainly engaged in grain-growing and cattle-breeding. It is of interest that 3 out of 8 patients with PBC from the Viljandi County, were living in Abja-Paluoja, a small town with 1747 inhabitants. Thus we could calculate an extremely high PBC prevalence for this town — 1717 per million. In some respect it could be explained by a relatively stable population situation in this area. All those patients have been living in the Abja-Paluoja area almost all their life. Some environmental factors could have played a role during such a long period. Certainly, genetic factors could also be taken into account. One familial case: daughter and mother have been found in our study population. Both live in Abja-Paluoja.

It has been supposed that PBC is more frequent in industrial areas (Hamlyn *et al.* 1983). However, no higher prevalence of PBC was found in northeast Estonia if compared with the overall prevalence of PBC in Estonia. Evidently, relatively unfavourable environmental situation does not seem to play a noticeably significant role in the occurrence of PBC.

In the Estonian population high female prevalence (18.4:1) was revealed if compared with the female/male ratio commonly found in Western Europe (Sherlock & Scheuer 1973; Triger *et al.* 1984; Nyberg & Loof 1989; Danielsson *et al.* 1990). It is not easy to explain such a discrepancy. However, since asymptomatic PBC is prevalent in men and the course of the disease is frequently atypical in men (Triger *et al.* 1984), the low number of asymptomatic cases in our material could be one of the reasons for such discrepancy.

The clinical presentation of PBC in males was similar to that of females, except that men had less fatigue noticed. As only one man died due to PBC during the follow-up period, the comparison of the clinical course of male and female patients was impossible.

It is also important to note that in postmenopausal patients the disease differed in several aspects if compared with premenopausal females. Obviously in postmenopausal patients PBC had progressed already into a more advanced stage as they had more often splenomegaly, esophageal varices and weight loss and their histological stage was worse at the moment of the diagnosis. Probably the overall duration of the disease was longer in postmenopausal patients. It could be added that at the same time cytolysis was much more expressed in premenopusal females. It is difficult to explain it, but higher estrogen levels could be one of the possible causes.

The clinical features of PBC patients in Estonia were typical of those previously reported (Sherlock & Scheuer 1973; Triger 1980; Christensen *et al.* 1980; Jeffery *et al.* 1990; Danielsson *et al.* 1990; Ilan & Shouval 1992). Pruritus was the most prevalent symptom in patients with PBC. As the symptomatic period preceding the diagnosing of PBC was relatively long (more than 2 years), PBC had developed into an advanced stage already e.g. 59.8% of patients were jaundiced already at the time of the PBC diagnosis and in 65.4% of patients

liver histology data indicated the third or fourth stage by Scheuer (1988). Before PBC was diagnosed by gastroenterologists, most of the patients had consulted once or more other physicians (e.g. neurologist, endocrinologist, psychiatrists, dermatologists) due to intensive pruritus.

The clinical association of PBC with other autoimmune disorders is in agreement with earlier reports (Christensen *et al.* 1980; Nyberg & Loof 1989; Jeffrey *et al.* 1990). However, the frequency of keratoconjunctivitis sicca was lower than in other published series, possibly because Schirmer's test was not routinely performed in the assessment. Moreover, we found PBC associated with ankylosing spondylitis in a 53-year-old female. Such kind of an association has not been described earlier. It is important to note that in PBC patients arthralgias could precede before the other PBC symptoms up to 10 years and some patients had been treated in the Division of Rheumatology before. Therefore, in patients with long time arthralgias and abnormal liver functional tests, the occurrence of autoimmune liver disease can always be suspected. On the contrary, back pain which was found in 31 out of 97 patients with PBC during the course of PBC was rare at the time of diagnosis (13 out of 31). It was not more frequent in postmenopausal females than in premenopusal ones. Probably, it was mostly associated with the development of the advanced liver disease and in part due to a long-time prednisolone treatment.

In asymptomatic patients, only alkaline phosphatase was significantly lower than in symptomatic patients, but aspartate and alanine aminotransferase activities were comparable. However, in symptomatic patients the activity of alkaline phosphatase did not correlate with pruritus. Therefore, the other substances, released into the blood circulation due to cholestasis could be responsible for pruritus. Histamin could be one of them (Gittlen *et al.* 1990).

Most PBC patients had a very high alkaline phosphatase activity at the time of the diagnosis. A high percentage of patients with PBC had cytolysis, but it was at a moderate level. Therefore, high alkaline phosphatase values accompanied by pruritus in female patients are highly suggestive of PBC. Liver synthetic function (albumin synthesis) was decreased only in about 1/10 of patients at the time of diagnosing PBC.

HBsAg and HCV-antibodies were rare in PBC patients. Other studies have reached to a similar conclusion (Brind *et al.* 1990; Pohjanpelto *et al.* 1991).

It has been indicated that most asymptomatic patients with PBC become symptomatic during the course of the disease. Their prognosis is the same as in patients being symptomatic at the time of the PBC diagnosis (Neuberger 1989; Mitchison *et al.* 1990; Mahl *et al.* 1994). In our material, about 50% of patients developed symptoms during the follow-up period (mean 5.2 years) and three patients died. The survival time from the development of symptoms in asymptomatic PBC patients and in symptomatic patients did not differ significantly. The asymptomatic status of patients with PBC is not so favourable as was thought earlier and in most cases the disease will progress continuously.

In 2.1% of patients liver carcinoma (one hepatocellular and one cholangiocellular) was diagnosed during the course of the disease. Certainly, small and asymptomatic liver carcinoma could be underdiagnosed in PBC patients, but it is obvious that liver carcinoma is not a frequent complication in patients with PBC as compared with alcoholic or viral cirrhosis patients.

Breast cancer was not more frequent in our material than compared with some earlier published data (Wolke *et al.* 1984; Goudie *et al.* 1985). It is important to add that six patients had benign breast tumors. It could be explained that changes in female sex hormone system are responsible for this (Eriksson *et al.* 1989; Becker *et al.* 1991; Floreani *et al.* 1991; Pearce *et al.* 1993).

Liver biopsy is very important for staging PBC patients and in connection with this to prognosticate the course of the disease (Roll *et al.* 1983; Christensen *et al.* 1985; Christensen *et al.* 1993). Liver biopsies were done in most cases by laparoscopical method for the present study as the method allows to visualize the liver. The specimens were taken from the most impressive damaged area. The method also makes it possible to receive larger specimens than by needle biopsy, and as a result more portal tracts were found in histological samples. This all led to better staging opportunities, and only in 16.4% of cases it was impossible to decide the stage exactly (mostly specimens from the early study period) .

A further point of interest was that in 12 out of 97 cases (12.4%) the diagnosis of PBC delayed due to the finding of gallstone disease. When after a surgical intervention the liver functional tests did not normalize and the symptoms did not disappear one could suspect PBC. Thus, we recommend testing of AMA in all female patients before gallbladder surgery, especially if they have abnormal liver function tests before the operation.

Two survival periods were studied in patients with PBC: one from the time of the diagnosis of PBC and the other from the development of the first symptom. It was necessary as the period preceding the diagnosis of PBC was relatively long. The survival data of PBC patients were lower than in several published reports (Roll *et al.* 1983; Nyberg & Loof 1989; Jeffrey *et al.* 1990; Rydning *et al.* 1990; Mitchison *et al.* 1990; Wiesner *et al.* 1991; Mahl *et al.* 1994) as most patients were symptomatic at the moment of the PBC diagnosis. Patients in whom PBC was diagnosed in the period of 1973–1985 (before the distribution of the information circular) had an especially short survival period. Only 67.7% of the patients lived for more than 5 years. However, 87.5% of PBC patients diagnosed between 1986 and 1991 lived for over five years. Recently, PBC has more frequently been diagnosed in patients with better clinical conditions than earlier. Also the positive influence of UDCA treatment during the last two years to the survival could be assumed in some patients.

The reasons for death were similar to the earlier published results (Danielsson *et al.* 1990; Inoue *et al.* 1995). Most frequent causes were liver coma and haemorrhages from oesophageal varices, but liver carcinoma was

rare. Four patients, whose survival was difficult to be associated with liver diseases were excluded from the survival analysis. It is possible that in some respect those deaths are connected with PBC, especially those which were caused by stroke and heart arrhythmia.

Several markers correlated well with poor survival. Using stepwise regression model, the survival of patients with PBC was independently connected with old age and high bilirubin levels. In several models it has been found that those factors, besides other ones (e.g. histological stage), are important to prognosticate a poor outcome (Schapiro *et al.* 1979; Roll *et al.* 1981; Christensen *et al.* 1985; Dickson *et al.* 1989; Goudie *et al.* 1989; Rydning *et al.* 1990; Christensen *et al.* 1993; Inoue *et al.* 1995). It has been supposed that bilirubin is an especially useful marker and its measurement could be informative to follow up the course of PBC in discrete patients and to include them in a liver transplantation program (Neuberger *et al.* 1990). In the present study the histological stage also correlates with the survival period, but in the present multiple regression analysis it loses its significance. Perhaps in follow-up studies the role of liver biopsy (as an invasive method) will decrease in the future whereas PBC progression could be detected enough exactly with a serial determination of bilirubin. In addition, coagulation failure in an advanced liver disease and uneven liver damage could sometimes make it difficult to perform liver biopsy and interpret its data.

5. PART II: SIGNIFICANCE OF VARIOUS ANTINUCLEAR ANTIBODY PATTERNS AND ANTIMITOCHONDRIAL ANTIBODIES IN THE COURSE OF PRIMARY BILIARY CIRRHOSIS (PAPER II)

5.1. Patients and methods

This part of the study includes 69 patients with PBC, diagnosed in the period of 1978–1994 at the Department of Internal Medicine, University of Tartu, Estonia. 61 out of 69 patients were symptomatic at the time of the diagnosis. Such a number of patients were included in the study because the sera of those patients were available at that time (stored at –20°C at the serum bank of the Laboratory of Immunology). The mean age of patients was 55.0±10.4 years (range 34–84, 2 males, 67 females). Six patients were in the first, 16 in the second, 22 in the third and 25 in the fourth histological stage of the disease.

Immunosuppressive treatment with prednisolone (40 patients), or with a combination of prednisolone and azathioprine (29 patients) was started after the diagnosis.

In all 69 patients serum was taken at the time of the diagnosis. All patients were evaluated for symptoms and signs, and several biochemical and immunological tests were done. In 37 patients blood was examined at least two times, the mean time between the first and the second determination of antinuclear antibodies (ANA) was 3.0±0.6 years. Altogether, 126 sera were tested.

During the follow-up of patients (on the average 5.5±3.4 years), 32 out of 69 patients with PBC died, 2 of them due to non-liver associated causes (car accident, stomach cancer). Those two patients were excluded from the outcome analysis.

Also, 16 sera of the first degree relatives and one husband of the PBC patients were studied (mean age 39.4 years, 9 males, 8 females). Among the relatives were 1 father, 1 mother, 7 sons, 5 daughters and 2 sisters of the patients with PBC. None of them had AMA test positive when immunofluorescence method on tissue sections was used. None of them had pruritus or any other liver disease associated symptoms. Their liver functional tests were also normal. One person had gallstones (silent), one had alopecia of unknown etiology, one had primary amenorrhea and one lung cancer.

The control groups included 21 patients with autoimmune hepatitis (AH), 26 patients with alcoholic liver disease (ALD), 13 patients with systemic connective tissue diseases (SCTD) represented by systemic lupus erythematosus (SLE) mainly, and 27 persons with irritable bowel syndrome (IBS). IBS patients had normal liver functional tests. No viral hepatitis markers (B, C) and autoantibod-

ies were revealed. Other chronic diseases were absent. The diagnoses of AH, ALD, SLE and systemic sclerosis were based on conventional clinical, laboratory and histological criteria (Kelley *et al.* 1989; Leevy *et al.* 1994). Those control groups were all age- and sex-matched with the main study group.

All sera were stored at –20 C° until testing. ANA were detected using air-dried cryostat sections from rat kidney, mouse liver and stomach (Walker *et al.* 1965), and HEp-2 cells as substrate at serum dilution 1:40. HEp-2 cells were grown on cover slips. The cells were fixed with 3.7% p-formaldehyde at room temperature for five minutes, followed by 100% methanol fixation at –20 C° for 10 minutes and treated afterwards with 0.2% Triton-X at room temperature for five minutes. FITC conjugated goat antihuman IgG (γ chain specific) antibody $F(ab)_2$ fragment (Sigma, St. Louis, USA) at dilution 1:50 was used as the second antibody. All washings were done with sterile phosphate buffered saline (PBS), containing 0.05% Tween 20. After the final washing the preparations were covered with other cover slips, and examined by fluorescence microscope Univar (Reichert, Germany) and photographed.

The ANA patterns were divided as follows: multiple nuclear dots (MND), fine speckled, homogeneous, perinuclear, nucleolar and centromere (ACA) according to the description by Bernstein with co-workers (1984).

Statistical analysis was performed using the one-way analysis of variance (ANOVA) for parametric signs and the Kruskal-Wallis one-way ranks analysis for non-parametric signs. Differences between groups were tested with the Mann-Whitney U test. The life-time analysis of survival was performed by the Kaplan—Meier method (Kaplan & Meier 1958).

5.2. Results

Using tissue sections as an antigen substrate ANA were revealed in 20 (29%) and AMA in 64 (93%) of PBC patients at the moment of the diagnosis. ANA reaction on tissue sections was also revealed in AH (57%) and in SCTD (85%) patients. Using HEp-2 cells as antigen substrate, more ANA-positive cases were detected among the PBC and AH patients: 70% and 62%, respectively. ANA were found in all five AMA-negative PBC cases: with MND pattern in two, fine speckled in one, homogeneous with MND in one and perinuclear in one patient. MND pattern was found in 29 out of 69 patients with PBC (42%), but also in two AH patients, and in one SCTD patient (table 7). In 13 out of 48 PBC patients with ANA (27%), at least two different ANA patterns were seen. In addition to these findings, there was also a high frequency of homogeneous ANA pattern in AH (45%) and SCTD (77%) patients, and ACA pattern in SCTD patients (54%).

Table 7

ANA and AMA findings in various chronic liver diseases, systemic connective tissue disease and irritable bowel syndrome patients

	PBC (n=69)	AH (n=21)	ALD (n=27)	SCTD (n=13)	IBS (n=29)
Immunofluorescence on tissue sections					
Antinuclear antibody positive	20 (29%)	12 (57%)	1 (4%)	11 (85%)	2 (7%)
Antimitochondrial antibody positive	64 (93%)	1 (5%)	0	1 (8%)	0
Immunofluorescence on HEp-2 cells					
Antinuclear antibody positive (all patterns)	48 (70%)	13 (62%)	1 (4%)	11 (85%)	2 (7%)
Fine speckled	6 (9%)	2 (10%)	1 (4%)	0	1 (3.5%)
Homogeneous	7 (10%)	9 (43%)	0	10 (77%)	1 (3.5%)
Nucleolar	2 (3%)	3 (14%)	0	1 (8%)	0
Perinuclear	11 (16%)	0	0	1 (8%)	0
Centromeric	7 (10%)	3 (14%)	0	7 (54%)	0
Multiple nuclear dots	29 (42%)	2 (10%)	0	1 (8%)	0

PBC — primary biliary cirrhosis, AH — autoimmune hepatitis, ALD — alcoholic liver disease, SCTD — systemic connective tissue disease, IBS — irritable bowel syndrome

Clinical and laboratory data of ANA-negative and ANA-positive patients are presented in Tables 8 and 9. 2/3 of patients had itching and about half had jaundice at the time of testing ANA. ANA-negative patients had higher sedimentation rate ($p<0.05$), more frequently ascites ($p<0.05$) and variceal haemorrhages ($p<0.05$), but other clinical and laboratory features were similar ($p>0.05$). No differences in histological stages were found. There were no more associated autoimmune diseases in the ANA-positive group ($p>0.05$).

AMA-negative ANA-positive (5 patients) and AMA-positive patients with PBC (64 patients) did not differ in clinical, laboratory and histological features from each other ($p>0.05$).

ACA-positive patients had more frequently the Raynaud phenomenon and scleroderma than ACA-negative PBC patients ($p<0.05$). Sicca syndrome was not statistically significantly more frequent in MND-ANA-positive patients than in MND-ANA-negative ones. MND-ANA-positive patients complained of more fatigue ($p<0.05$). No associations between the perinuclear ANA and joint symptoms were found ($p>0.05$), but patients with perinuclear ANA had higher IgM values than perinuclear ANA-negative patients (9.1±5.1 g/l vs. 5.8±2.5, $p<0.05$).

Table 8

Clinical features of ANA-positive and ANA-negative PBC patients

	ANA-positive patients (n=48)	ANA-negative patients (n=21)	p
Pruritus	35 (73%)	16 (76%)	>0.05
Jaundice	24 (50%)	10 (48%)	>0.05
Fatigue	20 (42%)	13 (62%)	>0.05
Abdominal pain	21 (44%)	6 (29%)	>0.05
Hyperpigmentation	23 (48%)	8 (28%)	>0.05
Xanthelasmata	20 (42%)	7 (33%)	>0.05
Arthralgia	14 (29%)	9 (43%)	>0.05
Back pain	4 (8%)	2 (10%)	>0.05
Weight loss	12 (25%)	7 (33%)	>0.05
Hepatomegaly	28 (58%)	14 (67%)	>0.05
Splenomegaly	4 (8%)	2 (10%)	>0.05
Ascites	1 (2%)	2 (10%)	<0.05
Oesophageal varices	3 (6%)	4 (19%)	>0.05
Haemorrhages ex varices	0 (0%)	2 (10%)	<0.05
Oedema	4 (8%)	4 (19%)	>0.05
Length of symptom. period (years)	1.9±2.0	2.0±2.5	>0.05
Length of follow-up period (years)	5.7±3.4	4.8±3.3	>0.05

Table 9

Laboratory data on ANA-positive and ANA-negative PBC patients

	ANA-positive patients (n=48)	ANA-negative patients (n=21)	p
Age (years)	54±9	57±12	>0.05
Bilirubin (μmol/l)	52±40	60±57	>0.05
Alkaline phosphatase (U/l)	1490±1036	1464±917	>0.05
ASAT (U/l)	96±40	84±48	>0.05
ALAT (U/l)	112±84	88±76	>0.05
Cholesterol (mmol/l)	9.1±3.8	9.9±4.4	>0.05
Total protein (g/l)	88±9	88±9	>0.05
Albumin (g/l)	43±6	41±6	>0.05
Globulin (g/l)	45±8	47±9	>0.05
SR (mm/h)	37±14	47±14	<0.05
IgM (g/l)	5.6±3.5	7.4±7.0	>0.05
IgG (g/l)	16.7±6.7	20.1±7.9	>0.05
IgA (g/l)	3.0±1.7	3.1±1.5	>0.05
Fat excretion (g/24h)	9.6±8.6	14.1±13.2	>0.05

The survival period from the time of diagnosing PBC (and ANA measurement) was longer in ANA-positive patients than in ANA-negative ones, 6.3 and

3.3 years respectively ($p<0.02$). The Kaplan—Meier survival curve was different in those groups, especially during the first five years of the follow-up (Fig. 6). At the same time the period between the development of the first symptom and diagnosing of PBC was similar in both groups (1.9 and 2 years, $p>0.05$, respectively). There were no statistically significant differences in histological stages at the beginning of the study and in the treatment regime between ANA-negative and ANA-positive patients group ($p>0.05$) during the course of the disease.

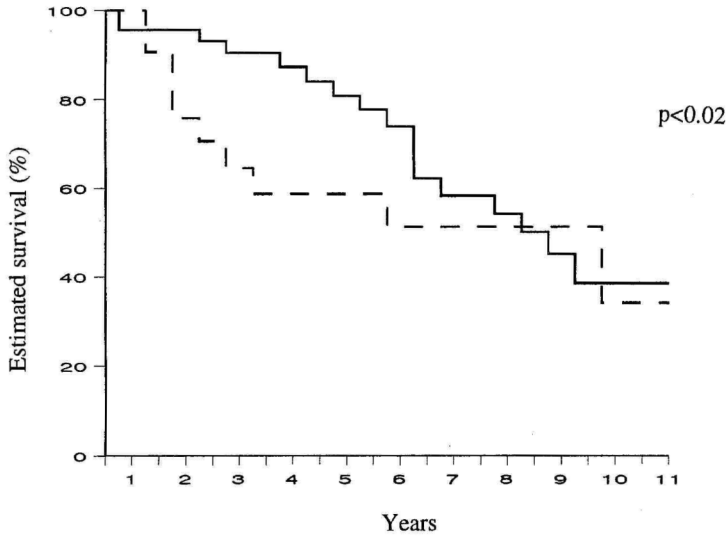

Fig. 6. Kaplan-Meier estimated survival curves for ANA-positive (—) and ANA-negative (·····) primary biliary cirrhosis patients (n=69).

In 37 patients (26 ANA-positive and 11 ANA-negative at the moment of the diagnosis) ANA were determined repeatedly during the course of the disease. ANA pattern did not change in 19 out of 26 (73%), changed in two out of 26 (6%), and ANA disappeared in five out of 26 persons (19%). During the follow-up period ANA did not develop in any patients who were ANA-negative at the time of the diagnosis. None of the patients developed liver cancer during the follow-up. No differences were found between those patients who lost or preserved ANA.

Two out of sixteen relatives of patients with PBC had ANA. In one case, homogeneous ANA pattern was found, another had MND-ANA. In the former case with the father of a PBC patient, lung cancer was diagnosed soon after the immunological investigation and the father died. In the second case, a 35-year-old son of a PBC patient, alopecia of unknown etiology has been diagnosed. Liver function tests were normal in both cases and liver biopsy was not performed for that reason.

5.3. Discussion

In the course of the study ANA were detected in PBC patients, using two substrates for immunofluorescence method, tissue sections and HEp-2 cell line. The results of the study confirm earlier investigations by other authors (Bernstein *et al.* 1984; Powell *et al.* 1984; Cassani *et al.* 1985; McMillan *et al.* 1987), indicating that ANA can be determined in chronic liver disease patients, especially in PBC sera more frequently if HEp-2 cells are used instead of frozen rodent tissue sections. Thus, the use of HEp-2 cells increases the ANA test sensitivity. In PBC, the use of HEp-2 cells as antigenic substrate for ANA is especially essential, because MND-ANA, thought to be characteristics of PBC, are hard to be detected in tissue sections. Another antibody staining pattern, detectable on HEp-2 cell line and invisible on tissue sections is ACA (Bernstein *et al.* 1984; Cassani *et al.* 1985).

In 70% of Estonian patients with PBC ANA were found by test with HEp-2 cell line. It is the highest percent described so far in the literature. MND-ANA were the most frequent ANA pattern in PBC patients and this was seen in 42 percent of cases. In other studies, the MND-ANA were reported in 13–44% of patients with PBC (Powell *et al.* 1984; Bernstein *et al.* 1984; Cassani *et al.* 1985; McMillan *et al.* 1987; Prost *et al.* 1987; Evans & Craft 1991; Fusconi *et al.* 1990; Szostecki *et al.* 1990). Additionally, MND-ANA were found in two AH patients and in one SLE patient. In those three cases the mean number of nuclear dots (four per cell) was smaller than that in patients with PBC (six per cell). One patient with AH had also additional fine speckled ANA, and another patient's MND were accompanied by a homogeneous pattern. Speckled and homogeneous ANA patterns are frequently found in AH (Cassani *et al.* 1985; Czaja *et al.* 1994). In those patients no clinical, biochemical or histological symptoms of cholestasis were revealed. One SLE patient had MND-ANA with a concomitant homogeneous ANA pattern but no clinical and biochemical signs of cholestasis. Some authors have also found MND-ANA pattern in diseases other than PBC (Hansen *et al.* 1991; Szostecki *et al.* 1987; Pawlotsky *et al.* 1992). It is possible that autoantigens or epitopes, at least in a part of the cases, are different from those recently cloned by Szostecki in PBC (Szostecki *et al.* 1990; Szostecki *et al.* 1992). The latter is directed against a soluble 100 kD protein (Sp-100 antigen) (Szostecki *et al.* 1987). Altogether, it should be pointed out that MND-ANA finding is highly suggestive for PBC. In the present study two out of five AMA-negative PBC patients had MND-ANA. Therefore, the test for MND-ANA could be an additional informative diagnostic test for PBC as it is highly specific. Moreover, MND-ANA were never observed in other chronic liver diseases, such as HBsAg-positive chronic hepatitis (Bernstein *et al.* 1984; Cassani *et al.* 1985; Szostecki *et al.* 1990), non A, non B hepatitis (Szostecki *et al.* 1990), primary sclerosing cholangitis (Szostecki *et al.*

1990) and alcoholic or cryptogenic cirrhosis (Cassani *et al.* 1985; McMillan *et al.* 1987). While other autoimmune diseases and overlap syndromes have frequently been found in patients with PBC, the possibility of asymptomatic PBC in a MND-ANA-positive SLE patient cannot be completely excluded before liver biopsy is performed. It should be added that those patients did not have AMA. In one study the specificity of MND-ANA for the diagnosis of PBC was 99.4% (Prost *et al.* 1987). The only study indicating that MND-ANA are not specific of PBC was done by Pawlotsky *et al.* (1992). He demonstrated that about 1/3 of patients with those antibodies had immunological disorders without liver involvement (Pawlotsky *et al.* 1992). As liver biopsy was not done in MND-ANA-positive patients during that research, the possibility of asymptomatic PBC in those patients or the development of this disease later cannot be excluded. The same hypothesis has been risen earlier as well (Szostecki 1990).

In the present study perinuclear ANA pattern was found in 18% of PBC patients. Such results are in accordance with the earlier performed studies of PBC (Ruffati *et al.* 1985; Lozano *et al.* 1988; Lassoued *et al.* 1990). Perinuclear pattern was also found in one patient with SLE. It has been indicated that perinuclear ANA pattern can also occur in AH and SLE patients as well, but in those cases antibodies could be directed against the other antigens (Lassoued *et al.* 1988; Wesierka-Gadek *et al.* 1988; Reeves *et al.* 1987; Chou *et al.* 1991). Therefore, using immunoblotting for the determination of the antigen molecular weight, it is possible to indicate a high probability of PBC. Experiments have shown that in PBC, perinuclear ANA recognize glycoprotein gp210 (Wozniak *et al.* 1989; Courvalin *et al.* 1990; Nickowitz & Worman 1993; Nickowitz *et al.* 1994) and lamin B receptor (Worman *et al.* 1988; Courvalin *et al.* 1990; Nickowitz *et al.* 1994).

Altogether, MND-ANA and perinuclear ANA were found in three out of five AMA-negative patients, and their testing could be of particular clinical use in diagnosing PBC when the AMA titre is low or AMA were not found.

Some authors have used the term autoimmune cholangitis for AMA-negative patients with PBC, although clinical, laboratory and histopathological characteristics are very similar to the AMA-positive PBC (Taylor *et al.* 1994; Mitchileti *et al.* 1994; Goodman *et al.* 1995; Lacerda *et al.* 1995). It seems that AMA-negative PBC has to be treated as a subtype of PBC as no differences have been revealed. Therefore, it is extremely necessary to determine ANA in cholestatic patients.

As the studied PBC patients were mostly in the third and fourth histological stages at the time of the diagnosis, the survival time of the patients was shorter than described in earlier studies (Roll *et al.* 1983; Jeffrey *et al.* 1990; Rydning *et al.* 1990; Mitchison *et al.* 1990; Wiesner *et al.* 1991; Mahl *et al.* 1994). According to the ANA test results, patients with PBC could be divided into two subgroups. The first group comprised ANA-positive patients with a relatively good prognosis, the second group included ANA-negative patients with a rela-

tively poor outcome. So far, it is difficult to explain such differences in the prognosis. Evidently, ANA-positive patients have better response to immunosuppressive treatment than ANA-negatives. From this aspect they could be similar to AH patients, although AH and PBC patients have different ANA patterns (Czaja *et al.* 1994).

It is unclear why and how ANA develop in so many patients with PBC, but this problem is still unresolved for other antibodies, as well including AMA. Most likely ANA do not cause liver damage directly in PBC, but will develop after the damage against the expressed and released liver cell structures has already started. Cellular immune mechanisms have been demonstrated to be essential in PBC (Vierling *et al.* 1992; Onishi *et al.* 1993; Bjorkland *et al.* 1994; Martinez *et al.* 1995; Jones *et al.* 1995).

Patients with PBC often have associated disorders (Crowe *et al.* 1980; Kaplan 1987; Nyberg & Loof 1989; Jeffery *et al.* 1990; Laurin & Lindor 1994; Inoue *et al.* 1995). The MND-ANA were considered to be present especially when PBC was associated with the sicca syndrome (Bernstein *et al.* 1984; Cassani *et al.* 1985). In the present study, the incidence of Sjögren's syndrome was much lower than expected. The incidence of MND-ANA was not connected with clinically expressed Sjögren's syndrome in the studied patients, but MND-ANA-positive patients complained of more of fatigue.

Nickowitz *et al.* (1994) found that by determining autoantibodies to integral proteins of the nuclear membranes it is possible to identify a subgroup of patients with PBC, predisposed to the development of rheumatoid arthritis. In the present study patients with perinuclear ANA had not more frequently rheumatoid arthritis than perinuclear ANA-negative patients. However, higher IgM values in perinuclear ANA-positive patients were found. It seems that a major part of perinuclear ANA is of the IgM type.

Follow-up studies on different ANA types have not been performed in patients with PBC so far. We found that as a rule ANA pattern did not change during the 3 years of treatment, and there was no ANA development in ANA-negative PBC cases. Although, immunosuppressive treatment was started after the diagnosis of PBC, only few patients changed or lost ANA during the course of the disease. Earlier it has been explained that the change of ANA pattern could be connected with cancerogenesis in liver diseases (Imai *et al.* 1993). None of patients with changed ANA patterns developed liver cancer in the present population study. As a matter of fact AMA did not disappear in PBC patients either. There were no clinical or outcome differences between the PBC patients with a changed ANA pattern compared to patients who preserved their ANA pattern. Maybe a longer investigation period is necessary to appraise the significance of this change for the outcome.

