Large-scale Characterization Study of Patients with Antimitochondrial Antibodies but Nonestablished Primary Biliary Cholangitis

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ABBREVIATIONS

AH: academic hospital

AID: autoimmune disease

ALP: alkaline phosphatase

ALT: alanine aminotransferase

AMA: antimitochondrial antibodies type 2

ANA: antinuclear antibodies

BMI: body mass index

CI: confidence interval

GGT: gamma-glutamyl transpeptidase

H: hospital

IgM: immunoglobulin M

PBC: primary biliary cholangitis

UDCA: ursodeoxycholic acid

ABSTRACT

The prevalence, clinical characteristics and outcomes of patients with antimitochondrial antibodies (AMA) but no clinical evidence of primary biliary cholangitis (PBC) are largely unknown. A prospective study of AMA incidence was conducted through a nationwide network of 63 French immunology laboratories. Clinical data from 720 out of 1318 AMA-positive patients identified in one year were collected. The patients were categorized as either newly diagnosed with PBC (n=275), previously diagnosed with PBC (n=216), or with non-established diagnosis of PBC (n=229). The latter group was specifically evaluated. Follow-up data were collected for up to 7 years after detection of AMA. The prevalence of AMA-positive patients without evidence of PBC was 16.1 per 100,000. These patients had the following characteristics: 78% female; median age 58 years; median AMA titre 1:160; extra-hepatic autoimmune disorders 46%; normal serum alkaline phosphatases (ALP) 74%; ALP above 1.5 times the upper limit of normal 13%; cirrhosis 6%. Compared to those newly diagnosed with PBC, the patients were slightly younger, had lower AMA titres, and lower sex-ratio imbalance. Among the patients with normal ALP and no evidence of cirrhosis, the 5-year incidence rate of PBC was 16%. Whereas no patients died from PBC, the 5-year survival rate was 75%, as compared to 90% in a control, standardized population matched for age and gender ($p<0.05$). *Conclusion:* Nearly half of the newly detected AMA in clinical practice does not lead to a diagnosis of PBC. PBC is unrecognized in 13% of those cases. Only 1 in 6 patients with AMA and normal ALP will develop PBC after 5 years. The mortality of AMA-positive patients without PBC is increased irrespective of the risk of PBC development.

INTRODUCTION

Primary biliary cholangitis (PBC) is a chronic cholestatic liver disease of unknown but presumably auto-immune origin characterized by elevated alkaline phosphatases (ALP) activity and presence of antimitochondrial antibodies (AMA) in the serum and highly suggestive histological lesions, namely granulomatous or lymphocytic non-suppurative destructive cholangitis of interlobular bile ducts, on liver biopsy.[1]

The standardization in clinical practice of AMA detection has led to earlier diagnosis and management of the disease, avoiding in most patients an evolution to end-stage liver disease at least in part because of the prescription of ursodeoxycholic acid (UDCA), which is the only first-line pharmacological treatment currently approved by drug agencies and international medical societies.[2, 3]

AMA are regarded as highly specific (≥ 95%) of PBC in people with abnormal ALP so that a diagnosis of PBC can be made with confidence without the use of a biopsy in patients with unexplained elevation of ALP and presence of AMA.[2, 4, 5] However, their detection in the absence of any other symptoms or signs of the disease has been widely reported, notably in patients with extra-hepatic autoimmune disorders such as scleroderma, Sjögren's syndrome or autoimmune thyroid disease, as well as in patients with hepatic or extra-hepatic non-autoimmune disorders such as chronic hepatitis C or hematologic malignancies.[6, 7] In such a situation, longitudinal studies seem to indicate AMA to be inevitably associated with PBC development, but these studies are rather old, all originated from a single UK center and limited area, and involve no more than 29 patients in total.[8-10]

So far, no large prospective study has been conducted to assess specifically the significance of AMA in the absence of clinical indications of PBC. The aim of the present study was therefore to evaluate, prospectively and at a large-scale level, the prevalence,

clinical characteristics and outcomes of patients with AMA but non-established diagnosis of PBC.

METHODS

Study design and population

This was a prospective nationwide observational study conducted in France during May 2006 to September 2013 in which an extensive network of clinical immunology laboratories, including 63 laboratories covering approximately 90% of the French metropolitan territory, was actively involved. The geographical location of the laboratories is shown in **Figure 1**.

The primary objective of the study was to assess the incidence of newly identified AMA-positive patients in France based on the tests routinely prescribed in clinical practice. For this purpose, every positive AMA tests identified between May 2006 and June 2007 were prospectively recorded in a secure web database through which the patients were registered in an anonymized format together with the name and address of the prescribing physician. This constituted the one-year census phase of the study.

All laboratories used the same first-line detection methods and diagnostic algorithms. In line with the international recommendations, serum AMA were first detected by indirect immunofluorescence (IF) on rat liver, kidney and stomach tissue sections. Every antibody titre equal to or higher than 1:40 was considered positive and subsequently tested by immunodotting or immunoblotting on mitochondrial recombinant or native antigens for confirmation. For the latter purpose, most laboratories used ready for use immunodot test commercial kits. The reference lab of Saint-Antoine hospital, Paris, used its own immunoblotting method, as previously described.[11] PBC-specific antinuclear antibodies

(ANA), i.e. ANA with either rim-like/membranous or multiple nuclear dots IF patterns, were detected on Hep-2 cells. A titre equal to or greater than 1:80 was considered significant.

After centralization and unanonymization of the data, the prescribing physicians were contacted and invited to complete a questionnaire on the past history, clinical, biochemical, and (when appropriate) histological features of their patient(s) and to state if a diagnosis of PBC was officially retained (diagnosis was based on physician opinion in real-life conditions). The items constitutive of this questionnaire are available in **Supplementary Table 1**. All patients were delivered an information form by their physician indicating that unless they notify their objection some of their medical data will be recorded for research purposes in the strict and specific field of autoimmunity.

According to the medical information thus received, the patients were categorized as being in one of the three following situations: 1) patients newly diagnosed with PBC (i.e. incident case of PBC); 2) patients previously diagnosed with PBC (i.e. pre-existing case of PBC); 3) patients non-diagnosed with PBC (i.e. non-established case of PBC).

For the purpose of the study, the focus was made on the latter group of patients. Prospective follow-up data were collected by asking the prescribing physicians to complete a second questionnaire 2, 4, 5 and 7 years, respectively, after the beginning of the census period and to state about the health and diagnosis status of their patient(s) over time. This constituted the follow-up phase of the study. The items constitutive of this second questionnaire are available in **Supplementary Table 2**. Survival and PBC incidence rates were directly estimated from these medical reports.

Statistical analysis

Descriptive statistics were expressed as median (range) or number (%). The incidence rates of AMA and PBC were calculated by dividing the number of incident cases registered

during the one-year census period by the total number of people in metropolitan France during the same period, taking into account the territory coverage of AMA screening, the participation rate of the prescribing physicians, and a 10% expected ratio of AMA-negative PBC.[12] The rates were calculated by gender and 10-year-age categories. Assuming that these rates were invariable in time, the point prevalence was estimated using the following formula: prevalence = incidence × life expectancy. Life expectancy was evaluated from a cohort of 378 patients with PBC of similar age structure followed-up in Saint-Antoine hospital, Paris between 1985 and 2005. Patient groups were compared using the Student's t-test, or the Mann-Whitney test when appropriate, for continuous variables and the Chi-square test, or the Fisher's exact test when appropriate, for categorical variables. Because survival data were determined between fixed intervals of time rather than using the exact date of deaths (a variable that was frequently missing), the survival rates were calculated using an actuarial method (i.e. life table analysis) with 1-year constant intervals. A standardized population matched for age, gender and follow-up period served as a control. The estimates of the survival rates of this population were obtained from the French official census tables.[13] Observed and expected survival rates were compared using the log-rank test. Prognostic variables were studied using a Cox regression model. The cumulative incidence function for PBC was estimated using a non-parametric method dealing with competing risk of death.[14] Were excluded from this analysis the patients who had serum phosphatase alkaline (ALP) activity higher than 1 times the upper limit of normal (ULN), pruritus, cirrhosis, or PBC-compatible lesions on histology, i.e. those with possible or presumed PBC.

Institutional review board approval

The study was approved by the French Advisory Committee for Data Processing in Health Research (CCTIRS) and the National Commission on Informatics and Liberty (CNIL) prior to data collection.

RESULTS

Incidence and prevalence of AMA-positive patients with or without established PBC

A total of 1367 positive AMA tests were registered in 1318 patients during the one-year census period of the study (**Figure 2**). The total number of AMA tests performed over this period was highly variable according to the laboratories (range: 64 – 18500; median: 563) but the percentages of positive results were similar between them, with an average of 2.5% of the whole tests (95% confidence interval: 1.6% - 3.5%; **Supplementary Table 3**). The prescribing physician was solicited for 1149 (87%) of the AMA-positive patients. Medical information could be collected in 772 (67%) patients but was really exploitable in 720 (63%). Among the latter were counted 275 (38%) incident cases of PBC, 216 (30%) prevalent cases of PBC, and 229 (32%) non-established cases of PBC (**Figure 2**).

The incidence rates per 100,000 inhabitant-years of AMA and PBC were 1.7 and 1.0, respectively. These rates varied as a function of age and gender (**Figure 3**). They were 4 (all AMA-positive patients) to 6 (PBC patients) times higher in females than in males. In both genders, incidence increased linearly with age from 20 years old to reach a peak during the 8th decade of life (**Figure 3**). The estimated prevalence rates per 100,000 inhabitants of AMA and PBC were 40.4 and 24.3, respectively. The prevalence rate of AMA-positive patients with non-established PBC was 16.1 per 100,000 inhabitants.

Characteristics of AMA-positive patients with non-established PBC

The clinical characteristics of the 229 patients with a positive AMA test but no established diagnosis of PBC are shown in **Table 1**. These patients were mainly females (78%) aged over 40 years old (median age, 58 years). The median titre of AMA was 1:160. Immunodot/blot tests, available in 150 (66%) patients, were positive in 91% of the tested individuals. The patients not tested with these second-line methods had similar AMA titres and demographic characteristics than those tested with (data not shown). The proportion of patients with PBC-specific ANA was 6%. In nearly half of the cases (46%), these antibodies were revealed in the evaluation of an autoimmune disorder. The most frequent autoimmune diseases were systemic lupus erythematosus (n=18; 8%), Sjögren's syndrome (n=14; 6%), and autoimmune hepatitis (n=10; 4%). In 12% of the cases, AMA were found in the background of a non-autoimmune liver disease. The most frequent of these diseases were chronic hepatitis C (n=8; 3%) and alcoholic liver disease (n=8; 3%). All the clinical settings in which AMA were evidenced are available in **Supplementary Table 4**. Regretfully, because of too few longitudinal data, the persistence of AMA positivity was not assessable.

