

# Rheumatic disorders and primary biliary cirrhosis: an appraisal of 170 Italian patients

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## Abstract

**Objective**—To establish the frequency of connective tissue diseases (CTD) in a cohort of Italian patients with primary biliary cirrhosis (PBC) and to evaluate the availability of a marker for the early identification of the more common associated CTD.

**Methods**—One hundred and seventy consecutive patients with histologically diagnosed PBC were screened for the presence of a CTD and/or Raynaud's phenomenon (RP). Patients were classified as having a CTD only if they fulfilled standardised criteria.

**Results**—Forty seven patients had a CTD. The most common CTD was systemic sclerosis (SSc), found in 21 patients. RP was present in 54 patients, most of whom (n=39) had an associated CTD. The most prevalent autoantibodies included antinuclear antibodies (ANA) with anticentromere (ACA) and speckled patterns (34 and 33 patients, respectively) and extractable nuclear antigens (ENA, 27 patients). However, while the frequencies of ACA and ENA were significantly higher in patients with an associated CTD ( $p < 0.0001$  and  $p < 0.005$ , respectively), no relationship was found for speckled ANA. ACA was the best predictor of a CTD in patients with PBC (odds ratio (OR) 24.5, 95% CI 5.5 to 108.8), followed by the presence of ENA (OR 23.9, 95% CI 5.6 to 101.0) and RP (OR 20.2, 95% CI 5.7 to 71.2).

**Conclusions**—Using strict standardised classification criteria we have found that SSc is the most common CTD associated with PBC and that ACA and ENA are strong markers for an associated CTD in patients with PBC.

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Primary biliary cirrhosis (PBC) is a chronic progressive cholestatic liver disease characterised by destruction of intrahepatic bile ducts causing fibrosis and eventually cirrhosis of the liver. The cause of this disease is unknown, although the immunological abnormalities and

the strong female preponderance point to an autoimmune aetiology.

Association with rheumatic diseases is well known.<sup>1,2</sup> The presence of an associated autoimmune disease has been suggested to be predictive of a poorer prognosis,<sup>3,4</sup> probably because, owing to improved management and liver transplantation, only the most severe cases of PBC die primarily from PBC. Since the associated conditions may be an important cause of morbidity and mortality, their early recognition is therefore mandatory for proper management. On the other hand, studies of the co-occurrence of autoimmune diseases may contribute to the knowledge of the still poorly understood mechanisms behind them.

The reported incidence and prevalence rates of PBC associated rheumatic diseases have often been conflicting, mainly because of methodological problems including diagnostic criteria and ethnic differences.<sup>5–7</sup>

The aims of this study were to assess the frequency of a number of connective tissue diseases (CTD) in a large cohort of Italian patients with PBC and to evaluate whether it is possible to find early marker(s) indicative of the co-occurrence of PBC and CTD. Moreover, since standardised definitions of diseases are necessary to compare data across studies, we evaluated only patients who fulfilled well accepted classification and/or diagnostic criteria.

## Methods

One hundred and seventy consecutive Italian patients (156 women, 14 men) of mean age 59 (range 30–84) with histologically confirmed PBC<sup>8</sup> who attended our centre between 1995 and 1998 entered the study after giving informed consent. The majority (90%) were from Northern Italy. Patients were classified as having systemic sclerosis (SSc), systemic lupus erythematosus (SLE), or rheumatoid arthritis (RA) if they fulfilled the American College of Rheumatology (ACR) accepted criteria.<sup>9–11</sup> Patients with Sjögren's syndrome (SS) fulfilled the criteria of Vitali *et al.*<sup>12</sup> Mixed connective tissue disease (MCTD) was diagnosed by the presence of the symptoms described by Sharp *et al.*<sup>13</sup> The diagnosis of polymyositis (PM) was based on Bohan and Peter's criteria.<sup>14</sup> Patients with features strongly suggestive of autoimmune rheumatic disease (Raynaud's phenomenon (RP), skin thickening limited to the fingers, finger oedema, serum anticentromere antibody (ACA), with no internal organ abnormalities), but not fulfilling the criteria for any disorder, were classified as undifferentiated CTD (UCTD).<sup>15</sup> Patients were diagnosed as

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Table 1 Prevalence of connective tissue disease (CTD) or Raynaud's phenomenon (RP) in 170 patients with primary biliary cirrhosis (PBC)

	Patients	Women	Mean (SD) age (years)
CTD– RP–	108 (63%)	97 (90%)	58 (12)
CTD+ RP–	8 (5%)	8 (100%)	59 (8)
CTD+ RP+	39 (23%)	37 (95%)	61 (9)
CTD– RP+	15 (9%)	14 (93%)	56 (11)

