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

Géraldine Dahlqvist, Farid Gaouar, Fabrice Carrat, Sofia Meurisse, Olivier Chazouillères, Raoul Poupon, Catherine Johanet, Christophe Corpechot

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AQ8 Géraldine Dahlqvist,[1,2] Farid Gaouar,[1] Fabrice Carrat,[3,4] Sofia Meurisse,[3,4] Olivier Chazouillères,[1,5] Raoul Poupon,[1,5] Catherine Johanet,[6] and Christophe Corpechot[1,5]; the French network of Immunology Laboratories

The prevalence, clinical characteristics, and outcomes of patients with antimitochondrial antibodies (AMAs), but no clinical evidence of primary biliary cholangitis (PBC), are largely unknown. A prospective study of AMA incidence was conducted through a nation-wide network of 63 French immunology laboratories. Clinical data from 720 of 1,318 AMA-positive patients identified in 1 year were collected. Patients were categorized as either newly diagnosed with PBC (n = 275), previously diagnosed with PBC (n = 216), or with nonestablished diagnosis of PBC (n = 229). The latter group was specifically evaluated. Follow-up data were collected for up to 7 years after detection of AMAs. 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 titer 1:160; extrahepatic autoimmune disorders 46%; normal serum alkaline phosphatases (ALP) 74%; ALP above 1.5 times the upper limit of normal 13%; and cirrhosis 6%. Compared to those newly diagnosed with PBC, the patients were slightly younger, had lower AMA titers, 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 sex ($P < 0.05$). *Conclusion:* Nearly half of the newly detected AMAs 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 AMAs 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. (HEPATOLOGY 2016; 00:000-000).

Primary biliary cholangitis (PBC) is a chronic cholestatic liver disease of unknown, but presumably autoimmune, origin characterized by elevated alkaline phosphatases (ALP) activity and presence of antimitochondrial antibodies (AMAs) in serum and highly suggestive histological lesions, namely, granulomatous or lymphocytic nonsuppurative 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]

Abbreviations: AID, autoimmune disease; AIH, autoimmune hepatitis; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMAs, antimitochondrial antibodies type 2; ANAs, antinuclear antibodies; CI, confidence interval; GGT, gamma-glutamyl transpeptidase; IF, immunofluorescence; IgM, immunoglobulin M; PBC, primary biliary cholangitis; UDCA, ursodeoxycholic acid; ULN, upper limit of normal.

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AMAs 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 AMAs.[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 extrahepatic autoimmune disorders, such as scleroderma, Sjögren's syndrome, or autoimmune thyroid disease, as well as in patients with hepatic or extrahepatic nonautoimmune disorders such as chronic hepatitis C or hematological malignancies.[6,7] In such a situation, longitudinal studies seem to indicate AMAs 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 AMAs 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 AMAs, but nonestablished diagnosis of PBC.

Patients and Methods

STUDY DESIGN AND POPULATION

This was a prospective, nation-wide, observational study conducted in France during May 2006-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 Fig. 1. F1

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 1-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 AMAs were first detected by indirect immunofluorescence (IF) on rat liver, kidney, and stomach tissue sections. Every antibody titer 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 immunodotting test commercial kits. The reference lab of Saint-Antoine hospital, Paris, used its own immunoblotting method, as described.[11] PBC-specific antinuclear antibodies (ANAs), that is, ANA with either rim-like/membranous or multiple nuclear dot IF patterns, were detected on Hep-2 cells. A titer 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 whether a

ARTICLE INFORMATION:

From the [1]Service d'Hépatologie, Centre de référence des Maladies Inflammatoires des Voies Biliaires (MIVB), Filière de Santé Maladies Rares du Foie de l'Adulte et de l'Enfant (FILFOIE), Hôpital Saint-Antoine, Assistance Publique–Hôpitaux de Paris (APHP), Paris, France; [2]Service d'Hépato-Gastroentérologie, Cliniques Universitaires Saint-Luc, Brussels, Belgium; [3]Service de Santé Publique, Hôpital Saint-Antoine, APHP, Paris, France; [4]Sorbonne Universités, INSERM, UPMC Université Paris 06, Institut Pierre Louis d'épidémiologie et de Santé Publique (IPLESP UMRS 1136), Paris, France; [5]UMR_S938, Faculté de Médecine Pierre et Marie Curie–Site Saint-Antoine, Université Pierre et Marie Curie–Paris 6, Paris, France; and [6]Laboratoire d'Immunologie, Hôpital Saint-Antoine, APHP, Paris, France. AQ2