Complete or partial biochemical data were available in 130 (57%) patients. The median serum levels of total bilirubin, ALP, alanine aminotransferase (ALT), and immunoglobulin M (IgM) were all in the normal range, while gamma-glutamyl transpeptidase (GGT) was slightly above the ULN (**Table 1**). Serum ALP and whole biochemical liver tests (i.e. bilirubin, ALP, GGT, and ALT) were normal in 74% and 44% of the patients, respectively. An ALP level above 1.5 × ULN without an alternative explanation (i.e. liver tumor, drug-induced liver injury, or any other well-identified liver diseases) to PBC was observed in 13% of the cases. A liver biopsy was performed in 28 (19%) out of the 148 patients for which data were available. None of the histological reports were suggestive of PBC as no bile duct lesions or granulomas were reported. Histological reports could include normal histology, mild portal inflammation, steatohepatitis, histological features compatible with autoimmune hepatitis, or

cirrhosis. Cirrhosis, whether diagnosed on histology or on clinical and ultrasound findings, was reported in 13 (6%) patients. In these patients, alcohol abuse was reported in half of the cases (n=7) while no aetiology was reported in four (30%). The patients with a negative immunodot/blot test (n=13) had similar characteristics than those with a positive one (n=137; **Supplementary Table 5**). Likewise, the patients with a low IF AMA titre (=1:40; n=36) did not differ significantly from those with a higher one (≥ 1:80; n=193; **Supplementary Table 6**).

The patients were compared to those (n=275, analysable=247) concomitantly diagnosed with PBC during the same period (**Table 2**). Compared to the latter group, they tended to be slightly younger and to have fewer symptoms. The proportion of patients with fatigue did not differ between groups but pruritus and jaundice were less frequently observed. The patients had a significantly lower titre of AMA, a lower proportion of PBC-specific ANA, a lesser sex ratio imbalance, and significantly lower serum levels of total bilirubin, ALP, GGT, ALT and IgM (**Table 2**). Also they were less frequently subjected to liver biopsy and were less likely to have cirrhosis. Finally, they had comparable personal and familial past histories of autoimmune disorders.

Clinical outcomes of AMA-positive patients with non-established PBC

Follow-up data were available in 92 (41%) out of the 222 patients still alive at the end of the census period. The mean duration of follow-up was 4.0 ± 1.8 years (range: 0.5 – 7.3 years). Taking all deaths into account (including those recorded during the census period), a total of 20 (9%) patients died during the whole study period. Considering the follow-up phase specifically, 17 (18%) out of the 92 followed-up patients died. The median age at death was 73.6 years (range: 42.4 – 90.0 years). The causes of death were the following: non-liver related cancers 5 (bronchial squamous cell carcinoma 1; pulmonary adenocarcinoma 1;

metastatic adenocarcinoma of undetermined origin 3), hematologic malignancies 4 (non-Hodgkin lymphoma 2; acute myeloid leukaemia 1; multiple myeloma 1), infectious diseases 3 (lung infection 2; infective endocarditis 1), cardio-vascular diseases 2 (multifactorial heart failure 1; chronic cor pulmonale 1), digestive diseases 3 (alcoholic chronic liver failure 2; severe acute pancreatitis 1), undetermined cause 3. No patients died officially from PBC. The actuarial and Kaplan-Meier survival curves are shown in **Figure 4**. The 1-, 3- and 5-year rates of survival were 95% (95% confidence interval: 92% – 98%), 90% (85% – 96%), and 75% (63% – 87%), respectively. These rates were significantly lower than those expected in the control population, specifically 99% (97% – 100%), 96% (92% – 100%) and 90% (83% – 98%), respectively ($p = 0.023$).

Development of PBC was reported in 9 (10%) out of the 92 followed-up patients, among whom 8 females (89%). The median age at PBC diagnosis was 62.1 years (range: 35.9 – 69.8 years). The incidence rates were calculated after excluding the patients with elevated ALP, pruritus or cirrhosis at baseline (i.e. from 66 out of 92 patients) and by taking care of the competing risk of death. The incidence curve is shown in **Figure 5**. The cumulative incidence rates of PBC at 1, 3, and 5 years were 2% (95% confidence interval: 0% - 7%), 7% (2% - 15%), and 16% (6% - 29%), respectively. Neither age, gender, AMA titre, PBC-specific ANA, personal or familial history of AID, or baseline serum levels of bilirubin, ALP, GGT, ALT or IgM were predictive of PBC development (**Table 3**). The incidence rate of PBC was not altered by the exclusion of patients with a negative dot/blot test (**Supplementary Figure 5**). In addition, no statistical difference was found between the low (= 1:40) and high (≥ 1:80) IF AMA titre groups, although no case of incident PBC was reported in the former group (**Supplementary Figure 6**).

DISCUSSION

In this study we showed that nearly half of the prospectively detected AMA in clinical practice was not related to a diagnosis of PBC. This observation raises again the question of the true significance of AMA in human pathology.[15, 16] Using classical diagnostic criteria, however, we showed that 13% of these patients had definite PBC, suggesting that the disease may be underdiagnosed by physicians, more likely internists and non-specialists of the liver as a large proportion of AMA are revealed in the settings of non-hepatic autoimmune diseases. Notwithstanding, taking care to exclude these false negative cases at inclusion, our follow-up data clearly showed that only a few number of patients eventually developed PBC over a mean follow-up of 4 years, whereas in the same time this population was shown to have an increased risk of mortality as compared to a matched control population.

PBC is asymptomatic for years and increasingly diagnosed incidentally on routine blood tests revealing mild chronic cholestasis. In this common situation, a positive AMA test is of major significance because of its high sensitivity and specificity for the diagnosis of PBC. AMA are one of the earlier hallmarks of the disease. They may antedate histological and biochemical manifestations by several years and persist thereafter throughout the course of the disease. This makes AMA test particularly attractive for assessing the extent of the PBC spectrum on a population scale. Our study was designed on such an AMA screening-based strategy. However, it was not comparable to a seroprevalence study because AMA tests were substantiated by clinical indications and thus the study was not able to capture all AMA-positive individuals. Consequently, our incidence and prevalence data should be regarded as low-end estimates of what reality is. Notwithstanding, it is emphasized that these estimates, which are the first epidemiological data available in France on both PBC and AMA, are quite comparable to those previously reported in Western Europe countries.[17-19]

Systematic screening of blood donors and healthy subjects shows AMA positive results in 0.07% to 9.9% of individuals depending on the technics and diagnostic thresholds used, as well as on the type, age and gender structures of the populations screened.[20-24] The relatively high rate (2.5% of all tests) of AMA positivity observed in our study may be related to the equivocal nature of low titers of AMA detected by IF but is more likely to be driven by the population concerned. The proportion of positive results is typically bigger in the female population above 40 years. The question is to know whether AMA in such circumstances are associated with underlying indolent, but nevertheless slowly progressive PBC that may necessitate UDCA treatment. Considering both PBC and AMA prevalences in the Japanese population, Shibata et al. inferred that only 0.73% of the AMA carriers in Japan should suffer from symptomatic PBC.[23] These data, which are in keeping with ours, point out that AMA-related conditions are a wide, poorly known area from which PBC, as the tip of the iceberg, would just be the known side.

In 1996, Metcalf et al. described a cohort of 29 asymptomatic patients who were positive for AMA without any other signs of the liver disease at first detection.[10] All the 29 patients were previously screened in a work-up for another autoimmune disease.[9] Liver biopsies were performed in the majority of them and at the screening, 24 patients had histological lesions compatible with or diagnostic of PBC, suggesting that before the advent of any clinical or biochemical manifestations those patients did have PBC. The median follow up was 17.8 years. During that period 76% developed symptoms of PBC and 83% had persistently abnormal liver tests showing cholestasis after a median time of only 5.6 years. No patients, however, developed portal hypertension or cirrhosis, and no patients died from PBC, pointing out that the progression of the disease in these patients was very slow.

Unlike the UK cohort, only a minority (19%) of our patients had a liver biopsy at AMA detection. The absolute number of biopsied patients, however, was similar (n=28). In contrast

with the UK experience, none of those patients displayed histological lesions suggestive of PBC. However, 13% of all patients met biochemical criteria for the disease. In addition, only 44% of the patients had their whole biochemical liver tests strictly normal at the time of AMA detection, thus suggesting that many of them did have a mild but unrecognized form of the disease. The fact that the patients were statistically younger and had lower AMA titres than those diagnosed with PBC at the same time supports the hypothesis of a very early, indolent phase of the disease. In contrast with the UK study, however, occurrence of PBC manifestations was reported in a minority (16%) of our patients after 5 years of follow-up. These inconsistent findings may result from inherent discrepancies between the populations studied and the methods used (single center retrospective selection vs. prospective nationwide screening). Notwithstanding, our data suggest that the breakdown of immune tolerance to PBC-specific mitochondrial epitopes does not necessarily result in disease emergence.

We sought to describe the mortality of this specific population. Our data showed that AMA carriers with no manifestations of PBC experienced an increased risk of mortality regardless of the PBC risk. The patients mainly died from non-hepatic primary cancers or hematologic malignancies whereas there is classically no such an increased risk of extra-hepatic malignant conditions in PBC.[25-27] Quite rightly, it may be argued that such a link between AMA and non-liver related mortality likely results from complications of concomitant diseases and/or treatments (immunosuppressive drugs) rather than from the consequences of AMA. Unfortunately, we could not obtain data from the patients who were tested AMA-negative during the same census period, which precludes any conclusions. However, whether AMA could directly or indirectly give rise to an increased risk of death is a hypothesis that cannot totally be excluded. This hypothesis is indeed supported by the increase in non-liver related mortality reported in some population-based studies,[26, 28] and

the growing evidence that patients with PBC may suffer from significant systemic dysfunctions.[29, 30]

The limitations of our study are inherent to its large-scale design mainly based on the good will of voluntary physicians and their declarative data. These include low rate of participation, incomplete exhaustiveness, frequent missing data, and residual uncertainties from unaudited data. The persistence of AMA positivity in time, for instance, could not be evaluated. However, the participation rate (67%) of physicians was quite satisfactory for such a large-scale study, just as was the mean exhaustiveness of data (64%). In addition, these intrinsic weaknesses are counterbalanced by the large amount of data collected and their representativeness in terms of population.

In conclusion, the present study highlights the relatively high proportion of AMA-positive patients with non-established PBC. The younger age and lower autoantibody titre of these patients together with the frequent mild abnormalities of their biochemical liver tests supports a very early, presymptomatic precholestatic stage of the disease. The incidence of clinical manifestations of PBC seems however much lower than previously reported. One the other hand, these patients may display an increased mortality risk whose link with AMA remains uncertain.

