Primary biliary cirrhosis (PBC) is a chronic inflammatory autoimmune disease that mainly targets the cholangiocytes of the interlobular bile ducts in the liver. The condition primarily affects middle-aged women. Without treatment, PBC generally progresses to cirrhosis and eventually liver failure over a period of 10–20 years. PBC is a rare disease with prevalence of less than 1/2000. PBC is thought to result from a combination of multiple genetic factors and superimposed environmental triggers. The contribution of the genetic predisposition is evidenced by the familial clustering. Several risk factors, including exposure to infectious agents and chemical xenobiotics, have been suggested. Ursodeoxycholic acid (UDCA) is currently the only FDA-approved medical treatment for PBC. When administered at doses of 13–15 mg/kg/day, a majority of patients with PBC have a normal life expectancy without additional therapeutic measures. One out of three patients does not adequately respond to UDCA therapy and may need additional medical therapy and/or liver transplantation. This review summarises current knowledge on the epidemiology, ethiopathogenesis, clinical, and therapeutic aspects of PBC.

Keywords: Antimitochondrial antibody; Apoptosis; Cholestasis; Inflammation; Fibrosis; Cirrhosis; Genetics; Xenobiotics; Autoimmunity; Innate immunity; Adaptive immunity; Epidemiology; Ursodeoxycholic acid; Immunosuppressive drugs; Pruritus; Hypercholesterolemia; Portal hypertension; Osteoporosis; Liver transplantation.

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Abbreviations: UDCA, ursodeoxycholic acid; PBC, primary biliary cirrhosis; NSDC, non-suppurative destructive cholangitis; AMA, antimitochondrial Antibody; AE2, Cl/HCO3; exchanger 2; NHE, Na+/H+ exchanger; MHC, major histocompatibility complex; NRAMP1, natural resistance-associated macrophage antigen 1; CTLA4, cytotoxic lymphocyte antigen-4; VDR, vitamin D receptor; M2Ab, M2 antibodies; AIH, autoimmune hepatitis; MBL, mannose binding lectin.

Epidemiology

In a systematic review of studies of PBC frequency [11], Prince and James identified 37 studies, 29 of which were performed after 1980. Incidence of PBC ranged from 0.7 to 49 per million per year in most recent studies. The point prevalence was estimated to range from 6.7 to 402 per million. In the French metropolitan area, a prospective study performed in 2006–7 shows that the incidence was 9 per million per year, with an estimate of 36 per million in women over 45 years. Assuming a life expectancy of 20 years after diagnosis, the point prevalence was estimated to be 207 per million, and for women above 45 years, 860 per million [12]. As in some other studies [13,14], the incidence of AMA positivity without evidence of liver disease was two times higher than the incidence of AMA positivity with evidence of liver disease. It has been observed that the incidence and prevalence rates are highest in Northern European countries (e.g., England and Scotland) [15,16] and in the Northern United States (e.g., Minnesota) [17]. The systematic review of PBC frequency [11] suggests that there is an increase incidence and prevalence of PBC worldwide. A good example comes from studies carried out in the region of Victoria (Australia). The first population-based study carried out in this region identified 84 cases, giving an estimate of PBC prevalence of 19.1 per million [18]. A subsequent study from the same region identified 249 cases, for a cumulative incidence that is almost 10-fold higher [19]. The reasons appear complex. Indeed, many explanations may be provided, e.g., true increase due to increased exposure to an
environmental factor, demographic changes with an increased elderly at-risk population, increase survival of already diagnosed persons, earlier diagnosis, improved care, and increase clinician as well patient awareness, among other reasons.

As discussed later, environmental factors preferentially affecting women may be implicated in the aetiopathogenesis of PBC. If changes in the incidence of PBC reflect a true epidemiological phenomenon, differing exposure to environmental risk factors could explain it. Marked geographic variation in the burden of PBC has been found in several studies [20–25]. In a study from Northeast England [22], there were very substantial anomalies in the spatial distribution of patients. Extraordinary clusters of disease were noticed in several urban areas (up to 13 cases/km²). No obvious demographic or geographical features were found to explain this variation, although they do suggest the presence of one or more unidentified environmental factor(s). North American studies have similarly localised clusters of PBC. A relationship between the location of toxic waste sites and increase prevalence of PBC, as well a relationship between individuals listed for liver transplantation and mean daily airborne pollutant concentrations was observed [23].

Etiologies

PBC is thought to result from a combination of multiple genetic factors and superimposed environmental triggers. All these factors may affect one or more components of the immune system and the tissue that is targeted by the disease (Fig. 1).

The contribution of the genetic predisposition is evidenced by the familial clustering [26–28] and the high concordance of monozygotic twins [29]. The prevalence of PBC is 100 times higher in first-degree relatives than in the general population.

