

EDITORIAL

## Concept on the pathogenesis and treatment of primary biliary cirrhosis

Vasily Ivanovich Reshetnyak

Vasily Ivanovich Reshetnyak, Scientific Research Institute of General Reanimatology, Russia Academy of Medical Sciences, Moscow, Russia

Correspondence to: Vasily Ivanovich Reshetnyak, Scientific Research Institute of General Reanimatology, 25-2, Petrovka str., Moscow 107031, Russia. v\_reshetnyak@yahoo.com  
Telephone: +7-495-2006505 Fax: +7-495-2006505  
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### Abstract

Primary biliary cirrhosis (PBC) is an organ-specific autoimmune disease that predominantly affects women and is characterized by chronic, progressive destruction of small intrahepatic bile ducts with portal inflammation and ultimately fibrosis, leading to liver failure in the absence of treatment. Little is known about the etiology of PBC. PBC is characterized by anti-mitochondrial antibodies and destruction of intrahepatic bile ducts. The serologic hallmark of PBC is the presence of auto-antibodies to mitochondria, especially to the E2 component of the pyruvate dehydrogenase complex (PDC). Current theories on the pathogenesis of PBC favor the hypothesis that the disease develops as a result of an inappropriate immune response following stimulation by an environmental or infectious agent. Some reports suggest that xenobiotics and viral infections may induce PBC. The pathogenetic mechanism is believed to be caused by a defect in immunologic tolerance, resulting in the activation and expansion of self-antigen specific T and B lymphocyte clones and the production of circulating autoantibodies in addition to a myriad of cytokines and other inflammatory mediators. This leads to ductulopenia and persistent cholestasis, by developing end-stage hepatic-cell failure. In this review are given our own and literary data about mechanisms of development of intrahepatic cholestasis and possible ways of its correction.

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### ETIOLOGY OF PRIMARY BILIARY CIRRHOSIS

Primary biliary cirrhosis (PBC) is a chronic cholestatic, granulomatous, and destructive inflammatory lesion of the interlobular and septal bile ducts, which is likely to be caused by an autoimmune mechanism with a potential tendency to progress to cirrhosis<sup>[1,2]</sup>.

Primary biliary cirrhosis is characterized by a T-cell-mediated destruction of bile duct epithelial cells that line the small intrahepatic bile ducts. This leads to ductulopenia and persistent cholestasis, by developing end-stage hepatic-cell failure.

The etiology of the disease is still unknown<sup>[3]</sup>. Many authors regard the disease as impaired immunoregulation with a loss of tolerance of histocompatibility antigen-enriched tissues. How and why the bile ducts are involved in this process remains unknown. Viruses<sup>[4,5]</sup>, bacteria, xenobiotics, and human immunoregulatory defect may be possible PBC triggers that initiate the immunopathological cascade<sup>[6-8]</sup>.

The development of PBC is preceded by a long asymptomatic period<sup>[9-11]</sup>. The wide use of computer-aided screening biochemical and immunological studies has significantly increased the detection of asymptomatic patients. In this period, there are generally no physical signs of PBC, at the same time anti-mitochondrial autoantibodies (AMA) are detectable in the serum of virtually all patients (95%)<sup>[12-15]</sup>. The fact that AMA are detectable many years before PBC manifests itself is indicative of their primary immunopathological role rather than a secondary phenomenon that occurs in the presence of cholestasis. The production of AMA is not an epiphenomenon, and an understanding of the mechanism of AMA induction will shed light on the etiology of PBC<sup>[3]</sup>.

The activity of antibodies to antigens of various specificity (exogenous and autologous genesis) is associated with different classes of immunoglobulins. In PBC, there is an elevated concentration of the immunoglobulin M class (IgM)<sup>[16]</sup>. The increase in serum IgM is the result of chronic B-cell activation induced *via* the toll-like receptor (TLR) signaling pathway<sup>[17]</sup>. The activity of tissue, bacterial, and viral antibodies are assumed to be associated with the biological properties of IgM. Testing for AMA and IgM are the most useful laboratory procedure in the diagnosis of PBC<sup>[18]</sup>.

Active searches are recently under way for autoantigens

whose expression induces an immune response that results in destruction of the biliary epithelium. The antigens reacting with AMA are located on the internal mitochondrial membrane. The targets of activated T-lymphocytes are the dihydrolipoamide acetyltransferase components of the 2-oxoacid dehydrogenase, enzyme complexes that are important in oxidative energy metabolism. Pyruvate dehydrogenase complex (PDC) is the best known of these. Among the events demonstrated to induce an antibody response cross-reactive with PDC are exposures to bacterial PDC or retroviral proteins or xenobiotics or microchimerism<sup>[2]</sup>. Its dihydrolipoamide acetyltransferase component is referred to as PDC-E2<sup>[6]</sup>. PDC-E1 and PDC-E2 antigens are sensitive (98%) and specific (96%) for the diagnosis of PBC<sup>[19,20]</sup>. A major question in understanding the pathogenesis of PBC is why PBC patients lose their tolerance to antigens that are found in virtually every cell in the body. The identification of anti-PDC responses (present in over 95% of PBC patients) has given rise to important questions pertinent to our understanding of the pathogenesis of PBC. How and why does immune tolerance break down to as highly conserved and ubiquitously expressed self-antigen as PDC? Why does the body stop recognizing individual components of the pyruvate dehydrogenase complex as proper proteins? Why does breakdown in tolerance to an antigen present in all nucleated cells result in damage restricted to the intra-hepatic bile ducts? How does the internal mitochondrial membrane antigen initiate the production of autoantibodies?