REFERENCES

1. Kaplan MM, Gershwin ME. Primary biliary cirrhosis. N Engl J Med 2005;353:1261-1273.

2. EASL EAftSotL. EASL Clinical Practice Guidelines: management of cholestatic liver diseases. J Hepatol 2009;51:237-267.

3. Lindor KD, Gershwin ME, Poupon R, Kaplan M, Bergasa NV, Heathcote EJ. Primary biliary cirrhosis. Hepatology 2009;50:291-308.

4. Walker JG, Doniach D, Roitt IM, Sherlock S. Serological Tests in Diagnosis of Primary Biliary Cirrhosis. Lancet 1965;1:827-831.

5. Munoz LE, Thomas HC, Scheuer PJ, Doniach D, Sherlock S. Is mitochondrial antibody diagnostic of primary biliary cirrhosis? Gut 1981;22:136-140.

6. Grimbert S, Johanet C, Bendjaballah F, Homberg JC, Poupon R, Beaugrand M. Antimitochondrial antibodies in patients with chronic hepatitis C. Liver 1996;16:161-165.

7. Zamfir O, Briaud I, Dubel L, Ballot E, Johanet C. Anti-pyruvate dehydrogenase autoantibodies in extrahepatic disorders. J Hepatol 1999;31:964-965.

8. James O, Macklon AF, Watson AJ. Primary biliary cirrhosis--a revised clinical spectrum. Lancet 1981;1:1278-1281.

9. 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.

10. Metcalf JV, Mitchison HC, Palmer JM, Jones DE, Bassendine MF, James OF. Natural history of early primary biliary cirrhosis. Lancet 1996;348:1399-1402.

11. Dubel L, Farges O, Bismuth H, Sebagh M, Homberg JC, Johanet C. Kinetics of anti-M2 antibodies after liver transplantation for primary biliary cirrhosis. J Hepatol 1995;23:674-680.

12. Galula G, Johanet C, Chazouilleres O, Poupon R. Quelle la fréquence réelle des cirrhoses biliaires primitives séronégatives? Gastroenterol Clin Biol 1999;23:947 A.

13. Taux de mortalité par sexe et groupe d'âges depuis 1962 : France métropolitaine. La situation démographique en 2013. In: Insee; 2015.

14. Lin G, So Y, Johnston G. Analyzing Survival Data with Competing Risks Using SAS® Software. In: Inc. SI, editor. SAS Global Forum: SAS Institute Inc. Cary, NC 27513; 2012. p. 1-8.

15. Onji M, Furukawa S. Significance of study on prevalence of AMA in healthy subjects. J Gastroenterol 2004;39:306-308.

16. Dahlqvist G, Corpechot C. [Are antimitochondrial antibodies the invariable hallmark of primary biliary cirrhosis?]. Presse Med 2014;43:1311-1313.

17. Metcalf JV, Bhopal RS, Gray J, Howel D, James OF. Incidence and prevalence of primary biliary cirrhosis in the city of Newcastle upon Tyne, England. Int J Epidemiol 1997;26:830-836.

18. Boberg KM, Aadland E, Jahnsen J, Raknerud N, Stiris M, Bell H. Incidence and prevalence of primary biliary cirrhosis, primary sclerosing cholangitis, and autoimmune hepatitis in a Norwegian population. Scand J Gastroenterol 1998;33:99-103.

19. 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.

20. Mattalia A, Quaranta S, Leung PS, Bauducci M, Van de Water J, Calvo PL, Danielle F, et al. Characterization of antimitochondrial antibodies in health adults. Hepatology 1998;27:656-661.

21. Omagari K, Rowley MJ, Whittingham S, Jois JA, Byron SL, Mackay IR. Autoantibodies to M2 mitochondrial autoantigens in normal human sera by immunofluorescence and novel assays. J Gastroenterol Hepatol 1996;11:610-616.

22. Turchany JM, Uibo R, Kivik T, Van de Water J, Prindiville T, Coppel RL, Gershwin ME. A study of antimitochondrial antibodies in a random population in Estonia. Am J Gastroenterol 1997;92:124-126.

23. Shibata M, Onozuka Y, Morizane T, Koizumi H, Kawaguchi N, Miyakawa H, Kako M, et al. Prevalence of antimitochondrial antibody in Japanese corporate workers in Kanagawa prefecture. J Gastroenterol 2004;39:255-259.

24. Liu HY, Wang LX, Liu YF. [Frequencies of autoantibodies specific for primary biliary cirrhosis in a general adult population group]. Zhonghua Gan Zang Bing Za Zhi 2008;16:922-925.

25. Howel D, Metcalf JV, Gray J, Newman WL, Jones DE, James OF. Cancer risk in primary biliary cirrhosis: a study in northern England. Gut 1999;45:756-760.

26. Jackson H, Solaymani-Dodaran M, Card TR, Aithal GP, Logan R, West J. Influence of ursodeoxycholic acid on the mortality and malignancy associated with primary biliary cirrhosis: a population-based cohort study. Hepatology 2007;46:1131-1137.

27. Floreani A, Franceschet I, Cazzagon N, Spinazze A, Buja A, Furlan P, Baldo V, et al. Extrahepatic autoimmune conditions associated with primary biliary cirrhosis. Clin Rev Allergy Immunol 2015;48:192-197.

28. Prince MI, Chetwynd A, Newman W, Metcalf JV, James OF. Survival and symptom progression in a geographically based cohort of paients with primary biliary cirrhosis: follow-up for up to 28 years. Gastroenterology 2002;123:1044-1051.

29. Jones DE, Hollingsworth K, Fattakhova G, MacGowan G, Taylor R, Blamire A, Newton JL. Impaired cardiovascular function in primary biliary cirrhosis. Am J Physiol Gastrointest Liver Physiol 2010;298:G764-773.

30. McDonald C, Newton J, Lai HM, Baker SN, Jones DE. Central nervous system dysfunction in primary biliary cirrhosis and its relationship to symptoms. J Hepatol 2010;53:1095-1100.

FIGURE LEGENDS

Figure 1. Map of the French immunology laboratories network. Sites of laboratories are represented as dark circles

Figure 2. Flow chart of the study

Figure 3. Distribution by age and gender of the incidence rate per 100,000 person-years of the patients with newly detected AMA (top) and of those with newly diagnosed PBC (down)

Figure 4. Actuarial (thick line) and Kaplan-Meier (thin line) survival curves of patients with newly detected AMA and non-established PBC compared to the actuarial survival curve of a standardized control population matched for age, gender and time period (dotted line)

Figure 5. Cumulative incidence curve of PBC (thick line) with 95% confidence interval boundaries (thin lines) in the subpopulation of patients with newly detected AMA and normal serum level of alkaline phosphatase at baseline

Supplementary Figure 1. Cumulative incidence curve of PBC (thick line) with 95% confidence interval boundaries (thin lines) in the subpopulation of patients with dot/blot test-confirmed AMA and normal serum level of alkaline phosphatase at baseline

Supplementary Figure 2. Comparison of PBC incidence rates between low (1:40; dotted curve) and high (≥ 1:80; plain curve) AMA-titre patients with normal serum level of alkaline phosphatase at baseline

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Table 1. Characteristics of AMA-positive patients with non-established PBC (n=229)

Variable	Available data	Median (range) / number (%)
Age (yr)	229	58 (15 – 90)
Female gender	229	179 (78%)
BMI ($kg.m^{-2}$)	92	24 (17 – 46)
Past history of AID	130	31 (24%)
Familial history of AID	102	11 (11%)
Clinical settings of AID	225	104 (46%)
AMA titre	229	1:160 (1:40 – 1:640 or higher)
Positive dot/blot test	150	137 (91%)
PBC-specific ANA	143	9 (6%)
Fatigue	148	82 (55%)
Pruritus	145	5 (3%)
Jaundice	145	6 (4%)
Ascites	144	3 (2%)
Total bilirubin (µmol/L)	110	10 (2 – 149)
ALP (× ULN)	119	0.7 (0.2 – 8.6)
GGT (× ULN)	127	1.1 (0.2 – 66.5)
ALT (× ULN)	130	0.6 (0.2 – 31.3)
IgM (× ULN)	41	0.6 (0.3 – 7.8)
Platelet (× 10^9/L)	119	247 (11 – 769)
Prothrombin index (%)	99	97 (20 – 110)
Liver biopsy	148	28 (19%)

Cirrhosis	229	13 (6%)

AID: autoimmune disease. ALP: alkaline phosphatase. ALT: alanine aminotransferase. AMA: antimitochondrial antibodies. ANA: antinuclear antibodies. BMI: body mass index. GGT: gamma-glutamyl transpeptidase. IgM: immunoglobulin M. PBC: primary biliary cholangitis.

Table 2. Comparison of established versus non-established PBC groups

	Established PBC group (n=247)		Non-established PBC group (n=229)		
Variable	**Available data**	**Results**	**Available data**	**Results**	***P***
Age (yr)	247	60 (20 – 91)	229	58 (15 – 90)	0.0567
Female gender	247	220 (89%)	229	179 (78%)	0.0012
BMI ($kg.m^{-2}$)	147	24 (17 – 45)	92	24 (17 – 46)	0.4440
History of AID	173	41 (24%)	130	31 (24%)	0.9168
Familial history of AID	144	13 (9%)	102	11 (11%)	0.8612
AMA titre*	247	5 (0 – 6)	229	3 (0 – 6)	<.0001
Positive dot/blot test	139	134 (96%)	150	137 (91%)	0.0551
PBC-specific ANA	142	18 (13%)	143	9 (6%)	0.0196
Fatigue	174	81 (47%)	148	66 (45%)	0.7253
Pruritus	172	41 (24%)	145	5 (3%)	<.0001
Jaundice	174	23 (13%)	145	6 (4%)	0.0050
Ascites	171	9 (5%)	144	3 (2%)	0.2365
Total bilirubin (µmol/L)	142	12.0 (2.0 – 357)	110	9.9 (2.0 – 149.0)	0.0358
ALP (× ULN)	161	1.7 (0.2 – 16.5)	119	0.7 (0.2 – 8.6)	<.0001
GGT (× ULN)	169	5.8 (0.3 – 71.9)	127	1.1 (0.2 – 66.5)	<.0001
ALT (× ULN)	166	1.4 (0.2 – 46.7)	130	0.6 (0.2 – 31.3)	0.2809
IgM (× ULN)	84	1.4 (0.2 – 5.5)	41	0.6 (0.3 – 7.8)	0.0069
Platelet (× 10^9/L)	157	249 (43 – 500)	119	247 (11 – 769)	0.9781
Prothrombin index (%)	152	100 (38 – 111)	99	97 (20 – 110)	0.1479
Liver biopsy	102	87 (85%)	148	28 (19%)	<.0001
Cirrhosis	168	21 (13%)	229	13 (6%)	0.0096