Allelic variations in MHC class II (DR, DQ), components of the innate (C4Q0, C4B'2, NRAMP1/SLC11A1, MBL, VDR) and of the adaptive (CTLA4, IL beta, TNF alpha, IL12A, IL12RB2) immune systems have been shown to be associated with the susceptibility of PBC [30–34]. The possible role of allelic variation of several components of the innate immune system suggests some disturbances of host resistance to microbial infection in PBC and their implication in the initiation or perpetuation of the inflammatory process. The same point can be made for the association with variants of the IL12 pathway since several data link inherited deficiencies of IL12, IL12R, and interferon gamma to increased susceptibility and severity of infectious diseases in particular mycobacterial diseases [35].

CTLA4 encodes a coinhibitory immunoreceptor that is a key regulator of self-tolerance with established genetic associations to multiple autoimmune diseases and in most, but not all, genetic studies of PBC [31,33,34,36–38]. Interestingly, there is an association of genetic variation at the VDR locus and susceptibility to PBC [32] thanks to the role of vitamin D not only in adaptive immunity but also in the regulation of innate immunity of the biliary tree [39].

The genetic basis of variability in disease progression is poorly understood. A French prospective study of genetic factors related to PBC severity has shown that two variants of TNF alpha and SLCA2/AE2 [33] were independent prognostic factors involved in the profile of PBC treated with UDCA. This human study, along with the recently reported Ae2a,b-deficient mouse model of PBC, provides further evidence for a pathogenic role of SLCA2 deficiency in PBC [40].

![Fig. 1. PBC results from a combination of multiple genetic and environmental factors that may affect the immune system and the liver.](image-url)
Regarding environmental factors, association with mucosal infections, particularly urinary tract infections, and cigarette smoking, have been consistently found [41-44]. The contribution of other risk factors, such as the use of cosmetics, frequency of pregnancy, and hormone replacement therapy, is not always clear [41,43].

Most xenobiotics are metabolised in the liver and some of them are known to induce autoimmunity and hepatitis. One of the best examples is the case of halothane hepatitis, which occurs in susceptible individuals who mount an immune response to trifluoroacetylated protein adducts [45]. Thus, it is tempting to imagine exposure to a xenobiotic, which is then transformed into a reactive intermediate that binds to proteins and results in neoantigen formation in PBC initiation. Moreover, using fluocicillin, an isoxazolyl-penicillin known to induce cholestasis and cholangiocyte injury, we have found that the molecule was bio-transformed by hepatocytes to a reactive intermediate eliminated in bile and responsible for cholangiocyte death, suggesting that biliary reactive intermediate could induce cholangiocyte damage and further auto-inflammation [46]. However, it should be noted that fluocicillin has never been implicated in PBC.

The lipoic acid binding domain of the 2-oxo-acid dehydrogenases, to which immune reactivity in PBC is seen, has a highly conserved domain structure containing a lipoic acid factor. The lipoic acid binding domain forms the core of the dominant autoepitope. Immunologic cross-reactivity between lipoic acid and structurally-related xenobiotics may represent a mechanism for breakdown tolerance against lipoic acid-containing autoantigens in PBC. Lipoic acid is exposed to the exterior part of the oxo-dehydrogenase complex, thus forming an ideal target for xenobiotic modifications. The role of xenobiotics is suggested by a series of experimental data. First, halogenated compounds, such as 6-bromohexanoic acid, can be incorporated naturally into PDC in place of lipoic acid [47]. Second, sensitisation with 6-bromohexanoic acid-modified bovine serum albumin can lead to the appearance of AMA and portal tract inflammation [48]. Third, specific organic structures attached to mitochondrial antigens are recognised by PBC sera with higher affinity than the native form of these antigens [49]. 2-Nonynoic acid, a compound found in several cosmetic products, is also recognised by PBC sera with high affinity [50]. Fourth, xenobiotic-induced PBC murine models made by immunisation with 2-octynoic acid of NOD.1101 or C57BL/6 strains have been reported [51,52].

AMA in PBC sera cross-reacts with a number of bacteria. This was the basis for the bacterial aetiology of PBC. This hypothesis remains highly uncertain because of a number of controversial data and the fact that there is no evidence of excess T cell response to bacterial proteins in PBC. The cross-reactivity between PBC sera and bacteria is explained by the highly conserved sequence of PDC throughout phylogeny. However, the Gerswihin group has recently provided some convincing evidence that a free living Gram-negative bacterium called *Novosphingobium aromaticivorans* (NA) could have a role in inducing PBC [53]. Indeed, NA has one of the highest degrees of homology with the human PDC2 autoepitope. NA is capable of inducing autoreactive AMA and chronic T cell-mediated autoimmunity against the small bile duct in a murine model of PBC [54].

There is also evidence that raises the possibility of a transmissible virus in PBC lymph nodes. Cholangiocytes, isolated from normal individuals incubated with homogenised lymph node from a PBC patient, develop aberrant mitochondrial antigen staining on plasma membranes, a phenotype trait characteristic of PBC [55,56]. Retroviral sequences cloned from the lymph nodes suggested a role for a retrovirus resembling the mouse mammalian tumour virus (MMTV). However, this finding has not been reproduced [57,58], and the data of a randomised control trial of zidovudine and lamivudine in PBC patients do not convincingly support the role of a retrovirus in PBC pathogenesis [59]. Nevertheless, it is worth noting that the NOD.c3c4 mouse model of PBC [60] has been found to express MMTV proteins in biliary epithelium [61]. These mice treated with anti MMTV had no evidence of the pathological traits of PBC, suggesting that MMTV triggers viral cholangitis in this experimental model [62].

