The limitations and hidden gems of the epidemiology of primary biliary cirrhosis

Mauro Podda a,*, Carlo Selmi b,c, Ana Lleo a, Luca Moroni a, Pietro Invernizzi a,c

a Liver Unit and Center for Autoimmune Liver Diseases, Humanitas Clinical and Research Center, Rozzano, Milan, Italy
b Division of Rheumatology and Clinical Immunology, Humanitas Clinical and Research Center, Rozzano, Milan, Italy
c Division of Rheumatology, Allergy and Clinical Immunology, University of California at Davis, Davis, CA, USA

1. Introduction

The etiopathogenesis of primary biliary cirrhosis (PBC) remains largely enigmatic despite the well established role of autoimmunity and genetic factors [1–3]. In such uncertainty, epidemiology becomes pivotal in suggesting clues to etiology and to shed light on putative environmental factors which may trigger the disease process in predisposed individuals, similarly to other autoimmune conditions [4–6]. It could be argued that epidemiological data are of major importance for all complex diseases, but we are convinced that this holds particularly true in the case of PBC for its scientific implications and to establish the real burden for healthcare resources of a disease once considered very rare and now significantly more common [7–9]. Indeed, the steep increase in PBC prevalence observed in data collected over time and the wide geographical variations have been the object of discussion [8] and we are convinced that a major role is due to the improved diagnostic accuracy and more complete data collection, favored by easier access to laboratory screening and availability of digitalized medical databases. Nevertheless, in some cases similar methodology changes led to widely variable data in different geographical areas or in groups with different ethnic background in a phenomenon coined geoepidemiology [9–12], thus leaving the crucial questions unanswered and warranting this critical reappraisal of epidemiology papers.

We herein report the results of critical evaluation of the available studies with a specific attention to the recurrent biases responsible for the variability and the limited value of the data. First and foremost, nearly all studies are aimed at establishing the number of patients with a reliable diagnosis of PBC in a definite area. It is therefore clear that the denominator, i.e. the population of the catchment area, may be easily and exactly obtained at any time...
from census registries, while the numerator, i.e. the number of PBC cases, is largely dependent on a plethora of factors including the location- and time-dependent access to diagnoses in the general population. As a result, we have the ambitious goal to critically discuss the published data and to suggest directions for future research.

2. Prevalence and incidence rates in population-based studies

A comprehensive systematic review of population-based studies for PBC epidemiology was recently published [13]. It followed the checklist proposed by the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group [14] to include 24 studies. The review reported PBC incidence and prevalence rates obtained from the population of well defined geographical areas with populations exceeding 100,000 inhabitants. We have updated this search over the next 23 months until December 2012 and included two additional articles fulfilling the above mentioned criteria [15,16]. All studies were case-finding studies, reporting the number of patients with an established diagnosis of PBC in a well defined geographic area, usually corresponding to the catchment area of one or a group of hospitals. Interestingly, in a single area, i.e. the Calgary Health region of Canada, cases were collected from administrative databases used for reimbursement and management purposes [17].