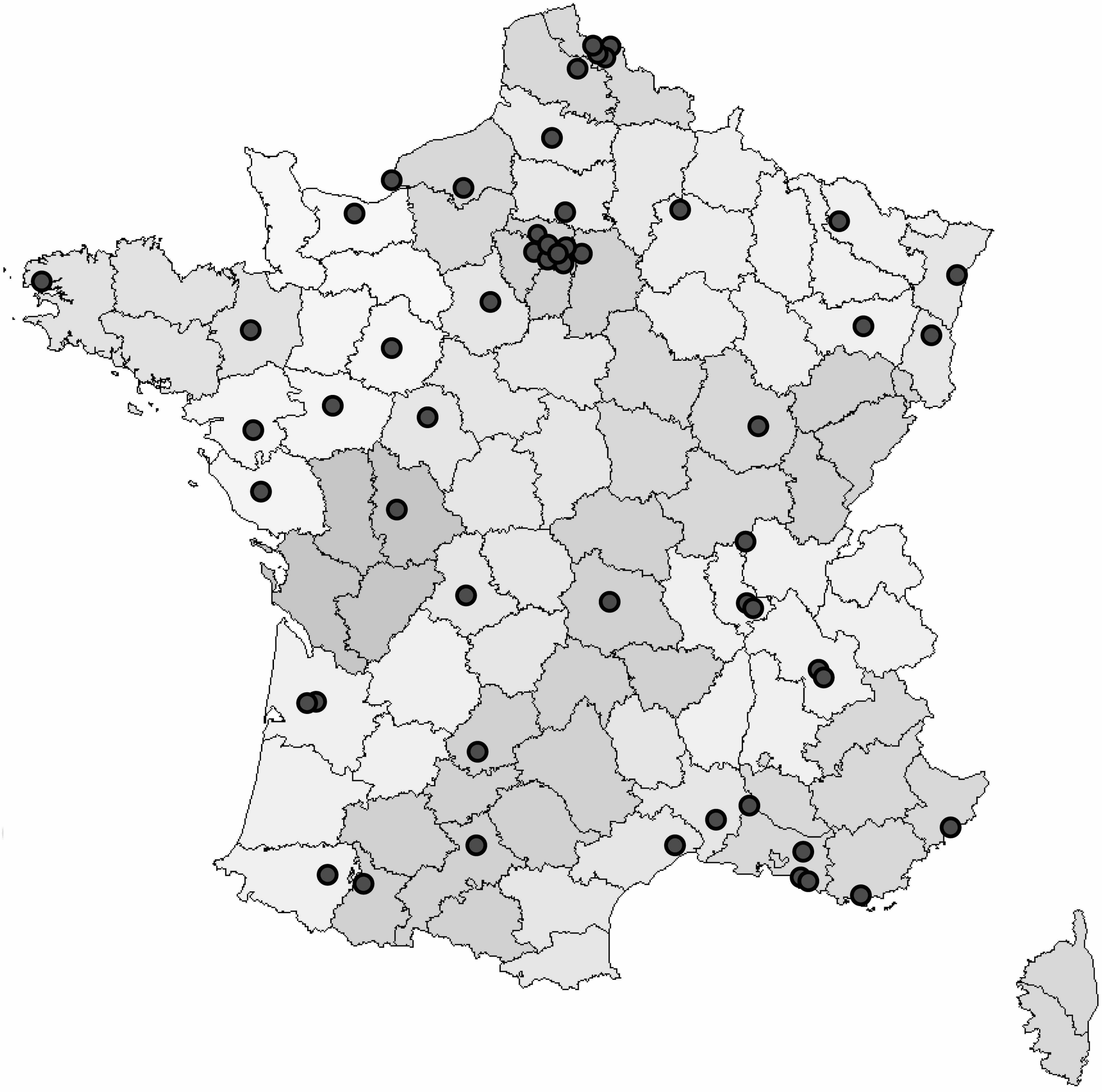
Variables are expressed as median (range) or number (% of available data). * AMA titre was evaluated according to the following semi-quantitative score: 1:40 = 1; 1:80 = 2; 1:160 = 3; 320 = 4; 1:640 = 5; > 1:640 = 6.

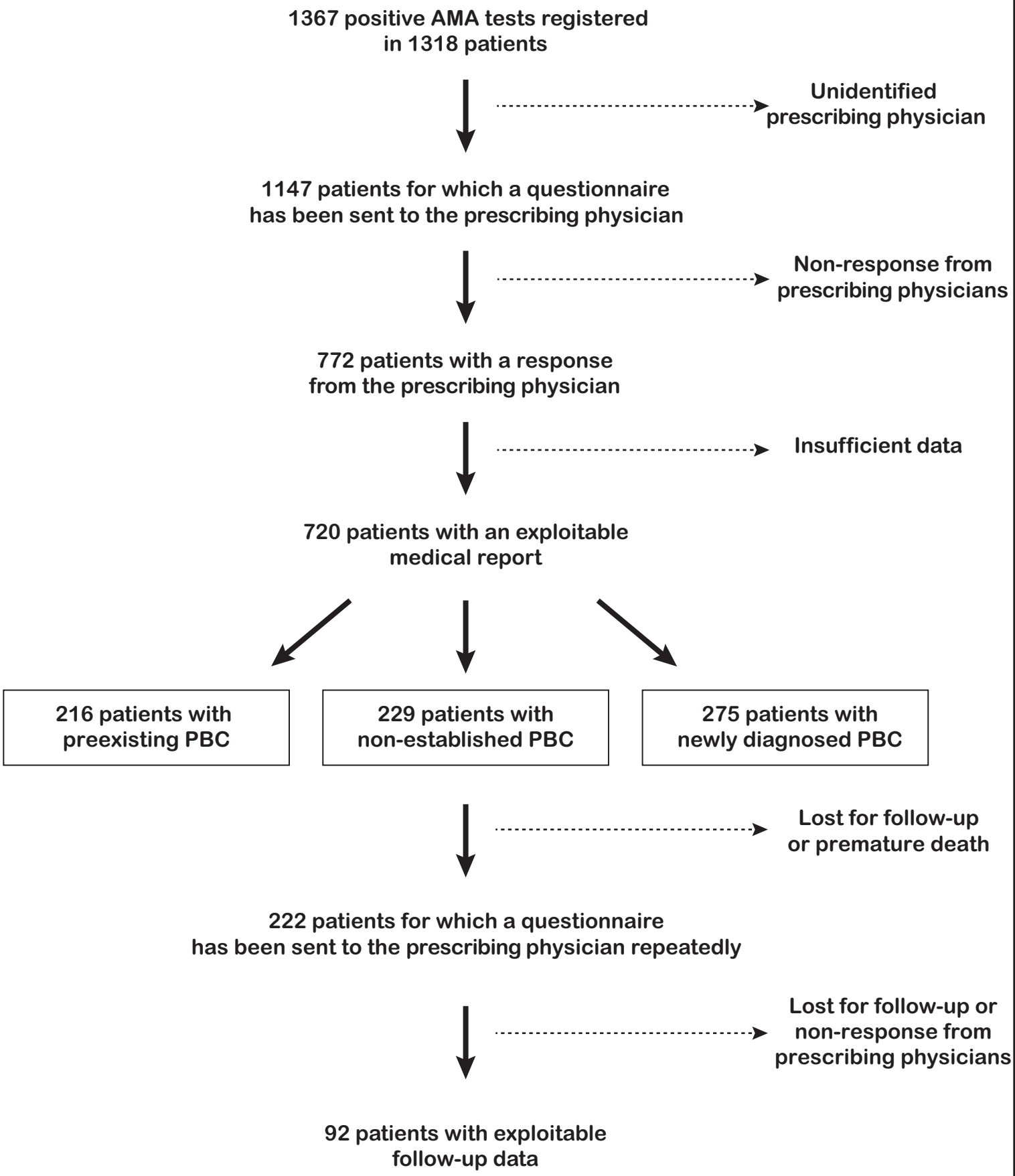
AID: autoimmune disease. ALP: alkaline phosphatase. ALT: alanine aminotransferase. AMA: antimitochondrial antibodies. ANA: antinuclear antibodies. BMI: body mass index. GGT: gamma-glutamyl transpeptidase. IgM: immunoglobulin M. PBC: primary biliary cholangitis.

Table 3. Univariate analysis of factors associated with PBC development in AMA-positive patients with normal alkaline phosphatase and available follow-up (n=66)

