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Abnormal lipoprotein-X

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Structure and Molecular Composition of Lipoprotein X

Lipoprotein X (LP-X) is an abnormal serum lipoprotein originating in several diseased conditions. It is a sub-fraction of low density lipoproteins (LDL), which does not react with antiserum against normal serum [86, 105]. LP-X is a lamellar spherical particle with mean diameter 69 nm (range 40 to 100 nm) as revealed by electron microscopy, that aggregates strongly [33, 47, 90]. Compared to other lipoproteins LP-X particles are rich in phospholipids (60-67%) and cholesterol (23-30%) but poor in cholesterol esters (0.5-2%), triglycerides (2-3%), and protein (3-7%) [25, 37, 68, 90]. The following phospholipids are mainly presented in LP-X. lysophosphatidylcholine (4.1%), sphingomyelin (14.2%), phosphatidylcholine (77.5%), and phosphatidylethanolamine (2.5%) [92]. Bile acids are also often present in LPX, among which lithocholic acid is the major component [66]. The protein component of LP-X is dominated by albumin located in core and apolipoprotein C (I, II and III) located on the surface of the particle [34, 71].

Albumin contains 40% of total protein components of LP-X. It is located in the internal water compartment of LP-X covered by lipids and becomes visible only after complete delipidation of LP-X. [11, 96]. The role of this protein relevant to LP-X is not clear, however it has been shown that albumin is required for binding to LP-X cholesterol metabolizing enzymes [68]. It has been shown that addition of albumin to bile lipoprotein converts it to LP-X. This may offer possible explanation on the origin of LP-X [57].

Apolipoprotein C was discovered in 1964 by Gustafson and co-workers [32], who separated this apolipoprotein from partially dilapidated very low density lipoproteins (VLDL). It was characterized by a high capacity for lipid binding, immunochemical properties, and peptide patterns different from other plasma apolipoproteins, and by serine and threonine as N-terminal amino acids [32]. Later, apolipoprotein C was isolated from chyled VLDL [1] and from all lipoprotein classes from plasma of healthy subjects [2]. Apolipoprotein C has the same common to other apolipoproteins structural feature – domain containing amphipathic helix. Hydrophobic amino acids are located at one side of the helix, and hydrophilic amino

acids at the other side. The hydrophobic part of the helix is thought to interact with acyl chains of phospholipids, whereas hydrophilic part interacts with phospholipid hydrophilic groups. Immunochemical analysis of apolipoprotein C has shown that the protein moiety consists of at least three antigenic determinants [49]. There are identified three distinct classes of this apolipoprotein, C1, C2 and C3, which are encoded by different genes [52, 77] and differ in signal peptide amino acid sequence [49]. C1 and C2 are encoded by gene located on chromosome 19, and C3 is encoded by gene located on chromosome 11. [52, 77]. The major function of the apolipoprotein C as well as other apolipoproteins is maintaining of lipoprotein stability, binding and transportation of lipids in the blood stream [85].

Fig 1 demonstrates schematic structure of LP-X. In tables 1–3 the molecular composition and apolipoprotein content of LP-X in comparison to other lipoproteins are presented.

Hydroxylapatite chromatography revealed that LP-X has a higher molecular weight compared to LDL [108]. In addition, proton magnetic resonance studies showed motion of acyl chains and/or cholesterol rings much more restricted in LP-X compared to its normal counterpart (LDL) [10]. One important characteristic feature of LP-X is its mobility toward the cathode on agar-gel electrophoresis [91]. This phenomenon is not the result of a positive charge on the particle at pH 8.6, but of a pronounced electro-endosmosis in this medium, which strongly affects the migration of this rather large particle with its relatively small protein component [33, 95].

Using zonal ultracentrifugation, LP-X can be divided into three isoforms. LP-X1, LP-X2, and LP-X3, differing in density, phospholipid content and apolipoprotein composition [77]. In addition to apolipoprotein C, LP-X2, and LP-X3 also contain apolipoprotein A1 and apolipoprotein E, absent in LP-X1 [77].

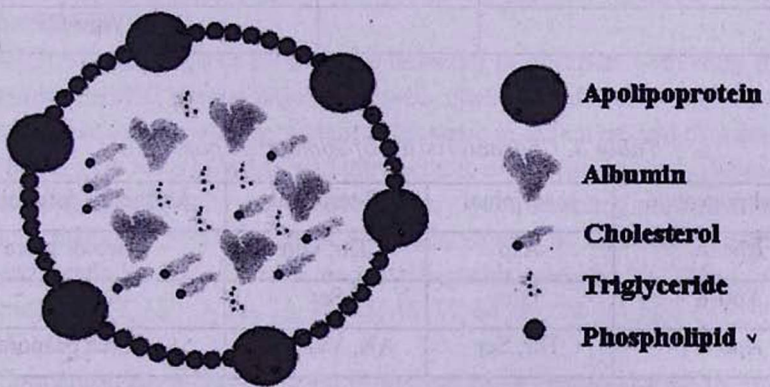

Figure 1. Schematic structure of LP-X

Table 1. Molecular composition of lipoproteins

Class	Diameter (nm)	Protein (%)	Cholesterol (%)	Phospholipid (%)	Triacylglycerol (%)
HDL	5-15	33	30	29	8
LDL	18-28	25	50	21	4
LP-X	40-100	6	27	65	2
VLDL	30-80	10	22	18	50
Chylomicrons	100-1000	<2	8	7	84

Table 2. Classes of apolipoproteins (Apo) in different lipoproteins

VLDL	LDL	LP-X	HDL
Major apolipoproteins			
Apo-B100	Apo-B100		Apo-A1
Apo-C1		Apo-C1	Apo-A2
Apo-C2		Apo-C2	
Apo-C3		Apo-C3	
Apo-E			
Minor apolipoproteins			
Apo-D		Apo-E	Apo-C1
		Apo-A1	Apo-C2
			Apo-C3

Table 3. Characteristics of some apolipoproteins

Apolipoprotein	N-terminal	C-terminal	Antigenic determinants
Apo-A	Asp	Thr, Gln	two or more
Apo-B	Glu	Ser	one
Apo-C	Thr, Ser	Ala, Val, Glu	three or more

LP-X in liver and biliary tract diseases

For the first time LP-X was detected, isolated from the blood, and characterized upon studying patients with liver disease with obstructive jaundice [86, 105].