Noteworthy is the assumption that infectious agents are involved in the etiopathogenesis of PBC. Based on the proposed role of microorganisms in the pathogenesis of the disease, Mao TK *et al* and Amano K *et al* hypothesize that patients with PBC possess a hyperresponsive innate immune system to pathogen-associated stimuli that may facilitate the loss of tolerance<sup>[21,22]</sup>. In PBC patients, AMA shows a cross-reaction with the subcellular components of gram-negative and gram-positive microorganisms<sup>[23]</sup>.

Recent studies have suggested that the induction of PBC is multifactorial, in which the primary player involves the xenobiotics modification of mitochondrial proteins or exposure to xenobiotics-modified bacterial mitochondrial protein homologs, leading to breaking of tolerance to the human mitochondrial autoantigens and eventually liver pathology in genetic susceptible individuals<sup>[3,24,25]</sup>. A possible cause is molecular mimicry between microbial agents and self-antigens<sup>[26-28]</sup>. Infection with or exposure to a microorganism whose PDC-E2 bears a close homology with human PDC-E2 could act as an immunological trigger that initiates the development of PBC. It is suggested that the mutant forms of *E. coli*<sup>[26,29,30]</sup> and mycobacteria<sup>[31-33]</sup> trigger an immunopathological process in PBC. Anti-PDC-E2 antibodies cross-react specifically with mycobacterial hsp65<sup>[34]</sup>. Immunization of rabbits with *E. coli* rough (R) mutants gives rise to PBC-specific AMA. The PBC patients' feces contain more or less counts of *E. coli* R-forms that specifically react with AMA<sup>[29]</sup>. Whether the intestinal R-forms are etiologically important for the development of PBC remains still unclear.

There has been recent evidence for the etiological

role of *Novosphingobium aromaticivorans* in the development of PBC<sup>[35-37]</sup>. *N. aromaticivorans* is a gram negative strictly aerobic bacterium that is found worldwide in soil, water, and coastal plain sediments. Its PDC-E2-like proteins have a higher degree of homology with the immunodominant region of human PDC-E2 than any microorganism thus far studied (100-1000 times greater than that of *Escherichia coli*)<sup>[35,37]</sup>. *N. aromaticivorans* can metabolize xenobiotics that are similar to the chemical compounds that react with sera from PBC patients. Some of these xenobiotics are immunologically related to lipoic acid, the cofactor that is at the active center of PDC-E2. Thus, *N. aromaticivorans* can theoretically break down self-tolerance in two ways: by molecular mimicry due to subclinical infection and by the metabolism of xenobiotics that are present in the environment.

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It was deduced that antibodies against *N. aromaticivorans* were found in 77 of 77 PBC patients from Milan, Italy, who had antibodies to PDC-E2 and that the titers to *N. aromaticivorans* proteins were similar to those to human PDC-E2<sup>[35,37]</sup>. Thirteen of 14 Icelandic PBC patients who were AMA positive reacted against at least one of the 2-oxoacid dehydrogenase-E2 complexes<sup>[36]</sup>. These observations provide additional evidence that exposure to *N. aromaticivorans* may trigger the development of PBC.

The epithelium of the bile ducts whose cell surface expresses antigens of the histocompatibility complex is considered to be the major target for AMA. The cross-interaction of AMA with the epitheliocytic antigens of bile cholangioles may damage the epithelium of ductules, by resulting in their obliteration. This leads to cholestasis in the minor canaliculi and bile capillaries with impaired bile excretion processes.

A morphologic study of liver biopsy specimens from asymptomatic patients with anti-mitochondrial autoantibodies detected during a screening makes it possible to diagnose one of the early stages of PBC. In stages I - II PBC, the biopsy specimens show different phases of lesion of bile canaliculi. Early disorders develop in the interlobular ducts, 45-75  $\mu\text{m}$  in diameter<sup>[38]</sup>. Dystrophy of ductal epitheliocytes should be considered to be the earliest disorder. Their cytoplasm becomes granular or homogeneous eosinophilic, swollen, and vacuolized, the nuclei get pycnotic. Later on, there is necrosis of a small segment of a canaliculus, but its outlines still remain and, finally, the wall is destroyed, that is a pattern of destructive cholangitis forms<sup>[39]</sup>.

The generation of immune responsiveness to self-antigen can result in pathogenic autoimmune damage of the intrahepatic biliary epithelial cells mediated by both humoral and cellular immune responses<sup>[2,40]</sup>. In PBC osteopontin is an important immune molecule in portal tracts, and contributes to the recruitment of mononuclear cells into epithelioid granuloma and also participates in bile duct injury *via* B-cell differentiation and plasma cell expansion<sup>[41]</sup>. PBC is characterized by chronic destructive cholangitis with a Th1-predominant cytokine milieu<sup>[42-44]</sup>.

CD8+ and CD69+ T cells were predominant in inflammatory infiltrates around damaged cholangiocytes;  $\beta_2$ -microglobulin conformational epitope and intercellular adhesion molecule-1 expression were enhanced in bile ducts and hepatocytes<sup>[45,46]</sup>. Toll-like receptor-3 and type I interferon signaling pathways are active in both the portal tract and liver parenchyma of early-stage PBC, and form the basis for hypothesis that these signaling pathways are involved in the pathophysiology of PBC<sup>[47]</sup>. Oxidative stress- and nitric oxide-mediated cellular senescence may be involved in bile duct lesions, which are followed by progressive bile duct loss in primary biliary cirrhosis<sup>[48-51]</sup>.