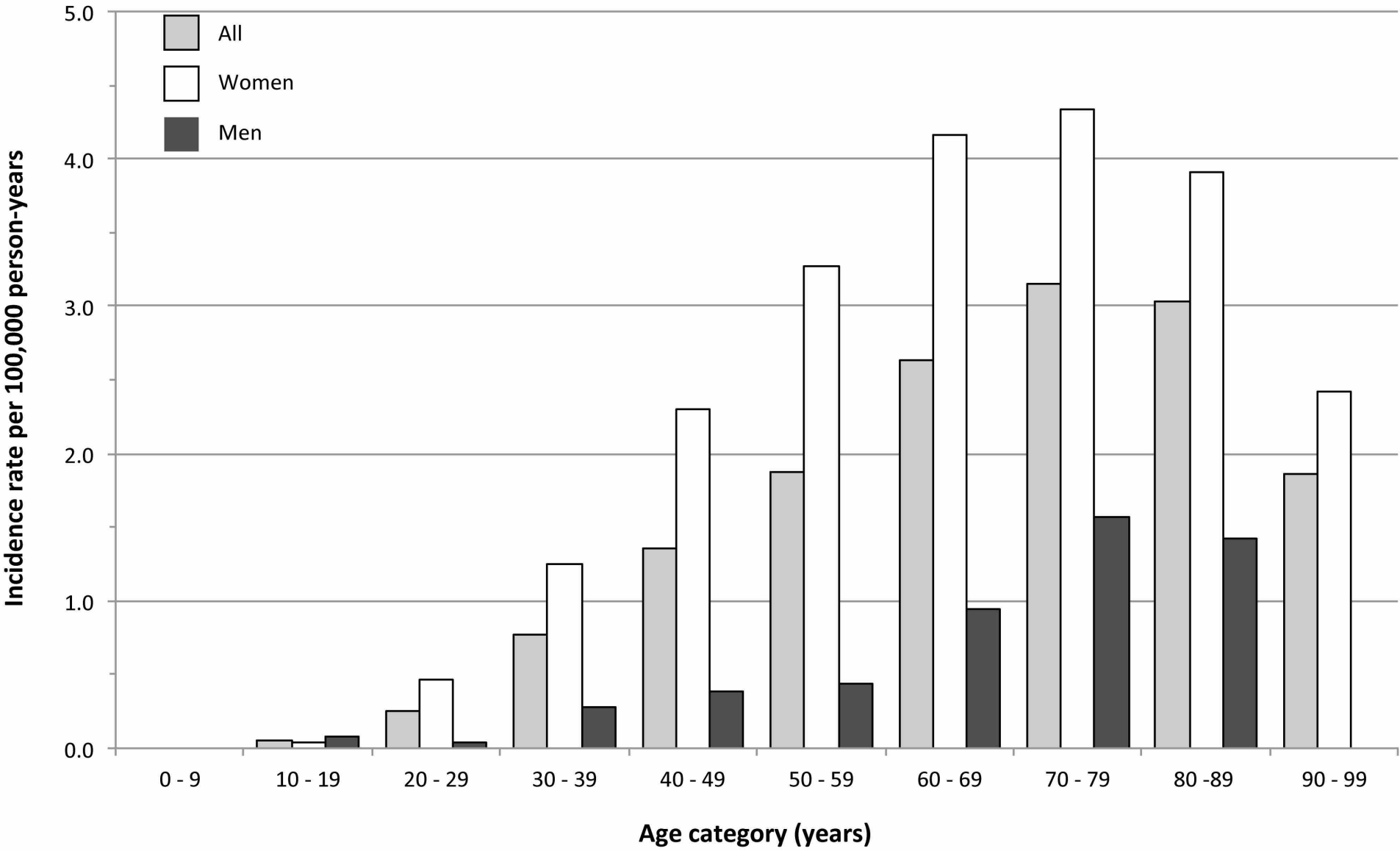
Variable	Hazard ratio (95% CI)	*P*
Age (yr)	0.98 (0.93 – 1.04)	0.4726
Male gender	0.44 (0.06 – 3.65)	0.4529
Past history of AID	0.54 (0.05 – 5.29)	0.5930
Familial history of AID	2.52 (0.26 – 24.46)	0.4245
AMA titre	1.19 (0.84 – 1.69)	0.3180
PBC-specific ANA	0.00 (0.00 – inf.)	0.9913
Total bilirubin (μmol/L)	0.92 (0.72 – 1.17)	0.4993
ALP (× ULN)	5.53 (0.00 – 40769)	0.7066
GGT (× ULN)	1.37 (0.79 – 2.40)	0.2631
ALT (× ULN)	0.91 (0.58 – 1.43)	0.6781
IgM (× ULN)	0.77 (0.19 – 3.06)	0.7093

AID: autoimmune disease. ALP: alkaline phosphatase. ALT: alanine aminotransferase. AMA: antimitochondrial antibodies. ANA: antinuclear antibodies. GGT: gamma-glutamyl transpeptidase. IgM: immunoglobulin M. PBC: primary biliary cholangitis.

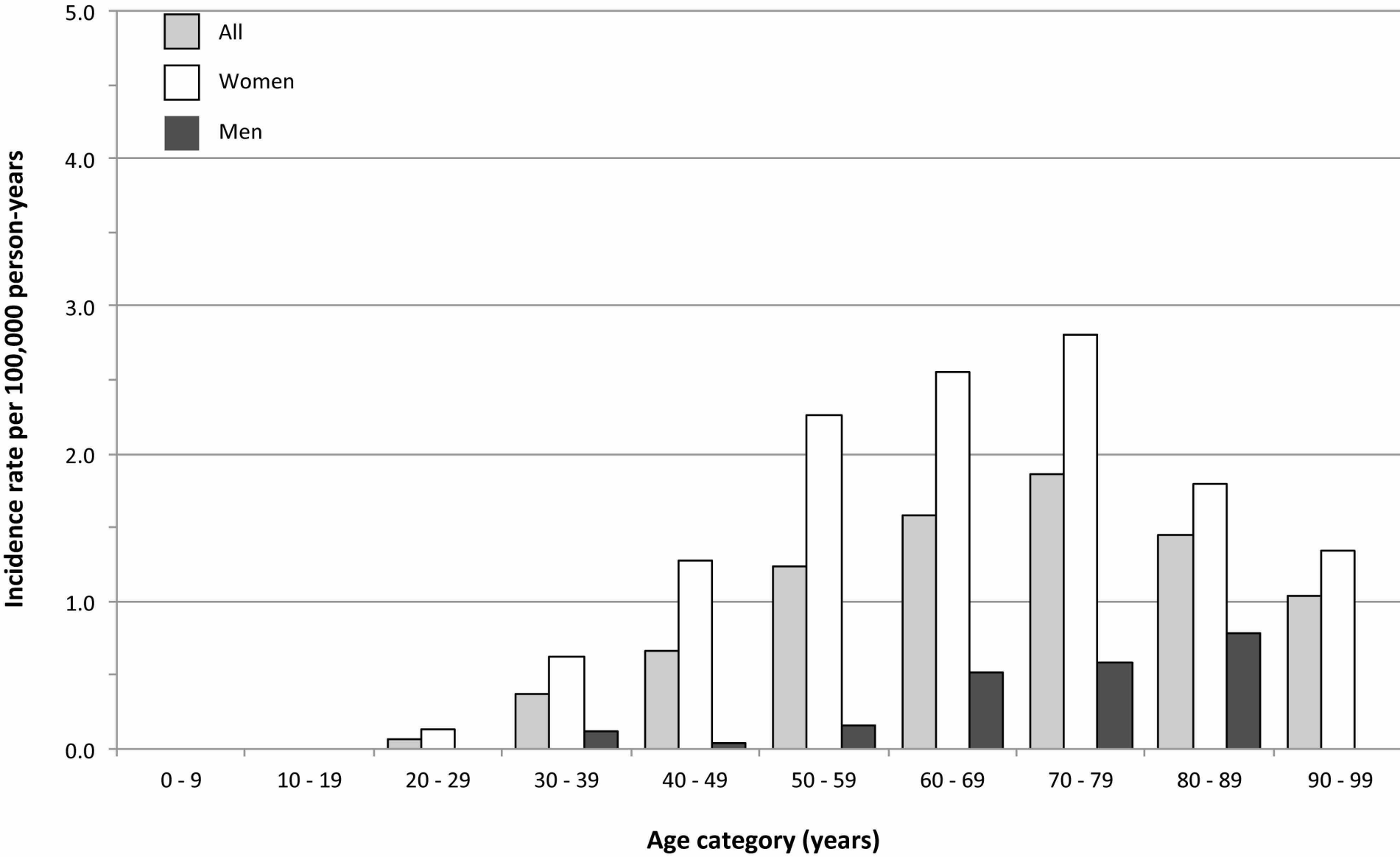


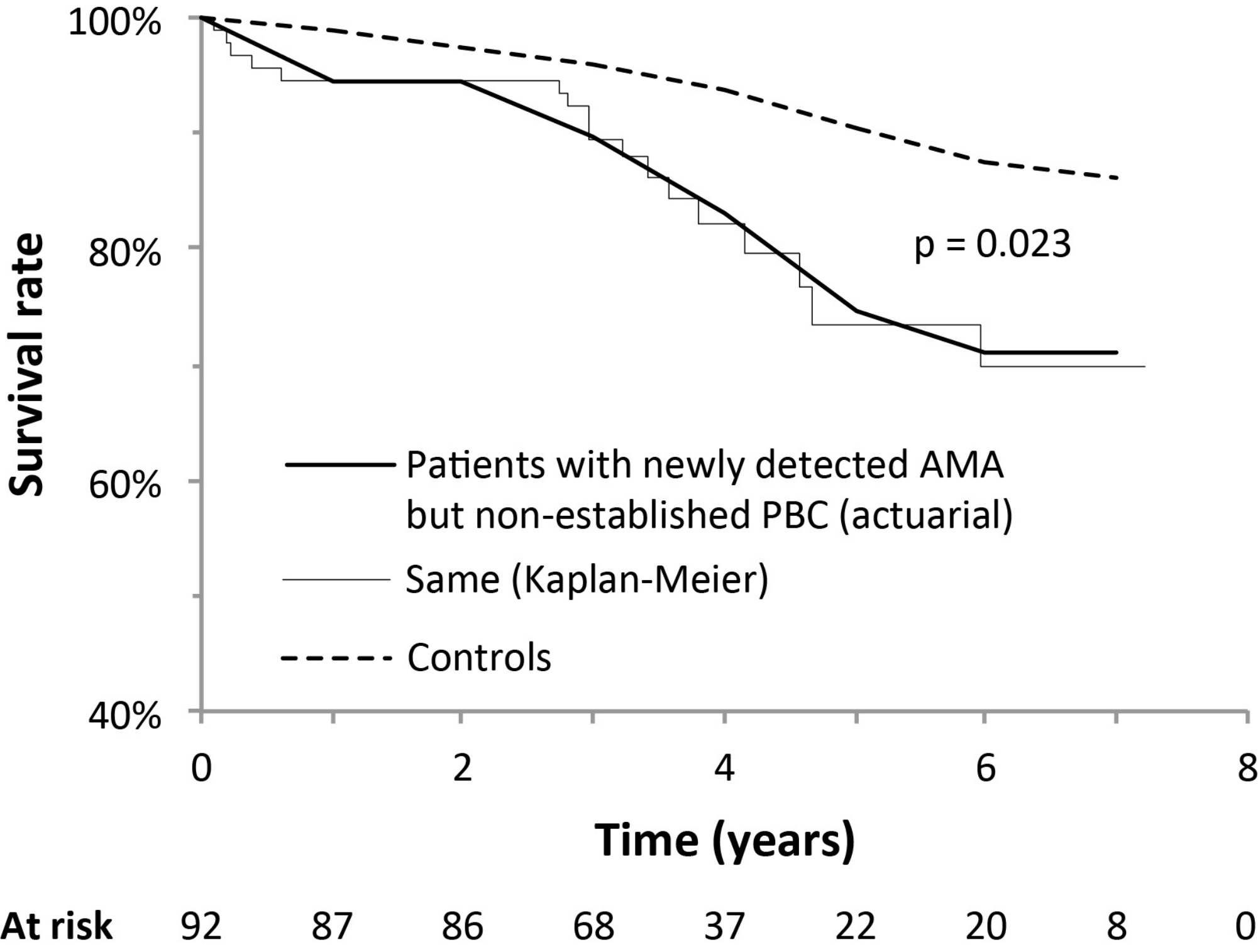

1367 positive AMA tests registered in 1318 patients
Unidentified prescribing physician
1147 patients for which a questionnaire has been sent to the prescribing physician
Non-response from prescribing physicians
772 patients with a response from the prescribing physician
Insufficient data
720 patients with an exploitable medical report
216 patients with preexisting PBC
229 patients with non-established PBC
275 patients with newly diagnosed PBC
Lost for follow-up or premature death
222 patients for which a questionnaire has been sent to the prescribing physician repeatedly
Lost for follow-up or non-response from prescribing physicians
92 patients with exploitable follow-up data