Several immunological and genetic abnormalities have been described in the relatives of patients with PBC (Galbraith *et al.* 1974; Salaspuro *et al.* 1976; Jaup & Zettergen 1980; Triger *et al.* 1989; Klein & Berg 1990; Caldwell *et al.*

1992; Tsuji *et al.* 1992; Harada *et al.* 1992; Maran *et al.* 1994; Bach & Schaffner 1994; Brind *et al.* 1995) and PBC is also more frequently diagnosed in them. Two out of 16 first-degree relatives of the PBC patients were ANA-positive. In one of them lung cancer was diagnosed later. It has been reported that in malignant processes ANA could be found in sera (Burnham 1972; Hodson & Turner-Warwick 1975). Probably ANA development in this case was also related to cancer. Another was a young man with alopecia. His liver function tests were normal. As liver biopsy was not done in that case, one cannot tell exactly whether that was an asymptomatic PBC case, but the follow-up has strongly indicated in the case.

6. PART III: AMINOTERMINAL PROPEPTIDE OF PROCOLLAGEN TYPE III AND HYALURONAN IN PRIMARY BILIARY CIRRHOSIS (PAPER III)

6.1. Patients and methods

Fifty-five patients with PBC (53 females, two males, mean age 54.7 years, range 34–84) were studied at the time of the diagnosis and were followed up for a mean period of 4.8 years (range 0.4–10.8 years). Such number of patients were included into the study whereas sera of those patients were available at that time. All studied patients were AMA-positive. 48 out of 55 patients were symptomatic at the time of the diagnosis.

None of those PBC patients had received immunosuppressive and/or antifibrotic treatment until the diagnosis. Treatment with prednisolone or with a combination of prednisolone and azathioprine was started in symptomatic patients after the diagnosis .

During the follow-up period 21 patients died (all symptomatic), 17 of them due to the liver disease, four due to the causes unrelated to the liver disease (car accident, cardiac arrhythmia, stroke, stomach cancer).

The control group was formed of 30 persons with irritable bowel syndrome. They had normal liver functional tests, no viral hepatitis markers (B, C) were established. Those persons did not have other chronic diseases. The control group was age- and sex-matched with the PBC group (29 females, 1 male, mean age 55.8 years, range 36–81).

Before starting the immunosuppressive treatment, samples of blood were collected by venipuncture in the morning (fasting state). Serum was separated by centrifugation soon and stored at -20^{0}C until testing. Procollagen III peptide (PIIINP) was measured by radioimmunoassay (Orion, Espoo, Finland) as described by Risteli (1988). Serum hyaluronan was assayed with a sequential radiometric assay (HA-test, Pharmacia Diagnostics, Uppsala, Sweden). This test is based on the use of specific hyaluronic acid-binding proteins isolated from bovine cartilage.

Biochemical and immunological tests were performed with the same blood samples (e.g. transaminases, cholesterol, alkaline phosphatase, gamma-glutamyltranspeptidase, bilirubin, albumin, globulin, fat excretion, immunoglobulins). At the time of collecting sera patients were examined for clinical signs and symptoms. All patients had also upper oesophagogastroduodenoscopy and ultrasound examination for the evaluation of the presence of portal hypertension.

In all patients liver biopsy was done at the time of the diagnosis. Biopsy specimens were fixed in 10% formalin and stained with hematoxylin-eosin. Histological data were staged according to the criteria of Scheuer (1988).

In twenty-six patients with PBC (all females, mean age 52, range 34–73), PIIINP and hyaluronan were studied dynamically. 23 out of 26 patients were symptomatic at the time of the diagnosis.

At the time of the diagnosis the blood samples were taken for the PIIINP and hyaluronan measurement. After diagnosing PBC, immunosuppressive treatment was started with prednisolone (16 patients) or with prednisolone-azathioprine combination (10 patients).

The second investigation of patients was done on the average 4.2±2.1 years later (range 1.5–8.1). After that the patients were followed up at least for 1 year (mean 2.4 years). During the follow-up period 11 patients died, 7 of them during the first year. All those patients died due to the liver disease (8 liver coma and 3 haemorrhages ex varices).

The patients of the 1st and 2nd histological stages at the time of the diagnosis were joined into one group (non-advanced PBC, 10 patients) and the 3rd and 4th to the other group (advanced PBC, 16 patients) for the following analysis. The data at the time of the diagnosis of PBC were marked with $index_I$ and the second measurements with $index_{II}$.

Differences between the groups were tested with the Mann-Whitney U test. Spearman's rank correlation coefficient was used for the correlation. The contributions of PIIINP and hyaluronan levels to a multivariate prediction of the outcome, using also bilirubin, albumin, age and histological stage as independent factors, were assessed by the logistic regression analysis according to the Cox model. $P<0.05$ was considered statistically significant.

6.2. Results

The upper limit of the normal value for serum levels of PIIINP (mean + 2SD) was 4.8 ng/ml, and in 45 (82%) patients the concentration exceeded this value. The upper limit of the normal value for serum hyaluronan was 74 µg/l and in 46 patients (84%) the concentration was over that. The mean PIIINP and hyaluronan concentrations of PBC patients were 8.8 ng/mL and 280 µg/l, respectively, with the highest concentration in patients of the fourth histological stage. The PIIINP sensitivity and specificity for diagnosing the cirrhotic stage were 0.87 and 0.56, respectively, and for diagnosing advanced PBC (3rd and 4th histological stage) were 0.84 and 0.7, respectively. The hyaluronan sensitivity and specificity for diagnosing cirrhosis were 0.94 and 0.52, respectively and for diagnosing advanced liver disease 0.91 and 0.64. Table 10 shows the PIIINP and hyaluronan values of the patients with PBC, according to the histological

stage. The highest PIIINP and hyaluronan values were found at the cirrhotic stage of PBC (4th stage), but the concentrations of both markers had already risen at the 2nd and 3rd stages. PIIINP concentrations were normal at the 1st histological stage of the PBC patients, but hyaluronan values were higher than in the control group. There was statistically significant correlation between the level of PIIINP and that of the histological stage ($r=0.44$, $p<0.01$), hyaluronan and histological stage ($r=0.56$, $p<0.00001$). The correlation between PIIINP and hyaluronan was 0.46 ($p<0.01$).

In symptomatic patients with PBC the values of PIIINP were higher than in asymptomatic ones (9.4±7.2 ng/mL, median 7.9, vs. 4.9±1.6 ng/mL, median 4.6, $p<0.05$). Hyaluronan was also higher in symptomatic patients (304±246 µg/l, median 201 vs. 111±119 µg/l. median 70, $p<0.01$). Three out of seven asymptomatic patients had both hyaluronan and PIIINP above the normal value. Two of them developed symptoms soon, but one remained asymptomatic during the follow-up period. The concentration of hyaluronan was in moderate correlation with the age of the patients ($r=0.33$, $p<0.05$).

PIIINP had no statistically significant correlation with the clinical symptoms of PBC.

Serum hyaluronan values correlated with pruritus ($r=0.32$, $p<0.05$), jaundice ($r=0.31$, $p<0.05$), fatigue ($r=0.41$, $p<0.01$), hepatomegaly ($r=-0.46$, $p<0.001$), esophageal varices ($r=0.34$, $p<0.01$) and weight loss ($r=0.29$, $p<0.05$). There were no correlations with pigmentation, abdominal pain, arthralgias, back pain, xanthomas, palmar erythema, spider naevi, splenomegaly, ascites, hemorrhages and oedemas. The hyaluronan concentration correlated with the length of the symptomatic period of the disease before the diagnosis of PBC ($r=0.43$, $p<0.01$).

Table 11 indicates that both, PIIINP and hyaluronan correlated positively with bilirubin, but hyaluronan correlated better. Hyaluronan correlated besides bilirubin also with the extent of fat excretion and inversely with albumin concentration. Correlations with other immunological and biochemical parameters were not found.

Using the Cox logistic regression analysis it was established that the survival was influenced only by the bilirubin concentration ($p<0.05$), but not by that of hyaluronan, PIIINP, age, albumin and histological stage ($p>0.05$).

Table 10

Values of PIIINP and hyaluronan in patients with primary biliary cirrhosis and healthy persons

	N.	PIIINP Increased N (%)	(ng/ml) p	Mean±SD	Range	Median	Hyaluronan Increased N (%)	(µg/l) p	Mean±SD	Range	Median
PBC	55	45(81)	<0.00001	8.8±6.8	2.7–45.3	7.3	46(84)	$<1.010^{-8}$	280±91	34–850	189
I stage	5	1(20)	0.3	4.0±0.9	2.8–5.1	4	1(20)	<0.05	66±34	34–122	56
II stage	18	14(78)	<0.0001	7.4±3.0	4.2–15.2	6.6	16(89)	<0.0000001	200±186	47–737	139
III stage	16	13(81)	<0.0001	8.0±4.1	2.7–17.8	7.6	14(88)	<0.000001	233±194	69–690	146
IV stage	16	14(88)	<0.0001	13.5±11.3	4.8–45.3	10	15(94)	<0.0000001	466±260	54–850	480
Control	30	2(7)		3.5±0.6	2.3–5.0	2.9	2(7)		39±18	13–76	22

Table 11

Spearman's rank correlations of PIIINP and hyaluronan with laboratory tests (n=55)

	PIIINP		Hyaluronan	
	r	p	r	p
Bilirubin	0.43	<0.01	0.54	<0.0001
Alkaline phosphatase	0.14	>0.05	0.006	>0.05
Cholesterol	0.12	>0.05	0.08	>0.05
ASAT	0.26	>0.05	0.002	>0.05
ALAT	0.03	>0.05	–0.003	>0.05
Total protein	0.05	>0.05	–0.25	>0.05
Albumin	–0.06	>0.05	–0.30	>0.05
Globulin	0.02	>0.05	–0.008	>0.05
γ-globulin	0.08	>0.05	0.07	>0.05
IgM	–0.005	>0.05	–0.2	>0.05
IgG	0.09	>0.05	0.001	>0.05
IgA	0.07	>0.05	0.23	>0.05
Steattorrhea	0.25	>0.05	0.53	<0.01

Clinical and laboratory characteristics of patients in a dynamic study have been presented in Tables 12 and 13. In the second examination, patients with PBC had less pruritus ($p<0.05$), more hyperpigmentation ($p<0.05$), more back pain ($p<0.01$), more hepatomegaly ($p<0.01$), more ascites ($p<0.05$), more esophageal varices ($p<0.01$) and more oedema ($p<0.05$) than at the time of the diagnosis (Table 12). Concerning the second laboratory tests, the PBC patients had an increase of hyaluronan but lower total protein ($p<0.001$), albumin ($p<0.001$) and IgM ($p<0.01$) levels than during the first investigation (Table 13). Bilirubin had also a positive trend for elevation, but had not acquired significance yet ($p=0.13$).

In the follow-up study of 20 (77%) patients the concentration of PIIINP was higher than normal at the time of the diagnosis. At the end of the investigation period PIIINP was above the normal value in 19 of the patients (73%) with PBC. Mean level of PIIINP was 8.1±2.74 (range 3.7–13.4, median 7.6) and 8.04±4.59 (range 3.9–20.2, median 6.3) at the time of the diagnosis and during the second measurement, respectively. For all the PBC patients, PIIINP values did not change significantly during the follow-up period, although the trend was towards a fall of concentration in advanced stage patients ($p=0.09$) (Table 14). In spite of immunosuppressive treatment either with prednisolone or with prednisolone/azathioprine combination, in both groups the values of PIIINP remained at a stable level ($p>0.05$).

Table 12

Clinical characteristics of 26 PBC patients at the time of diagnosis and in the second investigation. The second investigation of the patients was done on the average 4.2±2.1 years later (range 1.5–8.1).

Symptom or sign	At the time of diagnosis Number of positive findings (%)	In the 2nd investigation Number of positive findings (%)	p
Pruritus	20 (77)	10 (38.5)	<0.05
Jaundice	14 (54)	18 (69)	0.10
Fatigue	9 (35)	14 (54)	0.20
Pigmentation	12 (46)	18 (69)	<0.05
Abdominal pain	9 (35)	4 (15)	0.28
Arthralgia	7 (27)	5 (19)	0.61
Back pain	4 (15)	13 (50)	<0.01
Xanthomas	11 (42)	16 (61.5)	0.06
Weight loss	5 (19)	10 (38.5)	0.06
Palmar erythema	4 (15)	10 (38.5)	0.14
Spider naevi	5 (19)	6 (23)	0.8
Hepatomegaly	14 (54)	17 (65)	<0.01
Splenomegaly	1 (4)	2 (8)	0.60
Ascites	0 (0)	6 (23)	<0.05
Oesophageal varices	2 (8)	14 (54)	<0.01
Bleeding	0 (0)	3 (11.5)	0.18
Oedema	0 (0)	6 (23)	<0.05

Table 13

Laboratory characteristics of 26 PBC patients at the time of the diagnosis and during the second investigation

	At the time of diagnosis Mean±SD	In the 2nd measurement Mean±SD	p
PIIINP (ng/ml)	8.10±2.7	8.04±4.6	0.88
Hyaluronan (mg/l)	263.5±257.4	350.5±261.6	<0.05
Bilirubin (mmol/l)	50±38	70 ± 65	0.13
Alkaline phosphatase (IU/l)	1505±879	1213±443	0.24
Cholesterol (mmol/l)	8.9±2.5	8.4±2.6	0.16
ASAT (IU/l)	82±28	96±25	0.57
ALAT (IU/l)	90±40	95±44	0.76
Total protein (g/l)	90±7	82±4	<0.001
Albumin (g/l)	45±4	39±6	<0.001
IgM (g/l)	6.8±6.3	3.9±3.0	<0.01
IgG (g/l)	18.1±8.1	15.4±3.8	0.5
IgA (g/l)	3.3±1.9	3.1±1.0	0.78
Fat excretion (g/24h)	9.6±8.6	13.2±17.3	0.62

Hyaluronan was above the normal concentration in 19 (73%) patients at the time of the diagnosis and in 21 (81%) at the second examination. The mean hyaluronan concentration was 263.5±257.4 (range 34–850, median 161) at the time of diagnosing PBC and 350.5±261.6 (range 29–1117, median 304) in the repeated measurement. A significant elevation of hyaluronan concentration was found ($p<0.05$). It was mostly due to the increase of hyaluronan values in non-advanced PBC patients (Table 14). A later analysis indicated that in the prednisolone/azathioprine treated group the hyaluronan values did not change during the follow-up period (mean 247.7 vs. 280.7 μg/l, $p>0.05$). In the prednisolone-treated group hyaluronan concentration rose significantly (mean 285.7 vs. 404.8 μg/l, $p<0.05$). There were no differences at the histological stages between the prednisolone- and azathioprine/prednisolone-treated patients.

Table 14

Concentrations of PIIINP and hyaluronan related to the histological stages in PBC patients and healthy persons in the follow-up study (n=26)

	At the time of diagnosis			In the 2nd measurement			
	Mean ±SD	Range	Median	Mean ±SD	Range	Median	p
PIIINP							
Healthy persons (30)	3.5±0.6	2.3–5.0	2.9				
All patients (26)	8.1± 2.7	3.7–13.4	7.6	8.04±4.6	3.9–20.2	6.3	>0.05
I–II histological stage (9)	7.2 ± 2.1	4.2–9.6	6.7	9.2±6.4	5–20.2	6.4	>0.05
III–IV histological stage (17)	8.6±3.0	3.7–13.4	8.5	7.4±3.7	3.9–16.3	6.2	>0.05
Hyaluronan							
Healthy persons	39±18	13–76	22				
All patients	263±257	34–850	161	350±262	29–1117	304	<0.05
I–II histological stage (9)	134±86	34–276	121	317±279	54–850	210	<0.05
III–IV histological stage (17)	324±290	54–850	202	366±260	29–1117	315	>0.05

At the time of the diagnosis the PIIINP values correlated with $bilirubin_I$ ($r=0.4912$, $p<0.05$), but during the course of the disease this correlation disappeared ($p>0.05$). The $hyaluronan_I$ values correlated with $bilirubin_I$ ($r=0.5528$,

$p<0.01$) and preserved this correlation during the second measurement ($r=0.5912$, $p<0.01$). At the same time $hyaluronan_I$ correlated with $hyaluronan_{II}$ ($r=0.8115$, $p<0.001$), but $PIIINP_I$ did not correlate with $PIIINP_{II}$.

Patients who died during the one year after the second investigation had significantly higher hyaluronan ($p<0.05$) and bilirubin concentrations ($p<0.05$), lower total protein ($p<0.05$) and albumin values ($p<0.05$) than the survivors during the second investigation. They had also more ascites ($p<0.01$) and back pain ($p<0.01$) during the repeated examination. Data on the survivors and non-survivors are shown in Table 15.

Table 15

Clinical and biochemical markers of patients (n=7) who died within 1 year after the second investigation, compared with the indices of patients (n=19) surviving the period

	Non-survivors		Survivors		
	Mean±SD	range	Mean±SD	range	P
$PIIINP_I$	12.7±4.1	10–18.8	8.0±3.4	3.7–15.2	<0.05
$Hyaluronan_I$	481±290	161–823	179±192	34–850	<0.01
$Hyaluronan_{II}$	520±292	304–1117	273±223	29–788	<0.05
$Bilirubin_I$	80.6±46.9	42.7–177.8	38.0±27.2	8.9–97.6	<0.05
$Bilirubin_{II}$	105.3±61.6	56.4–210	58.1±64.7	10–270	<0.05
$Total\ protein_{II}$	74.1±4.5	69.9–80	86.2±8.8	68–98.3	<0.05
$Albumin_{II}$	35.1±3.5	30.2–38.1	41.0±5.6	32.2–48.9	<0.05
Length of symptomatic $period_I$	6.2±5.6	0.4–14.3	1.6±2.4	0–9.1	<0.05
Histological $stage_I$ adv/pt	7/7		9/19		<0.05
$Hepatomegaly_I$	6/7		8/19		<0.05
$Jaundice_I$	7/7		7/19		<0.01
$Ascites_{II}$	5/7		2/19		<0.01
$Back\ pain_I$	3/7		1/19		<0.05
$Back\ pain_{II}$	7/7		6/19		<0.01

6.3. Discussion

In the present study two fibrogenesis markers, PIIINP and hyaluronan, were measured in patients with PBC. It became evident that both, PIIINP and hyaluronan are highly sensitive, but not so specific for diagnosing advanced PBC. One-time measurement of those markers did not allow to differentiate advanced PBC patients (3rd or 4th histological stage: fibrosis and cirrhosis) from the 2nd histological stage patients, belonging to the non-advanced PBC group. In most patients of the 2nd histological stage fibrogenesis markers were high, too. However, in nearly all of the 1st stage patients PIIINP values were normal. Earlier some authors have shown that in the 1st histological stage patients the values of PIIINP could be significantly higher than in healthy persons (Babbs *et al.* 1988; Niemela *et al.* 1988; Nyberg *et al.* 1992; Ramadori *et al.* 1991). It has been assumed that the destruction of bile ducts, bile leakage, and chronic inflammatory cell infiltration of portal tracts could stimulate fibrogenesis (Seyer 1985). There is no apparent fibrosis yet at the 1st and 2nd histological stage (Scheuer 1988).

It has to be taken into account that the test system used during our research (worked out by Risteli with co-authors, 1988), mainly measures intact procollagen III, not its small degradation products and is more specific for the determination of fibrogenesis itself. In several other studies test systems were used, in which all procollagen molecules as well as their degradation products are measured. Therefore, they give information on fibrogenesis and fibrolysis in the liver simultaneously (Rohde *et al.* 1979). As in the patients with PBC both processes are accelerated, it was possible to measure higher PIIINP values already in non-advanced PBC patients (Babbs *et al.* 1988; Niemela *et al.* 1988; Plebani *et al.* 1990).The values of PIIINP measured by Orion's PIIINP assay are about 1/3 of those, measured by the previously available commercial assay (Risteli *et al.* 1988).

There can be various other reasons for the PIIINP elevation in the patients with PBC: diminished metabolism through the liver endothelial cells (Smedsrod 1988; Bentsen *et al.* 1989; Melkko *et al.* 1994) and impaired PIIINP excretion due to cholestasis (Raedsch *et al.* 1987; Babbs *et al.* 1988). It has been shown that significant changes in the endothelial cell structure and functions occur in the PBC patients and it appears to be a contributing factor to the progression of the disease (Babbs *et al.* 1990; Ueno *et al.* 1993). Such changes can occur already in the 1st and 2nd histological stage. On the other hand, we have to take into account the fact, that liver damage in patients with PBC is uneven (Scheuer 1988). There can be areas in the liver with inconsiderable and other areas with advanced liver damage. Advanced damage can be responsible for the high PIIINP values in some patients belonging to the 1st or the 2nd histological stage. Therefore, the measurement of PIIINP can be even more infor-

mative than liver biopsy, as it shows the activity of fibrotic process more precisely and correlates well with the histological stage of PBC. At fibrotic and cirrhotic stages when PIIINP is released abundantly due to the accumulation of collagen III and decreased clearance by liver endothelial cells, extremely high PIIINP concentrations can be measured in blood. Patients with high PIIINP values of the earlier histological stages probably develop active cirrhosis more quickly.

A-vitamin deficiency is another factor which can favour the synthesis of collagen III in cholestatic liver diseases (Takase *et al.* 1992).

Several studies have indicated that PIIINP correlates in chronic liver diseases with several biochemical indices (Bell *et al.* 1989; Gabrielli *et al.* 1989; Gonzalez-Reimers *et al.* 1990; Trinchet *et al.* 1991; Gonzalez-Reimers *et al.* 1992; Teran *et al.* 1994; Lin *et al.* 1995), but that is not the case with other studies (McCullough *et al.* 1987; Diodati *et al.* 1990). Several correlations have been found in PBC as well (Eriksson & Zettervall 1986; Babbs *et al.* 1988; Mutimer *et al.* 1989; Nyberg *et al.* 1992; Diaz *et al.* 1993). In our patients PIIINP did not correlate with the markers indicating cytolysis, cholestasis (alkaline phosphatase) or liver synthetic function. PIIINP non-correlations with alkaline phosphatase could be partly explained by the fact that a part of alkaline phosphatase is released into blood circulation from the skeleton due to metabolic bone diseases. It is not possible to differentiate them by traditional methods.

PIIINP did not prove to be an efficient marker to prognosticate short and long-time survival as its concentration was relatively stable during the course of the disease. It was not possible to prove an earlier established hypothesis that in the final stage PIIINP values decrease markedly in cirrhotic patients, although a trend towards it was found. PIIINP serial determination does not seem to give additional information besides other liver functional tests. Since PIIINP has a good correlation with every histological stage, its measurement can substitute liver biopsy to a certain extent, especially to indicate the presence of active fibrogenetic mechanisms. In those cases a more active immunosuppressive and antifibrotic treatment is necessary.

Hyaluronan concentrations were slightly increased in the 1st histological stage patients already, compared with the control group. Reasons for the hyaluronan elevation seem to be more complicated than in the case of PIIINP since hyaluronan correlated with markers indicating liver synthetic function, fibrotic changes, cholestasis, liver insufficiency and portal hypertension. Elevated serum hyaluronan levels are a sign of either an increased production and/or outflow from the tissue or of a decreased elimination of polysaccharides through the liver endothelial cells or through other parts of reticuloendothelial system or through the kidneys (Wu & Danielsson 1995). It has been assumed that the reduced metabolism of hyaluronan in the liver is a more important cause for the high values of hyaluronan than an increased synthesis in fibrotic areas in

chronic liver diseases (Frebourg *et al.* 1986), although hyaluronan correlated well with the histological stages of PBC in the present study.

When the liver endothelial cells are damaged (capillarization of sinusoids) the metabolism of hyaluronan can decrease (Babbs *et al.* 1990; Ueno *et al.* 1993). Diminished enzymatic activity pathways of hyaluronan can also be responsible for an increase of hyaluronan in liver diseases (Roden *et al.* 1989), but the receptor function is probably more significant. It is important to note that in patients with PBC the renal elimination of hyaluronan has increased (Laurent 1987). None of our PBC patients had renal insufficiency. Therefore renal damage cannot be responsible for high hyaluronan values. High hyaluronan concentrations can result from the damage of sinusoidal blood flow and blood shunting system in the liver (Gibson *et al.* 1992). Therefore, the dynamical determination of hyaluronan can reduce the need for serial liver biopsy in patients with PBC and render it possible to prognosticate the early development of portal hypertension in certain patients. In the present study hyaluronan correlated well with the presence of esophageal varices.

In this study hyaluronan had better correlation than PIIINP with every histological stage and bilirubin. Hyaluronan measurement had a very high sensitivity for diagnosing advanced PBC. However, its specificity was not so good as that of PIIINP. In our opinion hyaluronan is a better marker than PIIINP for the evaluation of the disease process in PBC patients since it gives more complex information about the liver functional status.

Hyaluronan concentration correlated significantly with PIIINP values in patients with PBC. This could be explained by a similar metabolic way (clearance through the liver endothelial cells), although different receptor-mediated structures are responsible for this (Laurent *et al.* 1986; Bentsen *et al.* 1987; Smedsrod 1988; Bentsen *et al.* 1989; Forsberg & Gustafson 1991; McGary *et al.* 1993; Melkko *et al.* 1994; McCourt *et al.* 1994; Dougherty *et al.* 1994). Acute hepatitis studies have concluded that the hyaluronan concentration is a fast changing marker, therefore it suits well for the dynamic evaluation of the liver damage (Bramley *et al.* 1991). It is not known yet whether it is the case for chronic hepatitis. Hyaluronan metabolism through the liver endothelial cells seems to get damaged earlier than the metabolism of PIIINP.

The PIIINP and hyaluronan concentrations were lower in asymptomatic patients than in symptomatic ones. Three out of seven asymptomatic patients had hyaluronan as well PIIINP above the normal value. Two of them developed symptoms soon. Those patients were all in advanced histological stages. We recommend the measurement of PIIINP and hyaluronan for the follow-up of asymptomatic patients to predict the development of symptoms. Higher PIIINP and hyaluronan values in asymptomatic PBC patients can indicate the subgroup of patients whose symptoms develop soon, and the treatment of those patients is certainly indicated.

Immunosuppressive treatment (prednisolone alone or prednisolone-azathioprine combination) did not affect PIIINP values significantly in the patients with PBC. In patients receiving only prednisolone therapy, the values of hyaluronan increased significantly in the dynamic study. In patients receiving combination therapy (prednisolone-azathioprine combination) hyaluronan remained at a stable level. Both those drugs inhibit the development of fibrogenesis in several inflammatory diseases (Weigand *et al.* 1984; Ballardini *et al.* 1984; Annoni *et al.* 1986; McCullough *et al.* 1987; Schuppan 1991; Oikarinen *et al.* 1992; Wu & Danielsson 1994). Earlier a research group has discovered that prednisolone treatment can decrease PIIINP in the patients with PBC (Mitchison *et al.* 1989). Other reseachers have suggested that in the PBC patients PIIINP levels are always elevated regardless of medical therapy (Savolainen *et al.* 1983; Weigand *et al.* 1984; Beukers *et al.* 1992). Those data have proved that D-penicillamine, prednisolone and cyclosporine A may have no favourable effect on the increased hepatic collagen formation involved in PBC. Because high PIIINP values indicate especially active fibrogenesis, the administered doses (10–15 mg prednisolone alone or in combination with 50 mg azathioprine per day) were not sufficient for the inhibition of fibrogenetic processes in the liver. Higher doses can be more effective, but can cause side-effects. In addition, prednisolone cannot affect hyaluronan metabolism in the liver. Probably combination therapy with other drugs (e.g. UDCA, colchicine) could be more efficient.

Despite of treatment, PBC progresses in most patients (Christensen *et al.* 1985; Balasubramaniam *et al.* 1990; Christensen *et al.* 1993; Inoue *et al.* 1995). In this study PIIINP and hyaluronan did not correlate with the mortality rate of the patients with PBC. When the prognostic values of PIIINP and hyaluronan were assessed, by using the Cox model, they were found not to influence the prognostication of survival. Therefore, our results are not in accordance with the results of some earlier published research in which the significance of PIIINP and/or hyaluronan was established for the prediction of the survival time of PBC patients (Eriksson & Zettervall 1986; Babbs *et al.* 1988; Niemela *et al.* 1988; Nyberg *et al.* 1992; Teare *et al.* 1993). As a matter of fact, the natural course and survival time of the studied patients with PBC can be influenced by the treatment with prednisolone or a combination of prednisolone-azathioprine. Consequently, there is a possibility that by using another treatment regime (UDCA, colchicine etc.), PIIINP and hyaluronan are essential for the longtime prognosis. Up to now, a study with UDCA, has already indicated that bilirubin loses its prognostic value in the Cox regression model and that PIIINP and hyaluronan are better markers for predicting the diminished survival (Poupon *et al.* 1994).

By using Spearman's rank correlation analysis for 26 patients, followed-up dynamically, mean hyaluronan concentrations were significantly higher at the time of the diagnosis and during the second examination in patients who died

within a year after the second investigation. In patients whose hyaluronan values were over 300μg/l, 1-year survival was not probable. Serial determination of hyaluronan was important in asymptomatic and symptomatic patients. In symptomatic patients the increase of hyaluronan indicated the progressing of the disease and could be useful for a timely inclusion of patients in the liver transplantation program. In asymptomatic patients the serial determination of hyaluronan makes it possible to predict the development of symptoms. It is important to add that hyaluronan preserved correlation with bilirubin during the follow-up period, but PIIINP did not. So far, bilirubin is used more frequently in prognostic models for PBC (Shapiro *et al.* 1979; Roll *et al.* 1983; Beswick *et al.* 1985; Christensen *et al.* 1985; Nyberg & Loof 1989; Goudie *et al.* 1989; Rydning *et al.* 1990; Christensen *et al.* 1993; Murtaugh *et al.* 1994). In the terminal stage PIIINP values did not decrease significantly in our patients. It could be associated with the fact that the activity of cirrhosis did not disappear completely in the PBC patients or the PIIINP metabolism was significantly diminished. Evidently, the serial determination of PIIINP does not give additional information on symptomatic patients since the interpretation of the results is frequently difficult. However it can be useful in the follow-up of asymptomatic patients with PBC.