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:

Christophe Corpechot, M.D.
Centre de référence des Maladies Inflammatoires des Voies biliaires,
Hôpital Saint-Antoine, Assistance Publique–Hôpitaux de Paris (APHP)
184 rue du Faubourg Saint-Antoine
75571 Paris Cedex 12, France
E-mail: christophe.corpechot@aphp.fr
Tel: +01 49 28 20 00 AQ3

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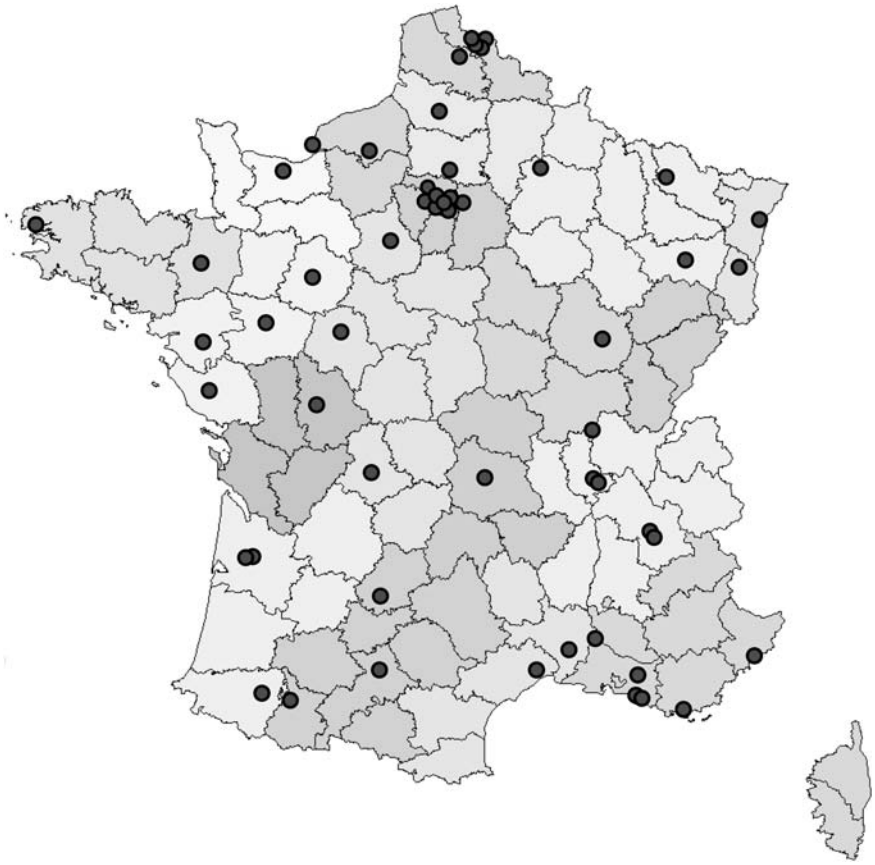

FIG. 1. Map of the French immunology laboratories network. Sites of laboratories are represented as dark circles.

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 Supporting Table S1. 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., preexisting case of PBC); and (3) patients nondiagnosed with PBC (i.e., nonestablished 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 Supporting Table S2. 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 AMAs and PBC were calculated by dividing the number of incident cases registered during the 1-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] Rates were calculated by sex and 10-year-age categories. Assuming that these rates were invariable in time, 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

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structure followed-up in Saint-Antoine hospital, Paris, between 1985 and 2005. Patient groups were compared using the Student *t* test or the Mann-Whitney U 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 death (a variable that was frequently missing), survival rates were calculated using an actuarial method (i.e., life table analysis) with 1-year constant intervals. A standardized population matched for age, sex, and follow-up period served as a control. Estimates of 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. Cumulative incidence function for PBC was estimated using a nonparametric method dealing with competing risk of death.[14] Excluded from this analysis were patients who had serum 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 and the National Commission on Informatics and Liberty before data collection.