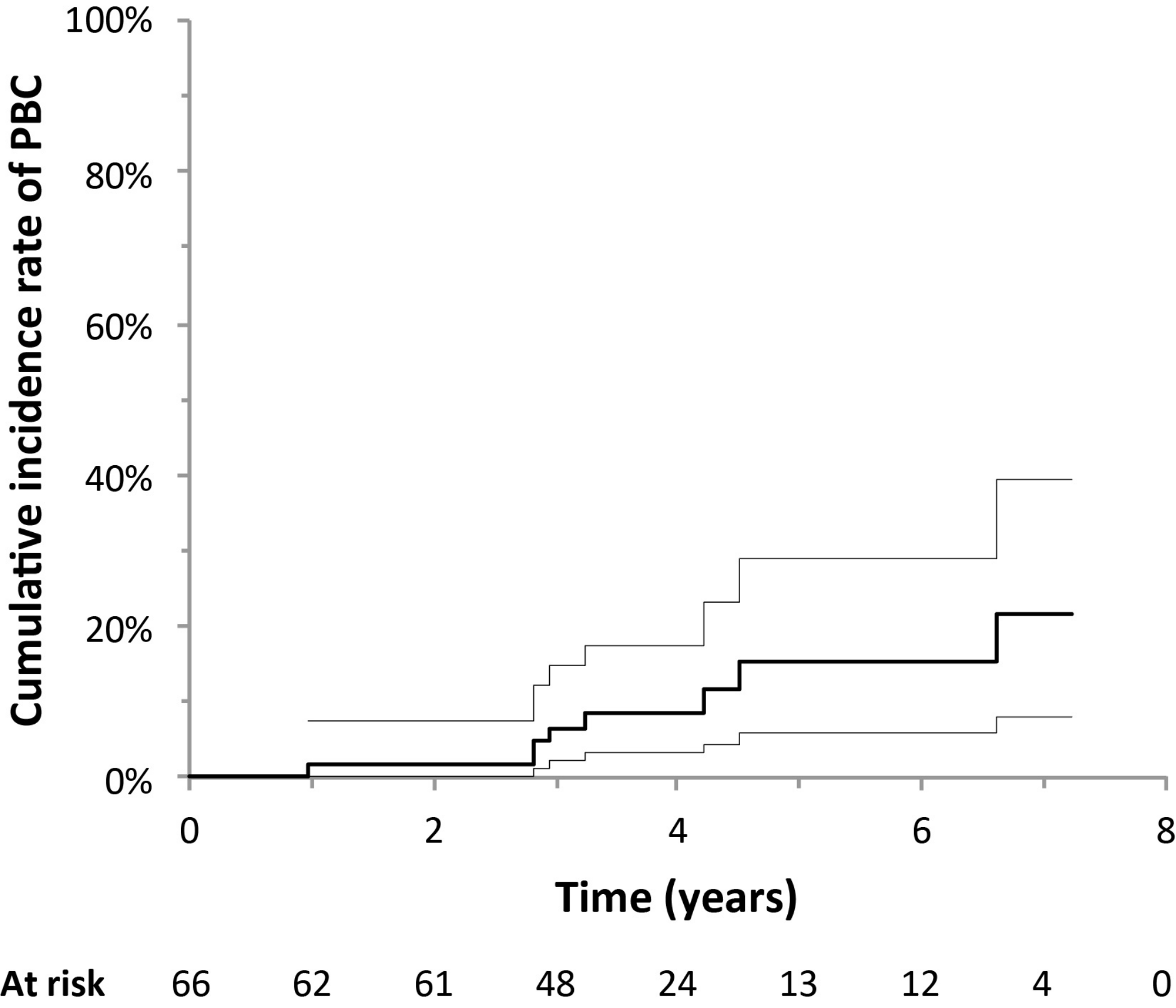
Patients with newly detected AMA



Patients with newly diagnosed PBC




100%
80%
60%
40%
Survival rate
p = 0.023
Patients with newly detected AMA but non-established PBC (actuarial)
Same (Kaplan-Meier)
Controls
0
2
4
6
8
Time (years)
At risk
92
87
86
68
37
22
20
8
0


100%
80%
60%
40%
20%
0%
Cumulative incidence rate of PBC
0
2
4
6
8
Time (years)
At risk
66
62
61
48
24
13
12
4
0

Supplementary Table 1. Items of the first questionnaire

1	Diagnosis of PBC: y/n/unk
2	If yes, date of diagnosis: yyyy/mm/dd
3	If no or unknown, disease condition for which AMA test was prescribed: plain text
4	Personal past history of autoimmune disease(s): y/n
5	If yes, which disease(s): plain text
6	Familial past history of autoimmune disease(s): y/n
7	If yes, which disease(s): plain text
8	Any other significant comorbity(ies): plain text
9	Weight (kg): number
10	Height (cm): number
11	Fatigue: y/n/unk
12	Pruritus: y/n/unk
13	Hepatomegaly: y/n/unk
14	Jaundice: y/n/unk
15	Ascites: y/n/unk
16	Total bilirubin (μmol/L): number
17	Alkaline phosphatases (U/L): number
18	Gamma glutamyltranspeptidase (U/L): number
19	Alanine aminotransferase (U/L): number
20	Platelet count (10^9/L): number
21	Prothrombin index (%): number
22	IgM level (g/L): number
23	Liver biopsy performed: y/n/unk
24	If liver biopsy performed, destructive cholangitis: y/n/unk
25	If liver biopsy performed, portal inflammation: y/n/unk
26	If liver biopsy performed, hepatic granulomas: y/n/unk
27	If liver biopsy performed, ductular reaction: y/n/unk
28	If liver biopsy performed, ductopenia: y/n/unk
29	If liver biopsy performed, bridging fibrosis: y/n/unk
30	If liver biopsy performed, cirrhosis: y/n/unk
31	If liver biopsy performed, Ludwig's or Scheuer's histological stage: 1/2/3/4/unk
32	Transient elastography (kPa): number
33	Oesophageal varices: y/n/unk
34	Initiation of ursodeoxycholic acid (UDCA) therapy: y/n/unk
35	Any other significant medical therapy(ies): plain text
36	Any comments: plain text

Y: yes. N: no. Unk: unknown.

Supplementary Table 2. Items of the second questionnaire

1	Patient still alive at last follow-up: y/n/unk
2	If patient died, date of death: yyyy/mm/dd
3	If patient died, cause of death: plain text
4	Patient lost to follow-up: y/n/unk
5	If patient lost to follow-up, date of last follow-up: yyyy/mm/dd
6	Diagnosis of PBC since last questionnaire: y/n/unk
7	If diagnosis of PBC, date of diagnosis: yyyy/mm/dd
8	AMA test confirmed since last questionnaire: y/n/unk
9	If AMA confirmed, date of confirmation: yyyy/mm/dd
10	If AMA confirmed, titre of AMA: number
11	Pruritus since last questionnaire: y/n/unk
12	Jaundice since last questionnaire: y/n/unk
13	Increase in alkaline phosphatases (ALP) since last questionnaire: y/n/unk
14	Increase in gamma glutamyltranspeptidase (GGT) since last questionnaire: y/n/unk
15	Increase in alanine aminotransferase (ALT) since last questionnaire: y/n/unk
16	Increase in IgM serum level since last questionnaire: y/n/unk
17	Liver biopsy performed since last questionnaire: y/n/unk
18	If liver biopsy performed, date of biopsy: yyyy/mm/dd
19	If liver biopsy performed, histological lesion suggestive of PBC: y/n/unk
20	If liver biopsy performed, histological lesion compatible with PBC: y/n/unk
21	Any comments: plain text

Y: yes. N: no. Unk: unknown.

Supplementary Table 3. Range of total and positive AMA tests registered over the census period in 28 out of the 63 laboratories participating in the study (laboratories were sorted in descending order of total tests performed)

Laboratory	Total No. of AMA tests performed	No. of positive tests	% positive tests
Mérieux. Lyon	18500	429	2.32
Saint-Antoine. Paris	9100	104	1.14
Grenoble	3446	20	0.58
Bichat, Paris	1994	26	1.30
Rennes	1571	13	0.83
Bordeaux	1493	43	2.88
Strasbourg	1419	34	2.40
Dijon	1418	12	0.85
Toulouse	1374	30	2.18
Montpellier	1296	10	0.77
Le Mans	1210	3	0.25
SMCB, Vizille	688	20	2.91
Pasteur, Lille	650	20	3.08
Henri Mondor, Créteil	594	9	1.52
Nantes	532	6	1.13
Poitier	359	10	2.79
Angers	344	9	2.62
Colmar	253	6	2.37
Pau	217	7	3.23
Creil	212	1	0.47
Argenteuil	198	8	4.04
Brest	187	17	9.09
Epinal	120	2	1.67
Béthune	116	2	1.72
Bry sur Marne	112	3	2.68
La Roche sur Yon	98	1	1.02
Lagny sur Marne	89	4	4.49
Ambroise Paré, Paris	64	7	10.94

Supplementary Table 4. Clinical settings in which AMA-M2 were evidenced

ID number	Non-hepatic autoimmune diseases
96	Systemic lupus erythematosus
122	Connective tissue disease (without further qualification)
140	Crohn's disease
143	Ankylosing spondylitis
168	Systemic lupus erythematosus
156	Type 1 diabetes
205	Multiple sclerosis
207	Systemic lupus erythematosus with antiphospholipid antibody syndrome
220	Periarteritis nodosa, MODY type 2 diabetes with chronic renal failure
221	Nonprogressive scleroderma
224	Type 1 diabetes, atopy
230	Sarcoidosis
234	Rheumatoid polyarthritis
253	Systemic lupus erythematosus, histologically proven cirrhosis
302	Systemic lupus erythematosus
380	Arthritis
382	Polyarthritis, type 1 diabetes
412	Type 1 diabetes
416	Multiple sclerosis
423	Kidney autoimmune disease
433	Sjögren's syndrome
442	Sjögren's diesase
447	Polymyalgia rheumatica
465	Stroke, systemic lupus erythematosus
492	Autoimmune thyroiditis
500	CREST syndrome
501	Systemic lupus erythematosus, type 1 diabetes
507	Fibromyalgia, Sjögren's syndrome
508	Lupus
513	Pemphigus vulgaris
515	Multiple sclerosis, Still's disease
532	Rheumatic disease
536	Biermer's disease, ischemic and valvular heart disease
546	Inflammatory rheumatic disease, endocarditis
551	Systemic lupus erythematosus
567	Sicca syndrome, goitre
588	CREST syndrome
619	Polymyalgia rheumatica
636	Bullous pemphigoid
691	Idiopathic thrombocytopenic purpura
699	CREST syndrome
713	Type 1 diabetes, poliomyelitis sequelae
718	CREST and Sjögren's syndrome
743	Sjögren's syndrome
752	Systemic lupus erythematosus, antiphospholipid antibody syndrome