Values of yearly incidence and point prevalence from the 26 papers are illustrated in Fig. 1 [7,15–30]. Both the yearly incidence (0.33–5.8/100,000) and point prevalence (1.91–40.2/100,000) rates manifest a wide variability, with median values of 1.55/100,000 and 13.7/100,000, respectively. Though different ethnic representations might contribute to the high data heterogeneity, we are convinced that methodological aspects and time trend play a preponderant role. This hypothesis is supported by later studies performed with different approaches in the Australian State of Victoria [31,32] and in the Canadian States of Ontario and Alberta [17,33] showing 3- to 10-fold prevalence increases. As somehow expected, the analysis of single articles indicated that higher values of incidence and prevalence were obtained in studies that applied a more rigorous methodology to trace PBC cases. In this regard, the search strategy applied and recommended by the Newcastle group in 1997 [34] is comprehensive and has been adopted by most investigators since its publication. This strategy has its cornerstone in the survey of serum antimitochondrial antibody (AMA) detection in regional laboratories and may have well contributed to the increase over time of PBC incidence and prevalence. Furthermore, medical awareness of the higher frequency of PBC also grew with time and stimulated appropriate testing in a wider number of subjects, usually with mild elevations of serum liver enzymes, even in the absence of symptoms classically associated with PBC such as pruritus. The introduction in many settings of digitalized medical records improved data collection tools and made this approach easier and more reliable in recent years [35]. We are convinced that these factors, taken altogether, should be credited at least partially for the time trend increase of both incident and prevalent cases in more recent papers as well as in those studies spanning through an extended number of years. On the other hand, we note that the improved management of advanced liver disease, the more frequent access to liver transplantation and the widespread use of ursodeoxycholic acid [3] at appropriate doses have clearly prolonged patient survival, thus ultimately affecting PBC prevalence. The obvious fact that these factors are not evenly distributed in different geographical areas reflects the discrepancy of access and expertise in healthcare and may further account for the wide data variability. As an indirect confirmation of this observation, it is noteworthy that the three studies reporting the highest rates for PBC point prevalence (Table 1) the search methods were similar and were performed in different well defined settings that were curiously corresponding to a whole city (Newcastle, UK) [25], county (Olmsted, MN) [28], and country (Iceland) [15]. Among these settings, the city of Newcastle hosts the Academic group which pioneered PBC epidemiology and set the standard for such studies with original and stimulating results, including the most recent one, indicating a seasonal variation in PBC identification [36]. Second, Olmsted country is the site of the Mayo Clinic which has established a network with other county hospitals to create a common database favoring the collaboration for epidemiologic investigations. Third, Iceland has unique conditions for an epidemiologic survey as AMA are measured in a single laboratory that makes it feasible “to select the entire country’s population as a study cohort” [15]. As an indirect confirmation of our hypothesis, remarkably similar point prevalence rates were reported in the three studies with 39.2 cases per 100,000 people in Newcastle, 40.2 in Olmsted County, and 38.3 in Iceland. We are convinced that this homogeneity within studies performed in different settings and at different latitudes but sharing an adequate methodology by highly experienced investigators, suggests that the highest values may represent standard values that can be presently obtained in case finding studies, at least in Westernized countries. Another peculiar aspect of these three studies is the commonly overlooked stability of PBC yearly incidence rates throughout the period of data collection, somehow in contrast with the significant trend of increasing values reported by the large majority of other studies [13] and confirms that in ideal conditions consistent data are found both between and within different studies. The only study based on administrative data in the State of Alberta [17] reported a frequency of PBC that is to be considered among the highest out of individual patient identification studies. Although the use of administrative data is applicable only where healthcare delivery is not fragmented, it represents a promising approach to collect a large volume of information for epidemiology and also for natural history of chronic diseases. This methodology, however, does not overcome the limits of case finding studies which will be discussed in the next section.

3. The limits of case-finding studies

All case finding studies are subject to obvious shortcomings which may influence the resulting incidence and prevalence rates to a variable extent. As already stated in the introduction, all problems reside in the numerator, i.e. the number of diagnoses made and detected in the population of a definite area (the
denominator). Potential biases of case finding studies have been extensively discussed for rheumatic diseases elsewhere [37], but these are more crucial in PBC research, due to the elusive and asymptomatic status of most patients, especially at earlier stages. Before overt disease becomes evident with clinical symptoms and/or liver decompensation two stages are traditionally recognized as part of PBC natural history [3]. The first stage is coined preclinical PBC, characterized by positive serum AMA, early but definite histological lesions. The second stage, asymptomatic PBC, features the two previous characteristics (positive AMA and histology) along with a cholestatic biochemical profile [3]. The preclinical and asymptomatic stages may last for decades [3], or most of PBC natural history, when diagnosis can be only incidental, usually via three different pathways. First, the probability of being diagnosed with PBC is largely dependent on the chance that an affected individual will seek medical advice for any iatrotropic reason with a workup including a liver screening. In the case of PBC, an abnormal laboratory test will lead to AMA determination. Second and more rarely, hepatomegaly at clinical examination or ultrasound is found and triggers the search for liver disease. Finally, the presence of an immune-mediated disease known to be associated with PBC, such as scleroderma or Sjogren syndrome, may induce the physician to order serum AMA or cholestasis indices. It is quite obvious that the limiting steps for an early PBC diagnosis are the former two and that the large variability in medical awareness of the disease and in the access to a laboratory performing accurate AMA determination are an issue, particularly in developing countries or in rural areas of Westernized countries. Indeed, this variability in different regions and settings, even with similar healthcare quality, is based on numerous scientific, cultural, social, and economic factors. When specific environmental factors are sought in PBC such variables are of seminal importance and cannot be overlooked.