Results

INCIDENCE AND PREVALENCE OF AMA-POSITIVE PATIENTS WITH OR WITHOUT ESTABLISHED PBC

A total of 1,367 positive AMA tests were registered in 1,318 patients during the 1-year census period of the study (Fig. 2). The total number of AMA tests performed over this period was highly variable according to the laboratories (range, 64-18,500; 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 [CI], 1.6-3.5; Supporting Table S3). The prescribing physician was solicited for 1,149 (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%) nonestablished cases of PBC (Fig. 2).

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

CHARACTERISTICS OF AMA-POSITIVE PATIENTS WITH NONESTABLISHED PBC

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 (median age, 58). The median titer of AMA was 1:160. Immunodotting/blotting tests, available in 150 (66%) patients, were positive in 91% of the tested individuals. Patients not tested with these second-line methods had similar AMA titers and demographic characteristics than those tested with (data not shown). The proportion of patients with PBC-specific ANAs 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 (AIDs) were systemic lupus erythematosus (n = 18; 8%), Sjögren's syndrome (n = 14; 6%), and autoimmune hepatitis (AIH; n = 10; 4%). In 12% of the cases, AMAs were found in the background of a nonautoimmune 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 AMAs were evidenced are available in Supporting Table S4. Regretfully, because of too few longitudinal data, persistence of AMA positivity was not assessable.

Complete or partial biochemical data were available in 130 (57%) patients. Median serum levels of total bilirubin, ALP, alanine aminotransferase (ALT), and immunoglobulin M (IgM) were all in the normal range, whereas gamma-glutamyl transpeptidase

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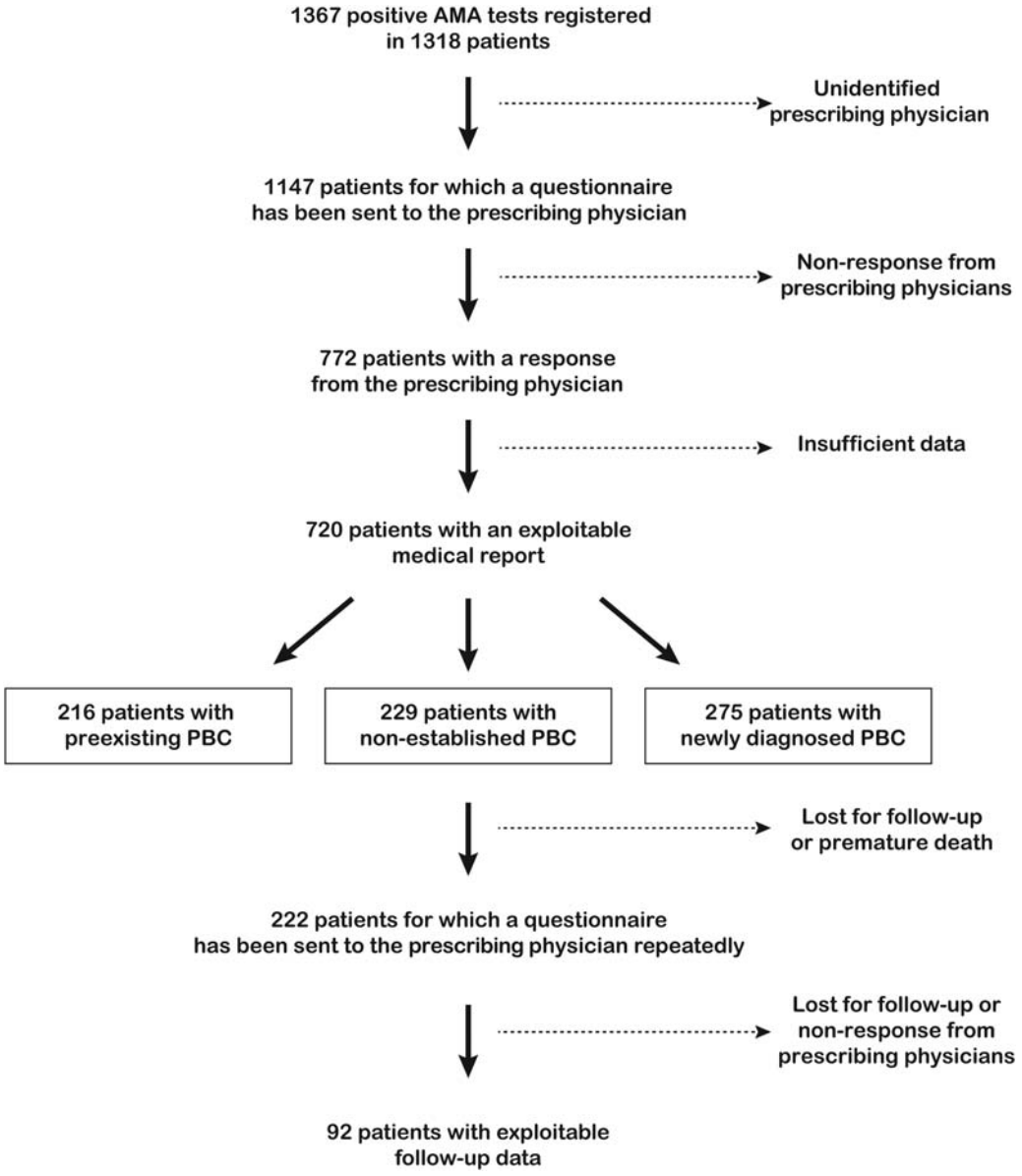