Any infectious agent present in the liver or bile in an immuno-genetically susceptible individual may generate a transient or chronic immune response that is cross-reactive with self PDC. A second hit (hormonal, viral, xenobiotics) in cholangiocytes, resulting in increased apoptosis and increased presentation of immunoreactive PDC via dendritic cells, may then be sufficient to convert this transient autoimmunity into autoimmune disease. The innate immune system relies on its capacity to detect pathogenic microbes and danger-associated molecular patterns released by injured cells through toll-like and nod-like receptors. Our knowledge of the complexity of the innate immune response is just beginning and could be crucial for the understanding of autoimmune diseases such as PBC.

Several spontaneous and induced animal models of PBC have been described in the last few years [40,60,63]. They all provide further evidence that abnormal immune regulation, with or without superimposed triggers, is involved in the breakdown of tolerance against PDC2 and early cholangitis. A mouse model, expressing a dominant negative form of transforming growth factor receptor restricted to T cells (dnTGFRII), develops an inflammatory biliary ductular disease with elevated serum levels of IL12p40. Interestingly, the IL12p40 KO dnTGFRII mice have a dramatic reduction in histological cholangitis and significant decrease in the levels of intrahepatic proinflammatory cytokines but similar levels of AMA compared to dnTGFRII controls [64]. These findings support recent genetic data implicating the IL12 pathway in susceptibility to human PBC [34]. As mentioned above, KO mice, with a disruption of most isoforms of AE2, develop features of PBC and a decreased number of T regulatory cells [40]. Genetic data in human PBC have also shown that one variant of the SLCA2/AE2 gene may be involved in evolving the profile of PBC treated with UDCA [33]. Whether AE2 dysfunction may lead to altered cholangiocyte homeostasis, increased rate of apoptosis, and subsequent enhanced presentation of antigenic epitopes or lymphocytes dysfunction, remains to be examined [65].

**Female preponderance of PBC**

The ratio of women to men with the disease is 10–1. The female preponderance may hold an important key to PBC aetiology. Potential mechanisms for the gender imbalance may include an increased risk of exposure to a disease-promoting factor (such as urinary infection or 2-octynoic acid) or altered response to a common trigger in females. There is no evidence that foetal microchimerism may contribute to disease pathogenesis [66-70].

Although none of the currently identified disease-associated loci for PBC are X or Y chromosome encoded, recent data have
suggested that defects in the X chromosome are detectable in PBC patients. There is a significantly higher frequency of monosomy of the X chromosome in peripheral leukocytes in women with PBC [71] compared to age-matched control women. Systemic sclerosis and autoimmune thyroiditis also show the same difference [72]. X loss in PBC was not random and more frequently affected the parentally-inherited chromosome [73].

Pathophysiology

Although a unified theory of PBC pathogenesis may not be possible currently, a paradigm that considered the pathobiological events contributing to disease progression may be presented (Fig. 2).

The preclinical phase is marked by autoimmunity. Autoimmunity is manifested by M2 antibodies (M2Ab) that specifically react with the lipoyl domain of the E2 subunits of the 2-oxo-acid dehydrogenase complexes of the mitochondria. The T and B cells infiltrating the liver in PBC are specific for the M2 antigen. The M2 epitope is detectable in cholangiocytes undergoing apoptosis and in the luminal domain of the biliary cells of the small bile ducts [74–76]. This luminal staining precedes the expression of BB1/B7 and MHC II molecules, suggesting that aberrant expression occurs early in the natural history of PBC [77]. The aberrant expression may be present in cholangiocytes in allografts of patients with recurrent PBC following transplantation [78], suggesting the role of an exogenous factor. However, others were unable to document this finding in the allograft in either the presence or absence of PBC recurrence [79].

In contrast to other cell types, cholangiocytes undergoing apoptosis fail to bind glutathione to the lysine-lipoyl residue of the dehydrogenase and thereby fail to cleave the autoreactive epitope [80]. Neighbouring cholangiocytes have the ability to destroy the apoptotic cells by phagocytosis and to express the M2 epitope [81,82]. This is a rational explanation for the tissue specificity of the autoimmune process.