The limitations that have been discussed appear to act only in one way, that is by decreasing the number of PBC cases that are properly diagnosed or otherwise detected, thus reducing both incidence and prevalence rates. The risk of overdiagnosing PBC, on the other hand, is negligible as objective and validated criteria have been established and are universally applied in all studies [38,39]. There is no reliable estimate of the magnitude of these underestimates in PBC epidemiology as we cannot expect that all asymptomatic patients with PBC have undergone the appropriate workup while the opposite, i.e. that not all symptomatic PBC cases have been diagnosed especially in areas of low medical awareness, is significantly more likely. Independent of the magnitude, two conclusions may be drawn following the previous considerations.

First, we are convinced that there is an inverse relationship between the incidence and prevalence rates and the degree of PBC understimation of ‘submerged’ data. Second, the PBC incidence and prevalence increase over the years, reported in most studies, is likely to derive from the easier access and the more appropriate use of diagnostic tools (as in the case of serum AMA, among others) for PBC differential diagnosis [40,41], in analogy with other chronic liver diseases [42]. As the natural subsequent step in this dissertation, we will now discuss the data of AMA prevalence in the general population; these observations are of major importance in this context, as AMA-positive subjects may well indicate the highest hypothetical number of PBC cases in a defined population subset despite the minor underestimation of AMA-negative PBC [40,41].

4. Serum AMA prevalence

It should be now clear that case-finding studies are prone to the risk of underestimating the prevalence of rare diseases. Conversely, the search for AMA-positive subjects in unselected population samples has the potential to identify the largest number of patients who have or may develop PBC, with the minor discrepancy of the small percentage of patients with AMA-negative PBC [43,44]. The assumption that asymptomatic subjects with normal laboratory values and an isolated positivity for AMA have preclinical PBC or will develop the disease was derived from the seminal paper of the Newcastle group [45]. The authors demonstrated that 75% of 29 asymptomatic individuals with an incidental finding of serum AMA positivity at indirect immunofluorescence and normal liver function tests eventually had PBC at baseline histology or developed the disease after a median follow-up of 18 years. This proportion became 100% when the frozen baseline serum samples of 21 subjects were tested years later by ELISA using the recently developed AMA recombinant autoantigens. This breakthrough discovery allowed higher specificity and reproducibility compared to indirect immunofluorescence, and the screening of large population samples became feasible at lower costs. However, only a few studies based on this technology have been reported thus far, usually on a small number of subjects who in most cases were not submitted to adequate clinical investigations to establish if they actually had PBC (Table 2).

Addressing in details the molecular and diagnostic characteristics of different antigens and laboratory techniques is beyond the aims of this article but it should be noted that this variable increases the complexity of the scenario and that AMA prevalence data should be evaluated also on this basis.

### Table 1
Features of the three population studies with the highest PBC prevalence.

<table>
<thead>
<tr>
<th>Area</th>
<th>Period</th>
<th>Population</th>
<th>PBC cases</th>
<th>Incidence per 10^5 per yr</th>
<th>Prevalence per 10^5</th>
<th>Male (%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olmsted County, USA</td>
<td>1975–1995</td>
<td>–</td>
<td>46</td>
<td>2.70</td>
<td>40.2</td>
<td>11</td>
<td>[28]</td>
</tr>
</tbody>
</table>