Subsequent studies demonstrated that the presence of LP-X is detected in 45% of cases of liver disease with cholestatic features, having raised levels of serum lipids, hypercholesterinemia, and a hyperlipoproteinemia, and was not detected in cases of liver disease without cholestasis [84, 91]. It was also shown that the incidence of LP-X in different causes of cholestatis varied, and while it was commonest in cases of extrahepatic obstruction of recent onset, occurring in 75% of cases, it was also found in primary biliary cirrhosis in 48% [84].

Further investigations performed by a number of research groups, including case- and large-scale studies of patients with different types of diseases of liver and bile tract, demonstrated presence of LP-X in the blood of patients with intrahepatic cholestasis (primary biliary cirrhosis, cholangitis, hepatic cirrhosis, hypercholesterolemia associated with pseudohyponatremia, graft-versus-host disease of the liver after allogeneic bone marrow transplantation, hepatic malignancy, etc.) and extrahepatic cholestasis (extrahepatic biliary obstruction by tumors and choledocholithiasis, cholestasis of pregnancy, etc.) [9, 35-40, 48, 58, 62, 70, 72, 80, 81, 87, 88, 99, 100, 109, 114, 117, 120, 121, 126].

In patients suffering with different liver diseases the LP-X test was positive in 82-100% in whom histological evidence of cholestasis was observed, and negative in 95-98% in whom histological examination was negative [26, 27, 61, 69]. Notably that concentrations of LP-X in the patients with extrahepatic cholestasis were significantly higher than in those with intrahepatic cholestasis [9, 14, 39, 40, 82, 90, 94, 117].

LP-X was also detected in the blood of patients with acute viral and toxic hepatitis. Positive results were found in 96% of patients with hepatitis A and in 82% of hepatitis B. It was shown that in acute phase of viral hepatitis, LP-X is the most specific test in determining the presence of cholestasis [54, 82, 106, 122]. Increased blood levels of LP-X were reported in patients with drug-induced cholestatic hepatitis [104, 63], chronic cholestatic hepatitis [72] as well as viral hepatitis NANB [97].

High blood levels of LP-X were detected in children with liver diseases (progressive familial intrahepatic cholestasis, chronic graft-versus-host disease of the liver) and in infants with persistent cholestatic jaundice caused by biliary atresia and biliary agenesis of extra- and intrahepatic origin (prolonged jaundice, choledochal cysts, hypoplastic extrahepatic biliary tract, absence of extrahepatic biliary tracts, mechanical occlusion of bile ducts caused by a rhabdomyoblastoma, inborn enzymatic liver dysfunction, neonatal hepatitis, neonatal cholestasis, Alagille syndrome, etc.) [7, 15, 18, 20, 22, 30, 45, 46, 51, 64, 65, 73-76, 107, 111, 112, 118, 119, 124, 130].

Concentration of LP-X in the blood has been considered as an important and informative marker of the above mentioned liver and biliary tract diseases, and determination of LP-X blood level has been widely used for clinical diagnostics of cholestasis and for monitoring the efficiency of relevant therapeutic measures [3, 6, 9, 19, 20, 22, 26, 27, 42, 43, 51, 54, 58, 60, 61, 66, 69, 70, 74, 78, 89, 94, 103, 106, 122, 123, 125, 127]. The plasma concentration of LP-X was significantly corre-

lated to the plasma activity of alkaline phosphatase and serum bilirubin, but seemed to be superior to these two parameters in the differentiation between intrahepatic and extrahepatic cholestasis. Plasma levels of LP-X above 400 mg/100 ml are highly indicative of extrahepatic biliary obstruction [80].

In experimental models of cholestasis, LP-X may be detected in the plasma within the first 20 hours. The calculated fractional catabolic rate of LP-X was found to be 0.450 ± 0.069 for dogs and 1.553 ±0.096 for rats corresponding to a mean biological half life of 37.7 ± 6.4 h or 10.7 ±0.6 h, respectively [93].

The cause of the appearance of lipoprotein X is unknown, but the analysis of associated biochemical features suggested its relationship to physical biliary obstruction rather than a derangement of liver cell function [84].

Antiatherogenic properties of LP-X

The fact that hypercholesterolemia increases atherosclerosis incidence in the general population but not in patients with primary biliary cirrhosis, a cholestatic liver disease associated with marked increases in plasma LDL cholesterol [37, 41], raises the question on antiatherogenic properties of LP-X. Despite the highly increased cholesterol levels, prospective observation for a median of 7.4 years of 312 patients with primary biliary cirrhosis of various stages found no increased incidence of atherosclerotic death compared with age and sex-matched controls [17]. Based upon these results it was proposed that bioactivities of LP-X, may be responsible for this phenomenon by preventing origination of oxidized LDL products and thus reducing LDL atherogenicity [101]. This suggestion has been recently confirmed by *in vitro* study performed by Chang and co-workers [16]. This study revealed that after prolonged incubation with copper, LPX containing LDL isolated from the blood of patients with primary biliary cirrhosis failed to increase the oxidation index or electrophoretic mobility noted in control LDL. An admixture of LP-X containing LDL or LP-X with control LDL prevented oxidation of the latter in a dose-dependent manner. Furthermore, LP-X containing LDL isolated from the blood of patients with primary biliary cirrhosis was also noncompetitive against copper-oxidized LDL (oxLDL) for binding with a murine monoclonal anti-oxLDL antibody in a competitive ELISA. OxLDL exerts its proapoptotic and antiangiogenic effects in part by inhibiting fibroblast growth factor 2 (FGF2) expression. Preincubation of oxLDL with LP-X, containing LDL, but not control LDL, attenuated the inhibitory effects of oxLDL on FGF2 expression in cultured bovine aortic endothelial cells. Notably, the antioxidant and prosurvival properties of LP-X containing LDL isolated from the blood of patients with primary biliary cirrhosis diminished after the patients underwent orthotopic liver transplantation [16]. These results suggest that LP-X reduces LDL atherogenicity by preventing LDL oxidation to protect endothelial cells integrity in the presence of hypercholesterolemia [16, 99]. They also suggest that altering LDL composition may be as important as reducing LDL concentration in preventing or treating atherosclerosis.