The canals of Hering (CoH) are destroyed in PBC in concert with the destruction of small bile ducts. This destruction appears to be an early event, because CoH numbers are lowest around stage 0 portal tracts, which still contain normal bile ducts<sup>[52]</sup>. The canals of Hering (CoH), converging from the hepatic lobule onto the portal tract, connect bile canaliculi to the interlobular bile ducts, and represent the most proximal portion of the bile drainage pathway with a cholangiocyte lining. *De novo* expression of intercellular adhesion molecule-1 (ICAM-1) both on mature cholangiocytes in CoH and epithelial cells in bile ductules in PBC implies that lymphocyte-induced destruction through adhesion by ICAM-1 and binding of lymphocyte function-associated antigen-1 expressing activated lymphocytes takes place not only in the bile ductules, but also in the CoH<sup>[53,54]</sup>.

## **PATHOGENESIS OF INTRAHEPATIC CHOLESTASIS**

Thus, autoimmune pathological processes gradually leading to ductulopenia develop in asymptomatic PBC<sup>[55-57]</sup>. The increased number of desolating bile ductules gradually result in impairment of bile excretion and in deficient entry of bile acids into the duodenum. In response to this, the hepatocyte increases the synthesis of bile acids. But ductulopenia does not diminish and bile secretion fails to restore. The reduction in the intestinal level of bile acids through the feedback system induces a compensatory hepatocytic increase in the biosynthesis of bile acids and cholesterol, the major substrate for their biosynthesis. The progressively increased bile acid biosynthesis occurring with the participation of cytochrome P<sub>450</sub> of the smooth endoplasmic reticulum and mitochondria leads, on the one hand, to hypertrophy of the endoplasmic reticulum and then to its hyperplasia and vacuolization, and, on the other, to the biosynthetic involvement of primary bile acids, other than specific P<sub>450</sub>-dependent monooxygenase and non-specific P<sub>450</sub>-dependent monooxygenase<sup>[58]</sup> that can oxidize cholesterol by the peroxide mechanism<sup>[59]</sup> to "atypical" metabolites. The latter may be further oxidized to "nonphysiological", "atypical" bile acids in the mitochondria. Moreover, the hepatocytic concentration of bile acids gradually elevates and their entry into the intestinal lumen remains insufficient, giving rise to a closed vicious circle that results in the accumulation of bile acids in the liver cell. Due to the elevated hepatocytic level of bile acids, their reabsorption from the portal venous bed decreases, leading to the entry and progressive

accumulation of bile acids in systemic circulation. This triggers the development of intrahepatic cholestasis that shows up as immunological, morphological, and biochemical signs when the disease is asymptomatic.

## **MECHANISMS OF DEVELOPMENT OF BIOCHEMICAL AND MORPHOLOGICAL SIGNS**

To inactivate the detergent effect of excess bile acids on the cell membrane apparatus, their sulfation and glucuronidization occur and the hepatocytic biosynthesis of cholesterol and phospholipids that are able to generate micellar-lamellar structures wherein bile acids are inserted diminishes. The biosynthesis of phospholipids occurs with the use of orthophosphate formed by hydrolysis of organic phosphorus compounds under the action of phosphatases: alkaline phosphatase (AP), and 5'-nucleotidase. The activity of these enzymes increases in patients with PBC just in the asymptomatic stage of the disease<sup>[16,60,61]</sup>. The increased activity of AP and 5'-nucleotidase is presumed to be due to their accelerated biosynthesis in the hepatocytes<sup>[62-64]</sup>. To enhance the synthesis of these enzymes, it is necessary to increase delivery of amino acids to the cells with the participation of  $\gamma$ -glutamyltransferase (GTP). A.S. Loginov<sup>[60]</sup> has noted that the change in blood  $\gamma$ -glutamyltransferase activity in patients with PBC outstrips the enhancement of the activities of AP and 5'-nucleotidase.

It is theorized that AP and 5'-nucleotidase give rise to orthophosphate where the latter is required<sup>[65]</sup>. Based on this theory, the enhanced activity of AP and 5'-nucleotidase in PBC is indicative of the higher hepatocytic need for orthophosphate.

High-resolution <sup>31</sup>P NMR spectroscopy has established the level of phosphate-containing compounds in the native bile of healthy individuals and PBC patients. There are lower levels of lecithin and orthophosphate in the patients' hepatic bile portion (Table 1).

The data given in the table show the average statistical integral intensity of the corresponding <sup>31</sup>P NMR spectral signals in conditional units.

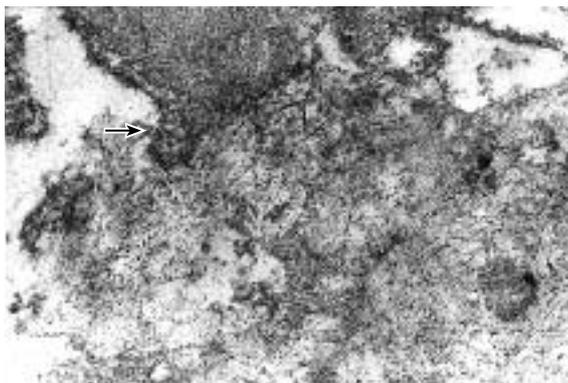
The change in the concentration of one of the native bile components-phosphatidylcholine (lecithin) suggests its altered acid-dependent (dependent on the secretion of bile acids) secretion by hepatocytes<sup>[66]</sup>. Biochemical studies have revealed that hepatic bile portions from PBC patients contain lower levels of not only lecithin, but also bile acids and cholesterol (Table 1). These findings suggest that hepatocytic bile secretion of the study components is decreased and that the relationship is retained between the secretion of bile lipid components (lecithin and cholesterol) and bile acids. At the same time there is an irregular reduction of bile acids and lipid components in the hepatic bile portion from patients with PBC. Thus, the levels of bile acids, lecithin, and cholesterol are decreased by 6, almost 5, and only 2.4 times, respectively, which is indicative of higher bile lithogenicity in these patients (Table 1).