Table 2 Prevalence of systemic sclerosis (SSc), undifferentiated connective tissue disease (UCTD), Sjögren's syndrome (SS), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), mixed connective tissue disease (MCTD) and polymyositis (PM) in 170 patients with primary biliary cirrhosis

	SSc	UCTD	SS	RA	SLE	MCTD	PM
No of patients	21	12	6	3	3	1	1
%	12.3	7.1	3.5	1.8	1.8	0.6	0.6

Table 3 Results of the best fitting logistical model

Variable	Odds ratio	p Value	95% confidence intervals	
			Lower	Upper
ACA	24.5	0.0001	5.5	108.8
ENA	23.9	0.0001	5.6	101.0
RP	20.2	0.0001	5.7	71.2
ACA-ENA	0.02	0.0112	0.0	0.4
Criterion	Intercept only	Intercept and covariates	$\chi^2$ for covariates	
-2 logL score	187.4	83.4	104.1 with 4df (p=0.0001) 93.3 with 4df (p=0.0001)	

having primary RP if they had typical episodic ischaemic attacks in response to cold or emotional stimuli for at least 2 years without clinical or serological evidence of CTD.

Antinuclear antibodies (ANA) were assayed by indirect immunofluorescence with HEp-2 cells (Immunoconcepts, Sacramento, CA, USA). Antimitochondrial antibodies (AMA) were assayed by indirect immunofluorescence on rat liver, kidney, and stomach sections (The Binding Site, Birmingham, UK). AMA negativity was confirmed by immunoblotting analysis of the PBC specific serum autoantibody against the M2 component of the mitochondrial inner membrane, as previously described.<sup>16</sup> The following ANA patterns were considered: homogeneous, speckled, centromere, nucleolar. Antibodies to extractable nuclear antigens (ENA), including RNP, SS-A, SS-B, Jo-1, anti-topoisomerase I (ATA), and Sm, were detected by an ELISA method (Diamedix Corp, Miami, FL, USA) and confirmed by immunoblotting analysis (Euroimmun GmbH, Gross Groenau, Germany).

#### STATISTICAL ANALYSIS

ANOVA followed by the Scheffé test was used to test differences between groups. The  $\chi^2$  test was used for categorical data. Logistic regression models were used to predict a binary dependent variable from a set of independent variables. The results are expressed as odds ratios (ORs) with 95% confidence intervals (CI).

Data are mean (SD) unless otherwise specified. p Values of <0.05 were considered significant.

#### Results

Sixty two patients (36.5%) had a CTD and/or RP. Table 1 shows the prevalence of CTD and/or RP and the sex and age distribution among the groups. The most common CTD was SSc (table 2). Fifteen patients with SSc had the limited form of the disease (lc-SSc) and six had the diffuse form (dc-SSc). Fifty four patients (51 women) had RP, most of whom (39 patients, 72%) presented with an associated CTD (SSc (n=21); UCTD (n=12); SS (n=4); SLE and PM (n=1 each)). Most

patients with UCTD had features of SSc; all had RP and ACA, three had skin thickening limited to the fingers, and three had telangiectasia. No patient had visceral involvement typical of SSc, and none had three or more of the CREST syndrome features (three patients could have been classified as RS, three as RT, and six as R). RP appeared before (1–52 years, mean 19 years, n=26) or after (1–16 years, mean 5 years, n=23) the diagnosis of PBC. In five patients PBC and RP occurred almost simultaneously.

One hundred and sixty three patients with PBC had autoantibodies. However, excluding AMA which is typical of PBC and was found in 164 patients, autoantibodies were observed in 56% of patients with PBC, particularly if a CTD co-existed (87% PBC with CTD v 44% PBC without CTD, p<0.0001). The most prevalent autoantibodies included ANA with anticentromere (n=34) and speckled (n=33) patterns and ENA (n=27). The frequencies of ACA and ENA were significantly higher in patients with PBC and CTD (ACA: 82% PBC with CTD v 18% PBC without CTD, p<0.0001; ENA: 69% v 30%, p<0.005), while the presence of the speckled pattern was independent of any autoimmune rheumatic conditions (37% v 63%, p=0.4018). Most of the ACA positive patients (n=27, 79%) had RP, but RP was unrelated to the presence of ACA (27/54 with RP had ACA and 27 did not). ACA were particularly frequent in UCTD and in SSc, with no significant differences between lc-SSc or dc-SSc (n=9 and n=4, respectively). The frequencies of ACA and ENA significantly differed between SSc and UCTD (ACA: SSc=13 patients positive v UCTD=all patients positive, p<0.02; ENA: SSc=7 patients positive v UCTD=all patients negative, p<0.05). ENA were particularly frequent in patients with SS (n=4 out of 6). SS-A, the most common ENA (n=16), was unrelated to rheumatic conditions (seven patients had PBC with no associated conditions, seven had a CTD, two had an isolated RP). SS-B was found in four patients, and in three it coexisted with SS-A (two patients had SS, one with no CTD). ATA was more frequent in CTD (three patients had dc-SSc, one had RA, one had PM, and one had PBC without rheumatic diseases). RNP was found in eight patients, five of whom had CTD; none of the patients had Sm or Jo-1. The ANA homogeneous pattern, found in 11 patients, was unrelated to CTD.