LP-X in familial lecithin-cholesterol acyltransferase deficiency

Lecithin-cholesterol acyltransferase (LCAT) (also called phosphatidylcholine-sterol O-acyltransferase, EC 2.3.1.43) is an enzyme which converts free cholesterol into cholesteryl ester (a more hydrophobic form of cholesterol) which is then sequestered into the core of a lipoprotein particle, eventually making the newly synthesized lipoproteins spherical and forcing the reaction to become unidirectional, since the particles are removed from the surface. The enzyme is bound to both HDL and LDL in the blood plasma [128]. Familial LCAT deficiency is a monogenic autosomal recessive trait affecting cholesterol esterification, developed due to several allelic mutations of polymorphic gene on chromosome 16 (16q22.1) encoded LCAT [5, 12, 13, 28, 29, 44, 59, 110]. A deficiency of LCAT causes accumulation of unesterified cholesterol in certain body tissues. The disease is characterized by diffuse corneal opacities, target cell hemolytic anemia (normochromic normocytic anemia), and renal dysfunction (proteinuria with renal failure) [13, 44]. Familial LCAT deficiency is accompanied by low levels of HDL and LDL and the accumulation of LP-X in the plasma of patients [31, 44, 83, 113,]. Plasma concentration of LP-X in familial LCAT deficiency ranges from 43 mg/100 ml to 251 mg/100 ml with a mean of 127 mg/100 ml. This is above the mean level of LP-X found in a group of patients with intrahepatic cholestasis (49mg/100 ml) and below the mean level found in patients with extrahepatic cholestasis (341 mg/100 ml) [82].

Pathogenic effects of LP-X in familial LCAT deficiency have been addressed in several human and animal studies, *in vivo* and *in vitro*. It has been demonstrated that high levels of LP-X cause glomerular capillary endothelial damage [98] and lead to progressive renal impairment and end-stage renal failure [31, 129]. The results presented by Lynn et al. suggest that LP-X participates in the pathogenesis of glomerulosclerosis and subsequent renal failure in familial LCAT deficiency by stimulating monocyte infiltration via a mechanism involving the expression MCP-1 by mesangial cells [53]. MCP-1 is an important chemoattractant for monocytes [115], and one key event in the pathogenesis of glomerulosclerosis is the infiltration of monocytes into affected glomeruli [24].

It was shown that the familial LCAT deficiency is characterized by the decreased catabolism of LP-X [67], however, the mechanisms for LP-X accumulation in this disease are unclear yet.

LP-X in other diseased conditions

Our own recent studies, for the first time have demonstrated the presence of the LP-X in the blood of patients with ischemic stroke and familial Mediterranean fever. The studies included 120 patients with ischemic stroke (each patient was examined at different time points after stroke onset) and 50 patients with familial Mediterranean fever (each was examined in remission stage and in acute stage of the disease). We found that in the blood of patients with ischemic stroke LP-X is present in both free form and cryoglobulin-bound form on days 1-7 after stroke onset [55, 56], and in the blood of patients with familial Mediterranean fever

a free form of LP-X was detected (unpublished data). Figures 2, 3 demonstrate the results of our investigations.

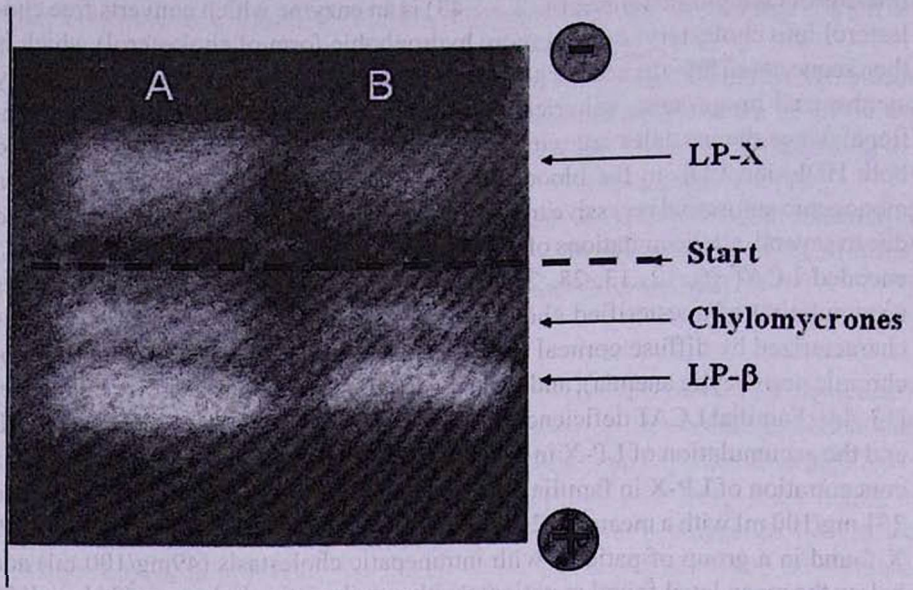

Fig. 2. Typical electrophoretic patterns of LDL detection in the blood serum of patients with familial Mediterranean fever (A) and ischemic stroke (B)

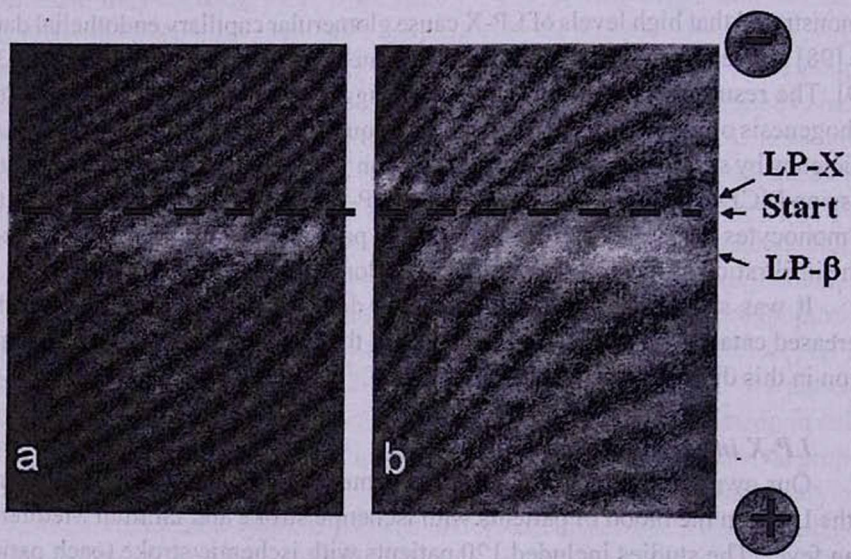

Fig. 3. Typical electrophoretic patterns of LDL detection in cryoglobulins isolated from the blood of healthy subjects (a) and patients with ischemic stroke (b)