The decreased quantity of orthophosphate in the hepatic bile portion from PBC patients points to its

**Table 1** Levels of bile acids, cholesterol, lecithin, and orthophosphate in the hepatic bile portion and serum of patients with PBC ( $n = 16$ ) and controls ( $n = 14$ )

Parameters		Values		
		Controls (mean <sub>1</sub> ± SE)	Patients with PBC (mean <sub>2</sub> ± SE)	mean <sub>1</sub> / mean <sub>2</sub>
Bile acids (g/L)	Bile	3.9 ± 0.8	0.65 ± 0.02	6.0
	Blood	0.012 ± 0.008	0.054 ± 0.008	-0.2
Cholesterol (μmol/L)	Bile	0.91 ± 0.06	0.38 ± 0.08	2.4
	Blood	4.2 ± 0.6	11.8 ± 1.6	-0.4
Lecithin <sup>a</sup>	Bile	2.1 ± 0.3	0.5 ± 0.1	-4.6
Orthophosphate <sup>a</sup>	Bile	1.2 ± 0.3	0.30 ± 0.07	4.0

<sup>a</sup> The data given in the table show the average statistical integral intensity of the corresponding <sup>31</sup>P NMR spectral signals in conditional units.



**Figure 1** Hepatocytic ultrastructure. Digitonin-treated biopsy specimen. The specimen is contrasted with aqueous uranyl acetate and lead citrate. Deposits of cholesterol-digitonin complexes (the view of "tender spoon-drift clouds") are seen in the cytoplasm close to the mitochondria. The mitochondrial membrane forms pseudopodia here and there (indicated by arrow).

hepatocytic secretion in bile excretion and its simultaneous reduction in its liver cell content since the secretion of orthophosphate, as other inorganic ions, is passively effected by the concentration gradient and does not depend on that of bile acids. The reduced hepatocytic concentration of orthophosphate (despite the enhanced activities of AP and 5'-nucleotidase) indicates the intensive uptake of a phosphorus group in the metabolic processes taking part in the liver cell in PBC.

Biosynthesis of phospholipids is one of the possible ways of using orthophosphate<sup>[67]</sup>. The hepatic biopsy specimens of PBC show a 1.5-fold increase in the total amount of phospholipids<sup>[68]</sup>. This increase occurs, by elevating the content of lecithin in the cell membranes. Moreover, the same membranes contain the lower levels of lysophosphatidylcholine, sphingomyelin, phosphatidylserine, phosphatidylinositol, and phosphatidylethanolamine<sup>[68]</sup>. There is a 2-fold decrease in the ratio of free cholesterol to phospholipids in the cell membranes. Major alterations in protein status and lipid composition occur in the erythrocyte membrane of patients with PBC<sup>[69]</sup>.

Along with this, segments of membrane cholesterolization and those of free cholesterol deposition in the cytoplasm (Figure 1) and Disse's space are detectable by electron histochemical studies of liver biopsy specimens from PBC patients, by using digitonin. All



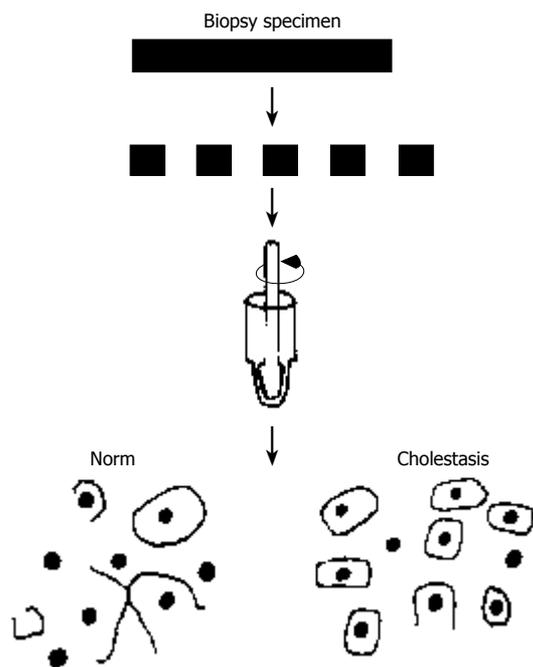
**Figure 2** Hepatocytic ultrastructure in PBC. Biopsy specimen. The specimen is contrasted with aqueous uranyl acetate and lead citrate. Arrows indicate myelin-like and multilamellar structures with increased osmiophilia.

cholesterol-digitonin complexes show a more or less significant osmiophilia, which points to the presence of phospholipids and proteins in these complexes.

Yokomori H *et al* demonstrate the increased expression of caveolins in proliferating bile ductules in PBC, which may be related to the homeostasis of cholesterol transport in regenerating bile ductules in PBC liver<sup>[70]</sup>. Caveolins are cholesterol-binding proteins involved in the regulation of several intracellular processes including cholesterol transport.

Electron microscopic studies of liver biopsy specimens from patients with PBC, including those with its early stage, have revealed ultrastructural alterations: hypertrophy and vacuolization of the smooth and rough endoplasmic reticulum, formation of lipid drops and myelin-like structures (Figure 2); the mitochondrial shape and structural changes characterized by the reduced number of internal membrane cristae, the appearance of external membrane "pseudopodia" (Figure 1), as well as local discrepancies of hepatocytes due to their intercellular space.

In the PBC biopsies, the enzymatic activity is increased in the bile canaliculi and is also present in the lateral membranes of the hepatocyte. Transmission electron microscopy of the lateral surface of the hepatocyte in normal livers showed a smooth surface without microvilli but in PBC livers a large number of microvilli were seen in the lateral membranes<sup>[71]</sup>. The localization of the enzymatic reaction, microvilli and Golgi apparatus at the PBC hepatocyte lateral membranes may represent a compensatory mechanism for derivation of bile flow and other components from the hepatocyte to the intercellular space.