FIG. 2. Flow chart of the study.

(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 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 cases. A liver biopsy was performed in 28 (19%) of the 148 patients for which data were available. None of the histological reports were suggestive of PBC given that no bile duct lesions or granulomas were reported. Histological reports could include normal histology, mild portal inflammation, steatohepatitis, histological features compatible with AIH, 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), whereas no etiology was reported in 4 (30%). The patients with a negative immunodot/blot test (n = 13) had similar characteristics than those with a positive one (n=137; Supporting Table S5). Likewise, the patients with a low IF AMA titer

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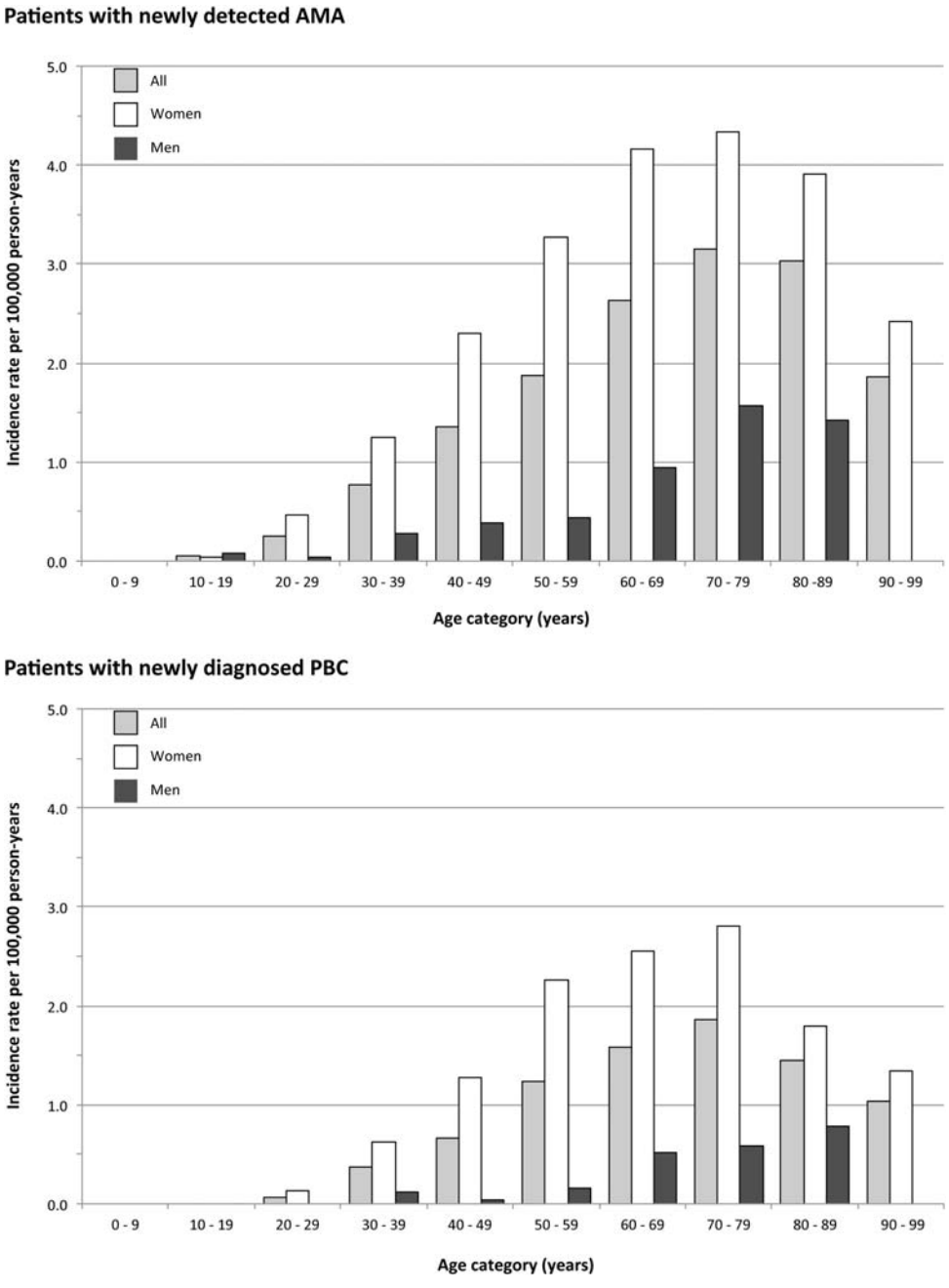