IgAM2ab may induce caspase and cholangiocyte injury in vitro [83], which suggests that cholangiocyte apoptosis occurs early in the natural history and participates in the initiation or amplification of the autoimmune process. Normal cholangiocytes lining the small bile ducts express Bcl2 and are more resistant to apoptosis than those lining large bile ducts [84]. However, stress-induced glutathione depletion in cholangiocytes markedly reduced Bcl2 expression, and activity and threshold of apoptosis [85,86]. In PBC, small bile duct-Bcl2 expression is reduced and associated with features of oxidative stress, replicative senescence, and apoptosis [87]. Thus, a simple pathogenetic model may rely on the presence of IgAM2ab with a superimposed acquired dysfunction or glutathione depletion in cholangiocytes of the interlobular bile ducts. This model could explain at least in part the beneficial effect of UDCA, a bile acid that reduces AMA titre and exerts antiapoptotic properties in cholangiocytes in the early natural history of PBC [10,88,89].
Inflammation and cholestasis are early events in the clinical phase of the disease. In the nonoductopenic stage, inflammation is associated with similar rates of apoptosis and proliferation of the biliary cells [90–92]. Cytolytic T cells, mainly, CD8, CD4, and NKT cells are attracted to the target-cells by the release of several chemokines [87,93–98]. The killing of biliary cells is mainly mediated by activation of TNF, CD40, and Fas receptors [99]. Under the pressure of this environment, cholangiocytes proliferate to compensate for death. Mediators possibly involved in this process include those of the cholinergic pathway, the IGFI system, and oestrogens acting through their alpha receptors [100,101].

The inflammatory process spreads into the lobule in two ways, through the lymphocytic piecemeal necrosis and the biliary piecemeal necrosis [89,102–104]. The piecemeal lymphocytic necrosis observed in PBC is an inflammatory destruction of a group of hepatocytes associated with lymphohistocytic cells, similar to that found in autoimmune hepatitis (AIH) (Fig. 3A). Lymphocytes invade or are close in contact with hepatocytes. Hepatocytes show degenerative features, swelling, shrinkage, and apoptosis while some other liver cells seem to survive, becoming hyperplastic while growing in a tubular fashion (so-called rosetting). As a result, the limiting plates are replaced by newly form connective tissue, sometimes accompanied by ductular profiles. The biliary piecemeal necrosis is marked by a strikingly increase in the number of ductular profiles extending in the periportal area accompanied by oedema, neutrophil infiltration, periductular fibroplasia, and features of hepatocellular death associated with cholate stasis (Fig. 3B). This form is usually associated with severe ductopenia. The interface hepatitis is a turning point in the natural history of PBC because its severity constitutes the signal that announces the onset of fibrosis and eventually cirrhosis [89,103,105]. A schematic view of the putative sequences involved in the inflammatory process is depicted in Fig. 4. Hepatocellular death triggers expansion of progenitor cells marked by the appearance of reactive ductules, which have the potential to secrete a series of mediators that may attract and activate fibroblasts of different origins, monocytes, bone marrow-derived cells, epithelial–mesenchymal transition, stellate cells and periductal portal fibroblasts [106–113].

Cholestasis is a key feature of PBC. Cholestasis in early PBC cannot be attributed exclusively to the loss of bile ducts because serum markers of cholestasis and pruritus may already be present before the onset of significant ductopenia, indicating a certain functional component. Cholestasis in early PBC is mainly a ductal cholestasis and secondarily a canaliculic cholestasis as the consequence of the outflow functional blockade. One of the main features of the signalling pathways involved in ductal cholestasis is its ability to induce alkalisation and dilution of canaliculic bile upon stimulation by secretin and several neuropeptides [114–117]. This unique property is lost in PBC and is associated with defective regulation of cholangioyte AE2 (SLCA2) and NHE (SLCA9A3) exchanger activities and expression [118,119], and loss of the expression of inositol 1,4,5-triphosphate receptors involved in Ca2+-mediated bicarbonate secretion [120]. Inflammatory cytokines inhibit cAMP-dependent fluid secretion in cholangiocytes and impair the barrier function of biliary epithelia [121,122], an effect that may be mediated by nitric oxide [123]. UDCA therapy partially restores AE2 activity and expression as well as secretin-induced bicarbonate and fluid secretion [124]. Dilution and alkalisation of bile are critical for the defence of the biliary epithelium against microbes and pathogen-associated molecular patterns. The impaired generation of a bicarbonate-rich choleresis may hinder the activity of antimicrobial peptides because of the high salt concentration of bile [125]. The absence of an alkaline pH in bile could also alter the activity of the biliary alkaline phosphatase, thus inhibiting its ability to dephosphorylate endotoxin and to prevent LPS-induced inflammation [126]. Circumstantial evidence supporting this view includes the simultaneous effects of UDCA therapy on dilution and alkalisation of bile, the accumulation of endotoxins in cholangiocytes [127], and serum markers of cholestasis and immune reaction against endotoxins [128].

Alterations in hepatocellular transporter gene expression evolve in PBC in stage-dependent fashion. In the early anicteric stage, no changes in bile salt and bilirubin transporters are detectable [129–131]. With disease progression, OATP2 and NTCP expression is down-regulated while BSEP, MDR3 and MRP2 expression is up-regulated or maintained. These changes may represent adaptive mechanisms to limit bile acid burden in chronic cholestasis. As these changes do not sufficiently counteract cholestatic liver damage, therapeutic strategies should aim at stimulating the bile acid detoxification pathways.
Natural history

PBC progresses through three irreversible states: (a) cirrhosis; (b) a terminal phase defined when serum bilirubin reaches 100 μmol/L (6 mg/dl) with or without GI bleeding, ascites, or encephalopathy; and (c) death unless OLT is performed.

The patterns of clinical disease and natural history have changed significantly in the last two decades.