**Figure 3** The scheme of liver biopsy specimen homogenization for quantitative adhesiometric study.

The results of electron microscopic studies of intercellular contact impairments in cholestasis agree well with the estimates of cell adhesion, by using the quantitative method developed by the authors to evaluate adhesive interactions in hepatocytes<sup>[72]</sup>. The essence of the method is as follows: tight junction (TJ) ensures a mechanical strength of interhepatocytic interactions and when liver tissue disperses with strong intercellular contacts, the plasma membrane breaks and the cell nuclei release. Decreased intercellular contacts lead to that in mechanical dispersion of liver tissue, the hepatocytes move away from each other, without rupturing the plasma membrane. A great number of single cells and much fewer cell nuclei are detectable (Figure 3).

After homogenization of a liver tissue biopsy specimen, single hepatocytes and free cell nuclei are detectable by light microscopy. Then the dissociation coefficient ( $C_d$ ) is the ratio of the number of single cells ( $N_c$ ) to the amount of single cells and the cell nuclei ( $N_n$ ) isolated after liver tissue dispersion is calculated by the formula:  $C_d = N_c / (N_c + N_n)$ .

A study of liver biopsy specimens indicated a statistically significant increase in  $C_d$  in patients with PBC as compared with those with chronic liver diseases, which were similar (Table 2). There is a 1.5-2-fold decrease in the size of hepatocytes. In PBC, the increase in  $C_d$  is associated with decreased intercellular contacts, particularly at the site of tight junction, probably due to cholestasis and its related elevated pressure in the bile capillaries. Substantial alteration in the TJ protein occurs predominantly in bile ducts and in hepatocytes in PBC, suggesting increased paracellular permeability along different paracellular routes for bile regurgitation in these chronic cholestatic liver diseases<sup>[73]</sup>. Melero S *et al* provide functional evidence that PBC cholangiocytes exhibit a widespread failure in the regulation of carriers involved in transepithelial  $H^+$ /

**Table 2** Arithmetic means of the dissociation coefficient in different nosological forms of chronic diffuse hepatic lesions

Liver lesion	$C_d$ (mean $\pm$ SD)	$C_d$ ranges
PBC ( $n = 11$ )	$0.61 \pm 0.03^b$	$0.41 \pm 0.84$
CH ( $n = 20$ )	$0.14 \pm 0.04$	0.02-0.24
LC ( $n = 3$ )	$0.18 \pm 0.03$	0.14-0.24
UH ( $n = 8$ )	$0.11 \pm 0.02$	0.00-0.16
FH ( $n = 11$ )	$0.07 \pm 0.02$	0.00-0.14
Controls ( $n = 16$ )	$0.09 \pm 0.02$	0.00-0.18

CH: Chronic hepatitis of various etiology; PBC: Primary biliary cirrhosis; UH: Gilbert's unconjugated hyperbilirubinemia; FH: Fatty hepatitis; LC: Liver cirrhosis; <sup>b</sup> $P < 0.001$  vs both the controls and other groups of the disease.

$HCO_3^-$  transport, thus, providing a molecular basis for the impaired bicarbonate secretion in this cholestatic disease<sup>[74]</sup>.

There is no direct evidence for elevated hepatocytic levels of bile acids since methods for their determination within the liver cell are lacking. However, many indirect experimental data lead to the conclusion that liver cell lesion observed in chronic cholestasis is at least partially caused by the hepatocytic accumulation of excessive cellular toxic bile acids<sup>[75-77]</sup>.

Osmiophilic myelin-like and cholesterol-digitonin structures detectable in the liver biopsy specimens by electron microscopic study appear to be intracellular located complexes of cholesterol, phospholipids, and bile acids.

The blood of patients with early-stage PBC contains higher levels of phospholipids, cholesterol<sup>[78,79]</sup>, and bile acids<sup>[80-82]</sup> (Table 1). The change in the content of cholesterol, phospholipids, and bile acids in the blood of patients with PBC is associated with their increased formation in the liver and regurgitation into blood flow. Down-regulation of basolateral uptake systems and maintenance/up-regulation of canalicular and basolateral efflux pumps may represent adaptive mechanisms limiting the accumulation of toxic biliary constituents<sup>[83]</sup>. The elevated levels of phospholipids and cholesterol in the serum of patients with PBC seem to be determined by the necessity of neutralizing the detergent effect of excess bile acids entering the systemic circulation. Cholesterol and phospholipids are able to bind bile acids, by inactivating their solubilizing effect. This gives rise to the so-called micellar-lamellar structures that are water soluble.

## MECHANISM OF CLINICAL SIGN DEVELOPMENT

Protracted and steady-state elevations in the concentration of cholesterol in the blood of patients with PBC give rise to xanthelasmas. There is a relationship between the development of cutaneous xanthelasmas and the elevated serum levels of cholesterol. According to the data obtained by HG Kunkel & EH Ahrens<sup>[84]</sup>, cutaneous xanthelasmas appear when the blood concentration of cholesterol is more than 450 mg/dL. Moreover, this cholesterol level should persist for at least 3 mo. Hyperlipidemia in PBC does not seem to be associated with an increased risk of atherogenesis<sup>[85]</sup>.

The normal (or slightly enhanced) activity of aminotransferases for several years<sup>[86]</sup> suggests the preserved integrity and normal permeability of the cytoplasmic membrane of hepatocytes in most patients with PBC.