759	Subacute lupus
769	Uncategorized connective tissue disease
773	CREST syndrome
788	Lupus erythematosus
841	Type 1 diabetes, IgA deficiency
827	Mixed connective tissue disease
832	Hemorrhagic rectocolitis
843	Polymyalgia rheumatica
851	Systemic lupus erythematosus
858	Cutaneous lupus, alopecia areata
861	Mixed connective tissue disease
864	Sjögren's syndrome
865	Systemic lupus erythematosus, autoimmune haemolytic anemia
868	Fibromyalgia
877	Systemic lupus eryhrmatosus
878	Ankylosing spondylarthritis
880	Idiopathic thrombocytopenic purpura
894	Rheumatoid polyarthritis, Felty's syndrome
924	Crohn's disease
936	Necrotizing angiitis, polyneuropathy
959	Hashimoto's disease
963	Bullous pemphigoid
969	Idiopathic thrombocytopenic purpura
981	Uncategorized rheumatism
1000	Suspected multiple sclerosis, diabetes
1006	Sclerodermia, Raynaud's syndrome
1018	Sjögren's syndrome, transient ischemic attack
1061	Ankylosing spondylarthritis
1065	Fibromyalgia
1069	Biermer's anemia
1076	Lupus erythematosus, chronic renal failure
1096	Lupus-like connective tissue disease
1112	Mild hypothyroidism
1121	Sjögren's syndrome
1162	Raynaud's syndrome
1165	Hashimoto's disease, liver tests abnormalities
1168	Bullous pemphigoid, hypothyroidism
1195	Vasculitis
1212	Autoimmune glomerulonephritis
1265	Multiple sclerosis
1314	Polyarthitis
1337	Antiphospholipid antibody syndrome, vasculitis
1349	Sjögren's syndrome, Hashimoto's disease
1435	Polyarthritis
1457	Systemic scleroderma, recent myocardial infarction
1461	Hashimoto's disease, arterial hypertension, type 2 diabetes
1475	Inflammatory polyarthritis, breast cancer in remission
1479	Sjögren's syndrome, ulcerative colitis

1493	Sjögren's syndrome

ID number	Primary hepatic abnormalities or diseases
118	Steatohepatitis with cirrhosis
131	Liver tests abnormalities, prostate adenoma
161	Liver tests abnormalities, Alzheimer's disease
165	Drug-induced liver injury, epilepsy
176	Liver tests abnormalities, arterial hypertension,
204	Cirrhosis and thromboembolic disease in a young man
231	Possible autoimmune hepatitis
252	Steatohepatitis
267	Autoimmune hepatitis, Sjögren's syndrome
274	Inactive hepatitis B virus infection
277	Acute alcoholic hepatitis, cirrhosis, hepatocarcinoma
293	Cryptogenic cirrhosis, mitral valve stenosis
310	Liver tests abnormalities
337	Abdominal pain, liver tests abnormalities
402	Alcoholic cirrhosis
417	Autoimmune cirrhosis
514	Chronic hepatitis with interface hepatitis at liver biopsy
516	Budd-Chiari's disease, hepatocarcinoma
531	Liver tests abnormalities
554	Anicteric cholestasis
557	Autoimmune hepatitis, fibromyalgia
558	Anicteric cholestasis, primary ovarian failure
561	Hepatitis C virus infection
639	Chronic hepatitis C
643	Chronic hepatitis C, chronic renal failure, hemodialysis
646	Chronic hepatitis C
648	Chronic hepatitis C
836	Autoimmune hepatitis
850	Liver neoplasia, diabetes
899	Unexplained cholestasis, pulmonary arterial hypertension
911	Toxic hepatitis, cryptogenic cirrhosis
915	Drug-induced liver injury
940	Chronic hepatitis C
979	Liver allograft for alcoholic cirrhosis
983	Unexplained cholestasis, severe steroid dependent asthma
991	Chronic hepatitis C
995	Regenerative nodular hyperplasia
1012	Hepatocarcinoma (no further details), arrhythmia
1022	Autoimmune hepatitis
1033	Chronic increase in gamma-glutamyltranspeptidase activity
1050	Autoimmune hepatitis, prostate adenoma
1052	Liver tests abnormalities
1099	Autoimmune hepatitis
1150	Chronic hepatitis C

1152	Dysmetabolic hepatosiderosis
1154	Alcoholic cirrhosis
1160	Hepatic polyadenoma, steatosis, obesity
1170	Acute hepatitis A
1177	Chronic hepatitis B and D
1210	Drug-induced liver injury
1229	Elevated transaminases, hyperthyroidism
1278	Liver tests abnormalities
1311	Autoimmune hepatitis
1323	Liver steatosis, arterial hypertension
1343	Gallbladder stones
1436	Autoimmune hepatitis
1484	Alcoholic cirrhosis
1500	Alcoholic cirrhosis, pulmonary arterial hypertension
ID number	**Non-hepatic oncological diseases**
98	Non Hodgkin lymphoplasmocytic lymphoma
112	Primary bronchial adenocarcinoma with liver metastases
144	Small bowel carcinoid tumor with liver metastases
276	Non Hodgkin lymphoma, dysglobulinemia
429	Bone marrow allograft versus Host disease
480	Monoclonal gammopathy, tachyarrhythmia,
539	Multiple myeloma
634	Mycosis fongoides (cutaneous T-cell lymphoma)
677	Bone marrow allograft for acute myeloblastic leukemia
724	Adenocarcinoma (without further qualification), CREST syndrome
750	Hairy cell leukemia
845	Primary bronchial cancer, alcoholic cirrhosis
860	Uterine sarcoma
891	Metastatic melanoma, Gougero-Sjögren's syndrome
982	Undifferentiated adenocarcinoma, uncategorized rheumatism
1100	Metastatic pulmonary neoplasia, pruritus
ID number	**Non-hepatic infectious diseases**
172	Secondary syphilis
211	Infectious mononucleosis
538	Human immunodeficiency virus infection
740	Syphilis
802	Recurrent pneumonia, Parkinson's disease
1432	Syphilis, severe acute colitis, liver tests abnormalities
1460	Pyosalpinx, peritonitis
ID number	**Other disease settings**
88	Heart failure, mitral valve stenosis
106	Raynaud's phenomenon, chronic cough
123	Colonic polyps
157	Pancytopenia

237	Spastic paraparesia
243	Corticobasale degenerescence, hyperferritinemia
287	Amenorrhea
370	Miscarriage
410	Acute coronary syndrome
432	Nodular skin lesions
461	Pulmonary embolism, chronic pancreatitis
506	Kidney allograft
510	Axonal sensitive neuropathy
529	Atypical Raynaud's syndrome
545	Ischemic heart disease
570	Nephroangiosclerosis with chronic renal failure
583	Alcoholism
587	Chronic renal failure
613	Inflammatory syndrome
616	No information
623	Stroke
635	Abdominal pain
638	Kidney allograft for polycystic kidney disease
642	Acute ileitis
645	Chronic renal failure
652	Chronic respiratory failure, obesity
669	Lower limbs pain
695	Cushing's syndrome, renal failure
701	Epilepsy
703	Raynaud's syndrome
728	Suspected but not confirmed Sjögren's syndrome
761	Hip monoarthritis
785	Polyalgia
796	Pulmonary embolism, thrombocytopenia
856	Vestibular neuronitis
879	Kidney allograft
917	Distal polyneuropathy
952	Thromboembolic disease
976	Type 2 diabetes, arrhythmia
987	Inflammatory anemia
997	Dyslipidemia
1025	Pulmonary embolism
1055	Unexplained infertility
1132	Metabolic syndrome
1140	Metabolic syndrome
1293	No information
1373	No information
1446	No information
1458	Nephroangiosclerosis
1459	Corneal ulcer
1462	Drug eruption
1490	Hyperuricemia, no further information

1491	Urticaria, deep edema
1497	Osteoarthritis

Supplementary Table 5. Comparison of negative versus positive dot/blot test (DBT) groups

	Negative DBT group (n=13)		Positive DBT group (n=137)		
Variable	**Available data**	**Results**	**Available data**	**Results**	***P***
Age (yr)	13	45 (17 – 87)	137	61 (15 -90)	0.0614
Female gender	13	9 (70%)	137	107 (78%)	0.4924
BMI ($kg.m^{-2}$)	6	24 (21 – 31)	50	23 (17 – 46)	0.2130
History of AID	7	1 (14%)	80	23 (29%)	0.6680
Familial history of AID	6	0 (0%)	56	6 (11%)	1.0000
AMA titre*	13	3 (1 – 6)	137	3 (1 – 6)	0.7601
PBC-specific ANA	8	0 (0%)	97	9 (9%)	1.0000
Fatigue	9	3 (33%)	91	36 (40%)	1.0000
Pruritus	9	0 (0%)	90	5 (6%)	1.0000
Jaundice	9	0 (0%)	89	2 (2%)	1.0000
Ascites	9	0 (0%)	89	2 (2%)	1.0000
Total bilirubin (µmol/L)	9	10 (4 – 32)	69	10 (2 – 67)	0.3301
ALP (× ULN)	9	0.7 (0.3 – 2.5)	74	0.7 (0.2 – 8.6)	0.2614
GGT (× ULN)	9	1.4 (0.5 – 16.6)	77	0.8 (0.2 – 34.0)	0.1119
ALT (× ULN)	9	0.9 (0.6 – 6.0)	79	0.6 (0.2 – 5.4)	0.0114
IgM (× ULN)	2	0.6 (0.5 – 0.6)	29	0.5 (0.3 – 7.8)	0.9358
Platelet (× 10^9/L)	9	225 (26 – 347)	73	250 (11 -769)	0.0839
Prothrombin index (%)	6	77 (72 – 100)	61	98 (22 – 110)	0.0205
Liver biopsy	9	0 (0%)	91	14 (15%)	0.3524
Cirrhosis	13	0 (0%)	137	4 (3%)	1.0000

Variables are expressed as median (range) or number (% of available data). * AMA titre was evaluated according to the following semi-quantitative score: 1:40 = 1; 1:80 = 2; 1:160 = 3; 320 = 4; 1:640 = 5; > 1:640 = 6.