### Table 2
AMA prevalence in the general population.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>Region</th>
<th>Source</th>
<th>Subjects</th>
<th>Cases</th>
<th>M %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Tot</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lazaridis [48]</td>
<td>2005</td>
<td>US</td>
<td>Routine health check</td>
<td>196</td>
<td>20</td>
<td>176</td>
</tr>
<tr>
<td>Liu [44]</td>
<td>2006</td>
<td>Southern China</td>
<td>Routine health check</td>
<td>8128</td>
<td>4248</td>
<td>3878</td>
</tr>
<tr>
<td>Mattial [47]</td>
<td>1998</td>
<td>Northern Italy</td>
<td>Blood donors and healthy subjects</td>
<td>1530</td>
<td>555</td>
<td>975</td>
</tr>
<tr>
<td>Turcany [46]</td>
<td>1997</td>
<td>Estonia</td>
<td>Unselected general population</td>
<td>1565</td>
<td>666</td>
<td>899</td>
</tr>
<tr>
<td>Shibata [54]</td>
<td>1999</td>
<td>Japan</td>
<td>Yearly health check</td>
<td>1714</td>
<td>959</td>
<td>755</td>
</tr>
</tbody>
</table>
When data are cumulatively considered a surprisingly high prevalence rate ranging from 0.43 to 1% is gathered from the general population. At present, the diverse nature of the studies, the limited number of subjects included, and the lack of an adequate diagnostic work-up do not allow to draw firm conclusions on the frequency and clinical significance of AMA positivity in otherwise healthy subjects. On these grounds, discussing specific studies is instrumental in explaining the limitations of the available data.

The screening of a random population of two neighboring Estonian villages identified 14 AMA positive individuals out of 1565 subjects [46]; of these, 8 were available for follow up and none agreed to undergo a liver biopsy. Over 9 years of follow up, 3 of the 8 subjects developed abnormal tests of liver biochemistry. Interestingly, a case finding study by the same group in the whole country of Estonia [24] indicated for PBC a point prevalence of 2.69/100,000 corresponding to a ratio of about 1 diagnosis of PBC to 350 AMA positive subjects.

Mattalia and Colleagues [47] investigated a large number of sera that included a majority of young blood donors and was mainly focused on the characterization of the antibody pattern in healthy subjects. In this Italian cohort 0.59% of subjects were AMA positive and 8/9 were retested after one year with the detection of a wider AMA pattern compared to baseline.

Quite surprisingly, 2 subjects tested positive for AMA in a small sample of 196 healthy subjects selected as matched controls for first degree relatives of patients with PBC [48].

The study with the largest number of cases combined an initial indirect immunofluorescence screening with subsequent ELISA and immunoblotting with PBC specific antigens [49]. Of the 8445 subjects reporting to a Southern China hospital for annual check up, 35 tested positive at indirect immunofluorescence and 22 of them confirmed their positivity with a more specific assay.

In conclusion, if adequate population studies in terms of patient selection and series, with proper diagnostic work-up and sufficient duration of follow up of positive cases will confirm the findings of the Newcastle group, PBC will no more be considered a rare disease but rather a relatively common condition. As a direct consequence, geoepidemiology, risk factors, and environmental associations need to be established on more solid grounds and an agreement between PBC and serum AMA should be expected as an indirect proof of the chosen approach.

5. Sex differences

A significant female preponderance is a widely accepted feature of PBC clinical description and the median ratio in case finding studies corresponds to approximately 9–10:1. This value, albeit not surprising, is among the highest for autoimmune diseases [12,50,51]. We note that PBC sex ratio is shared with frequently coexisting conditions such as Sjogren syndrome and autoimmune thyroid disease and the putative mechanisms influencing female preponderance have been recently reviewed for both PBC and autoimmune diseases and are outside the aims of this discussion [12,51,52]. As previously discussed, case finding studies are based on clinical recognition and the initial clues leading to the suspicion of PBC may be more easily appreciated in women than in men as suggested by the “anchoring bias” of behavioral economics. The subtle onset and long persistence of PBC at asymptomatic stages leaves wide space to mere heuristics for early diagnostic suspicion, which obviously privilege a more straightforward approach to diagnostic tests such as autoantibodies in women. The opposite bias, favoring men, has been reported in a paradigmatic condition of male-predominant disease, i.e. coronary artery disease, and one study demonstrated that general physicians tend to inquire about typical symptoms in men and women to support different evaluation processes [53]. The resulting possibility that PBC could be underdiagnosed in men is supported by different lines of evidence. First, AMA positivity in the general population reports a female preponderance reduced to approximately 2:1 (Fig. 2 [46,47,49,54]) but the small size of these studies falls short to draw solid conclusions. Second, a 4.3:1 ratio among the people deceased for PBC during a 20 year period [55] although this may also represent a more rapid progression rate or a worse response to treatment in men. Third, women outnumber men by a factor of 6 among cases of PBC undergoing liver transplantation in the UNOS dataset ([56] and Dr A.K. Singal personal communication). Taken altogether, these lines of evidence underline that, although female preponderance is confirmed in all studies, the universally accepted 9–10:1 ratio is likely an overestimate. Finally, and for more discussion, we note a recent symposium devoted entirely to gender, sex hormones, pregnancy and autoimmunity [12,57–101].