Skin hyperpigmentation is due to the excessive biosynthesis of melanin, as shown by S Sherlock<sup>[87]</sup>. The initial reaction of melanin biosynthesis is catalyzed by tyrosinase, the copper-containing enzyme. The liver is known to play an important role in the metabolism and regulation of copper homeostasis due to the formation of hepatocytic complexes of protein and copper and its bile excretion<sup>[39]</sup>. In normalcy, about 80% of the dietary copper is excreted into bile and eliminated with feces. Disturbance bile formation processes in PBC lead to the accumulation of copper in the body. Excess serum copper levels in patients with PBC may cause increases in the activity of tyrosinase and in the biosynthesis of melatonins that deposit in the skin, inducing hyperpigmentation. Simultaneous deposition of copper in the skin imparts a bronzed tint. Since copper is in ceruloplasmin-bound state, its elevated content exerts no toxic effect on the body, as in the Wilson-Konovalov disease.

Further progression of intrahepatic cholestasis results in the occurrence other of clinical signs of the disease.

Local or diffuse, moderate or severe skin itching is the earliest and permanent, and, occasionally for several months, sole symptom of the disease<sup>[88]</sup>. Epidermal deposition of bile acids resulting from cholestasis with PBC patients is a cause of skin itch<sup>[89]</sup>.

At the same time, all fractions of conjugated bile acids increase in the blood<sup>[90]</sup> while in the skin 50%-85% of bile acids are not conjugated with glycine or taurine<sup>[91]</sup> and less than 20% of bile acids are in the form of sulfoesters<sup>[92]</sup>. Sulfation and glucuronization of bile acids diminish their toxic properties and increase their urinary and fecal excretion. The processes of acid bile sulfation and glucuronization, which occur in PBC, seem to be a response to the toxic effect of excess bile acids and to aim at enhancing their elimination through the skin, kidney, and intestine and at lowering their detergent and irritant effect on cells, tissues, and organs. On entering the skin, bile acids induce skin itch. The latter's intensity depends on the ratio of sulfated (glucuronized) and nonsulfated (nonglucuronized) bile acids accumulating in the skin.

The sera of patients with PBC show a higher ratio of trihydroxy-/dihydroxycholanic acids and a lower glycine/taurine coefficient<sup>[90]</sup>. Garcia-Marin JJ *et al*<sup>[93]</sup> have indicated that taurine salts of bile acids stimulate the production of micelles with cholesterol and lecithin, promoting the inactivation of cholanic acids. Greim *et al*<sup>[94,95]</sup> have shown that cholic (trihydroxy-) bile acid has smaller detergent properties than dihydroxy- (deoxycholic, chenodeoxycholic) bile acids. The higher ratio of trihydroxy-/dihydroxy- bile acids in PBC as a compensatory detoxifying mechanism becomes apparent.

"Atypical", "nonphysiological" bile acids appear in the blood and urine of patients with PBC<sup>[96]</sup>. The appearance of "atypical" bile acids suggests that bile acid biosynthesis accomplished by the participation of 7- $\alpha$  and 12- $\alpha$  hydroxylases is impaired<sup>[80]</sup>. This may be due

to the involvement into the process of biosynthesis of primary bile acids, apart from specific and nonspecific P<sub>450</sub>-dependent monooxygenases<sup>[58]</sup>.

"Atypical" bile acids have a more potent irritant effect on the nerve receptors located in the skin. The intensity of skin itch also depends on the amount of "nonphysiological" ("atypical") bile acids in the skin of patients with PBC.

PBC affects mainly middle-aged, able-bodied females<sup>[97]</sup>. What is associated with is still unknown so far. Alterations in the content of sex hormones referring to as steroid ones are one of the possible causes of PBC in females<sup>[98]</sup>. There may be a change in the number or sensitivity of sex hormone receptors. The low expression of estrogen receptors-alpha in PBC and their disappearance in the advanced histological stages suggests that an estrogenic deficiency could favor the evolution of this disease toward ductopenia<sup>[99]</sup>.

A study of steroids hormones in the plasma of patients with PBC has demonstrated a statistically significant reduction in the levels of progesterone and cortisol<sup>[100]</sup>. The found changes seem to be associated with the delayed biosynthesis of steroid hormones in late-stage PBC. This may be due to the competitive inhibitions of monooxygenases (by enhancing the biosynthesis of bile acids from cholesterol) that participate in the biosynthesis of progesterone and cortisol. Changes in sex hormone profile are secondary to hepatic dysfunction in PBC<sup>[98]</sup>. Lower cortisol concentrations in the blood of patients with PBC may cause a change in the fluidity of membranes<sup>[101,102]</sup>, including hepatocytic cytoplasmic membranes. The changed fluidity of hepatocytic membranes leads to impaired bile excretion through the canalicular portion of a hepatocytic cytoplasmic membrane, which aggravates and enhances intrahepatic cholestasis.

Deficient entry of bile acids into the intestinal lumen substantially diminishes the absorption of fats and fat-soluble vitamins (A, D, and K)<sup>[103]</sup>. Along with insufficient vitamin D absorption, there is a lower formation of 1,25-dihydroxyvitamin D<sub>3</sub> on cytochrome P<sub>450</sub> (competitive inhibition of monooxygenases due to the enhanced biosynthesis of bile acids). This all brings about the inadequate calcium ion absorption in the small bowel and impaired phosphorus-calcium metabolism. Impaired hepatocytic calcium metabolism may affect the contractility of intracellular microtubules and microfilaments, which may cause a decrease in the contractility of bile capillaries and enhance cholestasis<sup>[77]</sup>.

Impaired phosphorus-calcium metabolism gradually results in osteodystrophy<sup>[104]</sup>. Signs of osteoporosis are most commonly detected in patients with end-stage PBC by bone X-ray studies<sup>[105,106]</sup> and morphological studies of bone biopsy specimens<sup>[87,107,108]</sup>.