Natural history in the pre-UDCA era

In 1979, Shapiro and colleagues [132] showed that after a relatively stable phase, serum bilirubin increased sharply in the months preceding death. In patients with serum bilirubin levels above 34 μmol/L, the mean survival was 4 years; in those with values above 102 μmol/L, it was 2 years. The multicentre European study, initially aimed at evaluating the efficacy of azathioprine in PBC, provided precise data on the natural history in this era [133]. At enrollment, 54% and 19% of a total of 236 patients had stage 1–2 and stage 3 disease, respectively. During a 4-year follow-up period, half of the patients developed histologically-proven cirrhosis and 25–35% acquired one or several criteria (see above) of the terminal phase of PBC. In the large community based study of 770 patients in Northeast England [134], 15% of them developed signs of liver failure during a 5-year follow-up period. The rate of histological progression, assessed in three large groups of patients in the absence of a therapeutically effective agent, has shown that the median time to develop extensive fibrosis was 2 years and the probability of remaining in the early stage was 29% (95% confidence interval: 15–52%) after 4 years of follow-up [133,135,136]. The rate of development of oesophageal varices, in a prospective study of 256 patients during a median follow-up of 5.6 years, was estimated to be 31% [137].

Natural history in the UDCA era

Patients receiving UDCA therapy have a delayed rate of histological progression to cirrhosis. In an early study [138], the rate of progression to cirrhosis after a follow-up period of 6 years was 13% in patients receiving UDCA and 49% in the control group of patients. In a French trial [136], UDCA therapy was associated with a 5-fold lower progression rate from early stage disease to extensive fibrosis or cirrhosis (7% per year under UDCA vs. 34% per year under placebo, p <0.002). At 4 years, the probability of UDCA-treated patients to remain in early stage disease was 76% (95% confidence interval: 58–88%), as compared to 29% (15–52%) in placebo-treated patients. The effect of UDCA therapy on the development of oesophageal varices was addressed in a prospective study of 180 patients followed up for 4 years [139]. The risk of developing varices was 16% for the UDCA-treated patients and 58% for those receiving the placebo.

Long-term observational studies and Markov modelling have been used to study the effect of UDCA on survival. In 262 patients who had received 13–15 mg/kg UDCA daily for a mean of 8 years
Clinical presentation, diagnosis investigation, and patient evaluation

Clinical presentation

There are three major forms of PBC (Fig. 5). The typical or classical form is represented by the slowly progressive decline of small bile ducts and parallel increase in liver fibrosis, leading to biliary cirrhosis over a period of 10–20 years. A second form, which affects 10–20% of patients, is characterised by the fluctuating or persistent presence of the features of AIH [144]. These patients have a more severe disease course, with early development of liver fibrosis and liver failure. A third form, which affects 5–10% of patients, is represented by the so-called premature ductopenic variant [145]. Its hallmark is a very rapid onset of ductopenia and severe icteric cholestasis, progressing very quickly towards cirrhosis in less than 5 years.

PBC is now diagnosed earlier in its clinical course than it was in the past. Half of the patients are asymptomatic at diagnosis. Fatigue and pruritus are the two symptoms of the early phase of the disease. Fatigue, a frequent complaint, is unrelated to the severity. Whether fatigue is a specific symptom or not remains unknown [146]. Nevertheless, it has a major impact on the quality of life of PBC patients [147–151]. It is associated with cognitive and emotional dysfunction, depression, sensory and autonomic abnormalities. It may be associated with excessive day-time somnolence [152,153]. A mild pruritus affects about half of the patients at diagnosis. It may be severe in the premature ductopenic variant, affecting the patients’ quality of life. In some patients, the diagnosis is made in the work-up of another autoimmune disease. In 5–10% of patients, the diagnosis is made because of signs of compensated or decompensated cirrhosis. In the variant associated with features of AIH, the diagnosis may be made in a patient with acute cytolytic hepatitis [154].

In the typical form, patient biochemistry is characterised by predominant elevation of serum alkaline and gammaglutamyltranspeptidase activities while serum activities of transaminases are mildly or moderately increased. In the premature ductopenic variant, there is great cholestasis and it is associated with considerable hypercholesterolemia affecting both HDL and LDL fractions, and the non-atherogenic lipid particle LPX [155]. In patients with both the features of AIH and PBC, serum activities of transaminases may be markedly elevated and are usually associated with marked increase of IgG. Increased IgM levels are a constant feature in all forms of PBC. Thrombocytopenia, polyclonal hyperglobulinemia, and hyperbilirubinemia are indices of cirrhosis. Low prothrombin index and hypoalbuminemia occurs usually in the late phase of the disease [156,157].

Diagnostic investigation

Good ultrasound investigation of the liver and biliary tree is mandatory in all patients presenting with liver abnormalities. If the biliary system appears normal by ultrasound and the AMA is positive, no further radiologic delineation of the bile ducts is necessary. If the diagnosis of PBC is uncertain, cholangiography may be necessary.

The major hallmark of PBC is the presence of AMA in serum. AMA that reacts with the E2 component of pyruvate dehydrogenase is diagnostic of PBC. A variety of antinuclear antibodies are associated with PBC. Of these, those reacting with the proteins of the pore complex (gp210, nucleoporin 62) and of the protein of the nuclear body (sp100) are specific.