The above mentioned study of Lynn and colleagues [54] demonstrated that LP-X is stimulating monocytes infiltration via a mechanism involving MCP-1 ex-

pression. Both animal and human studies suggest that MCP-1-driven migration of inflammatory cells are implicated in the pathogenesis of ischemic stroke [21]. Furthermore, we found increased serum levels of MCP-1 in ischemic stroke patients on days 1-7 after stroke onset [4, 8]. Therefore, it is reasonable to propose a possible involvement of LP-X in the inflammatory response occurring after stroke onset [21]. The same applies to familial Mediterranean fever, which is a genetic autosomal recessive autoinflammatory disease developed due to several allelic mutations of polymorphic gene coding a protein, pyrin, on chromosome 16 (16p13.3). Pyrin is a part of regulatory pathway of inflammation and normally assists in keeping inflammation under control by deactivating the immune response. Mutations in pyrin encoding gene lead to malfunctioning protein and uncontrolled inflammation [102].

Thus, in case of ischemic stroke and familial Mediterranean fever, as well as in LCAT deficiency, LP-X probably acts as a trigger of inflammation through induction of MCP-1 expression. Further studies will clear if there are relationships between LP-X and other inflammatory mediators.

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Аномальный липопротеин-Х

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Липопротеин-Х (ЛП-Х) представляет собой аномальный подкласс липопротеинов низкой плотности, характеризующийся высоким содержанием фосфолипидов и неэстерифицированного холестерина и низким содержанием эфиров холестерина, триглицеридов и белка. Среди белков, входящих в состав ЛП-Х, преобладают альбумин и аполипопротеин С. ЛП-Х был впервые обнаружен в крови больных механической желтухой. Дальнейшие исследования показали наличие ЛП-Х в крови при широком ряде заболеваний печени и желчных протоков, ассоциирующихся с холестазом. По этой причине наличие ЛП-Х в крови рассматривается как важнейший и информативный показатель холестаза. Высокие уровни ЛП-Х в крови были отмечены при семейном дефиците фермента лецитин-холестерин ацилтрансферазы. Кроме того, наши собственные недавно проведенные исследования позволили выявить наличие ЛП-Х в крови больных ишемическим инсультом и семейной средиземноморской лихорадкой. Исследования, проведенные *in vitro* и *in vivo*, показали, что ЛП-Х обладает как антиатерогенной, так и провоспалительной активностью. Причины образования ЛП-Х при вышеотмеченных заболеваниях до настоящего времени не выяснены. В настоящем обзоре обобщены данные литературы, а также результаты наших собственных

исследований, формирующие современные представления о структурно-функциональных свойствах, молекулярном составе ЛП-Х и его вовлечении в патомеханизмы различных заболеваний.

Անոմալ Х-լիպոպրոտեին

Ա.Ս. Քոյաջյան, Ա.Ա. Առաքելյան, Վ.Ա. Այվազյան, Գ.Մ. Սկրտչյան

Х-լիպոպրոտեինը (Х-ԼՊ) ցածր խտությամբ լիպոպրոտեիդների անոմալ ենթադասն է, որը հարուստ է ֆոսֆոլիպիդներով և ոչ եթերիֆիկացված խոլեստերինով, բայց աղքատ է խոլեստերինի եթերներով, եռգլիցերիդներով և սպիտակուցներով: Х-ԼՊ-ի սպիտակուցների կազմը հիմնականում ներկայացված է ալբումինով և С ապոլիպոպրոտեինով: Х-ԼՊ-ն առաջին անգամ հայտնաբերվել է մեխանիկական դեղնախտով զուգակցվող լարդի հիվանդություններով տառապող մարդկանց արյան մեջ: Հետագայում ցույց տրվեց արյան մեջ Х-ԼՊ-ի առկայությունը խոլեստազով զուգակցվող լարդի և լեղուղիների մեծ թվով հիվանդությունների ժամանակ: Այդ պատճառով այսօր Х-ԼՊ-ի առկայությունը արյան մեջ համարվում է խոլեստազի կարևոր և ինֆորմատիվ ցուցանիշ: Х-ԼՊ-ի բարձր մակարդակները արյան մեջ գրանցվել են լեցիտին-խոլեստերինի ացիլտրասֆերազ ֆերմենտի ընտանեկան անբավարարության ժամանակ: Մեր կողմից վերջին ժամանակներում կատարված ուսումնասիրությունները թույլ տվեցին հայտնաբերել Х-ԼՊ նաև իշեմիկ կաթվածով և ընտանեկան միջերկրածովյան տենդով տառապող հիվանդների մոտ: Х-ԼՊ-ի առաջացման պատճառը նշված հիվանդությունների ժամանակ մինչ այսօր պարզաբանված չէ: Համաձայն *in vitro* և *in vivo* հետազոտությունների Х-ԼՊ-ն կարող է ցուցաբերել և հակա-աթերոգեն, և հարբորբոքային ակտիվություն: Այս ակնարկում ամփոփված են գրականության և մեր սեփական ուսումնասիրությունների տվյալները Х-ԼՊ-ի կառուցվածքաֆունկցիոնալ հատկությունների, մոլեկուլային կազմի և տարբեր հիվանդությունների պաթոմեխանիզմներում ներգրավվածության վերաբերյալ:

References

1. *Alaupovic P., Furman R.H., Falor W.H. et al.* Isolation and characterization of human chyle chylomicrons and lipoproteins. Ann. N Y. Acad. Sci., 1968, 149(2), 791-807.
2. *Alaupovic. P.D., Lee M., McConathy W. J.* On the identification and separation of lipoprotein families in major density classes of normal human plasma lipoproteins. Circulation, 1969, 40 (Suppl. III), p.2.
3. *Alegre B., Carrasco D., Breto M.D. et al.* Semiquantitative determination of lipoprotein X (Lp-X). Its usefulness in differential diagnosis of jaundice. Rev. Clin. Esp., 1976, 143(1), p.79-81.
4. *Arakelyan A., Petrkova J., Hermanova Z. et al.* Serum levels of the MCP-1 chemokine in

patients with ischemic stroke and myocardial infarction. Mediat. Inflamm., 2005, 3, p.175-179.
5. *Azoulay M., Henry I., Tata F. et al.* The structural gene for lecithin-cholesterol acyl transferase (LCAT) maps to 16q22. Ann. Hum. Génet., 1987, 51(Pt. 2), p.129-136.
6. *Bartellini A., Melazzini F. Oliveras R.* Dyslipidemia in cholestasis. Acta Gastroenterol. Latinoam., 1981, 11(2), p.291-299.
7. *Bojanovski M., Lükermann R., Schulz-Falten J. et al.* Parameters of lipoprotein metabolism and cholestasis in healthy and cholestatic infants and children. Prog. Lipid. Res., 1991, 30(2-3), p.295-300.
8. *Boyajyan A.S., Arakelova E.A., Ayvazyan V.A., Manukyan LA.* Interleukins and chemokines in acute ischemic stoke complicated and non-complicated with diabetes. Cytokines Inflamm., 2008, 7(1), p.41-45.
9. *Brailski Kh., Dinkov L., Popov P. et al.* Differential diagnosis of jaundice. Vutr. Boles., 1987, 26(5), p.24-32.
10. *Brainard J.R., Cordes E.H., Gotto A.M.Jr. et al.* Lipoprotein-X proton and phosphorus-31 nuclear magnetic resonance studies on native, reconstituted, and model systems. Biochemistry, 1980, 19(18), p.4273-4279.
11. *Breckenridge W.C., Kakis G, Kuksis A.* Identification of lipoprotein X-like particles in rat plasma following Intralipid infusion. *Can. J. Biochem.*, 1979, 57(1), p.72-82.
12. *Bujo H., Kusunoki J., Ogasawara M. et al.* Molecular defect in familial lecithin.cholesterol acyltransferase (LCAT) deficiency. A single nucleotide insertion in LCAT gene causes a complete deficient type of the disease. Biochem. Biophys. Res. Commun., 1992, 181(3), p.933-940.
13. *Calabresi L.. Pisciotta L., Costantin A. et al.* The molecular basis of lecithin.cholesterol acyltransferase deficiency syndromes. a comprehensive study of molecular and biochemical findings in 13 unrelated Italian families. Arteriosclerosis, thrombosis, and vascular biology, 2005, 25(9), p.1972-1978.
14. *Calvo C., Arroqui I.* Quantitative determination of lipoprotein-X (LP-X) in extrahepatic cholestasis and intrahepatic cholestasis. Med. Chil., 1984, 112(3), p.233-237.
15. *Campbell D.P., Williams R.* Identification of the jaundiced infant who is likely to recover without surgical intervention. Ann. Surg., 1976, 184(1), p.89-96.
16. *Chang P.Y., Lu S.C., Su T.C. et al.* Lipoprotein-X reduces LDL atherogenicity in primary biliary cirrhosis by preventing LDL oxidation. J. Lipid. Res., 2004, 45, p.2116–2122.
17. *Crippin J.S., Lindor K.D., Jorgensen R. et al.* Hypercholesterolemia and atherosclerosis in primary biliary cirrhosis. what is the risk? Hepatology, 1992, 15, p.858–862.
18. *Davit-Spraul A., Pourci M.L., Atger V. et al.* Abnormal lipoprotein pattern in patients with Alagille syndrome depends on Icterus severity. Gastroenterology, 1996, 111(4), p.1023-1032.
19. *Deutsch J.* Value of percutaneous blind liver biopsy in preoperative diagnosis of atresia of the extrahepatic bile ducts. Monatsschr. Kinderheilkd., 1987, 135(11), p.763-769.
20. *Deutsch J., Kurz R., Müller W.D., Becker H.* Quantitative determination of LP-X in the differential diagnosis and treatment of direct hyperbilirubinemia in infancy. Z. Kinderchir., 1987, 42(4), p.230-234.
21. *Di Napoli M., Arakelyan A., Boyajyan A. et al.* The acute phase inflammatory response in stroke. Systemic inflammation and neuroinflammation. In. Pitzer J.A., – editor, Progress in Inflammation Research, Chapter III. USA. Nova Science Publishers Inc., 2005, p.95-145.
22. *Duchnowska A., Wach K., Poradowska W.* Lipoprotein X (LP-X) in the differential diagnosis of cholestasis in children, with special reference to biliary atresia. Probl. Med. Wieku Rozwoj., 1979, 8, p.84-91.
23. *Eder G, Sacher M., Frey E.* Lipoprotein-x determination in children beyond an age of 6 months. Padiatr. Pathol., 1977, 12(3), p.263-271.
24. *El Nahas A.M.* Glomerulosclerosis. insights into pathogenesis and treatment. Nephrol. Dial. Transplant., 1989, 4, p.843-853.
25. *Felker T.E., Hamilton R.L., Havel R.J.* Secretion of lipoprotein-X by perfused livers of rats with cholestasis. Proc. Natl. Acad. Sci. USA, 1978, 175(7), p.3459-3463.