FIG. 3. Distribution by age and sex of the incidence rate per 100,000 person-years of the patients with newly detected AMAs (top) and of those with newly diagnosed PBC (down).

(=1:40; n = 36) did not differ significantly from those with a higher one (≥1:80; n = 193; Supplementary Table S6).

Patients were compared to those (n = 275; analyzable = 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. Patients had a significantly lower titer of AMAs, a lower proportion of PBC-specific ANAs, 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.

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TABLE 1. Characteristics of AMA-Positive Patients With Nonestablished PBC (n = 229)

Variable	Available Data	Median (Range)/ No. (%)
Age (years)	229	58 (15-90)
Female sex (%)	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 titer	229	1:160 (1:40-1:640 or higher)
Positive dotting/blotting test (%)	150	137 (91)
PBC-specific ANAs (%)	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 ($\times$ULN)	119	0.7 (0.2-8.6)
GGT ($\times$ULN)	127	1.1 (0.2-66.5)
ALT ($\times$ULN)	130	0.6 (0.2-31.3)
IgM ($\times$ULN)	41	0.6 (0.3-7.8)
Platelet ($\times 10^9$/L)	119	247 (11-769)
Prothrombin index (%)	99	97 (20-110)
Liver biopsy (%)	148	28 (19)
Cirrhosis (%)	229	13 (6)

Abbreviation: BMI, body mass index.

CLINICAL OUTCOMES OF AMA-POSITIVE PATIENTS WITH NONESTABLISHED PBC

Follow-up data were available in 92 (41%) of the 222 patients still alive at the end of the census period. Mean duration of follow-up was 4.0 $\pm$ 1.8 years (range, 0.5-7.3). 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%) of the 92 followed-up patients died. Median age at death was 73.6 years (range, 42.4-90.0). Causes of death were the following: non-liver-related cancers, 5 (bronchial squamous-cell carcinoma, 1; pulmonary adenocarcinoma, 1; and metastatic adenocarcinoma of undetermined origin 3); hematological malignancies 4 (non-Hodgkin's lymphoma, 2; acute myeloid leukaemia, 1; and multiple myeloma, 1); infectious diseases, 3 (lung infection, 2; infective endocarditis, 1); cardiovascular diseases, 2 (multifactorial heart failure, 1; chronic cor pulmonale, 1); digestive diseases, 3 (alcoholic chronic liver failure, 2; severe acute pancreatitis, 1); and undetermined cause, 3. No patients died officially from PBC. Actuarial and Kaplan-Meier survival