Histological examination of the liver is only mandatory for diagnosis if the AMA is negative or if the patient has the biochemical picture of atypical PBC or is suspected of superimposed comorbidity. Nevertheless, it should be emphasised that histology is valuable for prognostic evaluation and treatment strategy.
The normal biochemistry is based on the following three criteria: (a) abnormal biochemical tests with preferential elevation of serum alkaline phosphatase and gammaglutamyltranspeptidase activities; (b) presence of antimitochondrial antibodies with M2 specificity as confirmed by ELISA or immunoblotting; and (c) evidence of NSCD at histology. Criteria a and b or c are sufficient for the diagnosis considering the high specificity of the anti-M2 antibody and NSDC [158,159].

Patients with AMA and normal biochemistry tests are at risk of developing true PBC. Patients with biochemical evidence of cholestasis but negative AMA may have PBC if NSCD or portal inflammation and ductopenia are demonstrated in the liver biopsy. The diagnosis is further supported in this setting if antinuclear antibodies against gp210, Nucleoporin 62, sp100 giving nuclear-rim or nuclear-dot pattern are present. NSDC is highly suggestive of PBC but is not pathognomonic since it may be present in patients with AIH, primary sclerosing cholangitis, lymphoma, and viral hepatitis C and E.

Variant forms of AIH may result from several concurrent features of PBC in AIH and may pose diagnostic difficulties. Specific autoantibodies to M2 autoantigen may be found in a minority of AIH [160–162]. Up to 24% of patients with AIH may have histological evidence of bile duct injury without biochemical cholestasis [163]. All of these patients respond to AIH-conventional therapy. Finally, up to 2.4% of patients with typical PBC develop an acute AIH-superimposed on their PBC [154,164]. In this context, the so-called PBC-AIH overlap syndrome is defined by the simultaneous or consecutive concurrent main characteristics of the two conditions. The following score has been proposed to establish the diagnosis, e.g., presence of two of the three of the following features: (1) ALT activity ≥ 5 times upper limits of normal; (2) IgG ≥ 2 times upper limits of normal and/or positive anti–smooth muscle antibody; and (3) liver biopsy with moderate or severe perportal or periseptal inflammation [158,159,165].

Patient evaluation

Mucosal infections, especially recurrent urinary infections and cigarette smoking, two potential risk factors of PBC, should be recognised, treated or prevented. About 20% of the patients exhibit other simultaneous or consecutive autoimmune diseases, the most frequent being AIH, CREST syndrome, and/or scleroderma and thyroiditis. Celiac disease is not so frequent but should be recognised because of the beneficial effect of the gluten free diet.

First-degree relatives of patients with PBC are at high risk of PBC or other autoimmune diseases. The patients and their relatives should be informed and evaluated for these conditions. While the pathogenesis of fatigue and pruritus is still unknown and their treatment empirical, these two symptoms should be carefully analysed and if possible, quantified.

The attention should focus on the severity or potential severity of the disease.

Is there evidence of cirrhosis? Asymptomatic cirrhosis is probable if splenomegaly is disclosed, prothrombin index is lower than 80%, serum albumin is lower than 38 g/L, serum bilirubin is more than 2 mg/dL, and the platelet count is less than 150,000/μL. In these patients, oesophageal varices should be evaluated and treated if they are large. The cirrhotic patients are at risk of developing hepatocellular carcinoma and as such need to be regularly investigated by appropriate imaging techniques.

Is there any evidence of indices of severity and predictors of poor response to UDCA therapy apart from cirrhosis? Fatigue and pruritus are in most cases not related to the severity of PBC and do not have major prognostic value. Serum bilirubin level is the best predictor of prognosis and of long-term response to UDCA treatment. Patients with even a mild elevation (more than 1 mg/dL) are at risk of developing extensive fibrosis or cirrhosis in the next 10 years. Patients with high serum bilirubin level (more than 3 mg/dL), high serum alkaline phosphatase activity, and cholesterol have usually severe ductopenia at presentation (the premature ductopenic variant of PBC) and will not respond to any medical therapy. These patients should be informed that liver transplantation is the only therapeutic alternative.

Is there any evidence of features of AIH? Patients with both the criteria of PBC and AIH need to be given both UDCA and glucocorticoids. High levels of serum bilirubin, together with high ALT and IgG as well as the presence of antiactine and anti-SLA antibodies, are very suggestive of this condition.

Liver histology may help to fully evaluate the prognosis and to establish the best medical treatment. Given the risk of sampling error, at least 10 portal tracts should be available. The individual lesions have to be graded. The Metavir score could be used to assess both fibrosis and interface hepatitis (under the form of lymphocytic piecemeal necrosis). The presence and extent of the NSCD should be noted. Ductopenia should be quantified. However, in case of heavy portal inflammation, this quantification may be difficult even with the use of cytokeratin histochemical staining. The presence and extent of biliary piecemeal necrosis should be assessed. Nodular regenerative hyperplasia, which is sometimes present, may indicate portal vascular damage by inflammatory cells. The presence of numerous fibrous septa, loss of more than 50% of the interlobular bile ducts, moderate to severe lymphocytic or biliary piecemeal necrosis are indices of severe prognosis.