7. CONCLUSION

1. Primary biliary cirrhosis (PBC) is a rare disease in Estonia as compared with other European countries. Nevertheless, during the 20-year research period its prevalence increased continuously, being 26.9 per million on December 31, 1992. Large regional differences in the distribution of the patients with PBC were found. The highest prevalence was discovered in the Viljandi County, being 123 per million at the end of the research period. However, no differences between the population groups of different ethnic background were found.

2. The incidence of PBC has been suprisingly stable since 1986 as approximately 6 new cases are diagnosed in Estonia every year.

3. Most PBC cases in Estonia have been diagnosed in advanced histological stages (3rd or 4th stage – 65.4%) whereas symptomatic period before the diagnosing of PBC was long. Most of 97 patients under the follow-up were symptomatic at the time of the PBC diagnosis. Over 50% of them were already jaundiced.

4. Associated autoimmune disorders were found in 34% of PBC patients, most frequent among them being keratoconjunctivitis sicca and Raynaud signs. 26.8% of the patients with PBC had gallstones. Antimitochondrial antibodies (AMA) is a highly specific and sensitive marker for PBC even if tested by indirect immunofluorescence test on rodent tissues. Therefore, AMA testing is important for patients with other autoimmune diseases and for gallstone patients with impaired liver functional tests.

5. About a half of asymptomatic patients with PBC developed symptoms during the follow-up period. Asymptomatic patients survival was similar to the survival of symptomatic PBC patients. Since the prognosis of asymptomatic patients was not good, extensive treatment could be indicated in this stage already.

6. The mean survival rate of patients with PBC in Estonia was significantly lower than that in recently performed studies in Europe, being 5.5 years from the time of the diagnosis and 7.8 years from the time of the development of the first symptom associated with the liver disease. Altogether, 78.1% of patients lived five years or more. High bilirubin concentration and old age were the only independent factors indicating an unfavourable outcome.

7. In PBC patients, living in Estonia, a high percentage of ANA-positive cases was found: 29% on tissue sections and 70% on HEp-2 cells. The most frequent ANA pattern was evaluated as multiple nuclear dots pattern and this was highly specific of PBC. ANA-positive patients had a significantly better prognosis than ANA-negative patients.

8. In all AMA-negative PBC cases ANA were found, characterised more frequently by the pattern of multiple nuclear dots. Therefore, ANA testing is important for the diagnosis of PBC, especially, in AMA-negative patients with chronic cholestasis. No differences in the clinical course of AMA-positive and AMA-negative patients were found.

9. In most patients with PBC two fibrogenetic markers, PIIINP and hyaluronan concentrations were increased in the blood, especially in the 3rd and 4th histological stages of the disease, being very sensitive for predicting advanced PBC. Hyaluronan and PIIINP correlated significantly with each other.

10. In symptomatic PBC patients the PIIINP and hyaluronan values were significantly higher than those in asymptomatic patients. Both fibrogenetic markers were elevated in most asymptomatic patients with PBC whose symptoms developed soon. Therefore, the measurement of PIIINP and hyaluronan allows to differentiate this subgroup of patients and diminish the necessity for invasive liver biopsy in asymptomatic patients.

11. Both PIIINP and hyaluronan correlated well with every histological stage of the disease and bilirubin. In addition, hyaluronan correlated positively with the extent of fat excretion, pruritus, jaundice, fatigue, esophageal varices, length of symptomatic period and weight loss, and negatively with albumin and hepatomegaly. Hyaluronan was a better marker to indicate the extent of liver damage and an advanced liver disease since it reflects changes in the liver more completely. However, none of those fibrogenetic markers was important for predicting a long-time outcome in the Cox regression model

12. The PIIINP values did not change significantly despite the applied treatment regime (prednisolone or prednisolone-azathioprine combination). The hyaluronan values remained in a stable level in the prednisolone/azathioprine-treated patients group, but increased significantly in the prednisolone group. Combination therapy in PBC patients seems more promising than monotherapy.

13. For predicting a short-time outcome (1-year survival) hyaluronan is a good marker. Its serial determination during the follow-up could be useful for patients with PBC.

8. REFERENCES

Ahrens EH, Rayne MA, Kunkle HG, Eisenmenger WJ, Blondeim SH. Primary biliary cirrhosis. Medicine 1950; 29: 299–364

Almdal T, Schaadt O, Vesterdad Jorgensen J, Lindgreen P, Ranek L. Vitamin D, parathyroid hormone, and bone mineral content of lumbar spine and femur in primary biliary cirrhosis. J Intern Med. 1989; 225: 207–213.

Annoni G, Cargnel A, Colombo M, Hahn EG. Persistent elevation of the aminoterminal peptide of procollagen type III in serum of patients with acute viral hepatitis distinguishes chronic active hepatitis from resolving or chronic persistent hepatitis. J Hepatol 1986; 2: 379–388.

Aycock RS, Seyer JM. Collagens of normal and cirrhotic human liver. Connect Tissue Res 1989; 23: 19–31.

Babbs C, Smith A, Hunt LP, Rowan BP, Haboubi NY, Warnes TW. Type III procollagen peptide: a marker of disease activity and prognosis in primary biliary cirrhosis. Lancet. 1988; 8593: 1021–1024.

Babbs C, Haboubi NY, Mellor JM, Smith A, Rowan BP, Warnes TW. Endothelial cell transformation in primary biliary cirrhosis: a morphological and biochemical study. Hepatology. 1990; 11: 723–729.

Bach N, Schaffner F. Familial primary biliary cirrhosis. J Hepatol. 1994; 20: 698–701.

Balasubramaniam K, Grambsch PM, Wiesner RH, Lindor KD, Dickson ER. Diminished survival in asymptomatic primary biliary cirrhosis. A prospective study [see comments]. Gastroenterology. 1990; 98: 1567–1571.

Ballardini G, Faccani A, Bianchi FB, Fallani M, Patrono D, Capelli M, Pisi E. Steroid treatment lowers hepatic fibroplasia, as explored by serum aminoterminal procollagen III peptide, in chronic liver disease. Liver 1984; 4: 348–352.

Bassendine MF, Yeaman SJ. Serological markers of primary biliary cirrhosis: diagnosis, prognosis and subsets [editorial; comment]. Hepatology. 1992; 15: 545–548.

Batta AK, Arora R, Salen G, Tint GS, Eskreis D, Katz S. Characterization of serum and urinary bile acids in patients with primary biliary cirrhosis by gas-liquid chromatography-mass spectrometry: effect of ursodeoxycholic acid treatment. J Lipid Res. 1989; 30: 1953–1962.

Baum H. Nature of mitochondrial antigens of primary biliary cirrhosis and their possible relationship to the etiology of the disease. Sem Liver Dis 1989; 9: 124–131

Bayerdorffer E, Lamerz R, Fliege R, Kopcke W, Mannes GA. Predictive value of serum procollagen-II-peptide for the survival of patients with cirrhosis. J Hepatol 1991; 13: 298–304.

Becker U, Almdal T, Christensen E, Gluud C, Farholt S, Bennett P, Svenstrup B, Hardt F. Sex hormones in postmenopausal women with primary biliary cirrhosis. Hepatology. 1991; 13: 865–869.

Bell H, Raknerud N, Orjasaeter H, Haug E. Serum procollagen III peptide in alcoholic and other chronic liver diseases. Scand J Gastroenterol 1989; 24: 1217–1222.

Ben Ari Z, Dhillon AP, Sherlock S. Autoimmune cholangiopathy: part of the spectrum of autoimmune chronic active hepatitis. Hepatology. 1993; 18: 10–15.

Benbassat C, Schlesinger M, Shoenfeld Y. The complement system and primary biliary cirrhosis. J Clin Lab Immunol. 1992; 38: 51–61.

Bengoa JM, Sitrin MD, Meredith S, Kelly SE, Shah N, Baker AL, Rosenberg IH. Intestinal calcium absorption and vitamin D status in chronic cholestatic liver disease. Hepatology. 1984 ; 4: 261–265.

Bentsen KD, Horn T, Risteli J, Engstrom-Laurent A, Horslev-Petersen K, Lorenzen I. Serum aminoterminal type III procollagen peptide and the 7S domain of type IV collagen in patients with alcohol abuse. Relation to ultrastructural fibrosis in the acinar zone 3 and to serum hyaluronan. Liver 1987; 7: 339–340.

Bentsen KD, Boesby S, Kirkegaard P, Hansen CP, Jensen SL, Horslev-Petersen K, Lorenzen I. Is the aminoterminal propeptide type III procollagen degraded in the liver? A study of type III procollagen peptide in serum during liver transplantation in pigs. J Hepatol 1988; 6: 133–150.

Berg PA, Klein R. Antimitochondrial antibodies in primary biliary cirrhosis and other disorders: definition and clinical relevance. Dig Dis. 1992; 10: 85–101.

Berg PA, Klein R. Antimitochondrial antibodies in primary biliary cirrhosis. A clue to its etiopathogenesis? J Hepatol. 1992; 15: 6–9.

Bernstein RM, Neuberger JM, Bunn CC, Callender ME, Hughes GRV, Williams R. Diversity of autoantibodies in primary biliary cirrhosis and chronic active hepatitis. Clin Exp Immunol 1984; 55: 553–560.

Beswick DR, Klatskin G, Boyer JL. Asymptomatic primary biliary cirrhosis. A progress report on long-term follow-up and natural history. Gastroenterology. 1985; 89: 267–271.

Beukers R, van Zanten RA, Schalm SW. Serial determination of type III procollagen aminopropeptide serum levels in patients with histologically progressive and non-progressive primary biliary cirrhosis. J. Hepatol. 1992; 14: 22–29.

Bird P, Calvert JE, Mitchison H, Ling NR, Bassendine M, James OF. Lymphocytes from patients with primary biliary cirrhosis spontaneously secrete high levels of IgG3 in culture. Clin Exp Immunol. 1988; 71: 475–40.

Bissell DM, Friedman SL, Maher JJ, Roll FJ. Connective tissue biology and hepatic fibrosis: a report of a conference. Hepatology 1990; 11: 488–498.

Bjorkland A, Loof L, Mendel-Hartvig I, Totterman TH. Primary biliary cirrhosis. High proportions of B cells in blood and liver tissue produce anti-mitochondrial antibodies of several Ig classes. J Immunol. 1994; 153: 2750–2757.

Bonsel GJ, Klompmaker IJ, van'T Veer F, Habbema JDF, Slooff MJ. Use of prognostic model for assessment of value of liver transplantation in primary biliary cirrhosis. Lancet 1990; 335: 493–497.

Borda F, Huarte MP, Zozaya JM, Jimenez C, del Cazo FJ, Lanas A, Tiberio G, Mu~noz M. Primary biliary cirrhosis in Navarra. An Med Interna. 1989; 6: 63–66.

Bramley PN, Rathbone BJ, Forbes MA, Cooper EH, Losowsky MS. Serum hyaluronate as a marker of hepatic derangement in acute liver damage. J Hepatol 1991; 13: 8–13.

Brenard R, Geubel AP. Antimitochondrial and antinuclear antibodies in primary biliary cirrhosis: an update in relation to their biochemical characterization and clinical significance. Acta Clin Belg. 1991; 46: 305–312.

Brind AM, Codd AA, Cohen BJ, Gabriel FG, Collins JD, James OF, Bassendine MF. Low prevalence of antibody to hepatitis C virus in north east England. J Med Virol. 1990; 32: 243–248.

Brind AM, Bray GP, Portmann BC, Williams R. Prevalence and pattern of familial disease in primary biliary cirrhosis. Gut 1995; 36: 615–617.

Burgeson RE, Nimni ME. Collagen types. Molecular structure and tissue distribution. Clin Orthop Rel Research. 1991; 282: 250–272.

Burnham TK. Antinuclear antibodies in patients with malignancies. Lancet 1972; 2: 436–437.

Burt AD. Liver fibrosis. B M J 1992; 305: 537–538.

Butler P, Hamilton-Miller J, Baum H, Burroughs AK. Detection of M2 antibodies in patients with recurrent urinary tract infection using an ELISA and purified PBC specific antigens. Evidence for a molecular mimicry mechanism in the pathogenesis of primary biliary cirrhosis? Biochem Mol Biol Int. 1995; 35: 473–45.

Caballero Plasencia AM, Lopez Callejas C, Valenzuela Barranco M, Montero Garcia M, Muros Navarro MC, de los Reyes Garcia MC, Sanchez Martinez H, Bellot Garcia V. [Epidemiology of primary biliary cirrhosis in the South area of Granada]. Med Clin (Barc). 1991; 96: 481–485.

Caldwell SH, Leung PS, Spivey JR, Prindiville T, de Medina M, Saicheur T, Rowley M, Reddy KR, Coppel R, Jeffers LJ, *et al.* Antimitochondrial antibodies in kinders of patients with primary biliary cirrhosis: antimitochondrial antibodies are unique to clinical disease and are absent in asymptomatic family members. Hepatology. 1992; 16: 899–905.

Camps J. Castilla A, Ruiz J, Civeira MP, Prieto J. Randomised trial of lymphoblastoid alpha-interferon in chronic hepatitis C. Effects on inflammation, fibrogenesis and vireamia. J Hepatol 1993; 17: 390–396.

Capra F, Casaril M, Gabrielli GB, Tognella P, Rizzi A, Dolci L, Colombari R, Mezzelani P, Corrocher 1R, De Sandre G. Alpha-interferon in the treatment of chronic viral hepatitis: effects on fibrogenesis serum markers. J Hepatol 1993; 18: 112–118.

Cassani F, Bianchi FB, Lenzi M, Volta U, Pisi E. Immunomorphological characterisation of antinuclear antibodies in chronic liver disease. J Clin Pathol 1985; 38: 801–805.

Chan CY, Lee SD, Huang YS, Wu JC, Tsai YT, Tsay SH, Lo KJ. Primary biliary cirrhosis in Taiwan. J Gastroenterol Hepatol. 1990; 5: 560–565.

Chan HL, Lee YS, Hong HS, Kuo TT. Anticentromere antibodies (ACA): clinical distribution and disease specificity. Clin Exp Dermatol. 1994; 19: 298–302.

Chojiker M, Brenner DA. Therapeutic strategies for hepatic fibrosis. Hepatology 1988; 176–182.

Chou MJ, Lee SL, Chen TY, Tsay GJ. Specificity of antinuclear antibodies in primary biliary. Ann Rheum Dis. 1995; 54: 148–151

Christensen E, Crowe J, Doniach D, Popper H, Ranek L, Rodes J, Tygstrup N, Williams R. Clinical pattern and course of disease in primary biliary cirrhosis based on an analysis of 236 patients. Gastroenterology 1980; 78: 236–240

Christensen E, Neuberger J, Crowe J, Altman DG, Popper H, Portmann B, Doniach D, Ranek L, Tygstrup N, Williams R. Beneficial effect of azathioprine and prediction of prognosis in primary biliary cirrhosis. Final results of an international trial. Gastroenterology. 1985; 89: 1084–1091.

Christensen E, Schlichting P, Andersen PK, Fauerholdt L, Schou G, Pedersen BV, Juhl E, Poulsen H, Tygstrup N. Updating prognosis and therapeutuc effect evaluation in cirrhosis with Cox's multiple regression model for time dependent variables. Scand J Gastroenterol 1986; 21: 163–174.

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: 1865–1876.

Clark AK, Galbraith R, Hamilton EDB, Williams R. Rheumatic disorders in primary biliary cirrhosis. Ann Rheum Dis. 1978; 37: 42–47

Clark M, Sack K. Deforming complicating primary biliary cirrhosis. J Rheumatol. 1991; 18: 619–621.

Cooper EH, Rathbone BJ. Clinical significance of the immunometric measurements of hyaluronic acid. Ann Clin Biochem 1990; 27: 444–451.

Courvalin JC, Lassoued K, Bartnik E, Blobel G, Wozniak R. The 200 kiloDalton nuclear envelope polypeptide recognized by human autoantibodies in primary biliary cirrhosis is the major glycoprotein of the pore complex. J Clin Invest 1990; 90: 181–186.

Courvalin JC, Lassoued K, Worman HJ, Blobel G. Identification and characterization of autoantibodies against the nuclear envelope lamin B receptor from patients with primary biliary cirrhosis. J Exp Med 1990; 172: 961–967.

Crippin JS, Lindor KD, Jorgensen R, Kottke BA, Harrison JM, Murtaugh PA, Dickson ER. Hypercholesterolemia and atherosclerosis in primary biliary cirrhosis: what is the risk? Hepatology. 1992; 15: 858–862.

Crippin JS, Jorgensen RA, Dickson ER, Lindor KD. Hepatic osteodystrophy in primary biliary cirrhosis: effects of medical treatment. Am J Gastroenterol. 1994; 89: 47–50.

Crowe JP, Christensen E, Butler J. *et al.* Primary biliary cirrhosis: the prevalence of hypothyreoidsm and its relationship to thyroid auto-antibodies and Sicca syndrome. Gastroenterology. 1980; 79: 1437–1441

Czaja AJ, Nishioka M, Morshed SA, Hachiya T. Patterns of nuclear immunofluorescence and reactivities to recombinant nuclear antigens in autoimmune hepatitis. Gastroenterology 1994; 107: 200–207.

Danielsson A, Boqvist L, Uddenfeldt P. Epidemiology of primary biliary cirrhosis in a defined rural population in the northern part of Sweden. Hepatology 1990; 11: 458–464.

Dickson ER, Grambsch PM, Fleming TR, Fisher LD, Langsworthy A. Prognosis in primary biliary cirrhosis: model for decision making. Hepatology 1989; 10: 1–7.

Diaz F, Collazos J, Genolla J. Study of serum procollagen type III peptide in patients with hepatic cirrhosis from a clinical point of view. Clin. Investig. 1993; 71: 416–418.

Diodati G, Bonetti P, Plebani M, Giacomini A, Rugge M, Realdi G, Burlina A. Cryptogenic chronic active liver disease. Evaluation of serum aminoterminal peptide of type III procollagen as a marker of histologic activity. Ric Clin Lab 1990; 20: 253–259.

Donaldson P, Doherty D, Underhill J, Williams R. The molecular genetics of autoimmune liver disease. Hepatology. 1994; 20 (1 Pt 1): 225–239.

Doniach D, Walker JG. Immunopathology of liver disease. Progr Liver Dis. 1972; 4: 381–402.

Engstrom-Laurent A, Loof L, Nyberg A, Schroeder T. Increased serum levels of hyaluronate in liver disease. Hepatology 1985; 5: 638–642.

Engstrom-Laurent A. Changes in hyaluronan concentration in tissues and body fluids in disease states. Ciba Found Symp. 1989; 143: 233–240.

Eriksson S, Lindgren S. The prevalence and clinical spectrum of primary biliary cirrhosis in a defined population. Scand J Gastroenterol. 1984; 19: 971–976.

Eriksson S, Zettervall O. The N-terminal propeptide of collagen type III in serum as a prognostic indicator in primary biliary cirrhosis. J Hepatol. 1986; 2: 370–378.

Eriksson S, Carlstrom K, Rannevik G. Sex steroids and steroid binding proteins in primary biliary cirrhosis. J Steroid Biochem. 1989; 32: 427–431.

Evans J, Craft J. PBC 95 K, a 95-kilodalton nuclear autoantigen in primary biliary cirrhosis. Arthr Reum 1991; 34: 731–736.

Farinati F, Floreani A, De Maria N, Fagiuoli S, Naccarato R, Chiaramonte M. Hepatocellular carcinoma in primary biliary cirrhosis. J Hepatol. 1994 ; 21: 315–316.

Floreani A, Titta M, Plebani M, Faggian D, Chiaramonte M, Naccarato R. Sex hormone changes in post-menopausal women with primary biliary cirrhosis and with cryptogenic chronic liver disease. Clin Exp Obstet Gynecol. 1991; 18: 229–234.

Floreani A, Zappala F, Mazzetto M, Naccarato R, Plebani M, Chiaramonte M. Different response to ursodeoxycholic acid (UDCA) in primary biliary cirrhosis according to severity of disease. Dig Dis Sci. 1994; 39: 9–14.

Forsberg N, Gustafson S. Characterization and purification of the hyaluronan-receptor on liver endothelial cells. Biochim Biophys Acta. 1991; 1078: 12–18.

Fraser JR, Engstrom-Laurent A, Nyberg A, Laurent TC. Removal of hyaluronic acid from the circulation in rheumatoid disease and primary biliary cirrhosis. J Lab Clin Med 1986; 107: 79–85.

Fraser JR, Laurent TC. Turnover and metabolism of hyaluronan. Ciba Found Symp 1989; 143: 41–53.

Frebourg T, Delpech B, Bercoff E *et al.* Serum hyaluronate in liver diseases: study by enzymoimmunological assay. Hepatology 1986; 6: 392–395.

Frei A, Zimmermann A, Weigand H. The N-terminal propeptide of collagen type III in serum reflects activity and degree of fibrosis in patients with chronic liver disease. Hepatology 1984; 4: 830–834.

Friedman SL, Roll JF, Boyles J, Bissell DM. Hepatic lipocytes: the principal collagen-producing cells of normal rat liver. Proc Natl Acad Sci USA 1985; 82: 8681–8685.

Friedman SL. The cellular basis of hepatic fibrosis. New Engl J Med 1993; 328: 1828–1835.

Fusconi M, Cassani F, Govoni M, Caselli A, Farabegoli F, Lenzi M, Ballardini G, Zauli D, Bianchi FB. Anti–nuclear antibodies of primary biliary cirrhosis recognize 78–92 kD and 96–100 kD proteins of nuclear bodies. Clin Exp Immunol 1991; 83: 291–297.

Gabrielli GB, Faccioli G, Caesaril M, Capra F, Bonazzi L, Falezza G, Tomba A, Barac-chino F, Corrocher R. Procollagen III peptide and fibronectin in alcohol-related chronic liver disease: correlations with morphological features and biochemical tests. Clin Chim Acta 1989; 179: 315–322.

Galbraith RA, Smith M, Mackenzie RM, *et al.* High prevalence of sero-immunologic abnormalities in relatives of patients with chronic active hepatitis and primary biliary cirrhosis. N Engl J Med. 1974; 290: 63–69.

Gershwin ME, Mackay IR. Primary biliary cirrhosis: paradigm or paradox for autoimmunity. Gastroenterology. 1991; 100: 822–833.

Gibson PR, Fraser JR, Brown TJ, Finch CF, Jones PA, Colman JC, Dudley FJ. Hemodynamic and liver function predictors of serum hyaluronan in alcoholic liver disease. Hepatology. 1992; 15: 1054–1059.

Gibson PR, Fraser JR, Colman JC, Jones PA, Jennings G, Dudley FJ. Change in serum hyaluronan: a simple index of short-term drug-induced changes in hepatic sinusoidal perfusion. Gastroenterology 1993; 105: 470–474.

Gittlen SD, Schulman ES, Maddrey WC. Raised histamine concentrations in chronic cholestatic liver disease. Gut. 1990; 31: 96–99

Goldberg DM, Brown D. Advances in the application of biochemical tests to diseases of the liver and biliary tract: their role in diagnosis, prognosis and the elucidation of pathogenetic mechanisms. Clin Biochem 1987; 20: 127–148.

Goldenstein C, Rabson AR, Kaplan MM, Canoso JJ. Arthralgias as a presenting manifestation of primary biliary cirrhosis. J Rheumatol. 1989; 16: 681–684.

Gonzalez-Reimers E, Brajin-Rodriguez MM, Rodriguez-Moreno F, Santolaria-Fernandez F, Batista-Lopez N, Alvarez-Arguelles H, Milena A, Rodriguez-Hernandez A. Clinical and prognostic value of serum procollagen levels in chronic alcoholic liver disease. Drug Alcohol Depend 1990; 25: 91–95.

Gonzalez-Reimers CE, Brajin-Rodriguez MM, Santolaria-Fernandez F, Diaz-Flores L, Conde-Martel A, Rodriguez-Rodriguez E, Essardas-Daryani H. Ito cells and fibrogenesis in chronic alcoholic liver disease. Drug Alcohol Depend 1992; 29: 225–230.

Goodman ZD, McNally PR, Davis DR, Ishak KG. Autoimmune cholangitis: a variant of primary biliary cirrhosis. Clinicopathologic and serologic correlations in 200 cases. Dig Dis Sci. 1995; 40: 1232–1242.

Goudie BM, Burt AD, Boyle P, Macfarlane G, Birnie GG, Mills PR,Gillis CR, MacSween RN, Watkinson G. Breast cancer in women with primary biliary cirrhosis. Br Med J (Clin Res Ed). 1985; 291: 1597–1598.

Goudie BM, Burt AD, Macfarlane GJ, Boyle P, Gillis CR, MacSween RN, Watkinson G. Risk factors and prognosis in primary biliary cirrhosis. Am J Gastroenterol. 1989; 84: 713–716.

Gores GJ, Moore SB, Fisher LD, Powell FC, Dickson ER. Primary biliary cirrhosis: associations with class II major histocompatibility complex antigens. Hepatology. 1987; 7: 889–892.

Gregory WL, Mehal W, Dunn AN, Cavanagh G, Chapman R, Fleming KA, Daly AK, Idle JR, James OF, Bassendine MF. Primary biliary cirrhosis: contribution of HLA class II allele DR8. Q J Med. 1993; 86: 393–399.

Gressner AM. Measurement of connective tissue parameters in serum for diagnosis and follow-up of liver fibrosis. Ann Clin Biochem. 1987; 24: 283–92.

Gressner AM, Haarmann R. Hyaluronic acid synthesis and secretion by rat liver fat storing cells. Biochem Biophys Res Commun 1988; 151: 220–229.

Gressner AM. Liver fibrosis: perspectives in pathobiochemical research and clinical outlook. Eur J Clin Chem Clin Biochem 1991; 29: 293–311.

Gressner AM, Bachem MG. Cellular communications and cell-matrix interactions in the pathogenesis of fibroproliferative disease: liver fibrosis as a paradigm. Ann Biol Clin 1994; 52: 205–226.

Gressner AM. Krull N, Bachem MG. Regulation of proteoglycan expression in fibrotic liver and cultured fat-storing cells. Path Res Pract 1994; 190: 864–882.

Gressner AM. Mediators of hepatic fibrogenesis. Hepatogastroenterology 1996; 43: 92–103.

Guanabens N, Peres A, Marinoso L *et al.* Factors influencing development of metabolic bone disease in primary biliary cirrhosis. Am J Gastroenterol 1990; 85: 1356–1362

Guechot J, Poupon RE, Giral P, Balkau B, Giboudeau J, Poupon R. Relationship between procollagen III aminoterminal propeptide and hyaluronan serum levels and histological fibrosis in primary biliary cirrhosis and chronic viral hepatitis C. J Hepatol. 1994; 20: 388–393.

Guechot J, Loria A, Serfaty L, Giral P, Giboudeau J, Poupon R. Serum hyaluronan as a marker of liver fibrosis in chronic viral hepatitis C: effect of alpha-interferon therapy. J Hepatol. 1995; 22: 22–26.

Hamlyn AN, Sherlock S. The epidemiology of primary biliary cirrhosis: a survey of mortality in England and Wales. Gut 1974; 15: 473–479

Hamlyn AN, Maklan AF, James O. Primary biliary cirrhosis: geographical clustering and symptomatic onset seasonality. Gut 1983; 23: 940–945.

Hansen BU, Eriksson S, Lindgren S. High prevalence of autoimmune liver disease in patients with multiple nuclear dot, anti-centromere and mitotic spindle antibodies. Scand J Gastroenterol. 1991; 26: 707–713.

Harada N, Dohmen K, Itoh H, Ohshima T, Yamamoto H, Nagano M, Iwata Y, Hachisuka K, Ishibashi H. Sibling cases of primary biliary cirrhosis associated with polymyositis, vasculitis and Hashimoto's thyreoiditis. Intern Med. 1992; 31: 289–293.

Hayasaka A, Schuppan D, Ohniski K, Okuda K, Hahn EG. Serum concentrations of the carboxyterminal cross-linking domain of procollagen type IV an the aminoterminal propeptide of procollagen type III in chronic liver disease. J Hepatol 1990; 10: 17–22.

Hayasaka A, Kohen H, Yoshikawa N, Iida S, Ohto M. The effect of interferon alpha and prednisolone on the serum concentrations of the aminoterminal domain of procollagen type III (PIIIP) and type IV (7-S collagen) in patients with chronic hepatitis B. J Hepatol. 1993; 19: 36–42.

Hodgson SF, Dickson ES, Wahner HW, Johnson KA, Mann KG, Riggs BL. Bone loss and reduced osteoblast function in primary biliary cirrhosis. Ann Intern Med 1985; 103: 855–860

Hodson ME, Turner-Warwick M. Autoantibodies in patients with bronchial carcinoma. Thorax 1975; 30: 367–370.

Hopf U, Moller B, Stemerowicz R, Lobeck H, Rodloff A, Freudenberg M, Galanos C, Huhn D. Relation between Escherichia coli R(rough)-forms in gut, lipid A in liver, and primary biliary cirrhosis. Lancet. 1989; 2: 1419–1422.

Horita M, Takahashi N, Seike M, Nasu S, Takaki R. A case of primary biliary cirrhosis associated with Hashimoto's thyroiditis, scleroderma and Sjogren's syndrome. Intern Med. 1992; 31: 418–421.

Ilan Y, Shouval D. Primary biliary cirrhosis in Israel. Isr J Med Sci. 1992; 28: 683–687.

Imai H, Nakano Y, Kiyosawa K, Tan EM. Increasing titers and changing specificities of antinuclear antibodies in patients with chronic liver disease who develop hepatocellular carcinoma. Cancer 1993; 71: 26–35.

Inoue K, Hirohara J, Nakano T, Seki T, Sasaki H, Higuchi K, Ohta Y, Onji M, Muto Y, Moriwaki H. Prediction of prognosis of primary biliary cirrhosis in Japan.Liver 1995; 15: 70–77.

James SP, Hoofnagle JH, Strober W, Jones E. Primary biliary cirrhosis: a model of autoimmune disease. NIH conference. Ann Intern Med 1983; 99: 500–512

James O, Macklon AF, Watson AJ. Primary biliary cirrhosis: a revised clinical spectrum. Lancet 1981; ii: 1278–1281.