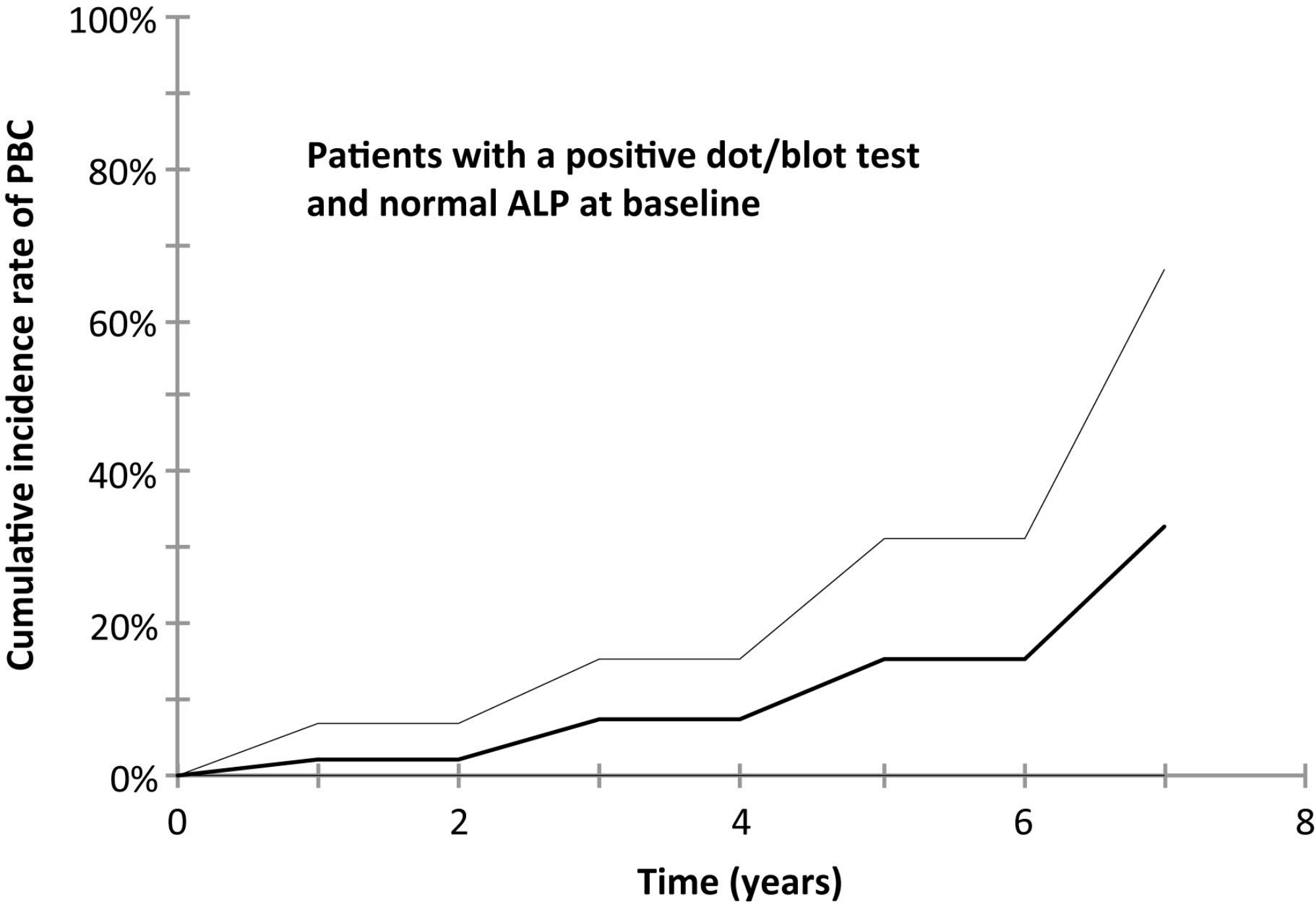
AID: autoimmune disease. ALP: alkaline phosphatase. ALT: alanine aminotransferase. AMA: antimitochondrial antibodies. ANA: antinuclear antibodies. BMI: body mass index. DBT: dot/blot test. GGT: gamma-glutamyl transpeptidase. IgM: immunoglobulin M. PBC: primary biliary cholangitis.

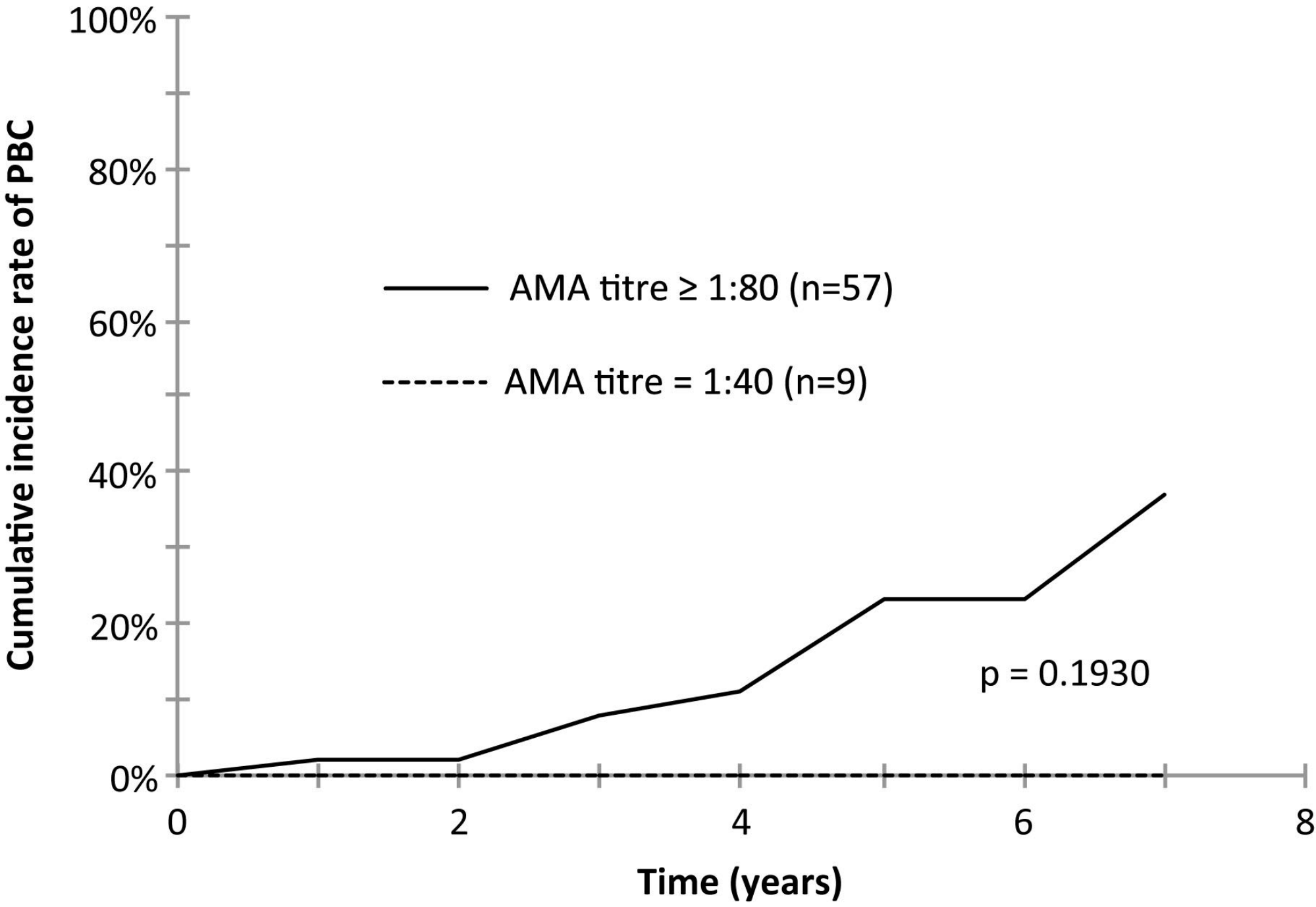
Table 2. Comparison of low (=1:40) versus significant (≥1:80) AMA titre groups

	Low titre (1:40) group (n=36)		Significant titre (≥1:80) group (n=193)		
Variable	**Available data**	**Results**	**Available data**	**Results**	***P***
Age (yr)	36	58 (15 – 90)	193	59 (28 – 90)	0.9508
Female gender	36	26 (72%)	193	153 (79%)	0.3471
BMI ($kg.m^{-2}$)	16	24 (18 – 38)	76	23 (17 – 46)	0.6879
History of AID	23	6 (26%)	105	25 (24%)	0.8174
Familial history of AID	15	2 (13%)	87	9 (10%)	0.6631
Positive dot/blot test	26	25 (96%)	124	112 (90%)	0.4679
PBC-specific ANA	25	3 (12%)	118	6 (5%)	0.1923
Fatigue	23	15 (65%)	125	51 (41%)	0.0304
Pruritus	23	2 (9%)	122	3 (2%)	0.1784
Jaundice	24	2 (8%)	121	4 (3%)	0.2589
Ascites	23	0 (0%)	121	3 (2%)	1.0000
Total bilirubin (µmol/L)	18	11 (5 – 125)	92	10 (2 – 149)	0.8235
ALP (× ULN)	18	0.6 (0.2 – 4.6)	101	0.7 (0.2 – 8.6)	0.3345
GGT (× ULN)	22	1.1 (0.2 – 66.5)	105	1.1 (0.3 – 34.0)	0.4733
ALT (× ULN)	21	0.7 (0.2 – 13.0)	109	0.6 (0.2 – 31.3)	0.7539
IgM (× ULN)	6	0.6 (0.3 – 1.6)	35	0.7 (0.3 – 7.8)	0.4827
Platelet (× 10^9/L)	19	245 (114 – 423)	100	248 (11 – 769)	0.7468
Prothrombin index (%)	16	91 (20 -100)	83	98 (22 – 110)	0.1580
Liver biopsy	23	7 (30%)	125	21 (17%)	0.1249
Cirrhosis	36	3 (8%)	193	9 (5%)	0.4084

Variables are expressed as median (range) or number (% of available data).

AID: autoimmune disease. ALP: alkaline phosphatase. ALT: alanine aminotransferase. AMA: antimitochondrial antibodies. ANA: antinuclear antibodies. BMI: body mass index. GGT: gamma-glutamyl transpeptidase. IgM: immunoglobulin M. PBC: primary biliary cholangitis.


Patients with a positive dot/blot test
and normal ALP at baseline
Cumulative incidence rate of PBC
100%
80%
60%
40%
20%
0%
0
2
4
6
8
Time (years)


100%
80%
60%
40%
20%
0%
Cumulative incidence rate of PBC
AMA titre ≥ 1:80 (n=57)
AMA titre = 1:40 (n=9)
p = 0.1930
0
2
4
6
8
Time (years)