The pathogenesis of osteoporosis in cholestatic lesions of the liver appears to be multifactorial<sup>[109]</sup> and involves impairments of vitamin D<sub>3</sub> absorption and metabolism<sup>[85]</sup>, decreased intestinal calcium ion absorption<sup>[108,110]</sup>, genetic predisposition<sup>[111]</sup>, and impact of corticosteroid therapy<sup>[112]</sup>.

Recent data suggest that serum leptin is associated with bone mineral density (BMD). F Szalay *et al* found a lower

serum leptin level and a higher soluble leptin receptor in patients with PBC, which could not be explained by the difference in body mass index. As leptin was associated with BMD, it may be hypothesized that leptin is involved in the complex regulation of bone metabolism in PBC<sup>[113]</sup>. There was a clear increase in serum leptin levels according to the histological stage of PBC<sup>[114]</sup>.

Glucocorticosteroids (GCSs) diminish intestinal calcium absorption, by reducing the production of 1, 25(OH)<sub>2</sub>-D<sub>3</sub>, increasing urinary calcium excretion, and depressing canalicular reabsorption. As a result, there is a compensatory increase in the production of parathyroid hormone (PTH) and bone resorption. In addition, GCSs directly increase PTH release and enhance its sensitivity. Furthermore, GCSs inhibit bone formation indirectly, by suppressing the synthesis of testosterone in gonads and by decreasing the production of growth hormone, insulin-like growth factor, and accordingly type 1 collagen, as well as directly by suppressing the function of osteoblasts<sup>[115,116]</sup>. Impaired fat absorption brings about steatorrhea - a daily fecal fat loss of more than 7 gram.

The altered composition of bile acids secreted into bile in patients with PBC leads to the impaired ratio of cholesterol, bile acids, and lecithin (in favor of cholesterol), which changes bile viscosity, and increases the bile lithogenicity index. This enhances evolving cholestasis in the bile capillaries and cholangioles. Blood bile reflux occurs. Another clinical sign of PBC, such as jaundice, develops. The development of jaundice is favored by attenuated interhepatocytic interactions and membrane fluidity changes leading to the impaired transport of bilirubin and copper through the canalicular portion of the hepatocytic cytoplasmic membrane. There is accumulation of copper in the body, activation of the copper-containing enzyme tyrosinase, an increase in the synthesis of melanins and in their skin deposition. Skin hyperpigmentation develops.

The serum concentration of bile acids increases and skin itch occurs in patients with PBC long before hyperbilirubinemia and jaundice develop in them. In end-stage PBC, an increase in the level of bilirubin occurs mainly due to its conjugated fraction. These data suggest that the development of hyperbilirubinemia in PBC is most likely to be associated with bile reflux from the bile capillary lumen or hepatocyte into blood and due to its impaired secretion through the apical portion of the hepatocytic cytoplasmic membrane. The altered fluidity of the liver cell membrane in PBC may promote impaired bilirubin transport and enhanced hyperbilirubinemia. Down-regulation of uptake transporters may contribute to the impaired hepatobiliary elimination in advanced PBC, and partially altered localization of MRP2 may reflect the onset of changes leading to icteric PBC<sup>[117]</sup>. It is clear to say that the glycosylating function of liver cells is retained at early stages of the disease.

At the late stage of the disease, hyperbilirubinemia may also develop due to erythrocytic hyperhemolysis caused by excess serum bile acids in patients with PBC. As potent detergents, bile acids (they are absent in systemic circulation in the normal state) can solubilize the cytoplasmic membranes of erythrocytes and other

blood formed elements, by causing their hemolysis. This in turn leads to the development of erythrocytopenia, thrombocytopenia, hypochromic anemia, and other hematological symptoms occurring in advanced stages of the disease with the significant increase in the serum bile acid levels<sup>[118]</sup>. The degree of hyperbilirubinemia is characterized by not only the conjugated fraction of bilirubin, but also by its unconjugated fraction.

Thus, an autoimmune pathological process occurring for unknown reasons triggers the development of intrahepatic cholestasis in PBC. A gradual progression of the latter contributes to the occurrence of immunological, biochemical, morphological, and clinical signs of the disease. The mechanisms responsible for triggering and developing the major signs of PBC are given in the scheme (Figure 4).

## TREATMENT OF PBC PATIENTS

The presented diagram is far from perfection. The diagram contains many debatable representations, but it gives an insight into the mechanism of development of major clinical signs in PBC and into their sequence and into that it is necessary to break a "closed vicious circle" in order to prevent the progression of the disease. This can be done by externally administering the missing content of bile acids into the intestinal lumen. The given scheme shows that use of ursodeoxycholic acid (UDCA) is a pathogenetically justified treatment for PBC, which promotes the stabilization of the disease process.

UDCA is a 7-beta-epimer of chenodeoxycholic acid (CDCA) (Figure 5). The OH group at carbonic atom 7 is at the alpha-position in CDCA, one of the primary bile acids, and that is at the beta-position in UDCA. It is precisely these structural differences that are at first glance minor, inducing essential dissimilarities of their chemical properties: UDCA is more hydrophilic and less hepatotoxic. With administration of UDCA agents, there is systemic improvement, diminished weakness, and reduced skin itch intensity. Moreover, it should be noted that some patients report greater itching in the first month of an agent's administration and when temperature drastically drops in the transition fall-winter period. Intensified skin itching at the beginning of a drug's administration seems to be associated with the incorporation of UDCA into enterohepatic circulation of bile acids and with their blood concentrations. However, then there is a gradual substitution of endogenous bile acids for administered UDCA and alleviation of skin itch.