26. *Fellin R., Manzato E., Zotti S. et al.* Lipoprotein-X and diagnosis of cholestasis. comparison with other biochemical parameters and liver biopsy. Clin. Chim. Acta, 1978, 85(1), p.41-47.
27. *Fischer M., Falkensammer C., Barouch G. et al.* The diagnosis of cholestasis. lipoprotein X (LP-X). Wien Klin. Wochenschr., 1975, 87(16), p.524-531.
28. *Funke H., VonEckardstein A., Pritchard P.H. et al.* Genetic and phenotypic heterogeneity in familial lecithin. cholesterol acyltransferase (LCAT) deficiency. Six newly identified defective alleles further contribute to the structural heterogeneity in this disease. J. Clin. Invest., 1993, 91(2), p.677-683.
29. *Gotoda T., Yamada N., Murase T. et al.* Differential phenotypic expression by three mutant alleles in familial lecithin.cholesterol acyltransferase deficiency. Lancet, 1991, 338(8770), p.778-781.
30. *Gottrand F., Clavey V., Fruchart JC., Farriaux JP.,* Lipoprotein pattern and plasma lecithin cholesterol acyl transferase activity in children with Alagille syndrome. Atherosclerosis, 1995, 115(2), p. 233-241.
31. *Guérin M., Dolphin PJ., Chapman M.J.* Familial lecithin:cholesterol acyltransferase deficiency: further resolution of lipoprotein particle heterogeneity in the low density interval. Atherosclerosis, 1993, 104(1-2), p.195-212.
32. *Gustafson A., Alaupovic P., Furman RH.* Studies of the composition and structure of serum lipoproteins. physical-chemical characterization of phospholipid-protein residues obtained from very-low-density human serum lipoproteins. Biochim. Biophys. Acta, 1964, 84, p.767-769.
33. *Hamilton R.L., Havel R.J., Kane J.P.* Cholestasis. Lamellar structure of the abnormal human serum lipoprotein. Science, 1971, 172(982), p.475-478.
34. *Hickman P.E., Dwyer K.P., Masarei J.R.* Pseudohyponatraemia, hypercholesterolaemia, and primary biliary cirrhosis. J. Clin. Pathol., 1989, 42(2), p.167-171.
35. *Higashi T., Carvalho L.C., Seki M. et al.* Frequency of lipoprotein-X (LP-X) in icteric patients. Comparison with some biochemical data. Arq. Gastroenterol., 1978, 15(3), p.117-122.
36. *Inamoto Y., Teramoto T., Shirai K. et al.* Severe hypercholesterolemia associated with decreased hepatic triglyceride lipase activity and pseudohyponatremia in patients after allogeneic stem cell transplantation. Int. J. Hematol., 2005, 82(4), p.362-366.
37. *Jahn C.E., Schaefer E.J., Taam L.A. et al.* Brewer HB. Lipoprotein abnormalities in primary biliary cirrhosis. association with hepatic lipase inhibition as well as altered cholesterol esterification. Gastroenterology, 1985, 89, p.1266-1278.
38. *Johnson P., Olegård R., Samsioe G. et al.* Studies in cholestasis of pregnancy. III. Fatty acid composition of serum phosphoglycerides. Acta. Obstet. Gynecol. Scand., 1975(a), 54(3), p.241-246.
39. *Johnson P., Samsioe G., Gustafson A.* Studies in cholestasis of pregnancy. Acta Obstet. Gynecol. Scand., 1975(b), 54(2), p.105-111.
40. *Johnson P.* Studies in cholestasis of pregnancy. IV. Serum lipids and lipoproteins inrelation to duration of symptoms and severity of the disease, and fatty acid composition of lecithin in relation to duration of symptoms. Acta Obstet. Gynecol. Scand., 1975, 54(4), p.307-313.
41. *Kaplan M.M.* Primary biliary cirrhosis. N. Engl. J. Med., 2005 335(12), p.1261–1273.
42. *Koga S., Yamamoto K., Ibayashi H.* Clinical significance of quantitative determination of lipoprotein X in patients with cholestasis. Nippon Shokakibyo Gakkai Zasshi, 1979, 76(2), p.231-238.
43. *Kostner G.M., Laggner P., Prexl H.J., Holasek A.* Investigation of the abnormal low-density lipoproteins occurring in patients with obstructive jaundice. Biochem J., 1976, 157(2), p.401-407.
44. *Kuivenhoven J.A., Pritchard H., Hill J. et al.* The molecular pathology of lecithin.cholesterol acyltransferase (LCAT) deficiency syndromes. J. Lipid. Res., 1997, 38(2), p.191-205.
45. *Lachmann D.* Abnormal lipoprotein (LP-X) in the first months of life with particular reference to obstructive jaundice. Wien Klin. Wochenschr. Suppl., 1977, 69, p.3-28.
46. Lachmann D. Abnormal lipoprotein (LP-X) in the first months of life with particular reference to obstructive jaundice. Fortschr. Med., 1978, 96(35), p.1750-1753.
47. Laggner P., Glatter O., Muller K. The Lipid Bilayer Structure of the Abnormal Human Plasma Lipoprotein X. Eur. J. Biochem., 1977, 77(1), p.165-171.