TABLE 2. Comparison of Established Versus Nonestablished PBC Groups

	Established PBC Group (n = 247)		Nonestablished PBC Group (n = 229)		
Variable	Available Data	Results	Available Data	Results	*P* Value
Age (years)	247	60 (20-91)	229	58 (15-90)	0.0567
Female sex (%)	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 titer*	247	5 (0-6)	229	3 (0-6)	<0.0001
Positive dotting/blotting test (%)	139	134 (96)	150	137 (91)	0.0551
PBC-specific ANAs (%)	142	18 (13)	143	9 (6)	0.0196
Fatigue (%)	174	81 (47)	148	66 (45)	0.7253
Pruritus (%)	172	41 (24)	145	5 (3)	<0.0001
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 ($\times$ULN)	161	1.7 (0.2-16.5)	119	0.7 (0.2-8.6)	<0.0001
GGT ($\times$ULN)	169	5.8 (0.3-71.9)	127	1.1 (0.2-66.5)	<0.0001
ALT ($\times$ULN)	166	1.4 (0.2-46.7)	130	0.6 (0.2-31.3)	0.2809
IgM ($\times$ULN)	84	1.4 (0.2-5.5)	41	0.6 (0.3-7.8)	0.0069
Platelet ($\times 10^9$/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)	<0.0001
Cirrhosis (%)	168	21 (13)	229	13 (6)	0.0096

Variables are expressed as median (range) or number (% of available data).
*AMA titer was evaluated according to the following semiquantitative score: 1:40 = 1; 1:80 = 2; 1:160 = 3; 320 = 4; 1:640 = 5; and >1:640 = 6.
Abbreviation: BMI: body mass index.

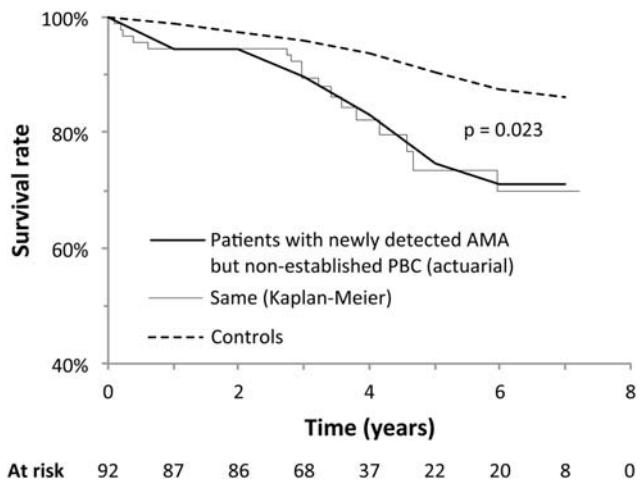

FIG. 4. Actuarial (thick line) and Kaplan-Meier (thin line) survival curves of patients with newly detected AMAs and nonestablished PBC compared to the actuarial survival curve of a standardized control population matched for age, sex, and time period (dotted line).

curves are shown in Fig. 4. The 1-, 3-, and 5-year rates of survival were 95% (95% CI, 92-98), 90% (95% CI, 85-96), and 75% (95% CI, 63-87), respectively. These rates were significantly lower than those expected in the control population, specifically, 99% (95% CI, 97-100), 96% (95% CI, 92-100), and 90% (95% CI, 83-98), respectively ($P = 0.023$).