Jaup BH, Zettergen LSW. Familial occurrence of primary biliary cirrhosis associated with hypergammaglobulinemia in descendants: a family study. Gastroenterology 1980; 78: 549–555.

Jeffrey GP, Reed WD, Shilkin KB. Primary biliary cirrhosis: clinicopathological characteristics and outcome. J Gastroenterol Hepatol. 1990; 5: 639–645.

Johnson SJ. Extracellular matrix protein and hepatic fibrosis. Hepatogastroenterology 1996; 43: 44–55.

Kaplan EL, Meier P. Non-parametric estimation from incomplete observations J Amer Stat Assoc 1958; 53: 457–481

Kaplan MM. Primary biliary cirrhosis. N Engl J Med 1987; 316: 521–528.

Kaplan MM. Primary biliary cirrhosis. Adv Intern Med. 1987; 32: 359–377.

Kaplan MM. Survival in asymptomatic primary biliary cirrhosis. Not as good as previously reported? Gastroenterology. 1990; 98: 1707–1709.

Keeffe EB. Sarcoidosis and primary biliary cirrhosis. Literature review and illustrative case. Am J Med. 1987; 83: 977–980.

Kelley WN, Harris ED, Ruddy S, Sledge CB. Textbook of Rheumatology. 3rd ed. 1989, Saunders, Philadelphia

Klein R, Berg PA. Demonstaration of 'naturally occurring mitochondrial antibodies in family members of patients with primary biliary cirrhosis. Hepatology 1990; 12: 335–341.

Krowka MJ, Grambsch PM, Edell ES, Cortese DA, Dickson ER. Primary biliary cirrhosis: relation between hepatic function and pulmonary function in patients who never smoked. Hepatology. 1991; 13: 1095–1100.

Lacerda MA, Ludwig J, Dickson ER, Jorgensen RA,Lindor KD. Antimitochondrial antibody-negative primary biliary cirrhosis. Am J Gastroenterol. 1995; 90: 247–249.

Lanspa SJ, Chan AT, Bell JS, Go VL, Dickson ER, DiMagno EP. Pathogenesis of steatorrhea in primary biliary cirrhosis. Hepatology. 1985; 5: 837–842.

Lassoued K, Guilly MN, Danon F, Andre C, Dhumeaux D, Clauvel JP, Brouet JC, Selligmann M, Courvalin JC. Antinuclear autoantibodies specific for lamins: characterization and clinical significance. Ann Intern Med 1988; 108: 829–833.

Lassoued K, Guilly MN, Andre C, *et al.* Autoantibodies to 200 kD polypeptide(s) of the nuclear envelope: a new serological marker of primary biliary cirrhosis. Clin Exp Immunol. 1988; 74: 283–288.

Lassoued K, Brenard R, Degos F, *et al.* Antinuclear antibodies directed to 200 kD polypeptide of the nuclear envelope in primary biliary cirrhosis. A clinical and immunological study of a series of 150 patients with primary biliary cirrhosis. Gastroenterology 1990; 90: 181–186.

Laurent TC, Fraser JR, Petroft H, Smedsrod B. Binding of hyaluronate and chondroitin sulphate to liver endothelial cells. Biochem J 1986; 234: 653–658.

Laurent TC. Biochemistry of hyaluronan. Acta Otolaryngol Suppl (Stockh). 1987; 442: 7–24.

Laurent TC, Lilja K, Brunnberg L, Engstrom-Laurent A, Laurent UB, Lindqvist U, Murata K, Ytterberg D. Urinary excretion of hyaluronan in man. Scand J Clin Lab Invest. 1987; 47: 793–799.

Laurent TC, Fraser JR. Hyaluronan. FASEB J. 1992; 6: 2397–2404.

Laurin JM, Lindor KD. Primary biliary cirrhosis. Dig Dis. 1994; 12: 331–350.

Leevey CM, Sherlock S, Tygstrup N, Zetterman R. Diseases of the liver and biliary tract. Standadization of nomenclature, diagnostic criteria, and prognosis. 1994, Raven Press, New York

Lehmann AB, Bassendine MF, James OF. Is primary biliary cirrhosis a different disease in the elderly? Gerontology. 1985; 31: 186–194.

Lin DY, Chu CM, Sheen IS, Liaw YF. Serum carboxy terminal propeptide of type I procollagen to aminoterminal propeptide of type III procollagen ratio is a better indicator than each single propeptide and 7S domain type IV collagen for progressive fibrogenesis in chronic viral liver diseases. Dig Dis Sci. 1995; 40: 21–27.

Lindor KD. Management of osteopenia of liver disease with special emphasis on primary biliary cirrhosis. Semin Liver Dis. 1993; 13: 367–373.

Lofgren J, Jarnerot G, Danielsson D, Hemdal I. Incidence and prevalence of primary biliary cirrhosis in a defined population in Sweden. Scand J Gastroenterol. 1985; 20: 647–650.

Loof L, Adami HO, Sparen P, Danielsson A, Eriksson LS, Hultcrantz R, Lindgren S, Olsson R, Prytz H, Ryden BO, *et al.* Cancer risk in primary biliary cirrhosis: a population–based study from Sweden. Hepatology. 1994; 20(1 Pt 1): 101–104.

Lozano F, Pares A, Borche L, Plana M, Gallart T, Rodes J. Autoantibodies against nuclear envelope-associated proteins in primary biliary cirrhosis. Hepatology 1988; 8: 930–938

Lucey MR, Neuberger JM, Williams R. Primary biliary cirrhosis in men. Gut. 1986; 27: 1373–1376.

Mahl TC, Shockcor W, Boyer JL. Primary biliary cirrhosis: survival of a large cohort of symptomatic and asymptomatic patients followed for 24 years. J Hepatol. 1994; 20: 707–713.

Manns MP, Bremm A, Schneider PM, Notghi A, Gerken G, Prager-Eberle M, Stradmann-Bellinghausen B, Meyer zum Buschenfelde KH, Rittner C. HLA DRw8 and complement C4 deficiency as risk factors in primary biliary cirrhosis. Gastroenterology. 1991; 101: 1367–1373.

Maran R, Dueymes M, Adler Y, Shoenfeld Y, Youinou P. Isotypic distribution of antipyruvate dehydrogenase antibodies in patients with primary biliary cirrhosis and their family members. J Clin Immunol. 1994 ; 14: 323–326.

Martinez OM, Villanueva JC, Gershwin ME, Krams SM. Cytokine patterns and cytotoxic mediators in primary biliary cirrhosis. Hepatology 1995; 21: 113–119.

Mattsson L, Lindqvist U, Weiland O, Aberg B. Serum levels of the aminoterminal propeptide of type III procollagen and hyaluronan during resolving and nonresolving posttransfusion non-A, non-B hepatitis. Scand J Infect Dis. 1990; 22: 11–17.

McCaughan GW, Feller RB. Osteoporosis in chronic liver disease: pathogenesis, risk factors, and management. Dig Dis. 1994 ; 12: 223–231.

McCourt PA, EKB, Forsberg N, Gustafson S. Intercellular adhesion molecule-1 is a cell surface receptor for hyaluronan. J Biol Chem. 1994; 269: 30081–30084.

McCullough AJ, Stassen WN, Wiesner RH, Czaja AJ. Serum type III procollagen peptide concentrations to severe chronic active hepatitis: relationship to cirrhosis and disease activity. Hepatology 1987; 7: 49–54.

McCullough AJ, Stassen WN, Wiesner RH, Czaja AJ. Serial determination of the amino-terminal peptide of type III procollagen in severe chronic active hepatitis. J Lab Clin Med 1987; 109: 55–61.

McGary CT, Yannariello-Brown J, Kim DW, Stinson TC, Weigel PH. Degradation and intracellular accumulation of a residualizing hyaluronan derivative by liver endothelial cells. Hepatology. 1993; 18: 1465–1476.

McHugh NJ, James IE, Fairburn K, Maddison PJ. Autoantibodies to mitochondrial and centromere antigens in primary biliary cirrhosis and systemic sclerosis. Clin Exp Immunol. 1990; 81: 244–249.

McMillan SA, Alderdice JM, McKee CM *et al.* Diversity of autoantibodies in patients with mitochondrial antibody and their diagnostic value. J Clin Pathol 1987; 40: 232–236.

Mehal WZ, Gregory WL, Lo YM, Cross SJ, Fleming KA, Bassendine MF, James OF, Campbell RD, Chapman RW, Rosenberg WM. Defining the immunogenetic susceptibility to primary biliary cirrhosis. Hepatology. 1994; 20: 1213–1219.

Melia WM, Johnson PJ, Neuberger J, Zaman S. Portmann B, Williams R. Hepatocellular carcinoma in primary biliary cirrhosis: detection by alphafetoprotein estimation. Gastroenterology. 1984; 87: 660–663.

Mella JG, Roschmann E, Maier KP, Volk BA. Association of primary biliary cirrhosis with the allele HLA-DPB10301 in a German population. Hepatology. 1995; 21: 398–402.

Melkko J, Hellevik T, Risteli L, Risteli J, Smedsrod B. Clearance of NH2-terminal propeptides of types I and III procollagen is a physiological function of the scavenger receptor in liver endothelial cells. J Exp Med. 1994; 179: 405–412.

Michieletti P, Wanless IR, Katz A, Scheuer PJ, Yeaman SJ, Bassendine MF, Palmer JM, Heathcote EJ. Antimitochondrial antibody negative primary biliary cirrhosis: a distinct syndrome of autoimmune cholangitis. Gut. 1994; 35: 260–265.

Miller JP. Dyslipoproteinaemia of liver disease. Baillieres Clin Endocrinol Metab. 1990 Dec. 4(4). P 807–832.

Minuk GY, Rascanin N, Paul RW, Lee PW, Buchan K, Kelly JK. Reovirus type 3 infection in patients with primary biliary cirrhosis and primary sclerosing cholangitis. J Hepatol. 1987; 5: 8–13.

Minuk GY, Boyd ND, Matheson DS, Fritzler MJ, Green BJ. Serum immunoglobulin E levels in patients with primary biliary cirrhosis. J Allergy Clin Immunol. 1989; 83: 462–466.

Misaki M, Shima T, Yano Y, Sumita Y, Kano U, Murata T, Watanabe S, Suzuki S. Basement membrane-related and type III procollagen-related antigens in serum of patients with chronic viral liver disease. Clin Chem 1990; 36: 522–524.

Mistry P, Seymour CA. Primary Biliary Cirrhosis — From Thomas Addison to the 1990s. Q J Med 1992; 82: 185–196.

Mitchison HC, Bassendine MF, Hendrick A, Bennett MK, Bird G, Watson AJ, James OF. Positive antimitochondrial antibody but normal alkaline phosphatase: is this primary biliary cirrhosis? Hepatology. 1986; 6: 1279–1284.

Mitchison HC, Malcolm AJ, Bassendine MF, James OF. Metabolic bone disease in primary biliary cirrhosis at presentation Gastroenterology. 1988; 94: 463–470.

Mitchison HC, Bassendine MF, Malcolm AJ, Watson AJ, Record CO, James OF. A pilot, double-blind, controlled 1-year trial of prednisolone treatment in primary biliary cirrhosis: hepatic improvement but greater bone loss. Hepatology. 1989; 10: P 420–429.

Mitchison HC, Lucey MR, Kelly PJ, Neuberger JM, Williams R, James OF. Symptom development and prognosis in primary biliary cirrhosis: a study in two centers. Gastroenterology. 1990; 99: 778–784.

Mitchison HC, Palmer JM, Bassendine MF, Watson AJ, Record CO, James OF. A controlled trial of prednisolone treatment in primary biliary cirrhosis. Three-year results. J Hepatol. 1992; 15: 336–344.

Modena V, Marengo C, Amoroso A, Rosina F, Costantini P, Bellando P, Coppo R, Rizzetto M. Primary biliary cirrhosis and rheumatic diseases: a clinical, immunological and immunogenetical study. Clin Exp Rheumatol. 1986; 4: 129–134.

Moreno Sanchez D, Cassinello Ogea C, Gonzalez Blanco P, Pulido Ortega F, Castellano Tortajada G. [Epidemiology of primary biliary cirrhosis in southern Madrid] Med Clin (Barc). 1990; 94: 564–569.

Morshed SA, Nishioka M, Saito I, Komiyama K, Moro I. Increased expression of Epstein-Barr virus in primary biliary cirrhosis patients. Gastroenterol Jpn. 1992; 27: 751–758.

Muller A, Krombholz B, Pott G, Machnik G, Vollandt R, Reinhardt M, Jorke D. Collagen peptidase and type III procollagen peptide serum levels in chronic liver diseases. Clin Chim Acta. 1991; 197: 59–66.

Murata K, Kuolo F. Motoyama T. Changes of collagen types at various stages of human liver cirrhosis. Hepatogastroenterology 1984; 31: 158–161.

Murawaki Y, Ikuta Y, Koda M, Kawasaki H. Serum type III procollagen peptide, type IV collagen 7S domain, central triple-helix of type IV collagen and tissue inhibitor of metalloproteinases in patients with chronic viral liver disease: relationship to liver histology. Hepatology. 1994; 20(4 Pt 1): P 780–787.

Murray-Lyon IM, Thompson RPH, Williams R. Scleroderma and primary biliary cirrhosis. B M J. 1970; ii: 258–259.

Murtaugh PA, Dickson ER, Van Dam GM, Malinchoc M, Grambsch PM,Langworthy AL, Gips CH. Primary biliary cirrhosis: prediction of short-term survival based on repeated patient visits. Hepatology. 1994; 20 (1 Pt 1): 126–134.

Mutimer DJ, Bassendine MF, Kelly P, James OF. Is measurement of type III procollagen aminopropeptide useful in primary biliary cirrhosis? J. Hepatol. 1989; 9: 184–189.

Myszor M, James OFW. The epidemiology of primary biliary cirrhosis in north-east England: an increasingly common disease? Q J Med 1990; 75: 377–385

Nakamura RM, Peebles CL, Rubin RL, Molden DO, Tan EM. Autoantibodies to nuclear antigens: advances in laboratory tests and significance in systemic rheumatic disease. 2nd ed. American Society of Clinical Pathology Press, Chicago 1985.

Nakanuma Y, Terada T, Doishita K, Miwa A. Hepatocellular carcinoma in primary biliary cirrhosis: an autopsy study. Hepatology. 1990; 11: 1010–1016.
Nakanuma Y, Saito K, Unoura M. Semiquantitative assessment of cholestasis and lymphocytic piecemeal necrosis in primary biliary cirrhosis: a histologic and immunohistochemical study. J Clin Gastroenterol. 1990; 12: 357–362.
Neuberger JM. Predicting the prognosis of primary biliary cirrhosis. Gut. 1989; 30: 1519–1522.
Neuberger JM, Gunson BK, Buckels JA, Elias E, McMaster P. Referral of patients with primary biliary cirrhosis for liver transplantation. Gut. 1990;. 31: 1069–1072.
Nickowitz RE, Worman HJ. Autoantibodies from patients with primary biliary cirrhosis recognize a restricted region within the cytoplasmic tail of nuclear pore membrane glycoprotein Gp210. J Exp Med. 1993; 178: 2237–2242.
Nickowitz RE, Wozniak RW, Schaffner F, Worman HJ. Autoantibodies against integral membrane proteins of the nuclear envelope in patients with primary biliary cirrhosis. Gastroenterology. 1994; 106: 193–199.
Niemela O, Risteli L, Sotaniemi EA, Risteli J. Aminoterminal propeptide of type III procollagen in serum in alcoholic liver disease. Gastroenterology 1983; 85: 254–259.
Niemela O, Risteli L, Sotaniemi EA, Stenback F, Risteli J. Serum basement membrane and type III procollagen-related antigens in primary biliary cirrhosis. J Hepatol. 1988; 6: 307–314.
Nouri Aria KT, Lombard M, Williams R. High serum levels of CD8 antigen in primary biliary cirrhosis: a possible cause of supressor cell dysfunction? Clin Exp Immunol. 1991; 86: 140–144.
Nyberg A, Engstrom-Laurent A, Loof L. Serum hyaluronate in primary biliary cirrhosis--a biochemical marker for progressive liver damage. Hepatology. 1988; 8: 142–146.
Nyberg A, Loof L. Primary biliary cirrhosis: clinical features and outcome, with special reference to asymptomatic disease. Scand J Gastroenterol. 1989; 24: 57–64.
Nyberg A, Lindqvist U, Engström-Laurent A. Serum hyaluronan and aminoterminal propeptide of type III procollagen in primary biliary cirrhosis: relation to clinical symptoms, liver histopathology and outcome. J Intern Med 1992; 231: 485–491.
O'Donohue J, McFarlane B, Bomford A, Yates M, Williams R. Antibodies to atypical mycobacteria in primary biliary cirrhosis. J Hepatol. 1994; 21: 887–889.
Oikarinen A, Autio P, Vuori J, Vaananen K, Risteli L, Kiistala U, Risteli J. Systemic glucocorticoid treatment decreases serum concentrations of carboxyterminal propeptide of type I procollagen and aminoterminal propeptide of type III procollagen. Br J Dermatol. 1992; 126: 172–178.
Onishi S, Sakamaki T, Maeda T, Iwamura S, Tomita A, Saibara T, Yamamoto Y. DNA typing of HLA class II genes; DRB10803 increases the susceptibility of Japanese to primary biliary cirrhosis. J Hepatol. 1994; 21: 1053–1060.
Pawlotsky JM, Andre C, Metreau JM, Beaugrand M, Zafrani ES, Dhumeaux D. Multiple nuclear dots antinuclear antibodies are not specific for primary biliary cirrhosis. Hepatology. 1992; 16: 127–131
Pearce S, Bassendine M, Dowsett M. Sex hormone binding globulin and non-protein-bound oestradiol in postmenopausal patients with primary biliary cirrhosis and normal controls. J Steroid Biochem Mol Biol. 1993; 44: 273–276.

Plebani M, Giacovini A, Floreani A. *et al.* Biochemical markers of hepatic fibrosis in primary biliary cirrhosis. Ric. Clin. Lab. 1990; 20: 169–174.

Pohjanpelto P, Tallgren M, Farkkila M, Miettinen T, Nuutinen H, Vuoristo M, Heinala P, Makela T. Low prevalence of hepatitis C antibodies in chronic liver disease in Finland. Scand J Infect Dis. 1991; 23: 139–42.

Poupon RE, Balkau B, Guechot J, Heintzmann F. Predictive factors in ursodeoxycholic acid-treated patients with primary biliary cirrhosis: role of serum markers of connective tissue. Hepatology. 1994; 19: 635–40.

Powell F, Schroeter AL, Dickson ER. Antinuclear antibodies in primary biliary cirrhosis. Lancet 1984; i: 288–289.

Powell FC, Schroeter AL, Dickson ER. Primary biliary cirrhosis and the CREST syndrome: a report of 22 cases. Q J Med. 1987; 62: 75–82.

Prost AC, Abuaf N, Rouquette-Gally AM, Homberg JC, Combrisson A. Comparing HEp-2 cell line with rat liver in routine screening test for antinuclear and antinucleolar autoantibodies in autoimmune diseases. Ann Biol Clin (Paris). 1987; 45: 610–617.

Raedsch R, Stiehl A, Sieg A, Walker S, Kommerell B. Biliary excretion of procollagen peptide in healthy humans and in patients with alcoholic cirrhosis of the liver. Gastroenterology 1983; 85: 1265–1270.

Ramadori G, Zohrens G, Manns M. *et al.* Serum hyaluronate and type III procollagen aminoterminal propeptide concentration in chronic liver disease. Relationship to cirrhosis and disease activity. Eur. J. Clin. Invest. 1991; 21: 323–330.

Reeves WH, Chaudhary N, Salemo A, Blobel G. Lamin B autoantibodies in sera of certain patients with systemic lupus erythematosus. J Exp Med 1987; 165: 750–762.

Risteli J, Niemi S, Trivedi P, Maentausta O, Mowat AP, Risteli L. Rapid equilibrium radioimmunoassay for the amino-terminal propeptide of human type III procollagen. Clin Chem. 1988; 34: 715–718.

Roberts-Thomson PJ, Shepherd K. Low molecular weight IgM in primary biliary cirrhosis. Gut. 1990; 31: 88–91.

Robson SC, Hift RJ, Kirsch RE. Primary biliary cirrhosis. A retrospective survey at Groote Schuur Hospital, Cape Town. S Afr Med J. 1990; 78: 19–22.

Roden L, Campbell P, Fraser JR, Laurent TC, Pertoft H, Thompson JN. Enzymatic pathways of hyaluronan catabolism. Ciba Found Symp. 1989; 143: 60–76.

Rohde H, Vargas L, Hahn E, Kalbfleisch H, Bruguera M, Timpl R. Radioimmunoassay for type III procollagen peptide and its application to human liver disease. Eur J Clin Invest 1979: 9: 451–459.

Rojkind M, Martinez-Paloma A. Increase in type I and type III collagens in human alcoholic liver cirrhosis. Proc Natl Acad Sci USA 1976; 73: 539–543.

Rojkind M, Giambrone MA, Biempica L. Collagen types in normal and cirrhotic liver. Gastroenterology 1979; 76: 710–719.

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: 1–7.

Ros E, Garcia-Puges A, Reixach M, Cuso E, Rodes J. Fat digestion and exocrine pancreatic function in primary biliary cirrhosis. Gastroenterology. 1984; 87: 180–187.

Rosenson RS, Baker AL, Chow MJ, Hay RV. Hyperviscosity syndrome in a hypercholesterolemic patient with primary biliary cirrhosis. Gastroenterology. 1990; 98: 1351–1357.

Rubel LR, Rabin L, Seeff LB, Licht H, Cuccherini BA. Does primary biliary cirrhosis in men differ from primary biliary cirrhosis in women? Hepatology. 1984; 4: 671–677.

Ruffati A, Arslan P, Floreani A. Nuclear membrane-staining antinuclear antibody in patients with primary biliary cirrhosis. J Clin Immunol 1985; 5: 357–361.

Rydning A, Schrumpf E, Abdelnoor M, Elgjo K, Jenssen E. Factors of prognostic importance in primary biliary cirrhosis. Scand J Gastroenterol. 1990; 25: 119–126.

Salaspuro MP, Laitinen OI, Lehtola J. Immunological parameters, viral antibodies and biochemical and histological findings in relatives of patients with chronic active hepatitis and primary biliary cirrhosis. Scand J Gastroenterol 1976; 11: 313–320.

Salupere V, Remmel H, Uibo R. Experiences in diagnosing and treatment of primary biliary cirrhosis. Ter arhiv (in Russian) 1987; 2: 112–113.

Sasaki H, Inoue K, Higuchi K, Yasuyama T, Koyata H, Kuroki T, Yamamoto S, Ichida F. Primary biliary cirrhosis in Japan: national survey by the Subcommittee on Autoimmune hepatitis. Gastroenterol Jpn. 1985; 20: 476–485.

Savolainen ER, Miettinen TA, Pikkarainen P, Salaspuro MP, Kivirikko KI. Enzymes of collagen synthesis and type III procollagen aminopropeptide in the evaluation of D-penicillamine and medroxyprogesterone treatments of primary biliary cirrhosis. Gut 1983; 24: 136–142.

Scheuer PJ. Primary biliary cirrhosis. Proc R Soc Med 1967; 60: 1257–60.

Scheuer PJ. Liver biopsy interpretation. 4th ed. London: Bailliere Tindall, 1988.

Schuppan D. Structure of the extracellular matrix in normal and fibrotic liver: collagens and glycoproteins. Semin Liver Dis 1990; 10: 1–10.

Schuppan D. Connective tissue polypeptides in serum as parameters to monitor antifibrotic treatment in hepatic fibrogenesis. J Hepatol. 1991; 13 Suppl: S17–25.

Seyer JM, Hutcheson ET, Kang AH. Collagen polymorphism in normal and cirrhotic human liver. J Clin Invest 1977; 59: 241–148.

Seyer JM. Mediators of increased collagen synthesis in fibrosing organs. Fund. Appl. Toxicol. 1985; 5: 228–239.

Seki T, Kiyosawa K, Ota M, Furuta S, Fukushima H, Tanaka E, Yoshizawa K, Kumagai T, Mizuki N, Ando A, *et al.* Association of primary biliary cirrhosis with human leukocyte antigen DPB10501 in Japanese patients. Hepatology. 1993; 18: 73–78.

Shapiro JM, Smith H, Schaffner F. Serum bilirubin: a prognostic factor in primary biliary cirrhosis. Gut 1979; 20: 137–140.

Sherlock S, Scheuer PJ. The presentation and diagnosis of 100 patients with primary biliary cirrhosis. New Engl J Med 1973; 289:674–678

Shibata M, Ueno Y, Yoshida N, Yamamuro W, Kubo S, Shimada N, Okada T, Sato G, Hatori T, Onozuka Y.[Clinical study of male patients with primary biliary cirrhosis (PBC)] Nippon Shokakibyo Gakkai Zasshi. 1990; 87:P 1846–1850.

Shiomi S, Kuroki T, Masaki K, Takeda T, Nishiguchi S, Nakajima S, Seki S, Kobayashi K, Okamura T, Ochi H. Osteopenia in primary biliary cirrhosis and cirrhosis of the liver in women, evaluated by dual-energy X-ray absorptiometry. J Gastroenterol. 1994; 29: 605–609.

Smedsrod B. Aminoterminal propeptide of type III procollagen is cleared from the circulation by receptor-mediated endocytosis in liver endothelial cells. Coll Relat Res. 1988; 8: 375–388.

Spiteri MA, Clarke SW. The nature of latent pulmonary involvement in primary biliary cirrhosis. Sarcoidosis. 1989; 6: 107–110.

Spiteri MA, Johnson M, Epstein O, Sherlock S, Clarke SW, Poulter LW. Immunological features of lung lavage cells from patients with primary biliary cirrhosis may reflect those seen in pulmonary sarcoidosis. Gut. 1990; 31: 208–212.

Stellon AJ, Webb A, Compston J, Williams R. Low bone turnover state in primary biliary cirrhosis. Hepatology. 1987; 7: 137–142.

Stemerowicz R, Hopf U, Moller B, Wittenbrink C, Rodloff A, Reinhardt R, Freudenberg M, Galanos C. Are antimitochondrial antibodies in primary biliary cirrhosis induced by R(rough)-mutants of enterobacteriaceae? Lancet. 1988; ii: 1166–1170.

Sundewall AC, Lefvert AK. Acetylcholine receptor antibodies in primary biliary cirrhosis: characterization of antigen and idiotypic specificity. Scand J Immunol. 1990; 31: 477–484.

Surrenti C, Casini A, Milani S, Ambu S, Ceccatelli P, D'Agata A. Is determination of N-terminal procollagen type III peptide (sPIIIP) a marker of hepatic fibrosis? Dig. Dis. Sci. 1987; 32: 705–709.

Szostecki C, Krippner H, Penner E, Bautz FA. Autoimmune sera recognize a 100 kD nuclear protein antigen (sp-100). Clin Exp Immunol 1987; 68: 108–116.

Szostecki C, Guldner HH, Netter HJ, Will H. Isolation and characterization of cDNA encoding a human nuclear antigen predominantly recognized by autoantibodies from patients with primary biliary cirrhosis. J of Immunology 1990; 145: 4338–4347.

Szostecki C, Will H, Netter HJ, Guldner HH. Autoantibodies to the nuclear Sp100 protein in primary biliary cirrhosis and associated diseases: epitope specificity and immunoglobulin class distribution. Scand J Immunol. 1992; 36: 555–564.

Takase S, Enyama K, Takada A, Tsutsumi M. Effects of vitamin A on collagen metabolism by cultured rat liver cells. Gastroenterol Jpn. 1992; 27: 354–363.

Tartakovsky F, Worman HJ. Detection of Gp210 autoantibodies in primary biliary cirrhosis using a recombinant protein containing the predominant autoepitope. Hepatology. 1995; 21: 495–500.

Taylor SL, Dean PJ, Riely CA. Primary autoimmune cholangitis. An alternative to antimitochondrial antibody-negative primary biliary cirrhosis. Am J Surg Pathol. 1994; 18: 91–99.

Teare JP, Sherman D, Greenfield SM. *et al.* Comparison of serum procollagen III peptide concentrations and PGA index for assessment of hepatic fibrosis. Lancet 1993; 342: 895–898.

Teran JC, Mullen KD, Hoofnagle JH, McCullough AJ. Decrease in serum levels of markers of hepatic connective tissue turnover during and after treatment of chronic hepatitis B with interferon-alpha. Hepatology 1994; 19: 849–856.

Thompson NP, Leader S, Jamieson CP, Burnham WR, Burroughs AK. Reversible jaundice in primary biliary cirrhosis due to hyperthyroidism. Gastroenterology. 1994; 106: 1342–1343.

Timpl R, Glanville RW. The aminopropeptide of collagen. Clin Othop 1981; 158: 224–242.

Torres-Salinas MM, Pares A, Caballeria J *et al.* Serum procollagen III peptides as a marker of hepatic fibrogenesis in alcoholic hepatitis. Gastroenterology 1986; 90: 1241–1246.

Triger DR. Primary biliary cirrhosis: an epidemiological study. B M J 1980; 281: 772–775

Triger DR, Berg PA, Rodes J. Epidemiology of primary biliary cirrhosis. Liver. 1984; 4: 195–200.

Triger DR, Muggleton RJ, Carter ND. IgM abnormalities in the families of patients with primary biliarycirrhosis. J Gastroenterol Hepatol. 1989; 4 Suppl 1: 58–59.

Trinchet JC, Hartmann DJ, Pateron D, Laarif M, Callard P, Ville G, Beaugrand M. Serum type I collagen and N-terminal peptide of type III procollagen in chronic hepatitis. Relationship to liver histology and conventional liver tests. J Hepatol. 1991; 12: 139–144.

Trinchet JC, Hartmann DJ, Pateron D, Munz-Gotheil C, Callard P, Ville G, Beaugrand M. Serum type I collagen and N-terminal peptide of type III procollagen in patients with alcoholic liver disease: relationship to liver histology. Alcohol Clin Exp Res. 1992; 16: 342–346.

Tsianos EV, Hoofnagle JH, Fox PC, Alspaugh M, Jones EA, Schafer DF, Moutsopoulos HM. Sjogren's syndrome in patients with primary biliary cirrhosis. Hepatology. 1990; 11: 730–734.