Because the prognosis of PBC is far better than two or three decades ago, two associated conditions deserve particular attention. Hypercholesterolemia with increased LDL cholesterol is observed in about 20% of the patients. Accordingly, the risk of cardiovascular disease should be evaluated and medical therapy possibly proposed. Osteoporosis and osteopenia might be more frequent in women with PBC than in a control population, although this remains controversial [166]. Nevertheless, metabolic bone disease should be assessed and prevented in particular in those at risk of receiving glucocorticoids.

Treatment

Specific therapy

UDCA therapy

All PBC patients with abnormal liver biochemistry should be considered for specific therapy. UDCA, at the dose of 13–15 mg/kg/day, is currently considered the mainstay of therapy for PBC. Randomised, double-blinded, placebo-control trials have consistently shown that UDCA improves parameters of liver biochemistry including serum bilirubin, the major prognostic marker in PBC. UDCA delays the progression of fibrosis and histological stage. A combined analysis of three randomised-controlled trials, including 548 patients with PBC, showed improved survival without liver transplantation in patients with moderate to severe disease treated with UDCA at doses of 13–15 mg/kg/day for up to 4 years [167]. Long-term observational studies have shown that
UDCA therapy provides a better survival rate than that predicted by the Mayo model [140–142,168,169]. The survival rate of UDCA-treated patients in early stages of disease is similar to that in a control population [142,143]. In both Europe and North America, the number of liver transplants for PBC is falling in parallel with the increased use of UDCA therapy [170,171]. Some meta-analysis (but not all) including short-term trials with low doses of UDCA (<12 mg/kg/day) has questioned the efficacy of UDCA. However, based on all available data, it is currently recommended to treat PBC with UDCA using doses of at least 13 mg/kg/day and to start early [158,159]. UDCA could be taken in divided doses or as a single dose. Because UDCA is an acid, it can produce gastric discomfort, burning sensation and symptomatic reflux in some patients. These symptoms are easily managed with proton pump inhibitors or by ingesting the bile acid at the end of meals.

Overall, UDCA is extremely safe. The majority of the patients note a weight gain (2 kg, on average). Whether this is due to changes in endogenous bile acid composition and subsequently on signalling through the TGR5 pathway remains to be explored [172]. The weight gain may be more significant in patients who have stopped smoking. In patients with pruritis and frank cholestasis, UDCA may increase pruritus if given at a dose of 13–15 mg/kg/day. In these patients it is recommended to start UDCA therapy at a low dose, 200–400 mg/day, and to progressively increase the daily dosage over a period of 4–8 weeks.

The aim of UDCA therapy is to provide the normalisation of the serum bilirubin, alkaline phosphatase, and ALT or AST levels during the first year of therapy. Patients with complete normalisation of serum bilirubin, ALT or AST, and alkaline phosphatase have a null risk to progress to cirrhosis (unpublished data). In many patients, this cannot be achieved. We defined an optimal response to UDCA when serum bilirubin is less than 1 mg/dl, AST less than two times the upper limit of normal, and alkaline phosphatase less than three times the upper limit of normal at the end of the first year of therapy. Indeed, patients with these criteria have a 10-year life expectancy without liver transplantation similar to that of the control population [142]. Others have observed that, patients with a decline of serum alkaline phosphatase of more than 50% achieve an excellent long-term prognosis [141]. In a subset of patients, the daily dose of 13–15 mg is not sufficient to achieve the best biochemical response. In the patients who do not respond adequately, measurement of the blood and biliary enrichment in UDCA by LC–MS may be useful. In those with a low enrichment of total serum bile acids with UDCA (less than 40%) a trial with daily doses up to 20 mg/kg/day may be proposed to achieve a better response.

About 40% of our patients have a suboptimal response to UDCA. These patients need an adjuvant therapy.

Adjuvant therapies
Patients with features of AIH, severe interface hepatitis, abnormal serum bilirubin level or suboptimal response to UDCA as defined above, require trials with adjuvant therapies. Currently, glucocorticoids (prednisone or budesonide) and methotrexate could be considered for these patients. The rationale for the use of glucocorticoids is based on the following arguments: glucocorticoids and specifically budesonide have been shown to provide benefit in patients treated with UDCA. A combination of both leads to better biochemical response and better histological response in terms of inflammation and fibrosis in de novo PBC patients [173,174].

Budesonide is given at 6–9 mg/day in non-cirrhotic patients. The drug is contraindicated in patients with cirrhosis. Preliminary studies in our patients indicate that patients with a suboptimal response to UDCA and frank biliary and periportal inflammation benefit from the combination of UDCA and glucocorticoids (in particular budesonide) in terms of survival without liver transplantation. In this setting, the use of glucocorticoids sparing agents, such as azathioprine or mycophenolate mofetil, may be recommended.