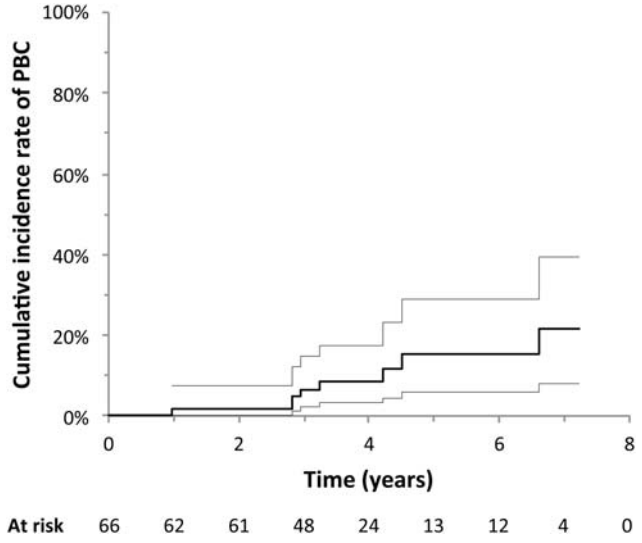

FIG. 5. Cumulative incidence curve of PBC (thick line) with 95% CI boundaries (thin lines) in the subpopulation of patients with newly detected AMAs and normal serum level of ALP at baseline.

TABLE 3. Univariate Analysis of Factors Associated With PBC Development in AMA-Positive Patients With Normal ALP and Available Follow-up (n = 66)

Variable	Hazard Ratio (95% CI)	*P* Value
Age (years)	0.98 (0.93-1.04)	0.4726
Male sex	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 titer	1.19 (0.84-1.69)	0.3180
PBC-specific ANAs	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

Development of PBC was reported in 9 (10%) of the 92 followed-up patients, among whom 8 were female (89%). Median age at PBC diagnosis was 62.1 years (range, 35.9-69.8). Incidence rates were calculated after excluding patients with elevated ALP, pruritus, or cirrhosis at baseline (i.e., from 66 of 92 patients) and by taking care of the competing risk of death. The incidence curve is shown in Fig. 5. Cumulative incidence rates of PBC at 1, 3, and 5 years were 2% (95% CI, 0-7), 7% (95% CI, 2-15), and 16% (95% CI, 6-29), respectively. Neither age, sex, AMA titer, PBC-specific ANAs, 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 exclusion of patients with a negative dotting/blotting test (Supporting Fig. S5). In addition, no statistical difference was found between the low (=1:40) and high (≥1:80) IF AMA titer groups, although no case of incident PBC was reported in the former group (Supporting Fig. S6).

Discussion

In this study, we showed that nearly half of the prospectively detected AMAs in clinical practice was not related to a diagnosis of PBC. This observation raises again the question of the true significance of AMAs 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 nonspecialists of the liver given that a large

proportion of AMAs are revealed in the settings of nonhepatic AIDs. Notwithstanding, taking care to exclude these false-negative cases at inclusion, our follow-up data clearly showed that only a few 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 diagnosis of PBC. AMAs 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 the 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 AMAs, 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%-9.9% of individuals, depending on the techniques and diagnostic thresholds used, as well as on the type, age, and sex 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 AMAs 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 AMAs, 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 AMAs without any other signs of the liver disease at first detection.[10] All the 29 patients were previously screened in a workup for another AID.[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. 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 titers than those diagnosed with PBC at the same time supports the hypothesis of a very early, indolent phase of the disease. In contrast to 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 nation-wide 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 PBC risk. Patients mainly died from nonhepatic primary cancers or hematological malignancies, whereas there is classically no such

increased risk of extrahepatic malignant conditions in PBC.[25-27] Quite rightly, it may be argued that such a link between AMAs and non-liver-related mortality likely results from complications of concomitant diseases and/or treatments (immunosuppressive drugs) rather than from the consequences of AMAs. 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 AMAs 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 goodwill 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 nonestablished PBC. The younger age and lower autoantibody titer 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. On the other hand, these patients may display an increased mortality risk whose link with AMAs remains uncertain.