Tsuji H, Murai K, Akagi K, Fujishima M. Familial primary biliary cirrhosis associated with impaired concanavalin A-induced lymphocyte transformation in relatives. Two family studies. Dig Dis Sci. 1992; 37: 353–360.

Uddenfeldt P, Danielsson A. Evaluation of rheumatic disorders in patients with primary biliary cirrhosis. Ann Clin Res. 1986; 18: 148–153.

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: 443–448.

Ueno T, Inuzuka S, Torimura T, Tamaki S, Koh H, Kin M, Minetoma T, Kimura Y, Ohira H, Sata M, *et al.* Serum hyaluronate reflects hepatic sinusoidal capillarization. Gastroenterology. 1993; 105: 475–481.

Ueno T, Inuzuka S, Sata M, Koh H, Tamaki S, Kin M, Sugawara H, Sakata R, Torimura T, Tanikawa K. Serum hyaluronate predicts response to interferon alpha therapy in patients with chronic hepatitis C. Hepatogastroenterology 1995; 42: 522–528.

Underhill J, Donaldson P, Bray G, Doherty D, Portmann B, Williams R. Susceptibility to primary biliary cirrhosis is associated with the HLA-DR8-DQB10402 haplotype. Hepatology. 1992; 16: 1404—1408.

Van Berkum FN, Beukers R, Birkenhager JC, Kooij PP, Schalm SW, Pols HA. Bone mass in women with primary biliary cirrhosis: the relation with histological stage and use of glucocorticoids. Gastroenterology. 1990; 99: 1134–1139.

van Zanten RA, van Leeuwen RE, Wilson JH. Serum procollagen III N-terminal peptide and laminin P1 fragment concentrations in alcoholic liver disease and primary biliary cirrhosis. Clin Chim Acta 1988; 177: 141–146.

Vierling JM. Immune disorders of the liver and bile duct. Gastroenterol Clin North Am. 1992; 21: 427–449.

Vilagut L, Vila J, Vi~nas O, Pares A, Gines A, Jimenez de Anta MT,Rodes J. Cross-reactivity of anti-Mycobacterium gordonae antibodies with the major mitochondrial autoantigens in primary biliary cirrhosis. J Hepatol. 1994; 21: 673–677.

Walker JG, Doniach D, Roitt IM, Sherlock S. Serological tests in diagnosis of primary biliary cirrhosis. Lancet 1965; i:827–831

Wallace JG Jr, Tong MJ, Ueki BH, Quismorio FP. Pulmonary involvement in primary biliary cirrhosis. J Clin Gastroenterol. 1987; 9: 431–435.

Wallaert B, Bonniere P, Prin L, Cortot A, Tonnel AB, Voisin C. Primary biliary cirrhosis. Subclinical inflammatory alveolitis in patients with normal chest roentgenograms. Chest. 1986; 90: 842–848.

Watson RGP, Angus PW, Dewar M, Goss B. Low prevalence of primary biliary cirrhosis in Victoria, Australia. Gut. 1995; 36: A927.

Weigand K, Zaugg PY, Frei A, Zimmermann A. Long-term follow-up of serum N-terminal propeptide of collagen type III levels in patients with chronic liver disease. Hepatology. 1984; 4: 835–838.

Wesierka-Gadek J, Penner E, Hitchman E, Sauermann G. Antibodies to nuclear lamins in autoimmune liver disease. Clin Immunol Immunopathol 1988; 49: 107–115.

Wiesner RH, Porayko MK, Dickson ER, Gores GJ, LaRusso NF, Hay JE, Wahlstrom HE, Krom RA. Selection and timing of liver transplantation in primary biliary cirrhosis and primary sclerosing cholangitis. Hepatology. 1992; 16: 1290–1299.

Wills MR, Savory J. Vitamin D metabolism and chronic liver disease. Ann Clin Lab Sci. 1984; 14: 189–197.

Witt-Sullivan H, Heathcote J, Cauch K, Blendis L, Ghent C, Katz A, Milner R, Pappas SC, Rankin J, Wanless IR. The demography of primary biliary cirrhosis in Ontario, Canada. Hepatology. 1990; 12: 98–105.

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: 1075–1078.

Worman HJ, Yuan J, Blobel G, Georgatos SD. A lamin B receptor in the nuclear envelope. Proc Natl Acad Sci USA 1988; 85: 8531–8534.

Wozniak R, Bartnik E, Blobel G. Primary structure analysis of an integral membrane glycoprotein of the nuclear pore. J Cell Biol 1989; 108: 2083–2092.

Wu J, Danielsson A. Inhibition of hepatic fibrogenesis: a review of pharmacological candidates. Scand. J. Gastroenterol. 1994; 29: 385–391.

Wu J, Danielsson A. Detection of hepatic fibrogenesis: a review of available techniques. Scand J Gastroenterol 1995; 30: 817–825.

PRIMAARNE BILIAARNE MAKSATSIRROOS EESTIS: EPIDEMIOLOOGIA, KLIINILISED ISEÄRASUSED NING HAIGUSE KULU PROGNOOSIMINE

Kokkuvõte

Primaarne biliaarne maksatsirroos (PBT) on krooniline, progresseeruva kuluga, kolestaasiga kulgev maksahaigus, mille etioloogia pole siiani teada. Kuna haigestunutel on leitud terve hulk muutusi nii humoraalses kui ka rakulises immuunsuses, eelkõige mitokondriantikehade olemasolu (AMA), siis arvatakse, et tegemist on autoimmuunse haigusega. Peamiselt haigestuvad menopausis naised ning haiguse progresseerudes tekib maksas fibroos ja tsirroos. Haiged surevad maksapuudulikkuse või söögitoru veenikomude verejooksu tõttu.

PBT-d on diagnoositud kõikjal maailmas. Kõige suurem esinemissagedus on leitud Rootsis: 154 haigusjuhtu 1 miljoni elaniku kohta. Haigestumissageduses on piirkonniti suuri erinevusi, mille põhjused pole praeguseks täpselt teada. Siiani puuduvad andmed PBT-sse haigestumuse kohta Ida-Euroopa maades.

Töö eesmärgiks oli:

- uurida PBT levimust ja haigestumust ning nende eripära Eestis;
- iseloomustada Eesti PBT haigete kontingenti;
- analüüsida haiguse pikaajalist kulgu ja selle prognoosimise võimalusi mitmesuguste markerite abil;
- määrata immunofluorestsentsmeetodiga tuumavastaseid antikehi koelõigul ja HEp-2 rakkudel, selgitamaks, kas mõni tuumavastaste antikehade muster on oluline PBT diagnoosimisel, eriti mitokondriantikehade puudumisel;
- kindlaks teha kliinilisi, laboratoorseid ja prognostilisi erinevusi tuumavastaseid antikehasid omavate ja mitteomavate patsientide vahel;
- selgitada, kas prokollageen III ja hüaluronaan korreleeruvad PBT puhul mõne kliinilise, laboratoorse või histoloogilise näitajaga ja leida nende tähendus haiguse lühi- ja kaugprognoosi ennustamisel.

Käesolevas uurimistöös analüüsiti PBT-sse haigestumust Eestis ajavahemikus 1. jaanuar 1973 – 15. märts 1996. Uuring algas retrospektiivsena ja jätkus 1992. a. sügisest prospektiivsena. Kuna PBT on oma kulult suhteliselt heterogeenne ning haiguse kulu prognoosimine on sageli raske, siis uuriti PBT-haigetel mitmesuguseid immunoloogilisi (tuumavastased antikehad) ja fibrogeneesi näitavaid markereid (hüaluronaan, prokollageen III), et prognoosida

haiguse kulgu ja tulevikus kasutada seda haigete õigeaegsel suunamisel maksatransplantatsioonile. Töö koosneb kolmest osast.

I. Epidemioloogiline uuring

Uuringus kasutati mitut informatsiooniallikat: 1986. aastal saadeti kõikidesse maakonna- ja linnahaiglatesse haigust tutvustav prospekt, palvega saata haigusekahtlased haiged edaspidisteks uuringuteks Tartu Ülikooli sisekliiniku gastroenteroloogia osakonda. Teiseks, vaadati üle kõik üld- ja molekulaarpatoloogia instituudi immunoloogia laboratooriumis leitud mitokondrite autoantikehadega (AMA) haigusjuhud (ainuke autoantikehade määramise koht Eestis kuni 1994 aastani). Need haiged, kes vastasid PBT diagnostilistele kriteeriumitele, lülitati uuringusse. Hiljem (alates 1994. a.) lülitati uuringusse ka Tallinna Keskhaiglas diagnoositud PBT juhud, mille puhul AMA oli määratud Tallinna Keskhaigla immunoloogia laboratooriumis.

1973–1992 diagnoositi Eestis 69 PBT juhtu. Aastas diagnoositi keskmiselt 2,27 uut juhtu 1 miljoni elaniku kohta ja epidemioloogilise uuringu selle osa lõpuks, 31. detsembriks 1992, oli esinemissagedus 26,9 juhtu 1 miljoni elaniku kohta. Selgus, et haigestumus oli märkimisväärselt suurem Viljandi maakonnas, kus esinemissagedus oli 123 juhtu 1 miljoni elaniku kohta. Põhjused, mis võiksid viia sellise suure haigestumuseni, ei ole praeguseks teada. Samas puudusid erinevused eri etnilise taustaga inimeste haigestumuses.

Edaspidi uuringud jätkusid kuni 15. märtsini 1996. Selle aja jooksul diagnoositi 28 uut PBT juhtu. Seega on analüüsil kasutatud kokku andmeid 97 haige kohta.

Meeste ja naiste suhe Eesti PBT-i haigetel oli 1:18,4. Keskmine vanus haiguse diagnoosimise momendil oli 55,6±10,4 aastat (32–84). Enne haiguse diagnoosimist olid haigete kaebused kestnud keskmiselt 2,1 aastat, peamiselt nahasügelemine. Seetõttu olid nad külastanud erisuguseid arste. Haiguse diagnoosimise momendil olid sagedasemateks sümptoomideks nahasügelemine (79,4%), hepatomegaalia (59,8%), ikterus (52,5%), hüperpigmentatsioon (48,4%), väsimus (47,2%) ja kõhuvalu (43,4%). Kaasuvaid autoimmuunse geneesiga sündroome ja haigusi leiti 34%-l haigetest. Neist sagedasemad olid Sjögreni ja Raynaud sündroomid. Haiguse kulu jooksul kurtis seljavalu 33% ja liigesvalu 37% haigetest.

Ainult 15 patsiendil (15,5%) puudusid haiguse diagnoosimise momendil sümptoomid, neist 7-l tekkisid sümptoomid jälgimisperioodi jooksul ja 3 suri.

91,6%-l haigetest leiti immunofluorestsentsuuringul AMA. Alkaalse fosfataasi aktiivsus oli tõusnud enamusel haigetest markantselt (üle 2 korra normist suurem). Tsütolüüs oli tunduvalt vähem väljendunud kui kolestaas (87%). Haiguse diagnoosimise momendil oli hüpoalbumineemiat harva (12,4%).

78 haigel 93-st oli maksabiopsia abil võimalik jaotada PBT histoloogilistesse staadiumitesse: 6 juhtu oli I. histoloogilises staadiumis, 21 juhtu II. staadiumis, 27 juhtu III. staadiumis ja 24 juhtu IV. staadiumis. Need andmed koos sümptoomideta haigusjuhtude vähese arvuga osutavad haiguse hilisele diagnoosimisele Eestis.

Gastroenteroloogia osakonnas jälgiti patsiente keskmiselt 2,85 aastat (0,1–20,4). Selle aja jooksul 38 haiget suri: 22 maksapuudulikkusesse, 7 söögitoru veenikomude, verejooksu tõttu 2 maksavähki. Nelja haige surm polnud seotud otseselt maksahaigusega. Keskmine elulemus haiguse diagnoosimise momendist oli 5,5 aastat (0,1–20,4 aastat) ja esimese sümptoomi tekkest, mis oli seotud maksahaigusega (nahasügelemine, ikterus jt.) 7,8 aastat (1,4–22,3 aastat). Viie aasta elulemus oli 78,1%. Mitmene regressioonianalüüs näitas, et patsientide eakus ja bilirubiini kõrge kontsentratsioon olid need markerid, mis viitasid halvale prognoosile.

II. Tuumavastaste antikehade esinemine ja nende tähendus

Uuriti 69 haiget, kasutati kahte substraati: külmutatud koelõike ja HEp-2 rakke, määramaks immunofluorestsentsmeetodil tuumavastaste antikehade (ANA) esinemist. Külmutatud koelõigul oli võimalik leida ANA 29%-l PBT-haigetest, HEp-2 rakkude kasutamisel oli ANA tiitris 1:40 positiivne 70%-l haigetest. ANA esines kõikidel AMA-negatiivsetel juhtudel. Sagedasimaks ANA variandiks PBT-i haigetel oli hulgine täppmuster (MND). MND-ANA leiti ka kahel autoimmuunse hepatiidi ja ühel luupusehaigel. Kuna viimasena nimetatul normaalsete maksafunktsioonitestide tõttu maksabiopsiat ei tehtud, siis täielikult ei saa sel haigel välistada asümptomaatilise PBT-i võimalust. Teine ANA variant, mis oli üsna spetsiifiline primaarsele biliaarsele tsirroosile oli perinukleaarse mustriga ANA, mida leiti 16%-l haigetest. Selgus, et AMA-negatiivsed PBT-haiged ei erine ühestki uuritud näitaja aspektist AMA-positiivsetest haigetest.

ANA-positiivsete haigete elulemus oli märkimisväärselt parem kui ANA-negatiivsetel (vastavalt 6,3 ja 3,3 aastat, $p<0,02$), kuigi nende haiguse histoloogilised staadiumid olid haiguse diagnoosimise momendil sarnased ja hiljem rakendatud ravi põhimõtteliselt ei erinenud.

ANA puhul on tegemist stabiilse markeriga. 73%-l haigetest, kel ANA määrati korduvalt, ANA muster ei muutunud. Mitte ühelgi ANA-negatiivsel haigel ei tekkinud ANA haiguse käigus.

III. Aminoterminaal-propeptiid-kollageen III ja hüaluronaan primaarse biliaarse tsirroosi haigetel

Selles töö osas uuriti 55-l PBT-haigel kahte fibrogeneesi markerit: prokollageen III ja hüaluronaani, selgitamaks nende seost haiguse iseärasustega ja prognoosiga. Vastavate markerite sisaldus määrati seerumis radiomeetrilisel meetodil, kasutades kahte standardtestsüsteemi (*Orion*, Soome ja *Pharmacia Diagnostics*, Rootsi). Tehti nii ühemomentne (55 haiget) kui ka dünaamiline uuring (26 haiget).

Selgus, et 82%-l haigetest oli prokollageen III ja 84%-l hüaluronaani sisaldus seerumis üle normi. Kõige kõrgemad väärtused saadi neljandas histoloogilises staadiumis olevatel PBT haigetel: prokollageen III keskmiselt 8,8 ng/ml ja hüaluronaan 280 µg/l. Nii prokollageen III kui ka hüaluronaanil oli kõrge tundlikkus, kuid madal spetsiifilisus kaugelearenenud PBT diagnoosimisel, misjuures hüaluronaan oli tundlikum näitaja. Nii prokollageen III kui ka hüaluronaan korreleerusid hästi haiguse histoloogilise staadiumiga (vastavalt $p<0,01$ ja $p<0,00001$).

Haigussümptoomidega PBT-i haigetel oli prokollageen III ja hüaluronaani väärtused oluliselt kõrgemad kui asümptomaatilistel haigetel. Neil haigetel kellel uurimisperioodi jooksul tekkisid sümptoomid olid mõlemad väärtused üle normi.

Seerumi prokollageen III korreleerus oluliselt bilirubiiniga ($p<0,01$), kuid mitte teiste kliiniliste ja laboratoorsete näitajatega. Hüaluronaani kontsentratsioon korreleerus positiivselt nahasügelemise ($p<0,05$), ikteruse ($p<0,05$), väsimuse ($p<0,01$), söögitoru veenikomude olemasolu ($p<0,01$), kehamassi vähenemise ($p<0,05$), bilirubiininivoo ($p<0,0001$), steatorröa raskuse ($p<0,01$), sümptomaatilise perioodi pikkusega ($p<0,01$) ning negatiivselt hepatomegaalia ($p<0,001$) ja seerumi albumiinisisaldusega ($p<0,05$).

Kasutades Coxi regressioonanalüüsi, selgus, et pikaajalise prognoosi tegemisel ei olnud kummalgi neist sidekoemarkeritest sõltumatut prognostilist tähendust. Samas aga oli oluline marker bilirubiin ($p<0,05$).

Prokollageen III taseme uurimisel dünaamikas selgus, et nii prednisolooni kui ka prednisoloon-asatiopriinravi grupis väärtused oluliselt ei muutunud. Hüaluronaani kontsentratsioon tõusis märkimisväärselt neil patsientidel, kes olid algul haiguse I–II histoloogilises staadiumis. Prednisoloon-asatiopriinravi saavatel haigetel jäid hüaluronaani väärtused ravile vaatamata stabiilsele tasemele, märkimisväärselt tõusis aga hüaluronaani tase ainult prednisoloonravi saavatel haigetel. Kui algul korreleerus prokollageen III bilirubiiniga ($p<0,05$), siis hiljem see korrelatsioon kadus. Hüaluronaan säilitas oma korrelatsiooni bilirubiiniga ka hiljem (algul $p<0,01$, hiljem $p<0,001$).

Jälgides haigete üheaastast elulemust sidekoe markerite viimase määramise momendist, selgus, et nendel haigetel, kes selle perioodi jooksul surid, olid

märkimisväärselt kõrgem hüaluronaani kontsentratsioon ($p<0,05$), kõrgem bilirubiini väärtus ($p<0,05$), madalam üldvalgu ($p<0,05$) ja albumiini ($p<0,05$) kontsentratsioon.

Kokkuvõttes võimaldavad käesoleva töö resultaadid teha järgnevaid järeldusi:

1. Võrreldes teiste Euroopa riikidega on PBT Eestis harvaesinev haigus. 20-aastase uurimisperioodi jooksul suurenes haiguse levimus pidevalt, olles 31. detsembril 1992. aastal 26,9 juhtu 1 miljoni elaniku kohta. Leiti suured regionaalsed erinevused haigestumuses. Suurim levimus oli Viljandi maakonnas: 123 juhtu 1 miljoni elaniku kohta. Samal ajal puudusid erinevused eri etnilise taustaga isikutel.

2. Alates 1986. aastast on Eestis diagnoositud ligikaudu kuus uut haigusjuhtu aastas, mis osutab PBT üllatavalt stabiilsele esinemisele Eestis.

3. Enamus PBT juhtudest dignoositakse Eestis hilisstaadiumis (III ja IV histoloogilise staadiumi haigeid 65,4%), ehkki haiguse sümptoomidega perioodi kestus enne PBT diagnoosimist oli pikk. Üle 50%-l haigetest oli diagnoosimise momendiks tekkinud ikterus. Sümptoomideta haigete hulk oli väike.

4. Kaasuvaid autoimmuunse geneesiga haigusi ja sündroome leiti 34%-l haigetest. Sagedasemateks olid Sicca ja Raynaud' sündroomid. 26,8%-l haigetest leiti sapikivid sapipõies. AMA leidmine immunofluorestsentsmeetodil on spetsiifiline ja tundlik tunnus PBT diagnoosimiseks. Seega on AMA testimine oluline autoimmuunseid haigusi põdevatel isikutel ja sapikivitõvehaigetel, kel leitakse maksafunktsioonide muutused.

5. Ligikaudu pooltel sümptoomideta haigetest tekkisid haigusnähud jälgimisperioodi jooksul. Nende haigete elulemus sarnanes haigete elulemusega, kellel diagnoosimise momendil olid juba sümptoomid. Näib, et asümptomaatilistel haigetel oleks vaja alustada raviga kohe, kuna nende haigete elulemus pole oluliselt parem.

6. Eesti PBT-haigete elulemus oli oluliselt lühem kui teiste Euroopa riikide haigetel. Keskmiselt elas haige 5,5 aastat haiguse diagnoosimise momendist ja 7,8 aastat esimese sümptoomi tekkemomendist. 78,1% haigetest elas viis aastat või enam. Eakus ja kõrged bilirubiini väärtused näitasid teineteisest sõltumatult halba prognoosi.

7. Paljudel Eestis elavatel PBT-haigetel oli seerumis ANA. Vastavaid antikehi leiti 29%-l haigetest koelõigul ja 70%-l HEp-2-rakkudel (antigeeni substraadid). Kõige sagedasemaks ANA variandiks oli hulgine täppmuster (MND) olemasolu osutus spetsiifiliseks PBT-le. ANA-positiivsete haigete eluprognoos on oluliselt parem kui ANA-negatiivsetel.

8. Kõigil AMA-negatiivsetel haigetel leiti ANA, kusjuures MND-ANA oli sagedaim. Seepärast on PBT diagnoosimisel oluline ANA testimine, eriti AMA-negatiivsetel kroonilise kolestaasiga haigetel. Samal ajal puudusid kliinilised erinevused AMA-positiivsete ja AMA-negatiivsete haigete vahel.

9. Enamusel PBT-haigetest on fibrogeneesimarkerite (prokollageen III ja hüaluronaan) kontsentratsioonid veres kõrged, eriti III ja IV histoloogilises staadiumis. Seega on need markerid tundlikud hindamaks kaugelearenenud haiguse olemasolu. Hüaluronaan ja prokollageen III korreleerusid oluliselt teineteisega.

10. Sümptoomidega PBT-haigetel olid prokollageen III ja hüaluronaani väärtused kõrgemad kui asümptomaatilistel. Samal ajal olid mõlemate markerite väärtused kõrgemad just sellel osal asümptomaatilistest haigetest, kellel tekkisid peagi sümptoomid. Seega on prokollageen III ja hüaluronaani määramine oluline antud haigete alagrupi kindlakstegmisel. See võiks oluliselt vähendada maksabiopsia vajadust asümptomaatilistel haigetel.

11. Prokollageen III ja hüaluronaani kontsentratsioonid korreleerusid hästi histoloogilise staadiumiga ja bilirubiini kontsentratsiooniga. Lisaks korreleerus hüaluronaan positiivselt steatorröa raskusastmega, nahasügelemisega, ikterusega, väsimusega, söögitoru veenikomude olemasoluga, haiguse sümptomaatilise perioodi pikkusega ja kehakaalu vähenemisega ning negatiivselt albumiini kontsentratsiooni ja hepatomegaalia ulatusega. Hüaluronaan on parem marker näitamaks maksakahjustust ja kaugelearenenud maksahaigust, kuna ta peegeldab komplekssemalt maksa muutusi. Samal ajal ei olnud hüaluronaan ega prokollageen III olulised haiguse pikaajalise prognoosi ennustamisel.

12. Prokollageen III väärtused ei sõltunud ravist (prednisoloon üksi või kombineeritult asatiopriiniga). Hüaluronaani väärtused jäid esialgsele tasemele prednisoloon-asatiopriinravi grupis, kuid tõusid prednisoloonigrupis. Näib, et kombineeritud ravi on PBT-haiget lubavam kui monoteraapia.

13. Haiguse üheaastase lähiprognoosi kindlakstegemiseks on hüaluronaan hea marker. Tema määramine dünaamikas võiks olla oluline PBT-haigete haigusekulu hindamisel.

ACKNOWLEDGEMENTS

This long-time follow-up study was carried out at the Department of Internal Medicine, University of Tartu and at the Laboratories of Cell Biology and Immunology, Institute of General and Molecular Pathology, University of Tartu.

I wish to express my sincere gratitude and respect to:

My supervisor, Professor Vello Salupere, whose advice, support and encouragement have been invaluable to me through this study. His contribution has made this dissertation possible;

My supervisor, Professor Raivo Uibo, who introduced me to the immunology and for his skilful review of the manuscript and the suggestion to improve it;

Senior Scientist Andres Piirsoo, from the Laboratory of Cell Biology for his kind support and teaching me in immunofluorescence studies. This all made possible to finish immunological part of the study;

Mrs. Anu Kõiveer, from the Laboratory of Cell Biology, for her support with cell cultures;

Doctor Valve Hiir and the colleagues from the Tartu Endocrinological Hospital for their collaboration in PIIINP and hyaluronan studies;

Docent Riina Kallikorm for the enthusiastic and fruitful discussion, and help during this study;

All my collegues in the Department of Internal Medicine, especially to gastroenterologists, docent Riina Salupere, doctors Toomas Kutsar, Hele Remmel, Katrin Labotkin, Mart Riispere, Ingrid Kull and Toomas Kariis, who participated actively in this long-time follow-up study and for the kind atmosphere and helpfulness;

All collegues from the Department of Gastroenterology, Tallinn Central Hospital, especially doctors Aivi Koldits and Benno Margus for their help in data collection;

Mr. Georg Allik, for his skilful linguistic revision of the manuscript;

Mr. Alar Haukanõmm, for programming works;

All doctors who thought about the primary biliary cirrhosis possibility in their patient;

All patients for their kind cooperation during this follow-up study;

Finally, the warmest thanks belongs to my mother, doctor Hele Remmel for her everyday support. Her huge practical experience helped me to understand several essential things better.

This study was supported by the grant from Estonian Science Foundation

PUBLICATIONS

I

Remmel T., Remmel H., Uibo R., Salupere V. Primary biliary cirrhosis in Estonia: with special references to prevalence, incidence, clinical features and outcome. Scand. J. Gastroenterol. 1995, 4: 367–371.

Primary Biliary Cirrhosis in Estonia

With Special Reference to Incidence, Prevalence, Clinical Features, and Outcome

T. REMMEL, H. REMMEL, R. UIBO & V. SALUPERE
Dept. of Internal Medicine and Laboratory of Immunology, Institute of General and Molecular Pathology, University of Tartu, Tartu, Estonia

Remmel T, Remmel H, Uibo R, Salupere V. Primary biliary cirrhosis in Estonia. With special reference to incidence, prevalence, clinical features, and outcome. Scand J Gastroenterol 1995;30:367–371.

Background: Primary biliary cirrhosis (PBC) is a liver disease of unknown etiology, whose occurrence varies greatly between different regions. For a long time there have been no published data about the incidence and prevalence of PBC from Eastern Europe countries. *Methods:* The incidence and prevalence of PBC have been investigated in the Estonian population during the period 1973–92. Two sources of information were used: an information circular/questionnaire was sent to all district hospitals and gastroenterologists, and the case histories of all patients with a positive antimitochondrial antibody titer of 1:40 or more were reexamined. *Results:* During this period 69 cases of PBC were diagnosed. The male to female ratio was 1:22; 13% of the patients were asymptomatic. The mean annual incidence was 2.27 per million, and on 31 December 1992 the point prevalence was 26.9 per million. There were differences in prevalence among the various districts of Estonia. Associated autoimmune conditions were reported in 32% of the patients. Mean survival from the time of diagnosis was 52.5 months. *Conclusions:* The incidence of PBC in Estonia is at the lower end of the range reported in the world literature. This has probably partly been caused by a low percentage of asymptomatic and male patients.

Key words: Cirrhosis; epidemiology; liver disease; primary biliary cirrhosis

Vello Salupere, M.D., Dept. of Internal Medicine, University of Tartu, 18 Ülikooli Str., Tartu EE2400, Estonia (fax: +37 27-435-440)

Primary biliary cirrhosis (PBC) is a cholestatic liver disease of unknown etiology which occurs predominantly in middle-aged women (1). Although the etiology of the disease is still unknown, many lines of evidence suggest an autoimmune etiology for PBC, since a wide range of abnormalities of cellular and humoral immunity have been described (2). Epidemiologic studies of PBC conducted in the Western Europe, North America, and Japan (3–11) indicate that the prevalence of PBC varies substantially among various countries, being the highest in Sweden and North-East England (8, 10). Differences between districts of the same country have also been found (8, 12). Therefore, some environmental factors, such as the origin of the water supply (12), are believed to influence the PBC incidence. Although the disorder is rare, it is now diagnosed more frequently in asymptomatic patients by a positive serum antimitochondrial antibodies (AMA) test and an increased activity of serum alkaline phosphatase levels (10, 13, 14).

Since there are no published data about the epidemiology of PBC from Eastern Europe countries, the aims of the present study were 1) to determine the incidence and prevalence of PBC in Estonia during the past 20 years; 2) to explain the differences among the various parts of Estonia and national distribution; and 3) to analyze the clinical data and natural history of PBC cases diagnosed in Estonia.

PATIENTS AND METHODS

The investigation area was the Republic of Estonia. The population is fairly stable, and 1,526,177 inhabitants lived there on 31 December 1992. Of the inhabitants 62.6% are of Finno-Ugric origin, 35.2% of Slavic origin, and 2.2% of other origin according to data from the national registry of 1989.

We attempted to identify all patients in whom PBC had been suspected in years 1973–92. First, in 1986 information circulars were sent to all district hospitals with the request to send all patients with probable PBC to the Dept. of Internal Medicine, University of Tartu, for further investigations. Additionally, at the same time all gastroenterologists were asked to inform us about all PBC patients seen since 1973. Second, the case histories of all patients with a positive AMA result (titer more than 1:40) during the period 1978–92 were reexamined at the Laboratory of Immunology, University of Tartu. More than 9000 sera have been analyzed, and 83 were AMA-positive. Sixty-four fulfilled the conventional criteria for PBC. The diagnosis of PBC was based on the presence of established clinical, biochemical, and serologic criteria and diagnostic or compatible liver biopsy findings (1). Patients were classified as asymptomatic if no clinical symptoms or signs attributed to their liver disease (pruritus, jaundice, pigmentation, hepatomegaly, splenomegaly, ascites, and variceal bleeding) were found. Extrahepatic disease of the biliary tree was ex-

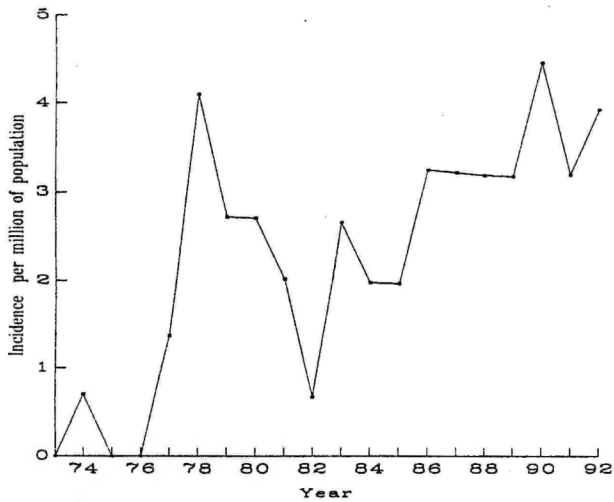

Fig. 1. Incidence of primary biliary cirrhosis in Estonia (1973–92).

cluded by endoscopic retrograde cholangiopancreatography (ERCP) and/or ultrasonography. Serum autoantibodies—AMA, antinuclear antibodies (ANA), smooth-muscle antibodies (SMA), and thyroid microsomal antibodies (TMA)—were detected by an indirect immunofluorescence technique in 67 of 69 patients. AMA were considered positive if the titer was higher than 1:40. The test was not performed in two cases because there were no possibilities to detect autoantibodies before 1978.