48. *LeRiche M., Burgess L.J., Marais A.D.* Pseudohyponatraemia in a patient with obstructive jaundice. Clin. Chim. Acta., 2006, 366(1-2), p.357-360.
49. *Lee D.M. Alaupovic P.* Studies of the composition and structure of plasma lipoproteins. Isolation, composition, and immunochemical characterization of low density lipoprotein subfractions of human plasma. Biochemistry, 1970, 9(11), p.2244-2252.
50. *Li W.H., Tanimura M., Luo C.C., Datta S., Chan L.* The apolipoprotein multigene family. Biosynthesis, structure, structure-function relationships, and evolution. J. Lipid. Res., 1988, 29(3), p.245-271.
51. *Lipsitz P.J., Yoss B., Schussheim A.* Lipoprotein-X and other tests in the diagnosis of obstructive jaundice in the infant. Am. J. Gastroenterol., 1979, 71(1), p.95-100.
52. *Lusis A.J., Heinzmann C., Sparkes R.S. et al.* Regional mapping of human chromosome 19. organization of genes for plasma lipid transport (APOC1, -C2, and -E and LDLR) and the genes C3, PEPD, and GPI. Proc. Natl. Acad. Sci. USA, 1986, 83(11), p.3929-3933.
53. *Lynn E.G, Siow Y.L., Frohlich J. et al.* Lipoprotein-X stimulates monocyte chemoattractant protein-1 expression in mesangial cells via nuclear factor-kappa B. Kidney Int., 2001, 60(2), p.520-532.
54. *Magnani H.N. Alaupovic P.* Utilization of the quantitative assay of lipoprotein X in the differential diagnosis of extraphepatic obstructive jaundice and intrahepatic diseases. Gastroenterology, 1976, 71(1), p.87-93.
55. *Manukyan L., Boyajyan A., Arakelyan A. et al.* Immunochemical composition of cryoglobulins generated in stroke. J. Clin. Immunol., 2009 29(3), p.274-281.
56. *Manukyan L.A., Boyajyan A.S., Arakelyan A.A. et al.* Molecular composition of cryoglobulins isolated from the blood of patients with ischemic stroke. Zh. Nevrol. Psikhiatr. im. S. S. Korsakova, 2007, Suppl. 19 (Stroke), p.56-62.
57. *Manzato E., Fellin R., Baggio G et al.* Formation of lipoprotein-X. Its relationship to bile compounds. J. Clin. Invest., 1976, 57(5), p.1248-1260.
58. *Mayr K.* The significance of lipoprotein X in the diagnosis of obstructive jaundice. comparison with other biochemical tests. Dtsch. Med. Wochenschr., 1975, 100(43), p.2193-2197.
59. *McLean J., Wion K., Drayna D. et al.* Human lecithin-cholesterol acyltransferase gene. Complete gene sequence and sites of expression. Nucleic Acids. Res., 1987, 14(23), p.9397-9406.
60. *Michel B., Ritter U.* The diagnostic importance of low-density lipoprotein (LP-X) for the diagnosis of cholestasis. *Z Gastroenterol*, 1976, 14(5), p.556-564.
61. *Milewski B., Palynyczko Z.* Significance of serum lipoproteid-X (LP-X) determination for the diagnosis of cholestasis in chronic liver diseases. *Pol. Med. Sci. Hist. Bull.*, 1975, 15(5-6), p.551-555.
62. *Mistilis S.P., Goren R., Tall A.R., Hickie J.R.* Plasma lipids in extra hepatic biliary obstruction. Aust. N. Z. J. Med., 1975, 5(6), p.540-543.
63. *Miyahara K., Kasahara N., Kondo Y. et al.* Changes in plasma lipids and abnormal lipoproteins in a patient with drug-induced cholestatic hepatitis. Jpn. J. Med., 1991, 30(4), p.354-359.
64. *Nagasaka H., Yorifuji T., Egawa H. et al.* Evaluation of risk for atherosclerosis in Alagille syndrome and progressive familial intrahepatic cholestasis. two congenital cholestatic diseases with different lipoprotein metabolisms. J. Pediatr., 2005, 146(3), p.306-307.
65. *Nagasaka H., Yorifuji T., Hirano K. et al.* Effects of bezafibrate on dyslipidemia with cholestasis in children with familial intrahepatic cholestasis-1 deficiency manifesting progressive familial intrahepatic cholestasis. Metabolism, 2009, 58(1), p.48-54.
66. *Narayanan S.* Biochemistry and clinical relevance of lipoprotein X. Ann. Clin. Lab. Sci., 1984, 14(5), p.371-374.
67. *Nishiwaki M., Ikewaki K., Bader G et al.* Human lecithin-cholesterol acyltransferase deficiency. in vivo kinetics of low-density lipoprotein and lipoprotein-X. Arterioscler. Thromb. Vasc. Biol., 2006, 26(6), p.1370-1375.
68. *O K., Frohlich J.* Role of lecithin.cholesterol acyltransferase and apolipoprotein A-I in cholesterol esterification in lipoprotein-X in vitro. J. Lipid. Res., 1995, 36(11), p.2344-2354.
69. *Ooi K., Shiraki K., Sakurai Y. et al.* Clinical significance of abnormal lipoprotein patterns in liver diseases. Int. J. Mol. Med., 2005, 15(4), p.655-660.

70. *Palynyczko Z., Milewski B.* Diagnostic importance of lipoprotein-X (LP-X) in the diagnosis of cholestasis. *Mater. Med. Pol.*, 1975, 7(4), p.327-333.
71. *Patsch J.R., Aune K.C., Gotto A.M.Jr., Morrisett J.D.* Isolation, chemical characterization, and biophysical properties of three different abnormal lipoproteins. LP-X1, LP-X2, and LP-X3. J. Biol. Chem., 1977, 252, p.2113–2120.
72. *Perales J., Lasunción M., Cano A. et al.* Changes in the lipid profile in chronic hepatopathies. Med. Clin. (Barc.), 1994, 102(10), p.364-368.
73. *Poley J.R., Alaupovic P., McConathy W.J. et al.* Diagnosis of extrahepatic biliary obstruction in infants by immunochemical detection of LP-X and modified 131 I-Rose Bengal excretion test. J. Lab. Clin. Med., 1973, 81(3), p.325-341.
74. *Poley J.R. Burdelski M.* Diagnostic value of laboratory and scintigraphic investigations in young infants with cholestatic jaundice. Leber. Magen. Darm., 1979, 9(2), p.55-59.
75. *Poley J.R., Caplan D.B., Magnani HN. et al.* Quantitative changes of serum lipoprotein-X after cholestyramine administration in infants with cholestatic biliary tract and liver disease. Eur. J. Clin. Invest., 1978, 8(6), p.397-404.
76. *Poley J.R., Smith E.I., Boon D.J. et al.* Lipoprotein-X and the double 131 I-rose bengal test in the diagnosis of prolonged infantile jaundice. J. Pediatr. Surg., 1972, 7(6), p.660-669.
77. *Protter A.A., Levy-Wilson B., Miller J. et al.* Isolation and sequence analysis of the human apolipoprotein C-III gene and the intergenic region between apoA-I and apoC-III genes. DNA, 1984, 3, p.449-456
78. *Raabo E., Franck C., Frey M., Ingwersen S.* Lipoprotein-X. Methods of analysis and diagnostic significance in cholestasis. Ugeskr. Laeger., 1980, 142(31), p.1986-1989.
79. *Radzevich I.M., Gromashevskaia L.L., Tat'ianko N.V.* The diagnostic significance of lipoprotein-X. Vrach. Delo, 1990, 1, p.116-119.
80. *Ritland S.* Quantitative determination of the abnormal lipoprotein of cholestasis, LP-X, in liver disease. Scand. J. Gastroenterol., 1975, 10(1), p.5-15.
81. *Ritland S., Blomhoff J.P., Elgjo K., Gjone E.* Lipoprotein-X (LP-X) in liver disease. Scand. J. Gastroenterol., 1973, 8(2), p.155-160.
82. *Ritland S., Gjone E.* Quantitative studies of lipoprotein-X in familial lecithin. cholesterol acyltransferase deficiency and during cholesterol esterification. Clin. Chim. Acta., 1975, 59(2), p.109-119.
83. *Ritland S., Stokke K.T., Gjone E.* Changes in the concentration of lipoprotein-X during incubation of postheparin plasma from patients with familial lecithin- cholesterol acyltransferase (LCAT) deficiency. Clin. Chim. Acta., 1976, 67(1), p.63-69.
84. *Ross A., Murphy G.M., Wilkinson P.A. et al.* Occurrence of an abnormal lipoprotein in patients with liver disease. Gut., 1970, 11(12),1035-1037.
85. *Rosseneu M.* Structure and function of apolipoproteins. USA. CRC Press, 1992, 437pp.
86. *Russ E.M., Raymunt J., Barr D.P.* Lipoproteins in primary biliary cirrhosis. J. Clin. Invest., 1956, 35, p.133-144.
87. *Samsioe G., Johnson P., Gustafson A.* Studies in cholestasis of pregnancy. VI. Fatty acid composition of glycero-phospholipids before and after delivery. Acta Obstet. Gynecol. Scand., 1977, 56(1), p.31-35.
88. *Sawaryn T.* Lipoprotein-X as an indicator of cholestasis in liver and biliary tract diseases. Wiad. Lek., 1984, 37(19), p.1522-1527.
89. *Schut J.M, Diaz D.P.* The value of combined determination of high molecular mass API and LP-X in the differential diagnosis of intrahepatic and extrahepatic obstruction. Clin. Chim. Acta., 1985, 148(3), p.221-227.
90. *Seidel D.* Lipoproteins in liver disease. J. Clin. Chem. Clin. Biochem., 1987, 25(9), p.541-551.
91. Seidel D., Alaupovic P., Furman R.H. A lipoprotein characterizing obstructive jaundice. I. Method for quantitative separation and identification of lipoproteins in jaundiced subjects. J. Clin. Invest., 1969, 48(7), p.1211-1223.
92. *Seidel D., Alaupovic P., Furman R.H., McConathy W.J.* A lipoprotein characterizing obstructive jaundice. II. Isolation and partial characterization of the protein moieties of low density lipoproteins. J. Clin. Invest., 1970, 49(12), p.2396-2407.