6. Familial PBC

Diagnosing multiple PBC cases within the same family has been coined familial PBC and anecdotal familial PBC clusters have been reported for the first time over 40 years ago, also including female [102] and even male [48] twins. Cumulatively, based on family studies of both PBC and serum AMA prevalence and especially from concordance rates in identical twins we can confidently draw the conclusion that there is a fundamental role played by genetic factors in the etiopathogenesis of PBC. In the 1990s the prevalence of patients with PBC having a positive family history, i.e. at least one affected relative, was investigated by several groups (Table 3) [103–109]. Nevertheless almost all these studies are burdened by numerous shortcomings, since the methodology was substantially based on case-note review and the criteria for case ascertainment

![Fig. 2. PBC and serum AMA female/male ratios [46,47,49,54].](image-url)

Table 3

<table>
<thead>
<tr>
<th>Study</th>
<th>PBC probands (n)</th>
<th>Affected families</th>
<th>First degree relatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bach</td>
<td>405</td>
<td>17 (4.3%)</td>
<td>–</td>
</tr>
<tr>
<td>Brind</td>
<td>736</td>
<td>8 (1.1%)</td>
<td>10 (1.3%)</td>
</tr>
<tr>
<td>Floreani</td>
<td>156</td>
<td>6 (3.8%)</td>
<td>5 (3.2%)</td>
</tr>
<tr>
<td>Jones</td>
<td>160</td>
<td>10 (6.4%)</td>
<td>8 (5.0%)</td>
</tr>
<tr>
<td>Tsuji</td>
<td>156</td>
<td>8 (5.1%)</td>
<td>8 (5.1%)</td>
</tr>
<tr>
<td>Corpechot</td>
<td>221</td>
<td>8 (3.6%)</td>
<td>8 (3.6%)</td>
</tr>
<tr>
<td>Gershwin</td>
<td>1032</td>
<td>57 (5.5%)</td>
<td>57 (3.5%)</td>
</tr>
</tbody>
</table>
were not uniform or clearly illustrated. Further, no attempt was made to calculate the size of the patient pedigree, and the relative proportion of affected members and all studies were performed in tertiary referral centers with the inherent “referral bias” favoring peculiar or interesting cases. Finally, in most studies no control group was available for comparison of the prevalence rate. Despite these limits, similar results were more recently reported by two case–control studies [108,109] in which the occurrence of familial PBC was self-reported.

Of note, the careful investigation by Jones et al. [106] was designed as a “geographically based population study” that avoided referral bias and compared prevalence rates within all family members with that of the area population [106]. Interestingly, the authors calculated a risk for a first-degree relative to have PBC as less than 1%, with higher values for female relatives (Table 4). The sibling relative risk, that is the odd ratio for PBC of a subject with a sibling affected by the disease is 10.5, surprisingly among the lowest for autoimmune diseases.