Biopsy specimens were available from 65 patients. All liver specimens were reevaluated, and the diagnosis confirmed with established histologic criteria (15) by one of the authors. Liver specimens were not available for four patients. If a liver specimen was not available, a positive AMA titer (>1:40) with an increasing activity of alkaline phosphatase more than twice as high as the upper reference value was required for PBC diagnosis. Patient survival data and causes of death were obtained from regional physicians or hospitals when the patients had died. All patients were treated with prednisone or with a combination of prednisone and azathioprine.

Mean and standard deviations were calculated with statistical methods, using the statistic program Statgraphics. The significance of differences was evaluated with the chi-square test.

RESULTS

During the period of 1973–92, 69 cases of PBC were diagnosed in Estonia. The mean annual incidence was 2.27 ± 1.4 per million, being 3.93 in the last year of investigation (Fig. 1). On 31 December 1992, 41 patients were alive, giving a point prevalence of 26.9 per million (Fig. 2). We have found significant differences among the various regions of Estonia. Most of the higher prevalence was in the Viljandi district, being 123 per million on 31 December 1992 (8 cases of PBC among 64,885 inhabitants of this district; $p < 0.00002$). There were no significant differences in PBC prevalence among the various nationalities in Estonia. Of the 69 PBC patients, 66 were women and 3 men, yielding a male to female ratio 1:22. Mean age at the time of diagnosis was 54.6 ± 10.2 years (range, 34–84 years) (Fig. 3).

The symptomatic period preceding the diagnosis of PBC

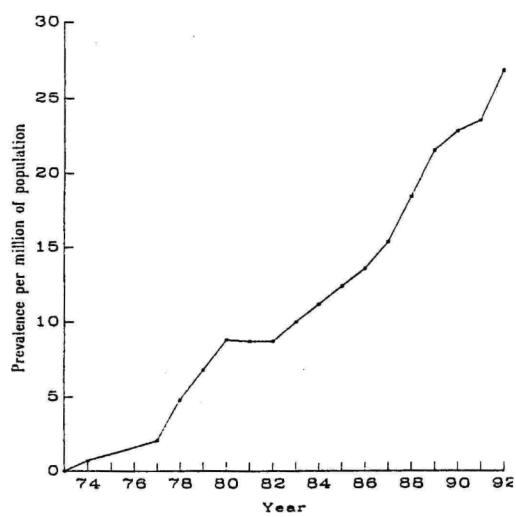

Fig. 2. Prevalence of primary biliary cirrhosis in Estonia (1973–92).

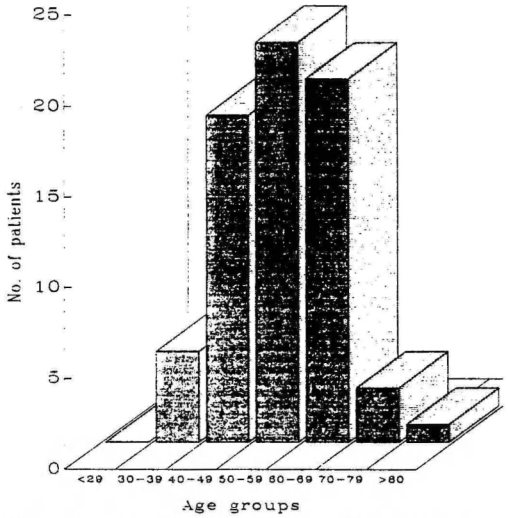

Fig. 3. Distribution of patients with primary biliary cirrhosis in age groups at the time of diagnosis (69 cases).

was 27.7 ± 26.4 months (range, 1–115 months). Table I summarizes the clinical signs and symptoms at the time of diagnosis. In 38 patients jaundice followed itching: in 39% during the 1st year, in 66% during the 3rd year, and in 84% during the 5th year. In six patients the itching and jaundice developed at same time, and in seven the jaundice was the first symptom of disease. Nine patients (13%) were classified as being asymptomatic at the time of PBC diagnosis. Associated autoimmune conditions were reported in 32% of PBC patients (Table II). Complaints about the musculoskeletal and joint systems were found in 22 (32%) and 29 (42%) patients, respectively. Altogether, seven patients gave a history of cancer: breast cancer in three, stomach cancer in one, cholangiocarcinoma in one, hepatocellular carcinoma

Table I. Clinical characteristics of primary biliary cirrhosis

Symptoms or signs	No. of patients	Percentage
Pruritus	55	79.7
Jaundice	44	63.8
Hepatomegaly	38	55.0
Fatigue	35	50.7
Pigmentation	30	43.5
Abdominal pain	30	43.5
Xanthomas	24	34.8
Joint symptoms	22	31.9
Weight loss	19	27.5
Back pain	7	10.1
Splenomegaly	5	7.2
Esophageal varices	5	7.2
Edema	4	5.8
Ascites	4	5.8

Table II. Associated autoimmune and other disorders

Disease or disorder	No. of patients	Percentage
Autoimmune disorders	22	32.0
Keratoconjunctivitis sicca	7	10.0
Lichen planus	5	7.2
Rheumatoid arthritis	4	5.8
Raynaud's signs	4	5.8
Autoimmune thyroid disease	3	4.3
Systemic sclerosis	3	4.3
Systemic lupus erythematosus	2	2.9
Fibrosing alveolitis	1	1.4
Hemolytic anemia	1	1.4
Psoriasis	1	1.4
Ankylosing spondylitis	1	1.4
Other disorders	5	7.2
Breast cancer	4	5.8
Hypernephroma	1	1.4
Stomach cancer	1	1.4

in one, and breast cancer together with hypernephroma in one patient. In 19 of the 69 patients (27.5%) gallstones were shown in the gallbladder at some point in the course of the disease. As a rule, these patients had no biliary colics (silent stones). In 10 of 19 patients the gallbladder had been removed before the PBC was diagnosed.

The activity of alkaline phosphatase was increased in 67 of the 69 (97%) patients at the time of diagnosis. Increased values of alanine and aspartate aminotransferase were found in 59 patients (85.5%). Bilirubin was higher than 34 μmol/l (2 mg/dl) in 44 (63.8%) and more than 69 μmol/l (4 mg/dl) in 20 patients (29%) at the time of diagnosis. Low albumin levels (<35 g/l) were determined in seven patients (10%). Of 67 64 (96%) were AMA-positive at a titer of 1:40 or more. ANA, SMA, and TMA were positive in 12 (18%), 23 (34%), and 9 (13%) patients, respectively, but they were very rare at a titer of more than 1:40. Immunoglobulins were determined in 55 patients. The IgM value was higher than normal in 42 (76.4%), IgG in 15 (27.3%), and IgA in 8 (14.5%). Hepatitis B surface antigen (HBsAg) was detectable in two patient sera, and hepatitis C virus (HCV) antibodies were found in four cases.

In 50 of 65 (77%) cases liver biopsy findings were diagnostic for PBC. There were no cases of PBC in stage 1, whereas stage 2 was found in 16 (32%), stage 3 in 16 (32%), and stage 4 in 18 (36%) patients. In 15 cases liver biopsy data were compatible with or suggestive of PBC.

Patients were followed up from 1 to 227 months (mean, 47.9 months). Twenty-eight patients died during this period: 16 of hepatocellular deficiency, 6 of bleeding of esophageal varices, 1 of hepatocellular carcinoma, and 2 of non-liver-associated disease (gastric cancer and ischemic heart disease). It was not possible to establish the cause of death in three cases. Mean survival from the diagnosis of PBC was 52.5 ± 41.6 months (range, 3–148 months) and that from the development of the first symptom associated with liver disease 82.7 ± 49.7 months (range, 17–175 months). The

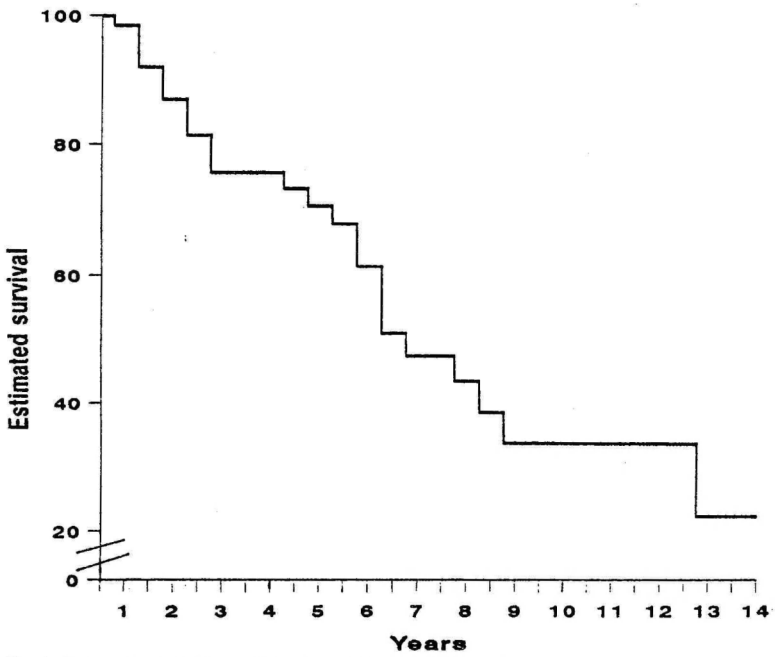

Fig. 4. Estimated survival data of 69 primary biliary cirrhosis patients.

5-year estimated survival rate was 68.1%. The estimated survival of PBC patients is indicated in Fig. 4.

DISCUSSION

This study from Estonia deals with the incidence, prevalence, and clinicopathologic features of PBC. We found that the prevalence and incidence of PBC are low in the Estonian population as compared with other European countries (4, 7, 8, 10). The reasons for such a low prevalence of PBC in Estonia could be explained by regional differences and partly by insufficient routine screening methods (AMA, alkaline phosphatase activity, and so forth) at district hospitals, which may have led to the underdiagnosing of asymptomatic cases of PBC. During the past 10 years the percentage of asymptomatic PBC cases has increased to 70% in studies in Western Europe (16), versus 13% in Estonia. A greater use of the screening methods could have increased the incidence and prevalence of asymptomatic PBC in Estonia.

The possibility of clustering of PBC cases for various unknown environmental or genetic reasons could not be completely ruled out. We found rather large differences in the distribution of PBC in Estonia. The highest prevalence was 123.3 per million in the Viljandi district in south Estonia. This region is rural and is mainly engaged in grain-growing and cattle-breeding. It is of interest that 3 of 8 PBC patients from the Viljandi district were living in Abja-Paluoja, a small town with 1747 inhabitants. Thus we could calculate an extremely high PBC prevalence for this town—1717 per million. There is region in northeast Estonia with a bad ecologic situation. One of the earlier research studies supposed that PBC is more frequent in industrial areas (3). However, we found no higher prevalence of PBC as compared with the overall prevalence of PBC in Estonia.

In the Estonian population we found a difference in the male to female ratio (1:22) as compared with the ratio commonly found in Western Europe—1:6 to 1:12 (1, 5, 10, 17). It is difficult to explain such a discrepancy. However, since asymptomatic PBC is prevalent in men, and the course of the disease is frequently atypical in men (5), the low number of asymptomatic cases in our material could have influenced our results. Therefore, larger epidemiologic studies with collection of blood samples for determination of AMA and alkaline phosphatase activity are needed. The clinical features of PBC patients in Estonia are typical of those previously reported (1, 12, 13, 18). As the symptomatic period preceding the diagnosing of PBC was more than 2 years, it had developed to an advanced stage already: 63.8% of the patients were jaundiced, and in 68% of the patients liver histology data indicated the third or fourth stage of Scheuer (15) at the time of PBC diagnosis.

The clinical association with other autoimmune disorders and with the frequent finding of other autoantibodies is in

agreement with previous reports (13, 16, 17). However, the frequency of keratoconjunctivitis sicca is lower than in other published series, possibly because Schirmer's test was not performed routinely in the assessment. Moreover, we found PBC associated with ankylosing spondylitis in a 53-year-old woman. Such an association has not been described earlier. A further point of interest was that in 10 of the 69 patients in our series (15%) the diagnosis of PBC was delayed because of the finding of gallstone disease. When, after surgical intervention, the liver functional tests did not normalize and symptoms did not disappear, one could suspect PBC. Nevertheless, we suggest testing AMA in all patients before operation.

We studied two periods of survival of our PBC patients: one from the time of diagnosis of PBC and the other from the development of the first symptom. This was necessary because the period preceding the diagnosis of PBC was relatively long. The survival data of our patients were lower than in recently published reports (16, 17), as most of our patients were symptomatic at the time of PBC diagnosis.

In conclusion, the incidence and prevalence of PBC in Estonia are at the lower end of the range reported in the world literature. Probably, this has partly been caused by the low percentage of asymptomatic and male patients in our series. Also, large differences in the distribution of PBC cases in Estonia were found, but it is not yet possible to explain this on the basis of environmental factors.

ACKNOWLEDGEMENT

This study was partly supported by a grant from the Estonian Science Foundation (no. 1055).

REFERENCES

1. Sherlock S, Scheuer PJ. The presentation and diagnosis of 100 patients with primary biliary cirrhosis. N Engl J Med 1973; 289:674–8.
2. James SP, Hoofnagle JH, Strober W, Jones EA. Primary biliary cirrhosis: a model autoimmune disease. Ann Intern Med 1983; 99:500–12.
3. Hamlyn AN, Macklon AF, James O. PBC: geographical clustering and symptomatic onset seasonality. Gut 1983;24:940–5.
4. Eriksson S, Lindgren S. The prevalence and clinical spectrum of PBC in a defined population. Scand J Gastroenterol 1984; 19:971–6.
5. Triger DR, Berg PA, Rodes J. Epidemiology of primary biliary cirrhosis. Liver 1984;4:195–200.
6. Sasaki H, Oinoue K, Higuchi K, Yasuyama T, Koyata H, Kuroki T, et al. PBC in Japan: national survey by the subcommittee on autoimmune hepatitis. Gastroenterol Jpn 1985;20:476–85.
7. Löfgren J, Järnerot G, Danielsson D, Hemdal I. Incidence and prevalence of primary biliary cirrhosis in a defined population in Sweden. Scand J Gastroenterol 1985;20:647–50.
8. Myszor M, James OFW. The epidemiology of primary biliary cirrhosis in north-east England: an increasingly common disease? Q J Med 1990;276:377–85.
9. Triger DR, Rodes J. Epidemiology of primary biliary cirrhosis in Europe: a ten year follow-up study. J Hepatol 1989;9 Suppl 1:238.
10. Danielsson A, Boquist L, Uddenfeldt P. Epidemiology of primary biliary cirrhosis in a defined rural population in the northern part of Sweden. Hepatology 1989;11:458–64.
11. Witt-Sullivan H, Heathcote J, Cauch K, Blendis L, Ghent C, Katz A, et al. The epidemiology of primary biliary cirrhosis in Ontario, Canada. Hepatology 1990;12:98–105.
12. Triger DR. Primary biliary cirrhosis: an epidemiological study. B M J 1980;281:772–5.
13. Christensen E, Crowe J, Doniach D, Popper H, Ranek L, Rodes J, et al. Clinical pattern and course of disease in primary biliary cirrhosis based on an analysis of 236 patients. Gastroenterology 1980;78:236–40.
14. Beswick DR, Klatskin G, Boyer JL. Asymptomatic primary biliary cirrhosis: a progress report on long-term follow-up and natural history. Gastroenterology 1985;89:267–71.
15. Scheuer PJ. Liver biopsy interpretation. London: Bailliere Tindall, 1980.
16. Nyberg A, Lööf L. Primary biliary cirrhosis: clinical features and outcome, with special reference to asymptomatic disease. Scand J Gastroenterol 1989;24:57–64.
17. Jeffrey G, Reed WD, Shilkin KB. Primary biliary cirrhosis: clinicopathological characteristics and outcome. J Gastroenterol Hepatol 1990;5:639–45.
18. Ilan Y, Shouval D. Primary biliary cirrhosis in Israel. Isr J Med Sci 1992;28:683–7.

Received 15 April 1994
Accepted 24 June 1994

II

Remmel T., Piirsoo A., Kõiveer A., Remmel H., Uibo R., Salupere V.
Clinical significance of different antinuclear antibodies patterns
in the course of primary biliary cirrhosis.
Hepato-Gastroenterology 1996, 43: 1135–1140.

Original Paper

Clinical Significance of Different Antinuclear Antibodies Patterns in the Course of Primary Biliary Cirrhosis

Triin Remmel, Andres Piirsoo[1], Anu Koiveer[1], Hele Remmel, Raivo Uibo[2], Vello Salupere

Department of Internal Medicine, [1]Laboratory of Cell Biology, [2]Laboratory of Immunology, Institute of General and Molecular Pathology, University of Tartu, Estonia

Abstract

Background/Aims: *The significance of antinuclear antibodies (ANA) in primary biliary cirrhosis (PBC) patient is still controversial in the literature. The purpose of this paper is to investigate the clinical significance of ANA in PBC patients.*

Methods and Materials: *Sixty-nine patients with PBC were investigated. Control groups included 21 patients with autoimmune hepatitis, 26 patients with alcoholic liver disease, 13 patients with systemic connective tissue disease and 27 healthy persons. ANA was detected by an immunofluorescence method on rat liver tissue sections and HEp-2 cells at serum dilution 1/40.*

Results: *In 48 out of 69 PBC patients (70%), ANA was positive in HEp-2 cell line, but in rat liver tissue sections only 29% of patients had positive ANA reactions. Most frequent patterns were multiple nuclear dots (MND) in 42% and perinuclear in 16%. MND-ANA was also found in two autoimmune hepatitis patients and in one systemic lupus erythematosus patient. Survival from the moment of developing first symptom(s) attributable to liver disease was longer in the ANA positive patients than ANA negative ones ($p<0.02$). Despite immunosuppressive treatment, in most of ANA positive patients (73%) ANA did not disappear. Most frequent ANA patterns in autoimmune hepatitis and systemic connective tissue diseases patients were homogeneous and anticentromere, respectively.*

Conclusions: *Immunofluorescence method on HEp-2 cell line for ANA detection is more sensitive than on rat liver tissue sections. In PBC patient's incidence of ANA, especially MND-ANA is a frequent immunological abnormality. ANA positive patients have better prognosis for survival.*

Hepato-Gastroenterology 43 (1996) (Pages 1135-1140)

Key words: primary biliary cirrhosis - antinuclear antibodies - liver cirrhosis - liver disease - prognosis

Abbreviations: anticentromere antibodies (ACA), autoimmune hepatitis (AH), alcoholic liver disease (ALD), antimitochondrial antibodies (AMA), antinuclear antibodies (ANA), healthy persons (HP), multiple nuclear dots (MND), primary biliary cirrhosis (PBC), phosphate buffered saline (PBS), systemic connective tissue diseases (SCTD), systemic lupus erythematosus (SLE)

Introduction

Primary biliary cirrhosis (PBC) is considered an autoimmune liver disease with progressive destruction of intrahepatic bile ducts and the presence of diverse immunological derangements (1). Among the latter, antimitochondrial antibodies (AMA) are most characteristic of PBC, and are widely used for the serodiagnosis of the disease (1-4). However, 25-58% of PBC patients have also antinuclear antibodies (ANA) (5-9). Among these, two distinct subtypes of antibodies, one giving the perinuclear pattern and the other multiple nuclear dots (MND) pattern by immunofluorescence, have been shown to be specifically associated with PBC and thus proposed as alternative serological markers for PBC in the AMA negative cases, particularly (4,5,7,10,11).

The role of ANA in PBC is not fully clear, especially the association with the course of PBC. Therefore, the aim of the present study was to examine the occurrence of different ANA types in PBC patients, and to evaluate their relationship to specific clinical features and the outcome of PBC.

Materials and Methods

The present study includes 69 patients with PBC diagnosed in the period of 1978-1994 at the Department of

Internal Medicine, University of Tartu, Estonia. The diagnosis of PBC was based on accepted clinical, biochemical, serological and morphological criteria (2). Sixty-one out of 69 patients were symptomatic at the time of diagnosis.

At the time of diagnosis, a liver biopsy was done and specimens were staged according to criteria of Scheuer (12). Six patients were in the 1st , 16 in the 2nd, 22 in the 3rd and 25 in the 4th histological stage.

Immunosuppressive treatment with prednisone (40 patients), or with combination of prednisone and azathioprine (29 patients) was started after diagnosis. Such treatment regime was used whereas there was no treatment of choice for PBC in seventies and eighties when most of PBC patients were included into present study.

All the patients were evaluated for symptoms and signs. Biochemical markers were measured by multiautoanalyzer (Hitachi, Japan). Immunoglobulins were detected by radial immunodiffusion method.

In all 69 patients, sera were taken at the time of diagnosis. In 37 patients, blood was examined many times, mean time between the first and the last determination of ANA was 3.0±0.6 years. All together, 126 sera were tested.

During the follow-up (meanly 5.5±3.4 years), 32 out of 69 PBC patients died, 2 of them due to the non-liver associated causes (car accident, stomach cancer). Those two patients were excluded from outcome analysis.

Control groups included 21 patients with autoimmune hepatitis (AH), 26 patients with alcoholic liver disease (ALD), 13 patients with systemic connective tissue diseases (SCTD) represented by systemic lupus erythematosus (SLE) mainly, investigated at the time of diagnosis, and 27 healthy persons (HP). These control groups were age and sex matched with the main study group.

All sera were stored at -20°C until testing. ANA and AMA were detected using air-dried cryostat sections from rat kidney, mouse liver and stomach (13), and HEp-2 cells as substrate at serum dilution 1/40.

HEp-2 cells were grown on cover slips. The cells were fixed with 3.7% p-formaldehyde at room temperature for five minutes, followed by 100% methanol fixation at -20°C for 10 minutes and treated afterwards with 0.2% Triton X at room temperature for five minutes. FITC conjugated goat antihuman IgG g chain specific antibody F(ab)2 (Sigma, St. Louis, USA)) at dilution 1:50 was used as the second antibody. All washings were done with sterile phosphate buffered saline (PBS), containing 0.05% Tween 20. After the final washing the preparations were covered with other cover slips, and examined in fluorescence microscope Univar (Reichert, Germany) and photographed. All preparations were examined independently by two authors and the results were compared. In cases of disagreement of results the preparations were reviewed until the consensus was reached.

The ANA patterns were divided as follows: multiple nuclear dots (MND), fine speckled, homogeneous, perinuclear, nucleolar and centromere (ACA) according to the description of Bernstein (5).

Statistical analysis was performed using one-way analysis of variance (ANOVA) for parametric signs and Kruskal-Wallis one-way ranks analysis for non-parametric signs. Life-time analysis of survival was performed by the Kaplan-Meier method (14).

This research has been approved by the Committee of Ethics at the University of Tartu, Estonia.

Results

Using tissue sections as antigen substrate ANA were revealed in 20 (29%) and AMA in 64 (93%) of PBC patients at the moment of diagnosis. ANA reaction on tissue sections was also revealed in AH (57%) and in SCTD (85%) patients. Using HEp-2 cells as antigen substrate, more ANA positive cases were detected among the PBC and AH patients: 70% and 62%, respectively. ANA were found in all five AMA negative PBC cases: with MND pattern in two, fine speckled in one, homogeneous with MND in one and perinuclear in one patient. MND pattern was found in 29 out of 69 (42%) PBC patients, but also in 2 AH patients, and in 1 SCTD patient (**Table 1**). In 13 out of 48 (27%) PBC patients with ANA, at least two different ANA patterns were seen. In addition to these findings, there was also high frequency of homogeneous ANA pattern in AH (45%) and SCTD (77%) patients, and ACA pattern in SCTD patients (54%).

Clinical and laboratory data of ANA-negative and ANA positive patients have given in **Table 2 and 3**. ANA-negative patients had higher sedimentation rate ($p<0.03$), more frequently ascites ($p<0.045$) and variceal hemorrhages ($p<0.03$). There were no more associated autoimmune diseases in ANA-positive group.

We do not find any differences in clinical, laboratory and histological features between AMA negative-ANA-positive (5 patients) and AMA positive PBC patients (64 patients).

The survival time from the time of diagnosing PBC (and ANA measurement) was longer in ANA-positive patients than in ANA-negative, 6.3 and 3.3 years respectively ($p<0.02$). Kaplan-Meier survival curve was different in those groups (**Figure 1**). At the same time the period between the development of first symptom and diagnosing of PBC was comparable in both groups (1.9 and 2 years, respectively). There were no statistically significant differences in histological stages, treatment regime and doses, between ANA-negative and ANA-positive patients group at the beginning of the study.

In 37 patients (26 ANA positive and 11 ANA negative at the moment of diagnosis) ANA were determined repeatedly during the disease course. In spite of immunosuppressive treatment, ANA pattern did not

TABLE 1. CHARACTERIZATION OF ANA AND AMA FINDINGS IN DIFFERENT CHRONIC LIVER DISEASES AND SYSTEMIC CONNECTIVE TISSUE DISEASES PATIENTS.

	PBC (69)	AH (21)	ALD (27)	SCTD (13)	HP (29)
Immunofluorescence on tissue sections					
Antinuclear antibody positive	20 (29%)	12 (57%)	1 (4%)	11 (85%)	2 (7%)
Antimitochondrial antibody positive	64 (93%)	1 (5%)	-	1 (8%)	-
Immunofluorescence on HEp-2 cells					
Antinuclear antibody positive (all patterns)	48 (70%)	13 (62%)	1 (4%)	11 (85%)	2 (7%)
Fine speckled	6 (9%)	2 (10%)	1 (4%)	-.	1 (3.5%)
Homogeneous	7 (10%)	9 (43%)	-	10 (77%)	1 (3.5%)
Nucleolar	2 (3%)	3 (14%)	-	1 (8%)	-
Perinuclear	11 (16%)	-	-	1 (8%)	-
Centromeric	7 (10%)	3 (14%)	-	7 (54%)	-
Multiple nuclear dots	29 (42%)	2 (10%)	-	1 (8%)	-

PBC primary biliary cirrhosis, AH autoimmune hepatitis, ALD alcoholic liver diseases, SCTD systemic connective tissue diseases, HP healthy persons

change in 19 out of 26 (73%), changed in two out of 26 (8%), and ANA disappeared in five out of 26 (19%). During the follow-up period ANA did not develop in any patients who were ANA negative at the time of diagnosis. None of the patients developed liver cancer.

TABLE 2. CLINICAL FEATURES OF ANA-POSITIVE AND ANA-NEGATIVE PBC PATIENTS.

	ANA-positive patients (48)	ANA-negative patients (21)
Itching	35 (73%)	16 (76%)
Jaundice	24 (50%)	10 (48%)
Fatigue	20 (42%)	13 (62%)
Abdominal pain	21 (44%)	6 (29%)
Hyperpigmentation	23 (48%)	8 (28%)
Xanthelasmata	20 (42%)	7 (33%)
Arthralgia	14 (29%)	9 (43%)
Back pain	4 (8%)	2 (10%)
Weight loss	12 (25%)	7 (33%)
Hepatomegaly	28 (58%)	14 (67%)
Splenomegaly	4 (8%)	2 (10%)
Ascites	1 (2%)	2 (10%)
Oesophageal varices	3 (6%)	4 (19%)
Haemorrhages ex varices	0 (0%)	2 (10%)
Oedema	4 (8%)	4 (19%)
Length of sympt. period (years)	1.9±2	2±2.5
Length of follow-up period (years)	5.7±3.4	4.8±3.3

TABLE 3. LABORATORY DATA OF ANA-POSITIVE AND ANA-NEGATIVE PBC PATIENTS.

	ANA-positive patients (48)	ANA-negative patients (21)
Age	54±9	57±12
Bilirubin	52±40	60±57
Alkaline phosphatase	1490±1036	1464±917
ASAT	96±40	84±48
ALAT	112±84	88±76
Cholesterol	9.1±3.8	9.9±4.4
Total protein	88±9	88±9
Albumin	43±6	41±6
Globulin	45±8	47±9
Sedimentation rate	37±14	47±14
IgM	5.6±3.5	7.4±7
IgG	16.7±6.7	20.1±7.9
IgA	3.0±1.7	3.1±1.5
Fat excretion	9.6±8.6	14.1±13.2

Discussion

This study confirms earlier investigations of other authors (5-7), showing that ANA could be determined more frequently if HEp-2 cells are used instead of frozen rodent tissue sections in chronic liver disease patients, especially in PBC sera.