The use of UDCA drugs in cholestatic liver lesions results in stabilization of clinical and biochemical signs of the disease. The stabilizing effect of UDCA in patients with PBC is associated with the delivery of missing bile acids into the small bowel (replacement therapy). The drug also inhibits the hepatocytic biosynthesis of endogenous bile acids from cholesterol. The "closed vicious circle" is discontinued. UDCA is particularly effective in early-stage<sup>[119,120]</sup> and asymptomatic PBC<sup>[121]</sup>. The early use of the drug prevents these histologic features of PBC<sup>[122]</sup>. In 30% of patients with PBC, UDCA causes full biochemical normalization, while 70% are incomplete responders<sup>[123]</sup>. In



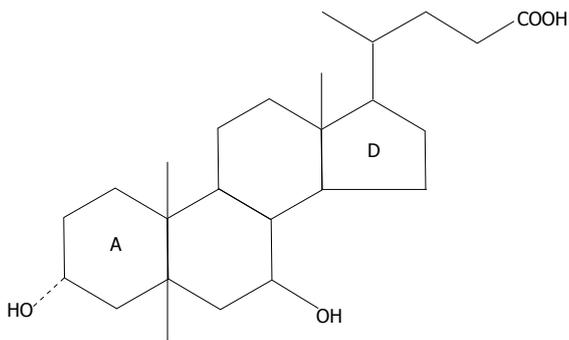


Figure 5 Ursodeoxycholic acid.

sulfated bile acids that, unlike nonsulfated ones, may be renally cleared from the body. Moreover, their content in the blood and tissues of the body may decrease, which should yield a positive therapeutic effect. S-adenosyl-L-methionine may also participate in the cellular biosynthesis of phospholipids. This may contribute to the formation of micellar-lamellar structures composed of phospholipids and bile acids. The formation of such structures enables the toxic effect of excess bile acids to be neutralized. Reasoning from these assumptions and the mechanism of development of intrahepatic cholestasis in PBC (see the scheme), the use of S-adenosyl-L-methionine in chronic liver diseases accompanied by intrahepatic cholestasis is pathogenetically justified. Its use is perhaps long and conceivably continuous, as the administration of UDCA agents.

It is advisable to co-administer UDCA and S-adenosyl-L-methionine<sup>[132]</sup>. This should lead to the potentiation of a therapeutic effect of each of the drugs individually.

Since the etiology and pathogenesis of cholestatic liver lesions remain unknown so far, their treatment is generally symptomatic and aimed at eliminating skin itching<sup>[133-137]</sup> and jaundice, diminishing steatorrhea, and simultaneously affecting osteoporosis<sup>[83,138-140]</sup>. The assumed autoimmune nature of PBC has served as the basis for using prednisolone<sup>[141]</sup> and other immunomodulators<sup>[142-144]</sup>. However, a rapid rise in bone changes, a great deal of side effects, and a low therapeutic efficacy of these agents have not permitted these drugs to be commonly used for the treatment of PBC<sup>[141]</sup>.

Liver transplantation currently remains to be the most radical treatment for PBC<sup>[145,146]</sup>. However, selection of patients for surgery is a difficult task. The patient generally gives his/her consent to surgery when his/her condition causes a drastic reduction in the quality of life. Severe weakness, intolerable skin itch, and persistent severe jaundice make the patient be at home and complicate his/her relations with his/her peoples and relatives. This condition is aggravated by the occurrence of ascitis, bleedings from the varicose veins of the esophagus and stomach, or encephalopathy<sup>[147]</sup>. Liver transplantation may be expected to show the best prognosis in this condition<sup>[148,149]</sup>. As a rule, the postoperative rehabilitative period is fair<sup>[150]</sup>.

After liver transplantation, there is a change in the titer of anti-mitochondrial autoantibodies: the AMA titers

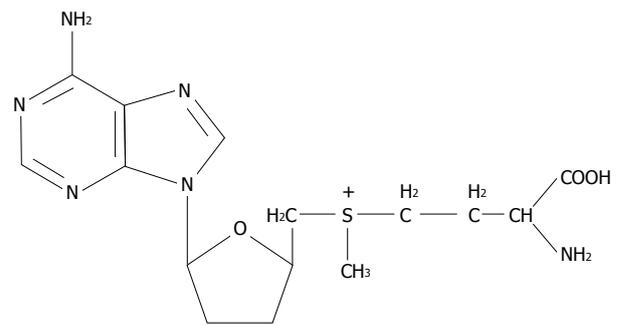


Figure 6 S-adenosyl-L-methionine.

generally drop in the first postoperative months then tend to return to their original values<sup>[9]</sup>.

Whether the disease recurs remains to be solved<sup>[151]</sup>. Histological changes typical of PBC are detectable in the needle liver biopsy specimens in 9 of 10 patients a year after surgery; of them, 4 patients are then observed to have skin itch and/or other clinical signs<sup>[152]</sup>. On the contrary, Demetris AJ *et al*<sup>[153]</sup> detected no histological data of a recurrence in patients with PBC after liver transplantation. Changes in the bile ducts may be induced by other causes: hepatitis C or G viruses; cytomegalovirus; a gradual rejection of the organ or problems associated with biliary anastomotic changes.

With this, 25% of patients needed liver retransplantation due to the development of "bile duct loss" syndrome.

In conclusion, a great body of new data on primary biliary cirrhosis has been recently accumulated. However, inadequate efficiency of its therapy has given impetus to further studies of the pathogenetic mechanisms of the disease<sup>[154]</sup> and on their basis to searches for new treatment options<sup>[155]</sup>. No specific therapy that effectively stops or reverses disease progression has been identified, thus it behooves investigators to aggressively pursue identification of the etiology of PBC<sup>[156]</sup>.

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