Once again, the search for serum AMA in apparently healthy first-degree relatives of patients with PBC is of major relevance, as discussed in a previous section. Furthermore, the presence of AMA in first-degree relatives is a strong indicator of the role of genetic factors in breaking tolerance towards mitochondrial antigens. Early studies based on immunofluorescence found a prevalence ranging from 4.9 to 7.4% in screened relatives [110,111], however almost half of them were children, while siblings were a minor fraction. In a more recent study, carefully selected first-degree relatives were screened for AMA by ELISA using recombinant mitochondrial autoantigens and an overall 13.1 prevalence was detected with higher rates, as expected, in siblings [48] (Table 5). Of note, a recent paper from Greece, employing the same study design and methodology, reported a prevalence rate of 18.1% [112]. These values are 15–20 times higher than those reported by Jones for clinical PBC [106], and reinforce the similar finding in the general population (Table 6).

Ultimately, we note that the reported 63% concordance rate among monozygotic twins compared to the null concordance among dizygotic pairs [113] together with growing evidences from genome wide association studies [114–117] demonstrated the importance of genetic factors for PBC [2,118].

### Table 5

AMA prevalence in first degree relatives of patients with PBC, modified from Lazarian et al. [48].

<table>
<thead>
<tr>
<th>Population group</th>
<th>AMA positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBC probands</td>
<td>88.0</td>
</tr>
<tr>
<td>PBC FDRs</td>
<td>13.1</td>
</tr>
<tr>
<td>Mother</td>
<td>15.1</td>
</tr>
<tr>
<td>Father</td>
<td>3.7</td>
</tr>
<tr>
<td>Sister</td>
<td>20.7</td>
</tr>
<tr>
<td>Brother</td>
<td>7.8</td>
</tr>
<tr>
<td>Daughter</td>
<td>9.8</td>
</tr>
<tr>
<td>Son</td>
<td>0.0</td>
</tr>
</tbody>
</table>

### Table 6

Data on coronary artery disease awareness with patients of both sexes may suggest a female bias in PBC diagnosis [53].

<table>
<thead>
<tr>
<th>Questions asked (n)</th>
<th>Men</th>
<th>Women</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>From ordering tests</td>
<td>90%</td>
<td>80%</td>
<td>0.025</td>
</tr>
<tr>
<td>Appropriate prescriptio</td>
<td>64%</td>
<td>52%</td>
<td>0.048</td>
</tr>
</tbody>
</table>

### 7. Concluding remarks and auspices

As we are confident should be clear from our review of the available evidence, PBC epidemiology is far from being a closed case and several issues remain to be addressed. First, prevalence rates of “clinically recognized” PBC as high as 40 per 100,000 should be considered the standard rather than the high extreme of prevalence. This is particularly true among Caucasians and in geographical areas characterized by a high level of clinical expertise, easy access to laboratory investigations, and optimal organization to retrieve information from medical databases. Second, reported rates underestimate the whole population of subjects with PBC which includes preclinical and “lantanthic” patients. Third, the exact prevalence of a predominantly asymptomatic disease such as PBC can be established only through an appropriate screening of an adequate sample of unselected population. Unfortunately, this approach would require high costs in view of PBC relative rarity and would raise ethical questions from at least two points of view. It would allow the diagnosis of a disease at a stage in which the prognosis and treatment are largely undetermined, with unclear consequences for the patient, and in case of positive AMA and normal alkaline phosphatase a liver biopsy would be needed to establish the diagnosis of PBC. The latter point would be applicable also for a more straightforward and less expensive approach based on the screening for serum AMA and alkaline phosphatase of blood samples collected for other purposes from sufficiently large population series. In case of positive AMA and normal alkaline phosphatase levels follow up could be informative in a subgroup, without the need of a liver biopsy. A fourth open issue is the variable sex ratio among studies with different methodologies, with case finding studies favouring a larger female predominance as compared to serum AMA prevalence, death certificates and liver transplantations. We cannot overlook the possibility that this may also be caused by a more severe progression in men with shorter survival and more frequent need for transplantation. Though early retrospective study did not find differences in survival [119] further accurate investigations are warranted. Fifth, the clinical significance of “isolated” AMA and the natural history of early PBC could also be clarified by follow up of subjects detected through the screening procedure.

In conclusion, the switch from descriptive to clinical epidemiology could offer principles of major relevance to “the basic science of the clinician” as clinical epidemiology was defined [120] and we encourage a strenuous multidisciplinary effort to tackle this goal as data mining tools become more powerful and effective.

### References