Some authors have used the term autoimmune cholangitis for AMA-negative PBC patients, although

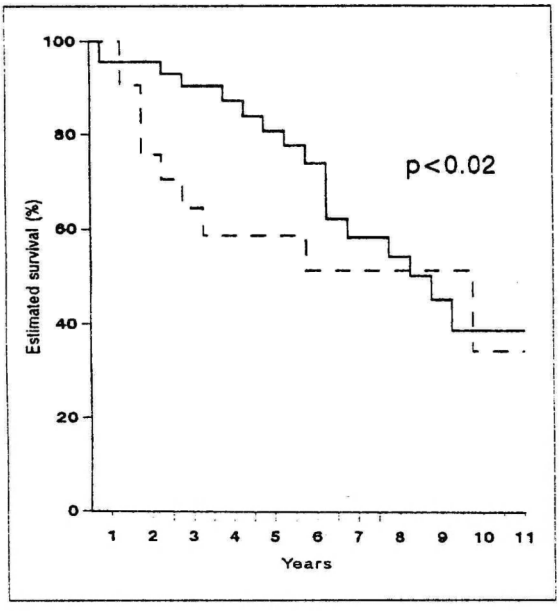

Figure 1 *Kaplan-Meier estimated survival in 20 ANA-negative (------) and in 47 ANA positive (———) PBC patients*

clinical, laboratory and histopathological characteristics are very similar with AMA-positive PBC (15-18). It seems to us that AMA-negative PBC has to be treated as subtype of PBC as we cannot find any differences between those groups. Therefore, it is extremely necessary to determine ANA in cholestatic patients.

In PBC, the use of HEp-2 cells as antigenic substrate for ANA is especially important, whereas MND-ANA characteristics for PBC are not detectable in tissue sections. Another antibody staining pattern detectable on HEp-2 cell line and invisible on tissue sections is ACA (5,7). The advantage of HEP-2 cell line is the large nucleus and rapid mitosis. This makes possible the differentiation between MND and ACA. The nuclear dots vary in size and remain dispersed throughout the nucleus during mitosis, which helps distinguish the dots from centromeres (5).

MND was the most frequent ANA pattern and seen in 42 per cent of PBC patients. Additionally, we found MND-ANA in two AH patients and in one SLE patient. In all those three cases the mean number of nuclear dots was smaller (mean four per cell) than that in PBC patients (mean six per cell). The first patient with AH had also additional fine speckled ANA, and the second MND accompanied by homogeneous pattern. In these patients no clinical, biochemical and histological symptoms of cholestasis were revealed. The SLE patient had MND-ANA with concomitant homogeneous pattern and no clinical and biochemical signs of cholestasis. Some authors have also found an MND-ANA pattern in diseases other than PBC (9,19,20). It is possible that autoantigens or epitopes, at least in part of the cases, are different from those ones recently cloned by Szostecki (21,22). Altogether, it should be mention that MND-ANA finding is highly suggestive for PBC possibility. In our study three out of five AMA negative PBC patients had MND-ANA. Therefore, we suggest the MND-ANA detection as another informative diagnostic test for PBC. Whereas other autoimmune diseases and overlap syndromes have been found frequently in PBC patients, we cannot also exclude a possibility of asymptomatic PBC in a MND-ANA positive SLE patient, as a liver biopsy was not performed.

We should mention that whereas our patients were mostly in the third and the fourth histological stages at

the time of diagnosis, the survival of the patients were lower than described in previous studies (23-27). At the same time the per cent of the patients in each histological stages in ANA-positive and ANA-negative groups did not differ. According to the ANA test results, PBC patients could be divided into two subgroups. The first group comprises ANA positive patients with relatively good prognosis, the second group includes ANA negative patients with relatively poor outcome. So far, it is difficult to explain such differences in prognosis. It could be that ANA positive patients have better response to immunosuppressive treatment than ANA negatives. Why and how ANA develop in so many PBC patients is not clear yet, but this problem is so far unresolved for other antibodies, including AMA.

To our knowledge, follow-up studies on different ANA types have not been performed so far in PBC patients. We found that as a rule ANA pattern did not change during 3 years of treatment, and there was no ANA development in ANA negative PBC cases. Also, none of ANA patterns changed patients developed liver cancer. Therefore it is impossible to explain the change of ANA pattern with carcinogenesis in our patients as reported earlier (28). There were no clinical and outcome differences between PBC patients with changed ANA pattern compared to patients preserved this. Maybe we need a longer investigation period to appraise the significance of this change.

In conclusion, ANA is a frequent immunological abnormality found in patients with PBC, especially if HEp-2 cell line is used as a substrate in indirect immunofluorescence test. From our study, it appears that PBC patients with positive ANA have a better prognosis. In most ANA positive cases the pattern of ANA did not change in spite of immunosuppressive treatment.

References

1 *Kaplan MM.* Primary biliary cirrhosis. N J Med 1987; 316: 521-528.

2 *Sherlock S, Scheuer PJ.* The presentation and diagnosis of 100 patients with primary biliary cirrhosis. N Engl J Med 1973; 289:674-678.

3 *James O, Macklon AF, Watson AJ.* Primary biliary cirrhosis: a revised clinical spectrum. Lancet 1981; ii:1278-1281.

4 *Bassendine MF, Yeaman SJ.* Serological markers of primary biliary cirrhosis: diagnosis, prognosis and subsets. Hepatology 1992;15:545-548.

5 *Bernstein RM, Neuberger JM, Bunn CC, Callender ME, Hughes GRV, Williams R.* Diversity of autoantibodies in primary biliary cirrhosis and chronic active hepatitis. Clin Exp Immunol 1984; 55:553-560.

6 *Powell F, Schroeter AL, Dickson ER.* Antinuclear antibodies in primary biliary cirrhosis. Lancet 1984; 1:288-289.

7 *Cassani F, Bianchi FB, Lenzi M, Volta U, Pisi E.* Immunomorphological characterisation of antinuclear antibodies in chronic liver disease. J Clin Pathol 1985; 38:801-805.

8 *Lassoued K, Brenard R, Degos F, et al.* Antinuclear antibodies directed to 200 kD polypeptide of the nuclear envelope in primary biliary cirrhosis. A clinical and immunological study of a series of 150 patient with primary biliary cirrhosis. Gastroenterology 1990; 90:181-186.

9 *Hansen BC, Eriksson S, Lindgren S.* High prevalence of autoimmune liver disease in patients with multiple nuclear dot, anti-centromere and mitotic spindle antibodies. Scand J Gastroenterol 1991; 26:707-713.

10 *Lassoued K, Guilly MN, Andre C, et al.* Autoantibodies to 200 kD polypeptide(s) of the nuclear envelope: a new serological marker of primary biliary cirrhosis. Clin Exp Immunol 1988;74:283-288.

11 *Nickowitz RE, Wozniak RW, Schatjner F, Worman HJ.* Autoantibodies against integral membrane proteins of the nuclear envelope in patients with PBC. Gastroenterology 1994;106:193-199.

12 *Scheuer PJ.* Liver biopsy interpretation. London: Bailliere Tindall, 1988.

13 *Walker JG, Doniach D, Roitt IM, Sherlock S.* Serological tests in the diagnosis of primary biliary cirrhosis. Lancet 1965;i:827-831.

14 *Kaplan EL, Meier P.* Non-parametric estimation from incomplete observations J Amer Stat Assoc 1958; 53:457-481.

15 *Taylor SL, Dean PJ, Riely CA.* Primary autoimmune cholangitis. An alternative to antimitochondrial antibody-negative primary biliary cirrhosis. Am J Surg Pathol 1994;18:91-99.

16 *Michiletti P, Wanless IR, Katz A, Scheuer PJ, Yeaman SJ, Bassendine MF, Palmer JM, Heathcote EJ.* Antimitochondrial antibody negative primary biliary cirrhosis: a distinct syndrome of autoimmune cholangitis. Gut 1994;35:26-265.

17 *Goodman ZD, MsNally PR, Davos DR, Ishak KG.* Autoimmune cholangitis: a variant of primary biliary cirrhosis. Clinicopathologic and serologic correlations in 200 cases. Dig Dis Sci 1995; 40:1232-1242.

18 *Lacerda MA, Ludwig J, Dickson ER, Jorgensen RA, Lindor KD.* Antimitochondrial antibody- negative primary biliary cirrhosis. Am J Gastroenterol 1995; 90:247-249.

19 *Szostecki C, Krippner H, Penner E, Bautz FA.* Autoimmune sera recognize a 100 kD nuclear protein antigen (sp-100). Clin Exp Immunol 1987;68:108-116.

20 *Pawlotsky J-M, Andre C, Metreau J-M, Beaugrand M, Zafrani E-S, Dhumeaux D.* Multiple nuclear dots antinuclear antibodies are not specific for primary biliary cirrhosis. Hepatology 1992;16:127-131.

21 *Szostecki C, Guldner HH, Netter HJ, Will H.* Isolation and characterization of cDNA encoding a human nuclear antigen predominantly recognized by autoantibodies from patients with primary biliary cirrhosis. J of Immunology 1990;145:4338-4347.

22 *Szostetcki C, Will H, Netter HJ, Guldner HH.* Autoantibodies to nuclear Sp100 protein in primary biliary cirrhosis and associated diseases: epitope specifity and immunoglobulin class distribution. Scand J Immunol 1992; 36:555-564.

23 *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; 308:1-7.

24 *Jeffrey GP, Reed WD, Shilkin KD.* Primary biliary cirrhosis: clinicopathological characteristics and outcome. J Gastroenterol Hepatol 1990; 5:639-645.

25 *Rydning A, Schrumpf E, Adbelnoor M, Elgjo K, Jenssen E.* Factors of prognostic importance in primary biliary cirrhosis. Scand J Gastroenterol 1990; 25:119-126.

26 *Mitchison HC, Lucey MR, Kelly PJ, Neuberger JM, Williams R, James OF.* Symptom development and prognosis in primary biliary cirrhosis: a study in two centres. Gastroenterology 1990; 99:778-784.

27 *Wiesner RH, Porayko HK, Dickson ER, et al.* Selection and timing of liver transplantation in primary biliary cirrhosis and primary sclerosing cholangitis. Hepatology 1991;16:1290-1299.

28 *Imai H, Nakano Y, Kiyosawa K, Tan EM.* Increasing titers and changing specificities of antinuclear antibodies in patients with chronic liver disease who develop hepatocellular carcinoma. Cancer 1993; 71:26-35.

Acknowledgement

This study has partly supported by grant number 1055 from Estonian Science Foundation.

Corresponding Author:
Vello Salupere, MScD,
Department of Internal Medicine,
University of Tartu, 18 Ülikooli Str.,
Tartu EE2400,
Estonia
Tel: 372-7-448-600
Fax: 372-7-448-607

III

Remmel T., Remmel H., Salupere V. Aminoterminal propeptide of type III procollagen and hyaluronan in patients with primary biliary cirrhosis: markers of fibrosis in primary biliary cirrhosis.
J. Gastroenterol. Hepatol. 1996, 11: (accepted)

Journal of Gastroenterology and Hepatology (1996) 11, 000-000

PRIMARY BILIARY CIRRHOSIS

Aminoterminal propeptide of type III procollagen and hyaluronan in patients with primary biliary cirrhosis: Markers of fibrosis in primary biliary cirrhosis

TRIIN REMMEL, HELE REMMEL AND VELLO SALUPERE

Department of Internal Medicine, University of Tartu, Tartu, Estonia

Abstract The aminoterminal propeptide of type III procollagen (PIIINP) and hyaluronan have previously been studied in different liver diseases. The results of these studies are controversial. The aim of the present study was to examine the relationship between PIIINP and hyaluronan levels and the clinical, biochemical and histological features of primary biliary cirrhosis (PBC) and its prognosis. Fifty-five PBC patients were studied at the time of diagnosis of PBC and were followed up for a mean of 58 months. During the follow-up period 21 patients died. In addition, 30 healthy subjects were examined in the present study. Hyaluronan and PIIINP were measured by radioimmunoassay and the levels of both PIIINP and hyaluronan were higher in PBC patients than in healthy volunteers ($P < 1.8X10^{-4}$ and $1.6X10^{-4}$, respectively). Hyaluronan and PIIINP levels were above normal values in 82 and in 84% of PBC patients, respectively. There were correlations between PIIINP and hyaluronan and the histological stage of PBC ($r = 0.44$, $P < 0.004$ and $r = 0.56$, $P < 0.00001$, respectively). The correlation between PIIINP and hyaluronan was 0.46 ($P < 0.0035$). In symptomatic patients, both PIIINP and hyaluronan values were higher than in controls ($P < 0.002$ and $P < 0.006$, respectively). The levels of PIIINP correlated significantly with bilirubin ($r = 0.43$, $P < 0.006$), while hyaluronan was correlated with age ($r = 0.33$, $P < 0.015$), pruritus ($r = 0.32$, $P < 0.02$), fatigue ($r = 0.41$, $P < 0.003$), hepatomegaly ($r = -0.46$, $P < 0.0008$), the presence of oesophageal varices ($r = 0.34$, $P < 0.002$), weight loss ($r = 0.29$, $P < 0.05$), bilirubin ($r = 0.54$, $P < 0.0001$), albumin ($r = -0.30$, $P < 0.04$), extent of fat excretion ($r = 0.53$, $P < 0.009$) and length of symptomatic period before diagnosis of PBC ($r = 0.43$, $P < 0.002$). Using Cox's logistic regression analysis, survival was found to be influenced by bilirubin concentration but not by hyaluronan, PIIINP, age, albumin or histological stage. Therefore, hyaluronan is a more sensitive marker for predicting advanced PBC than is PIIINP. However, neither hyaluronan nor PIIINP gave any indication of prognostic outcome.

Key words: fibrogenesis, hyaluronan, liver cirrhosis, liver disease, primary biliary cirrhosis, procollagen III.

INTRODUCTION

Primary biliary cirrhosis (PBC) is a clinically heterogeneous disease and predictions of the course of the disease are rather difficult and controversial. Thus far, bilirubin is the only biochemical parameter identified that is believed to have any prognostic importance for PBC.[1-4] Unfortunately, it is a late hallmark of the disease. This has lead to the necessity for an investigation of new indices that may be of use in determining the prognosis of PBC. One such factor, the aminoterminal propeptide of type III procollagen (PIIINP), is released during the extracellular processing of type III collagen, which is a major component of the hepatic extracellular matrix.[5] It has been suggested that in fibrotic liver disease, elevation of PIIINP may be connected with fibrogenesis,[6-8] hepatic inflammation and necrosis[9,10] and may also be influenced by cholestasis.[11] Another factor, hyaluronan, is a high molecular weight polysaccharide that is widely distributed in connective tissue and is produced mainly by mesenchymal cells.[12] It has been hypothesized that hyaluronan levels in serum reflect both increased synthesis by activated fibroblasts in fibrotic areas and reduced catabolism caused by hepatic failure.[13] Furthermore, some studies have shown that in PBC PIIINP and hyaluronan could be alternative prognostic markers in addition to bilirubin.[14-16] Others have found

Correspondence: Triin Remmel, Department of Internal Medicine, University of Tartu, Tartu EE2400, Estonia.
Accepted for publication 4 March 1996.

these factors do not provide any additional information with respect to the prognosis of PBC.[19–21]

The aims of the present study were, first, to investigate the relationship between PIIINP and hyaluronan levels and the clinical, biochemical and histological features of the disease and, second, to examine the significance of PIIINP and hyaluronan levels with respect to determining the prognosis for PBC.

METHODS

Fifty-five PBC patients (53 females, two males; mean age 54.7 years, range 34–84) were studied at the time of diagnosis of PBC and were followed up for a mean of 58 months (range 5–130). The diagnosis of PBC was based on conventional criteria[22] and all patients studied were AMA positive. Forty-eight of 55 patients were symptomatic at the time of diagnosis (without pruritus, jaundice, pigmentation, hepatomegaly, splenomegaly, ascites and/or variceal bleeding).

None of the PBC patients had received immunosuppressive and/or antifibrotic treatment until diagnosis. Treatment with prednisone or with a combination of prednisone and azathioprine was started in symptomatic patients after diagnosis. During the follow-up period 21 patients died (all symptomatic), 17 of them because of liver disease and four because of unrelated causes (car accident, cardiac dysrhythmia, stroke, stomach cancer).

The control group consisted of 30 healthy subjects who were age- and sex-matched with the patient group.

Before beginning immunosuppressive treatment, blood samples were collected by venipuncture in the morning (fasting state). Serum was separated by centrifugation and was stored at −20°C until testing. The mean storage time before assays was 4.5 years. Serum PIIINP was measured by radioimmunoassay (Orion, Espoo, Finland) as described by Risteli.[4] Serum hyaluronan was assayed with a sequential radiometric assay (HA-test; Pharmacia Diagnostics, Uppsala, Sweden). This test is based on the use of specific hyaluronic acid-binding proteins isolated from bovine cartilage.

Biochemical and immunological tests (e.g. transaminases, cholesterol, alkaline phosphatase, gamma-glutamyltranspeptidase, bilirubin, albumin, globulin, fat excretion, autoantibodies and immunoglobulins) were performed on the same blood samples.

At the time of collection of sera, patients were examined for clinical signs and symptoms by one doctor. Patients who complained of itching that disturbed their activities and who had excoriation marks on their skin were determined to be pruritic. The other subjective symptom, fatigue, was defined as positive when it disabled the daily activities of patients. All patients also underwent upper oesophagogas-troduodenoscopy and ultrasound examination.

Liver biopsy was performed at the time of diagnosis. Biopsy specimens were fixed in 10% formalin and were stained with haematoxylin–eosin. Data were staged according to the criteria of Scheuer.[23]

Differences between groups were tested with the Kruskal–Wallis test and the Mann–Whitney *U*-test. Spearman's rank correlation coefficient was used for correlation. $P < 0.05$ was considered to be statistically significant. The contributions of PIIINP and hyaluronan levels to a multivariate prediction of the disease outcome, using also bilirubin, albumin, age and histological stage as independent factors, were assessed by logistic regression analysis according the Cox model.

This research was approved by the Committee of Ethics at the University of Tartu, Estonia.

RESULTS

The upper limit of normal values for serum levels of PIIINP (mean + 2s.d.) was 4.8 ng/mL and in 45 (82%) patients the concentration exceeded this value. The upper limit of normal values for serum hyaluronan was 74 mg/L and in 46 (84%) patients the concentration was over this. The mean PIIINP and hyaluronan concentrations in PBC patients were 8.8 ng/mL and 280 mg/L, respectively, with the highest concentration found in patients with the fourth histological stage of PBC. PIIINP sensitivity and specificity for diagnosing cirrhotic stage were 0.87 and 0.56, respectively, while for the diagnosis of advanced PBC (third and fourth histological stage) the sensitivity and specificity were 0.84 and 0.7, respectively. Hyaluronan sensitivity and specificity for diagnosing cirrhosis were 0.94 and 0.52, respectively, while for the diagnosis of advanced liver disease, these values were 0.91 and 0.64, respectively. There was a statistically significant correlation between the level of PIIINP and the histological stage of PBC ($r = 0.44$, $P < 0.0037$) and between hyaluronan and the histological stage of PBC ($r = 0.56$, $P < 0.00001$). The correlation between PIIINP and hyaluronan was 0.46 ($P < 0.0035$). Table 1 shows the PIIINP and hyaluronan values of the PBC patients according to histological stage.

In symptomatic PBC patients the values of PIIINP were higher than in asymptomatic patients (9.4 ± 7.2 (median 7.9) *vs* 4.9 ± 1.6 ng/mL (median 4.6), respectively; $P < 0.02$). Hyaluronan levels were also higher in symptomatic than in asymptomatic patients (304 ± 246 (median 201) *vs* 111 ± 119 mg/L (median 70), respectively; $P < 0.006$). Three of seven asymptomatic patients had both hyaluronan and PIIINP levels above normal values. Two of these patients developed symptoms soon after, while one remained asymptomatic. The concentration of hyaluronan was moderately correlated to the age of patients ($r = 0.33$, $P < 0.015$).

PIIINP had no statistically significant correlation with clinical symptoms of PBC.

Serum hyaluronan values were correlated with pruritus ($r = 0.32$, $P < 0.02$), jaundice ($r = 0.31$, $P < 0.02$), fatigue ($r = 0.41$, $P < 0.003$), hepatomegaly ($r = -0.46$, $P < 0.0008$), oesophageal varices ($r = 0.34$, $P < 0.002$) and weight loss ($r = 0.29$, $P < 0.05$). There were no correlations with pigmentation, abdominal pain, arthralgias, back pain, xanthomas, palmar erythema, spider naevi, splenomegaly, ascites, haemorrhages or oedemas. In addition, the hyaluronan

Table 1 PIIINP and hyaluronan levels in primary biliary cirrhosis patients and healthy subjects

		PIIINP (ng/mL)					Hyaluronan (μg/L)				
	n	No. increased (%)	*P*	Mean ± s.d.	Range	Median	No. increased (%)	*P*	Mean ± s.d.	Range	Median
PBC*	55	45 (82)	1.8×10^{-11}	8.8 ± 6.8	2.7–45.3	7.3	46 (84)	1.6×10^{-9}	280 ± 91	34–850	189
I	5	1 (20)	0.3	4.0 ± 0.9	2.8–5.1	4	1 (20)	0.047	66 ± 34	34–122	56
II	18	14 (78)	1.8×10^{-5}	7.4 ± 3	4.2–15.2	6.6	16 (89)	6×10^{-6}	200 ± 186	47–737	139
III	16	13 (81)	9×10^{-5}	8.0 ± 4.1	2.7–17.8	7.6	14 (88)	7×10^{-7}	233 ± 194	69–690	146
IV	16	14 (88)	41×10^{-5}	13.5 ± 11.3	4.8–45.3	10	15 (94)	4×10^{-6}	466 ± 260	54–850	480
Control	30	2 (7)		3.5 ± 0.6	2.3–5.0	2.9	2 (7)		39 ± 18	13–76	22

Biopsy specimens were staged according to the criteria of Scheuer.[21]
PIIINP, aminoterminal propeptide of type III procollagen; PBC, primary biliary cirrhosis.

Table 2 Correlations of PIIINP and hyaluronan with laboratory tests

	PIIINP		Hyaluronan	
	r	*P*	*r*	*P*
Bilirubin	0.43	0.006	0.54	0.0001
Alkaline phosphatase	0.14	> 0.05	0.006	> 0.05
Cholesterol	0.12	> 0.05	0.08	> 0.05
ASAT	0.26	> 0.05	0.002	> 0.05
ALAT	0.03	> 0.05	-0.003	> 0.05
Total protein	0.05	> 0.05	-0.25	> 0.05
Albumin	-0.06	> 0.05	-0.30	0.035
Globulin	0.02	> 0.05	-0.008	> 0.05
G-globulin	0.08	> 0.05	0.07	> 0.05
IgM	-0.005	> 0.05	-0.2	> 0.05
IgG	0.09	> 0.05	0.001	> 0.05
IgA	0.07	> 0.05	0.23	> 0.05
Steattorrhoea	0.25	> 0.05	0.53	0.009

r, Spearman's rank correlation coefficient; *P* = significance of correlation coefficient.
PIIINP, aminoterminal propeptide of type III procollagen; ASAT, xxx; ALAT, xxx.

concentration was found to be correlated with the symptomatic period of the disease prior to diagnosis ($r = 0.43$, $P < 0.002$).

Table 2 shows that PIIINP and hyaluronan correlated positively with bilirubin. In addition, hyaluronan was correlated with extent of fat excretion and was inversely correlated with albumin concentration.

Using Cox logistic regression analysis, survival was found to be influenced only by bilirubin concentration ($P < 0.045$ and not by hyaluronan, PIIINP, age, albumin or histological stage ($P > 0.05$).

DISCUSSION

Hyaluronan and PIIINP together have been previously investigated in different liver diseases with the prospect of using them for diagnostic and follow up purposes.[17,18,24–28] Thus far, there is no clear indication that the routine determination of PIIINP and hyaluronan would give any additional information in PBC patients to that obtained by the performance of other laboratory tests and in addition to liver biopsy data.

Both PIIINP and hyaluronan were highly sensitive but hardly specific for the diagnosis of advanced PBC. It was not possible to differentiate advanced PBC (third or fourth histological stage) from second stage patients (belonging to the non-advanced PBC group) solely on the basis of the measurement of hyaluronan and PIIINP; furthermore, in most second stage patients collagen markers were also high. However, PIIINP values were normal in most first stage patients.

Some previous studies have shown that in first stage patients the values of PIIINP could also be significantly higher than in healthy subjects.[15–17,25] It has been assumed that destruction of bile ducts, bile leakage and chronic inflammatory cell infiltration of portal tracts could stimulate fibrogenesis.[26] There is no fibrosis in the first and second stages.[21] Therefore, the elevation of PIIINP could be due to diminished metabolism through

liver endothelial cells[30,31] and impaired PIIINP excretion due to cholestasis.[11,15] In contrast, it should be taken into account that liver damage in PBC patients is uneven. There may occur, simultaneously, in the liver areas with non-advanced and advanced liver damage. The latter could be responsible for high PIIINP values in some patients classified as being either in the first or second stage of the disease. Therefore, the measurement of PIIINP may be more informative than even liver biopsy, in that it shows the activity of fibrotic process.

Hyaluronan concentrations were already slightly increased in first stage patients, compared with the control group. It seems that the reasons for hyaluronan elevation are more complicated than for PIIINP and hyaluronan levels were found to be correlated with markers indicating liver synthetic function, fibrotic activity, cholestasis, liver insufficiency and portal hypertension. Hyaluronan levels were better correlated with histological stage and bilirubin than PIIINP. It is assumed that the reduced metabolism of hyaluronan in the liver is a more important cause for the increased levels in chronic liver disease than is the increased synthesis of hyaluronan in fibrotic areas[13].

In our opinion, hyaluronan is a better marker for the evaluation of the disease process in PBC patients than is PIIINP.

In asymptomatic patients (four in the first stage, one in the second and two in the fourth), both PIIINP and hyaluronan levels were lower than in symptomatic patients. Three of seven asymptomatic patients had both hyaluronan and PIIINP levels above normal values. In two patients, symptoms developed soon after diagnosis, while one patient remained asymptomatic. None of the asymptomatic patients with normal PIIINP values developed symptoms during the follow-up period. We recommend measurement of PIIINP and hyaluronan for follow-up asymptomatic patients for predicting the development of symptoms.

In the present study PIIINP and hyaluronan did not correlate with the mortality of PBC patients. When the prognostic value of PIIINP and hyaluronan were assessed, using multivariate stepwise regression analysis (Cox's model), they did not influence the prognosis of survival. Therefore, our results are not in agreement with results of some earlier published studies in which the significance of PIIINP and/or hyaluronan were established for predicting survival time.[14–18] Of course, the natural course and survival time of our PBC patients may have been influenced by treatment with prednisone or a combination of prednisone and azathioprine. These drugs both inhibit collagen synthesis in liver.[32] Therefore, the possibility exists that by using other treatment regimes (UDCA, colchicine etc.), PIIINP and hyaluronan could preserve their importance in determining long-term prognosis.

In conclusion, as hepatic fibrosis should be viewed as a dynamic process,[23] the repeated measurement of PIIINP and hyaluronan during the course of PBC could be more helpful than a single measurement only once during the course of the disease. We suggest that hyaluronan is a more sensitive marker for indicating the extent of changes in PBC.

ACKNOWLEDGEMENTS

This research was supported in part by a grant from the Estonian Science Foundation (no. 1055).

REFERENCES

1 Shapiro JM, Smith H, Schaffner F. Serum bilirubin: A prognostic factor in primary biliary cirrhosis. *Gut* 1979; 20: 137–40.
2 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. Med. J.* 1983; 308: 1–7.
3 Christensen E, Neuberger J, Crowe J *et al.* Beneficial effect of azathioprine and prediction of prognosis in primary biliary cirrhosis. Final results of an international trial. *Gastroenterology* 1985; 89: 1084–91.
4 Dickson ER, Grambsch PM, Fleming TR, Fisher LD, Langsworthy A. Prognosis in primary biliary cirrhosis: Model for decision making. *Hepatology* 1989; 10: 1–7.
5 Biagini G, Ballardini G. Liver fibrosis and extracellular matrix. *J. Hepatol.* 1989; 8: 115–24.
6 Risteli J, Niemi S, Trivedi P, Mäentausta O, Mowat AP, Risteli L. Rapid equilibrum radioimmunoassay for the amino-terminal propetide of human type III procollagen. *Clin. Chem.* 1988; 34: 715–18.
7 Frei A, Zimmermann A, Weigand H. The N-terminal propeptide of collagen type III in serum reflects activity and degree of fibrosis in patients with chronic liver disease. *Hepatology* 1984; 4: 830–4.
8 Torres-Salinas MM, Pares A, Caballeria J *et al.* Serum procollagen III peptides as a marker of hepatic fibrogenesis in alcoholic hepatitis. *Gastroenterology* 1986; 90: 1241–6.
9 Savolainen ER, Goldberg B, Leo MA, Velez M, Lieber CS. Diagnostic value of serum procollagen peptide measurements in alcoholic liver disease. *Alcohol. Clin. Exp. Res.* 1984; 8: 384–9.
10 Surrenti C, Casini A, Milani S, Ambu S, Ceccatelli P, D'Agata A. Is determination of N-terminal procollagen type III peptide (sPIIIP) a marker of hepatic fibrosis? *Dig. Dis. Sci.* 1987; 32: 705–9.
11 Raedsch R, Stiehl A, Sieg A, Walker S, Kommerell B. Biliary excretion of procollagen peptide in healthy humans and in patients with alcoholic cirrhosis of the liver. *Gastroenterology* 1987; 85: 1265–70.
12 Laurent UBG, Laurent TC. On the origin of hyaluronate in blood. *Biochem. Int.* 1981; 2: 195–9.
13 Frebourg T, Delpech B, Bercoff E *et al.* Serum hyaluronate in liver diseases: Study by enzymoimmuno-logical assay. *Hepatology* 1986; 6: 392–5.
14 Eriksson S, Zettervall O. The N-terminal propeptide of collagen III in serum as a prognostic indicator in primary biliary cirrhosis. *J. Hepatol.* 1986; 2: 370–8.
15 Babbs C, Smith A, Hunt LP, Rowan BP, Habouchi NY, Warnes TW. Type III procollagen peptide: A marker of disease activity and prognosis in primary biliary cirrhosis. *Lancet* 1988; ii: 1021–4.
16 Niemela O, Risteli L, Sotaniemi EA, Stenback F, Risteli J. Serum basement membrane and type III procollagen related antigens in primary biliary cirrhosis. *J. Hepatol.* 1988; 6: 307–14.