No relationship was observed between AMA negativity (n=6), the other autoantibodies, and the different rheumatic disorders.

A multivariate analysis found that ACA was the best marker of an associated rheumatic disease, followed by the presence of ENA and RP (table 3).

#### Discussion

This study confirms that a relatively large proportion of patients with PBC have an autoimmune rheumatic disease.

To avoid the effects of geographical factors which have a role in PBC as well as in autoimmune rheumatic diseases,<sup>17,18</sup> we performed

our study on a cohort of Italian patients sufficiently large to draw conclusions about the co-existence of CTD in a defined population. Furthermore, we included only those patients with PBC who fulfilled well defined diagnostic or classification criteria for rheumatic diseases, which made it easier to compare data across studies.

The most frequent autoimmune rheumatic disease we found in patients with PBC was SSc. The co-existence of PBC and SSc is well known with a frequency ranging from 3% to 50%.<sup>1 2 5 7</sup> Even if patients with a "scleroderma spectrum" disorder are excluded, and although epidemiological studies of the prevalence of SSc in an Italian population are lacking, the prevalence of SSc in PBC appears to be significantly greater than that found in the general population.<sup>19 20</sup> This finding may suggest some common aetiological mechanism, and certain peculiar aspects of the immune system reported in patients with PBC associated SSc seem to confirm this.<sup>21 22</sup> Whether PBC and SSc have a chronic graft-versus-host disease pathogenesis,<sup>23 24</sup> possibly related to the persistence of microchimeric fetal cells in the circulation,<sup>25 26</sup> has to be clarified. It is tempting to speculate that the co-existence of PBC and SSc may be a new distinct clinical syndrome.

Since the frequencies of SS, SLE, MCTD, and PM were similar to those reported in the general population,<sup>20 27-29</sup> the co-occurrence with PBC should be considered casual. The previously reported<sup>30</sup> high prevalence of SS in patients with PBC has not been confirmed in this study, probably because different classification criteria were used. The frequency of RA, which is similar to that observed in the general population,<sup>31</sup> appears particularly high if compared with that recently reported in an Italian epidemiological survey.<sup>32</sup> The previously reported greater frequency of RA in PBC<sup>1 6</sup> probably also reflects different classification criteria. In fact, patients with PBC often complain of articular symptoms which do not fulfil the ACR criteria for RA ("arthritis of PBC").<sup>2</sup>

Further studies over time are necessary to understand the significance of the co-occurrence of UCTD and PBC and, in particular, to evaluate whether these patients are at risk of SSc. It would be interesting to ascertain whether ACA, found in all UCTD, might identify a subset of patients with PBC who are destined to develop SSc. It is worth noting that ACA have been associated with severe vascular disease in SSc,<sup>33</sup> and that microvascular abnormalities have been found in PBC.<sup>34</sup>

The high frequency of RP is probably non-casual, although the prevalence of RP is difficult to assess.<sup>35</sup> The significance of RP in patients with PBC and the relationship between RP and ACA are difficult to understand since RP was unrelated to ACA but, although weaker, RP was also a good indicator of an associated CTD. Whether or not RP is the result of an endothelial dysfunction is an interesting question that has to be settled.

With the exception of ENA, the frequency of the other autoantibodies was not related to

CTD. The significance of ATA is intriguing since half of the ATA positive patients with PBC did not have SSc.

In conclusion, using strict standardised classification and diagnostic criteria, we have found that a number of Italian patients with PBC have autoimmune rheumatic diseases, with SSc being the most common. Since autoimmune diseases constitute a leading cause of death among women,<sup>36</sup> ACA and ENA, which are strongly indicative of a coexisting CTD, could be a useful prognostic test for patients with PBC. Further studies are needed to assess whether the combined occurrence of these diseases may provide new insight into their aetiopathogenesis.

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