Methotrexate improved biochemical test results and liver histological findings when it was added to UDCA in patients who had an incomplete response to UDCA [175,176]. However, other studies found no efficacy of methotrexate when used alone or in combination with UDCA [177–179]. In a 10-year study, survival was the same in patients taking methotrexate and UDCA as in those taking colchicine and UDCA and was similar to that predicted by the Mayo model. However, one-third of the patients had few signs of PBC after 10 years of treatment. No patient who was in the precirrhotic stage at baseline and receiving methotrexate showed progression to cirrhosis, suggesting that methotrexate could be useful in a small subset of patients [180]. Randomised control trials of cyclosporine monotherapy in PBC have shown that the drug was effective in terms of clinical, biochemical, and histological progression [181] and significantly prolonged the time to death or transplantation [182] despite the well-known detrimental influence of cyclosporine on hepatobiliary transport systems [183]. However, because of the high rate of side effects, there has been little enthusiasm to further examine the potential benefit of cyclosporine, particularly in association with UDCA, a hydrophilic bile acid able to prevent cyclosporine-induced cholestasis [184].

Several drugs such as colchicine, chlorambucil, penicillamine, azathioprine, mycophenolate mofetil, melotilate, and thalidomide have been evaluated for PBC treatment. Many of them are either ineffective or toxic. None of them have been shown to be effective in UDCA-treated patients at risk of development of cirrhosis or liver failure as defined above [185].

Taking into account the considerable progress made in the understanding of the pathobiology of PBC, many novel therapeutic approaches could be proposed to target patients with no or incomplete biochemical response to UDCA [186]. Among them, PPAR alpha agonists [187,188], FXR agonists [189] and biotherapies such as antiCD20 [190,191], GLP1 receptor agonists [192,193], and oestrogen-alpha receptor agonists [100] could be promising.

Liver transplantation
Liver transplantation has greatly improved the survival of patients with PBC [9]. It is the only effective treatment for those with decompensated cirrhosis or liver failure. Patients, who present with the features of the premature ductopenic variant of PBC, do not respond to any medical therapy and despite the absence of any decompensation draw a major benefit from liver transplantation. PBC recurs in about 20% of patients at 5 years [194]. Recurrence is more frequent in patients without a glucocorticoid and cyclosporine regimen. The beneficial long-term effect of UDCA in this setting remains unknown [195].

Treatment of symptoms and complications
Pruritus
Cholestyramine is widely used as first-line treatment, although the evidence to support this is limited. When both agents are
Review

used, UDCA and cholestryramine should be spaced a minimum of 4 h apart to prevent binding and loss of efficacy [196]. Rifampicin has a strong evidence base [197,198]. Daily dosage up to 600 mg/day may be used in patients receiving UDCA. However, the drug may induce hepatitis in some cases [199]. Other therapies include glucocorticoids, sertraline, and opiate antagonists [200]. Plasmapheresis or biliary drainage may be successful when other treatments fail. In very rare patients, resistant pruritus may be an indication for liver transplantation.

Fatigue

Fatigue may be multifactorial; causes other than PBC should be considered. Modafinil, a drug approved for the treatment of narcolepsy, has been reported in open studies to provide significant benefit in PBC patients with fatigue [201,202]. The drug used at doses up to 400 mg/day seems well tolerated and very effective in those with excessive fatigue and day-time somnolence.

Hypercholesterolemia

UDCA induces an average 15–20% decrease in total and LDL cholesterol at 1 year of therapy [203]. Statins are safe and effective in PBC [204,205].

Portal hypertension

A minority of patients with PBC develop presinusoidal portal hypertension before becoming cirrhotic. Management of portal hypertension in patients with PBC should be the same as that for other cirrhotic patients. Severe portal hypertension, even without any other sign of decompensation, is a good indication for liver transplantation. Liver transplantation should be preferred to TIPS [206].

Osteopenia and osteoporosis

Current treatments for osteopenia and osteoporosis, which affect up to 30% of PBC patients, included: physical activity, calcium and vitamin D supplementation to achieve at least 30 ng/ml of serum 25OH D3, bisphosphonates or oestrogens supplementation [158,159,166].

Key points:

- Progressive immune-mediated destruction of the small intrahepatic bile ducts.
- Affects predominantly middle-aged women.
- Risk factors: history of familial autoimmune disease, active or passive smoking, recurrent urinary tract infections.
- AMA, targeting the 2-oxo-acid dehydrogenase complex, is diagnostic.
- Associated with autoimmune hepatitis in 10% of the patients.
- UDCA (13–15 mg/kg/day) is recommended as first-line medical therapy by the 2009 EASL and AASLD practice guidelines.
- Absence or incomplete biochemical response to UDCA has major prognostic implication and represents a simple and useful tool in identifying patients requiring further therapeutic intervention.
- Liver transplantation is the only treatment for patient with decompensated cirrhosis or the features of the preducto-penic variant.

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References


Review


[176] Babatini MA, Sanai FM, Swain MG. Methotrexate therapy for the symptomatic treatment of primary biliary cirrhosis patients, who are biochemical incomplete responders to ursodeoxycholic acid therapy. Aliment Pharmacol Ther 2006;24:813–820.