17 Nyberg A, Lindqvist U, Engström-Laurent A. Serum hyaluronan and aminoterminal propeptide of type III procollagen in primary biliary cirrhosis: relation to clinical symptoms, liver histopathology and outcome. *J. Intern. Med.* 1992; 231: 485–91.
18 Poupon RE, Balkau B, Guechot J, Heintzmann F. Predictive factors in ursodeoxycholic acid-treated patients with primary biliary cirrhosis: Role of serum markers of connective tissue. *Hepatology* 1994; 19: 635–40.
19 Mutimer DJ, Bassendine MF, Kelly P, James. Is measurement of type III procollagen aminopropeptide useful in primary biliary cirrhosis? *J. Hepatol.* 1989; 9: 184–9.
20 Beukers R, van Zanten RA, Schalm SW. Serial determination of type III procollagen aminopropeptide serum levels in patients with histologically progressive and non-progressive primary biliary cirrhosis. *J. Hepatol.* 1992; 14: 22–9.
21 Diaz F, Collazos J, Genolla J. Study of serum procollagen type III peptide in patients with hepatic cirrhosis from a clinical point of view. *Clin. Invest.* 1993; 71: 416–18.
22 Sherlock S, Scheuer PJ. The presentation and diagnosis of 100 patients with primary biliary cirrhosis. *N. Engl. J. Med.* 1973; 289: 674–8.
23 Scheuer PJ. *Liver Biopsy Interpretation*, 4th edn. London: Bailliere Tindall, 1988.
24 Plebani M, Giacovini A, Floreani A *et al.* Biochemical markers of hepatic fibrosis in primary biliary cirrhosis. *Ric. Clin. Lab.* 1990; 20: 169–74.
25 Ramadori G, Zohrens G, Manns M *et al.* Serum hyaluronate and type III procollagen aminoterminal propeptide concentration in chronic liver disease. Relationship to cirrhosis and disease activity. *Eur. J. Clin. Invest.* 1991; 21: 323–30.
26 Guechot J, Poupon RE, Giral P, Balkau B. Relationship between procollagen III aminoterminal propeptide and hyaluronan serum levels and histological fibrosis in primary biliary cirrhosis and chronic viral hepatitis. *J. Hepatol.* 1994; 20: P388.
27 Floreani A, Zappala F, Mazzetto M, Naccarato R, Plebani M, Chiaramonte M. Different response to ursodeoxycholic acid (UDCA) in primary biliary cirrhosis. *Dig. Dis. Sci.* 1994; 39: 9–14.
28 Teare JP, Sherman D, Greenfield SM *et al.* Comparison of serum procollagen III peptide concentrations and PGA index for assessment of hepatic fibrosis. *Lancet* 1993; 342: 895–8.
29 Seyer JM. Mediators of increased collagen synthesis in fibrosing organs. *Fundam. Appl. Toxicol.* 1985; 5: 228–39.
30 Smedsrod D. Aminoterminal propeptide of type III procollagen is cleared from the circulation by receptor-mediated endocytosis in liver endothelial cells. *Coll. Rel. Res.* 1988; 8: 375–88.
31 Melkko J, Hellevik T, Risteli L, Risteli J, Smedsrod D. Clearence of NH_2-terminal propeptides is a physiological function of the scavenger receptor function in liver endothelial cells. *J. Exp. Med.* 1994; 179: 405–12.
32 Wu J, Danielsson A. Inhibition of hepatic fibrogenesis: A review of pharmacological candidates. *Scand. J. Gastroenterol.* 1994; 29: 385–91.

IV

Remmel T. Primaarne biliaarne tsirroos.
Eesti Arst 1994; 6: 486–490.

Eesti Arst, 1994, 6, 486—490

Primaarne biliaarne tsirroos

Triin Remmel

primaarne biliaarne tsirroos, autoimmuunhaigused, kolestaatilised maksahaigused, maksatsirroos

Primaarne biliaarne tsirroos on progresseeruv, kolestaasiga kulgev krooniline maksahaigus, mille põhjus on siiani olnud teadmata. Et haigetel on leitud nihkeid nii rakulises kui ka humoraalses immuunsuses, siis arvatakse, et tegemist on autoimmuunhaigusega (16).

Esmakordselt kirjeldasid haigust Thomas Addison ja William Gull 1851. aastal. Nad kirjeldasid kuut haiget, kellel olid nahakollasus, vitiliigo ja ksantoomid. Samuti oletasid nad, et kahjustus lokaliseerub maksas. Termini «primaarne biliaarne tsirroos» võttis esmalt kasutusele E. Ahrens 1950. aastal (2). See mõiste on teatud määral ekslik, sest tsirrootilised muutused maksas tekivad primaarse biliaarse tsirroosi korral alles haiguse lõppstaadiumis. Seetõttu on kirjanduses soovitatud kasutada ka sellist nimetust nagu krooniline mittemädane granulomatoosne kolangiit (20).

Haiguse tekkepõhjus ei ole siiani teada. On uuritud erinevaid baktereid (*Enterobacteriaceae, E. coli, Mycobacteriaceae*), kuid otsest seost kindla haigusetekitajaga ei ole õnnestunud leida (13, 25). Siiski on võimalik, et primaarse biliaarse tsirroosi tekkes on oluline mingi bakteri- või viirusevastaste antikehade ristreaktsioon maksa mitokondrite erinevate komponentidega struktuuride sarnasuse tõttu, mis põhjustab sapikapillaari rakkude molekulaarse koostise muutust ja immuunsüsteemi hüperergilist reaktsiooni.

Keskkonnafaktorite võimalikkusele haiguse tekkes viitab D. Trigeri töö 1980. aastast (27). Autor leidis, et primaarse biliaarse tsirroosi haigestumus erines ühe linna piires kümnekordselt. Seda seletas ta veevarustuspiirkondade erinevusega, kuid ühte konkreetset faktorit, mis viiks primaarse biliaarse tsirroosi tekkeni, ei õnnestunud tal leida.

Haiguse tekkes on oluline ka geneetiline foon. Neil haigetel on populatsioonis sagedamini HLA-DR8-DQB1*0402 haplotüüp (29). Geneetiliste faktorite osatähtsusele haiguse tekkes viitab ka haigusjuhtude olemasolu perekonniti. Ühes uurimuses oli see 2,4% (17).

Primaarset biliaarset tsirroosi põdejad moodustavad maksatsirroosi haigestunutest ligikaudu 2% (11). Naised haigestuvad 6...10 korda sagedamini kui mehed ja moodustavad ümmarguselt 90% haigestunutest (23). Kõige sagedamini haigestuvad 40...60-aastased, kuid vanusepiir on väga varieeruv, ulatudes 20...80. eluaastani.

Primaarset biliaarset tsirroosi on diagnoositud üle maailma. Üldhaigestumus 100000 inimese kohta varieerub 3,7...14,4 juhuni ja aastas diagnoositakse keskmiselt 5,8...15 uut haigusjuhtu ühe miljoni inimese kohta. Primaarse biliaarse tsirroosi haigestumus varieerub piirkonniti, olles madalaim Jaapanis ja Austraalias ning kõrgeim Rootsis ja Inglismaal (7, 17, 21, 28). Maa- ja linnaelanike vahel erinevust haigestumussageduses ei ole (7). Arvatakse, et Inglismaal on umbes 7000 primaarse biliaarse tsirroosi põdejat (17).

Kui varem oletati, et haigus on suhteliselt harva esinev, siis viimasel ajal on seda haigust maailmas diagnoositud aina rohkem. Haigestumise sagenemise põhjused ei ole täpselt teada. Võimalik, et haigestumus on reaalselt suurenenud, kuid arvestama peab ka järgnevate faktorite koosmõju võimalikkust: haiguse diagnoosimist rohkem asümptomaatilises staadiumis, arstiabi ja diagnoosimismeetodite paremat kättesaadavust (eelkõige mitokondritevastaste antikehade määramine) ning täiuslikumate haigusregistrite olemasolu (7).

Kahel kolmandikul haigetest on esmasteks sümptoomideks nahasügelus ja väsimus (23). Nahasügelus võib algul olla lokaalne, kuid hiljem hõlmab kogu keha. Nahasügelus on väga piinav, haiged ei saa

Triin Remmel — Tartu Ülikooli Sisekliinik

sageli öösel magada ja võivad seetõttu kõigepealt pöörduda dermatoloogi, psühhiaatri, neuroloogi, allergoloogi või terapeudi poole. Hiljem lisanduvad nahasügelusele nahakollasus ja naha hüperpigmentatsioon (*melanoderma*). Harvem tekivad kollasus ja nahasügelus üheaegselt (20%-l) või nahakollasus on esmaseks sümptoomiks (8%-l) (23). Üksikutel haigetel manifesteerub haigus esimest korda kas verejooksuna söögitoru veenilaienditest, astsiidi või entsefalopaatiana (11).

Tänapäeval on primaarset biliaarset tsirroosi üha rohkem diagnoositud asümptomaatilises staadiumis (7,17), mil haigetel nahasügelus, ikterus, portaalhüpertensioonisündroom või muud maksahaigusele viitavad nähud veel puuduvad. Nendel haigetel leitakse aga mitokondritevastaseid antikehi ja alkaalse fosfataasi või gamma-glutamüültranspeptidaasi aktiivsuse tõusu vereseerumis. Eriti varajases haigusstaadiumis võib ensüümide aktiivsus olla normis, kuid mitokondriaalsed antikehad on kindlasti olemas.

Haiguse diagnoosimisel kliiniliselt selgelt avalduvas staadiumis võib haigel leida ksantoome, ksantelasme, *melanoderma*'t, verevalumeid ja hepatomegaaliat, harvem splenomegaaliat ja astsiiti. Kolestaasist tingituna tekib malabsorptsioonisündroom, mis väljendub eelkõige steatorröa (rasvade rohke eritumine väljaheitega) ja rasvlahustuvate vitamiinide imendumise puudulikkusena (A-, D-, E-, K-vitamiin). Väga tõsiseks probleemiks haigetel on lülisamba osteoporoosist tingitud lülidemurrud koos tugeva valusümptoomiga (10). Lisaks malabsorptsioonisündroomist tingitud D-vitamiini- ja Cadefitsiidile on osteoporoosi tekkes oluline ka osteoblastide vähenenud aktiivsus, haigete vanus ja sugu. Enamik haigeid on naised pärast menopausi (12).

30...80%-l haigetest on peale primaarse biliaarse tsirroosi kaasnevana ka mõni muu immunogeneesiga haigus või sündroom. Kirjanduse põhjal on sagedasemaks Sjögreni sündroom, reumatoidartriit, sklerodermia, autoimmuunne türeoidiit ja fibroseeriv alveoliit. Harvem on leitud tsöliaakiat, bulloosset pemfigoidi, lamesammaspoolt, vaskuliiti, süsteemset erütematoosset luupust, membranoosset glomerulonefriiti, ultseroosset koliiti, polümüosiiti, difuusset toksilist struumat, sarkoidoosi ja autoimmuunset trombotsütopeeniat (24).

Sapieritushäireist, haigete soost ja vanusest tingituna leitakse neil haigeil sagedamini sapikivitõbe (23, 24). Kivid on tavaliselt nn. vaiksed kivid (sapipõies) ja vajadust kirurgilise ravi järele ei ole. Küll aga võib kivide avastamine viia selleni, et nahasügelust ja ikterust ning teisi sümptoome tõlgendatakse sapikivitõve sümptoomidena ning haiget opereeritakse. Pärast operatsiooni haigetel maksafunktsioon tavaliselt halveneb. Ravi on vajalik juhul, kui kivi paikneb ühissapijuhas, sel juhul peetakse eelistatavaks meetodiks papillotoomiat.

Laboratoorselt on primaarsele biliaarsele tsirroosile iseloomulik eelkõige kolestaasi tähised, s.o. alkaalse fosfataasi ja gammaglutamüültranspeptidaasi aktiivsuse 3...10-kordne tõus vereseerumis. Haiguse diagnoosimise ajal on bilirubiini kontsentratsioon tihti normis, kuid haiguse progresseerumisel bilirubiini kontsentratsioon vereseerumis enamikul haigeil suureneb, seda nii konjugeeritud kui ka konjugeerimata fraktsiooni arvel. Transaminaaside aktiivsuse tõus (tsütolüüs) ei ole nii tugevalt väljendunud.

Suurel osal haigetest esinevad hüperlipideemia ja hüperkolesteroleemia, mis on refraktaarne medikamentoossele ravile. Maksahaiguse progresseerumisel tekivad hüpoalbumineemia ja koagulopaatia. Osal haigetel puudub seos haiguse kliinilise pildi, laboratoorsete näitajate ja maksa bioptaadi histoloogilise leiu staadiumi vahel.

Primaarse biliaarse tsirroosi diagnoosimisel peetakse eriti vajalikuks mitokondritevastaste antikehade määramist vereseerumis. Mitokondritevastaseid antikehi on leitud 93...98%-l haigetest (7, 23, 28). On avastatud neli mitokondritevastast

antikeha, mis reageerivad mitokondrite membraani erinevate antigeenidega. Olulisemad neist on M2-tüüpi antikehad, mis on suunatud kolme ensüümi vastu mitokondrite sisemembraanil (30). Nendeks ensüümideks on püruvaadi dehüdrogenaasi E2 kompleks (15), 2-oksohappe dehüdrogenaas ja kõrvalahelaga 2-oksohappe dehüdrogenaas (9).

M4 antikehi leitakse sellise primaarse biliaarse tsirroosi variandi puhul, millel on sarnaseid jooni kroonilise aktiivse autoimmuunse hepatiidiga. Anti-M2 koos anti-M8-ga näitab haiguse halba prognoosi, anti-M9 leidumine vereseerumis on seevastu iseloomulik healoomulise kuluga haigusele (5). Anti-M9 võib leida ka haigete sugulastel ning arstide ja laboritöötajate vereseerumist, kes on töötanud primaarset biliaarset tsirroosi põdejate vereseerumitega (5).

Ehkki enamiku haigete vereseerumis on M2-tüüpi antikehade kõrge tiiter, arvatakse, et mitokondritevastased antikehad on haiguse tagajärg, mitte põhjus. Haiguse tekkeks on vajalik HLA II klassi molekulide ekspressioon sapikapillaaride epiteelile (normaalses maksas ekspresseeruvad HLA I klassi antigeenid), mis viivad abistajarakkude aktiveerumiseleja T-lümfotsüütide tsütotoksilise toime tekkele sapikapillaaride vastu (3).

Mitokondritevastaste antikehade kõrval võib primaarse biliaarse tsirroosi korral leida ka muid antikehi (tuuma-, silelihaskoe-, kilpnäärme mikrosoomidevastased jm.), kuid nende olemasolu ei ole primaarsele biliaarsele tsirroosile patognostiline (16, 28). Peale eespool toodu on neil haigetel vereseerumis leitud kõrget IgM-i kontsentratsiooni, hüpergammaglobulineemiat, hüpokomplementeemiat ja immuunkomplekse (16). Rakulise immuunsuse häiretele viitavad granuloomide olemasolu maksas ja defektne T-lümfotsüütide funktsioon nii perifeerses veres kui ka sapikapillaaride ümber (16).

Histoloogiliselt jaotatakse primaarne biliaarne tsirroos nelja staadiumi. Esimesele staadiumile on omane väikese ja keskmise suurusega sapijuhade destruktsioon, teisele sapikapillaaride proliferatsioon, kolmandale fibroos ja neljas on nn. tõelise tsirroosi staadium, mil sapikapillaare histoloogilises preparaadis on väga vähe (22). Haiguse varajasemates staadiumides (esimeses ja teises) võib preparaadis leida ka granuloome. Alati tuleb aga arvestada seda, et maksakahjustus ei ole täiesti difuusne ning seetõttu võib maksa eri osades olla erinevatesse histoloogilistesse staadiumidesse kuuluvaid muutusi.

Haiguse kulg primaarset biliaarset tsirroosi põdejail on väga varieeruv, sageli raskesti prognoositav. Sümptoomide puudumise korral on haigete elulemus samasugune kui vanuseliselt ja sooliselt representatiivsetel inimestel üldpopulatsioonis selle hetkeni, kuni neil tekivad sümptoomid. Haigus muutub aga kliiniliselt avalduvaks suuremal osal sümptoomideta haigetest, kuid selleks võib aega kuluda kuni 30 aastat.

Kliiniliselt sümptoomideta haigetel näitavad halba prognoosi, s.t. haiguse kiiret muutumist kliiniliselt väljendunuks, hepatomegaalia olemasolu, kaasneva autoimmuunse haiguse olemasolu ja histoloogiliselt III...IV staadiumile iseloomulike muutuste leidmine maksas (18). Kliiniliselt avaldunud haigusstaadiumis on haigete eluprognoos tavaliselt halb, keskmine elulemus diagnoosimise momendist on 10 aastat. Prognoos on eriti halb sellisel juhul, kui haigel on ikterus, astsiit, hüpoalbumineemia, tsirrootilised muutused maksas ja anamneesis hiljutine verejooks söögitoru veenikomudest (19). Kõige tähtsamaks prognostiliseks markeriks peetakse bilirubiini.

Primaarset biliaarset tsirroosi põdejate ravi on probleemiks kogu maailmas. Praegu puudub efektiivne medikamentoosne ravi. Medikamentoosse raviga võib saavutada küll sümptoomide kadumise, laboratoorsete analüüside teatava paranemise (normaliseerumist tavaliselt ei toimu), kuid muutused maksas progresseeruvad ja eluea pikenemist ei ole täheldatud.

Medikamentoosne ravi on oma olemuselt eelkõige immuunsupressiivne ja antifibrootiline. Haigete ravimisel on kasuta-

tud D-penitsillamiini, prednisolooni, asatiopriini, kolhitsiini, tsüklosporiini, metotreksaati, kloorambutsiili ja ursodeoksükoolhapet (4).

D-penitsillamiini ja asatiopriini kasutamine monoteraapiana on kliiniliste ravimitega tehtud katsete tulemusena tunnistatud mittetoimivaks (6, 14). Enamiku muude ravimite kasutamist piirab pikemaajalise ravi korral kõrvaltoimete teke (prednisoloon ja osteoporoos, tsüklosporiin ja neerukahjustus, metotreksaat ja vereloomekahjustus, asatiopriin ja vereloomekahjustus). Viimasel ajal on saadud kõige paremaid tulemusi ursodeoksükoolhappega (13...15 mg kehakaalu kilogrammi kohta päevas), mille täpset toimemehhanismi ei ole veel selgitatud. Arvatakse, et primaarse biliaarse tsirroosi puhuse maksakahjustuse tekkes on peale immuunmehhanismide oluline ka sekundaarne, endogeensete sapphapete kuhjumine maksa ja nende toksiline toime maksarakkudesse.

On teada, et ursodeoksükoolhape vähendab endogeensete, toksiliste sapphapete hulka maksas, kuna ta imendub paremini peensoolest (endogeensete, toksiliste sapphapete kontsentratsioon väljaheites suureneb, enterohepaatilises tsirkulatsioonis väheneb), parandab sapieritust maksas ning on immuunmoduleeriva ja hepatoprotektiivse toimega (1, 26). Kuid ka siin on võrdlevad kahekordsed pimekatsed ravimitega oma kestvuselt olnud liialt lühikesed, et saaks lõplikult deklareerida, et ainuõige ravim on lõpuks leitud.

Selleks, et vähendada ravimite kõrvaltoimet, on tulevik tõenäoliselt polüteraapia päralt, kusjuures parimad mõeldavad kombinatsioonid oleksid järgmised: prednisoloon ja asatiopriin, prednisoloon ja tsüklosporiin, tsüklosporiin ja kolhitsiin ning muud. Sümptomaatilise ravimina kasutatakse nahasügeluse puhul kolestüramiini ja D-hüpovitaminoosi puhul 25-hüdroksüvitamiini D süstetena.

Primaarset biliaarset tsirroosi põdejad on see haigete kontingent, kelle puhul maksa transplanteerimisel on saadud kõige paremaid lähi- ja hilistulemusi. Nii ilmnes Ameerika Ühendriikides Pittsburghis tehtud uurimusest, et primaarset biliaarset tsirroosi põdejaist, kellel oli tehtud maksatransplantatsioon, elas 70% üle viie aasta ja enam (8). Enamik surmajuhte registreeriti esimese 6 kuu jooksul. Ei ole leitud ka seda, et haigus tekiks transplanteeritud maksas uuesti, kuigi mitokondriaalsed antikehad jäävad verre tsirkuleerima (8). Muidugi piiravad maksa transplanteerimise laialdast kasutamist sobivate doonorite vähesus ja operatsiooni kallidus. Sellepärast jätkuvad uuringud ka uute ravimeetodite leidmiseks.

Kokkuvõtteks võib öelda, et primaarne biliaarne tsirroos on haigus, millesse haigestuvad töövõimelises eas inimesed. Väga oluline on haiguse varajane, juba asümptomaatilises staadiumis diagnoosimine ja nende haigete pidev jälgimine ning ravi korrigeerimine tüsistuste tekkimise korral. Et praeguseks on Tartu Ülikooli Sisekliinikul pikaajaline kogemus primaarse biliaarse tsirroosi diagnoosimise, ravi ja jälgimise alal (80 haiget on olnud ravil ajavahemikul 1973...1993) ning abiks on ka tõhus koostöö Tartu Ülikooli Üld- ja Molekulaarpatoloogia Instituudi immunoloogialaboriga (mitokondritevastaste antikehade määramine), siis on ülimalt soovitatav, et haiged, kellel kahtlustatakse primaarset biliaarset tsirroosi, suunataks uuringuteks ja ravi alustamiseks Tartu Ülikooli Sisekliinikusse.

KIRJANDUS: 1. *Abe, K., Nakadate, I., Shimotono, H.* Gastroenterology, 1992, 102, A770. — 2. *Ahrens, E. H., Rayne, M. A., Kunkle, H. G. a. o.* Medicine, 1950, 29, 299—364. — 3. *Ballardini, G., Mirakian, R., Bianchi, F. B. a. o.* Lancet, 1984, 11, 1009—1013. — 4. *Berg, C. L., Gollan, J. L.* Scand. J. Gastroenterol., 1992, 27, Suppl. 192, 43—49. — 5. *Berg, P. A., Klein, A.* Clinical and prognostic relevance of different mitochondrial antibody profiles in primary biliary cirrhosis. Mol. Aspects. Med., 1985, 8, 235—247. — 6. *Crowe, J., Christensen, E., Smith, M.* Gastroenterology, 1980, 78, 1005—1010. — 7. *Danielsson, A., Boquist, L., Uddenfeldt, P.* Hepatology, 1990, 11, 195—200. — 8. *Esquivel, C. O., van Thiel, D. H., Demetris, A. J.* Gastroenterology, 1988, 94, 1207—1216. — 9. *Fussey, S. P. M., Ali, S. T., Guest, J. R. a. o.* Proc. Natl. Acad. Sci. USA, 1990, 887, 3987—3991. — 10. *Guanabens, N., Peres, A., Marinoso, L.* Am. J. Gastroenterol., 1990, 85, 1356—

1362. — 11. *Hamlyn, A. N., Sherlock, S.* Gut, 1974, 15, 473—479. — 12. *Hodgson, S. F., Dickson, E. S., Wahner, H. W. a. o.* Ann Intern. Med., 1985, 103, 855—860. — 13. *Hopf, U., Moller, B., Stemerowicz, R.* Lancet, 1989, 11, 1419—1422. — 14. *James, O. F. W.* Gut, 1985, 26, 109—113. — 15. *James, O. F. W., Yeaman, S. J., Bassendine, M. F.* Hepatology, 1989, 10, 247—251. — 16. *James, S. P., Hoofnagle, J. H., Strober, W. a. o.* Ann. Intern. Med., 1983, 99, 500—512. — 17. *Myszor, M., James, O. F. W.* Q. J. Med., 1990, 75, 377—385. — 18. *Neuberger, J. M.,* Gut, 1990, 30, 1069—1072. — 19. *Roll, J., Boyer, J. L., Barry, D. a. o.* New Engl. J. Med., 1983, 308, 1—7. — 20. *Rubin, E., Schaffner, F., Popper, H.* Am. J. Pathol. 1965, 46, 387—407. — 21. *Sasaki, H., Oinoue, K., Higuchi, K. a. o.* Gastroenterol. Jpn., 1985, 20, 476—485. — 22. *Scheuer, P. J.* Proc. R. Soc. Med., 1967, 60, 1267—1270. — 23. *Sherlock, S., Scheuer, P. J.* New Engl. J. Med., 1973, 289, 674—678. — 24. *Sherlock, S.* In: Diseases of the liver and biliary system. London, 1990, 180—187. — 25. *Stemerovicz, R., Hopf., U., Moller, B.* Lancet, 1988, 11, 1166—1170. — 26. *Stiehl, A., Raedsh, R., Rudolph, G.* Gastroenterology, 1990, 98, 424—428. — 27. *Triger, D. R.* B. M. J. 1980, 281, 772—775. — 28. *Triger, D. R.* Liver, 1984, 4, 195—200. — 29. *Undrehill, J., Donaldson, P., Bray, G. a. o.* Hepatology, 1992, 16, 1404—1408. — 30. *Walker, J. G., Doniach, D., Roitt, I. M. a. o.* Lancet, 1965, 1, 827—831.

Summary

About the Primary Biliary Cirrhosis. Primary Biliary Cirrhosis is a cholestatic, progressive liver disease of unknown etiology which occurs predominantly in middle age females. Although the disorder is rare, it is diagnosed more frequently nowadays. The contemporary points of view about Primary Biliary Cirrhosis have been described in this paper.

CURRICULUM VITAE

CITIZIENSHIP:	Estonia
BORN:	8. August 1968, Jõgeva, Estonia
PERSONAL STATUS:	single
ADDRESS:	Anne 90–4, Tartu EE2400
PHONE:	+372-7-483-390
FAX:	+372-7-448-607

EDUCATION:

1975–1986	Elva Secondary School, *cum laude*
1986–1992	Medical Faculty, University of Tartu, *cum laude*
1992–	Department of Internal Medicine, University of Tartu, post-graduate student

SPECIAL COURSES:

1993	University of Amsterdam, Netherland
1994	University of Ljubljana, Slovenia

SCIENTIFIC WORKS:

Most studies are connected to the primary biliary cirrhosis, its epidemiology, peculiarities and prognostification of the course of the disease.
18 scientific publications and 6 presentations in the international meetings.
Member of the Estonian Society of Gastroenterology (1992).

ELULOOKIRJELDUS

KODAKONDSUS:	Eesti
SÜNDINUD:	8. august 1968, Jõgeva, Eesti
PEREKONNASEIS:	vallaline
AADRESS:	Anne 90–4, Tartu EE2400
TELEFON:	+372-7-483-390
FAX:	+372-7-448-607

HARIDUS:

1975–1986	Elva Keskkool, *cum laude*
1986–1992	Arstiteaduskond, Tartu Ülikool, *cum laude*
1992–	Sisekliinik, Tartu Ülikool, doktorant

ERIALANE TÄIENDUS:

1993	Amsterdami Ülikool, Holland
1994	Ljubljana Ülikool, Sloveenia

TEADUSLIK TEGEVUS:

Põhilised uuringud on seotud primaarse biliaarse tsirroosiga, tema epidemioloogia, iseärasustega ja haiguse kulu prognoosimisega.
Omab 18 teaduslikku publikatsiooni ja 6 ettekannet rahvusvahelistel konverentsidel.
Eesti Gastroenteroloogide Seltsi liige (1992).

DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS

1. **Heidi-Ingrid Maaroos.** The natural course of gastric ulcer in connection with chronic gastritis and Helicobacter pylori. Tartu, 1991.
2. **Mihkel Zilmer.** Na-pump in normal and tumorous brain tissues: Structural functional a. tumorigenesis aspects. Tartu, 1991.
3. **Eero Vasar.** Role of cholecystokinin receptors in the regulation of behaviour and in the action of haloperidol and diazepam. Tartu, 1992.
4. **Tiina Talvik.** Hypoxic-ischaemic brain damage in neonates (clinical, biochemical and brain computed tomographical investigation) Tartu, 1992.
5. **Ants Peetsalu.** Vagotomy in duodenal ulcer disease: A study of gastric acidity, serum pepsinogen I, gastric mucosal histology and Helicobacter pylori. Tartu, 1992.
6. **Marika Mikelsaar.** Evaluation of the gastrointestinal microbial ecosystem in health and disease. Tartu, 1992.
7. **Hele Everaus.** Immuno-hormonal interactions in chronic lymphocytic leukaemia and multiple myeloma. Tartu, 1993.
8. **Ruth Mikelsaar.** Etiological factors of diseases in genetically consulted children and newborn screening: dissertation for the commencement of the degree of doctor of medical sciences. Tartu, 1993.
9. **Agu Tamm.** On metabolic action of intestinal microflora: clinical aspects. Tartu, 1993.
10. **Katrin Gross.** Multiple sclerosis in South-Estonia (epidemiological and computed tomographical investigations). Tartu, 1993.
11. **Oivi Uibo.** Childhood coeliac disease in Estonia: occurrence, screening, diagnosis and clinical characterization. Tartu, 1994.
12. **Viiu Tuulik.** The functional disorders of central nervous system of chemistry workers. Tartu, 1994.
13. **Margus Viigimaa.** Primary haemostasis, antiaggregative and anticoagulant treatment of acute myocardial infarction. Tartu, 1994.
14. **Rein Kolk.** Atrial versus ventricular pacing in patients with sick sinus syndrome. Tartu, 1994.
15. **Toomas Podar.** Incidence of childhood onset type 1 diabetes mellitus in Estonia. Tartu, 1994.
16. **Kiira Subi.** The laboratory surveillance of the acute respiratory viral infections in Estonia. Tartu, 1995.
17. **Irja Lutsar.** Infections of the central nervous system in children (epidemiologic, diagnostic and therapeutic aspects, long term outcome). Tartu, 1995.
18. **Aavo Lang.** The role of dopamine, 5-hydroxytryptamine, sigma and NMDA receptors in the action of antipsychotic drugs. Tartu, 1995.
19. **Andrus Arak.** Factors influencing the survival of patients after radical surgery for gastric cancer. Tartu, 1996.
20. **Tõnis Karki.** Quantitative composition of the human lactoflora and method for its examination. Tartu 1996.
21. **Reet Mändar.** Vaginal microflora during pregnancy and its transmission to newborn. Tartu 1996.

ISSN 1024–395X
ISBN 9985–56–199–6