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Appendix

MEMBERS OF THE FRENCH NETWORK OF IMMUNOLOGY LABORATORIES WHO ACTIVELY PARTICIPATED IN THIS STUDY:

Dr. P. Aberer, hôpital Pasteur, Colmar; Dr. M.-A. Alyanakian, CHU Necker, Paris; Dr. C. André, CHU Henri-Mondor, Créteil; Dr. F. Aucouturier, CHU hôpital Saint-Louis, Paris; Dr. M. Audrain, CHU Nantes; Dr. I. Bahon- Riedinger, CHU Rennes; Dr. O. Bandin, hôpital Sainte-Camille, Bry-sur-Marne; Dr. C. Barthet, laboratoire Pasteur Cerba, Cergy Pontoise; Dr. A. Bayle, CH Macon; Dr. A. Beaume, CHU Poitiers; Dr. Benarroche, laboratoire Ronchèse, Nice; Dr. Z. Benseddik, CH Chartres; Dr. S. Benzaken, CHU hôpital Archet 1, Nice; Dr. C. Capron, hôpital Ambroise-Paré, Boulogne; Dr. P. Chrétien-Leprince, CHIC, Créteil; Dr. A. Chevailler, CHU hôpital Larrey, Angers; Dr. G. Chyderiotis, laboratoire Marcel-Mérieux, Lyon; Dr. E. Comby, CHU Caen; Dr. M.-F. Danjoux, CH de Bigorre, Tarbes; Dr. M.-C. Debarbentane, CHG Avignon; Dr. D. Degenne, CHRU hôpital Bretonneau, Tours; Dr. S. Dekeyser, hôpital Germon-Gauthier, Béthune; Dr. A.-S. Deleplanque, Institut Pasteur de Lille; Dr. S. Dubucquoi, CHRU Lille; Dr. P. Dumouchel, hôpital Laennec, Creil; Dr. A. Ebel, laboratoire Claude-Lévy (LCL), Yvry-sur-Seine; Dr. A. Escande, CHU Saint-Eloi, Montpellier; Dr. N. Fabien, CHU Lyon Sud; Dr. M. Fabriboule, laboratoire Alphabio, Marseille; Dr. F. Fevrier, hôpital Rougier, Cahors; Dr. F. Fortenfant, CHU hôpital Rangueil, Toulouse; Dr. C. Fourcade, hôpital Victor-Dupouy, Argenteuil; Dr. F. Frayssinet, CH du Pays-d'Aix, Aix-en-Provence; Dr. P. Ghillani-Dalbin, CHU Pitié-Salpêtrière, Paris; Dr. J. Goetz, CHU Hautepierre, Strasbourg; Dr. V. Gouilleux, CHU d'Amiens; Dr. C. Goulvestre, CHU hôpital Cochin, Paris; Dr. F. Guerber, Groupement de laboratoire SCMB 12, Vizille; Dr. C. Hamon, CH de Lagny, Lagny-sur-Marne; Dr. C. Heinemann, CH Hyères; Dr. M.-O. Jaubertau-Marchan, CHU Limoges; Dr. S. Jégo-Desplat, CHU hôpital de la Conception, Marseille; Dr. F. Jouen, CHU Rouen; Dr. N. Lalloyer, CHU Carémeau, Nimes; Dr. V. Lasserre, hôpital Robert-Ballanger, Aulnay-sous-Bois; Dr. C. Le Boterff, CH départemental de La Roche-sur-Yon; Dr. S. Lepers, laboratoire Biolille, Lille;

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Dr. N. Montaut, CH Pau; Dr. P. Nicaise, CHU hôpital Bichat, Paris; Dr. N.-O. Olsson, CHU hôpital du Bocage, Dijon; Dr. J.-Y. Peltier, CH Poissy Saint-Germain, Saint-Germain-en-Laye; Dr. F. Pineau-Vincent, CH Le Mans; Dr D. Ponard, CHU Grenoble; Dr. A.-M. Rouquette, CHU hôpital Tenon, Paris; Dr. A. Sarrat, CHU hôpital Pellegrin, Bordeaux; Dr. A. Schandelong, hôpital Flaubert, Le Havre; Dr. A. Scotton, hôpital Jean-Monet, Epinal; Dr. M.-H. Sumyuen, hôpital Robert-Debré, Reims; Dr. T. Tabary, CHR Metz-Thionville, Metz; Dr. M.-F. Taillefer, laboratoire Biocentre, Bondues; Dr. A. Tridon, CHU Clermont-Ferrand; Pr. P. Youinou, CHU hôpital Morvan, Brest.

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