93. *Seidel D., Büff H.U., Fauser U., Bleyl U.* On the metabolism of lipoprotein-X (LP-X). Clin. Chim. Acta., 1976, 66(2), p.195-207.
94. *Seidel D., Gretz H., Ruppert C.* Significance of the LP-X test in differential diagnosis of jaundice. Clin. Chem., 1973, 19(1), p.86-91.
95. *Seidel D., Agostini B., Muller P.* Sturucture of an abnormal plasma lipoprotein (LP-X) characterizing obstructive jaundice. Biochim. Blophys. Acta., 1972, 260(1), p.146-152.
96. *Seidel D.* Studies on the structure and metabolism of lipoprotein-X (LP-X), the abnormal plasmalipoprotein in cholestasis. Klin. Wochenschr., 1977, 55(13), p.611-623.
97. *Senn H.J., Orth M., Fitzke E. et al.* Altered concentrations, patterns and distribution in lipoproteins of serum gangliosides in liver diseases of different etiologies. J. Hepatol., 1990, 11(3), p.290-296.
98. *Sessa A., Battini G, Meroni M. et al.* Hypocomplementemic type II membranoproliferative glomerulonephritis in a male patient with familial lecithin-cholesterol acyltransferase deficiency due to two different allelic mutations. Nephron, 2001, 88(3), p.268-272.
99. *Sorokin A., Brown J.L., Thompson P.D.* Primary biliary cirrhosis, hyperlipidemia, and atherosclerotic risk. a systematic review. Atherosclerosis, 2007, 194(2), p.293-299.
100. *Sörös P., Böttcher J., Maschek H. et al.* Lipoprotein-X in patients with cirrhosis. its relationship to cholestasis and hypercholesterolemia. Hepatology, 1998, 28(5), p.1199-1205.
101. *Steinberg D., Witztum J.L.* Is the oxidative modification hypothesis relevant to human atherosclerosis? Do the antioxidant trials conducted to date refute the hypothesis? Circulation, 2002, 105, v2107–2111.
102. *Stojanov S., Kastner D.L.* Familial autoinflammatory diseases. genetics, pathogenesis and treatment. Curr. Opin. Rheumatol., 2005, 17(5), p.586-599.
103. *Studenik P.* Lipid disorders in liver diseases. Vnitr. Lek., 2000, 46(9), p.547-548.
104. *Suehira S., Baba T., Ichimura M., Tohge H.* Case of drug-induced hepatitis with hyperlipoprotein-X-emia and densitometric determination of lipoprotein-X in the patient's serum after agarose gel electrophoresis and enzymatic cholesterol staining. Japan. J. Clin. Pathol., 1984, 32(12), p.1365-1371.
105. *Switzer S.* Plasma lipoproteins in liver disease. I. Immunologically distinct low-density lipoproteins in patients with biliary obstruction. J. Clin. Invest., 1967, 46(11), p.1855-1866.
106. *Szczotka W., Sawaryn T., Wiczkowski A.* Clinical usefulness of lipoprotein-X (LP-X) as an indicator of intrahepatic cholestasis in viral hepatitis. Wiad. Lek., 1986, 39(11), p.739-748.
107. *Takaya J., Nakano S., Imai Y. et al.* Usefulness of magnetic resonance cholangiopancreatography in biliary structures in infants. a four-case report. Eur. J. Pediatr., 2007, 166(3), p.211-214.
108. *Tam S.P., Breckenridge W.C.* Apolipoprotein and lipid distribution between vesicles and HDL-like particles formed during lipolysis of human very low density lipoproteins by perfused rat heart. J. Lipid. Res., 1983, 24(10), p.1343-1357.
109. *Tanno H., Fay O., Palazzi J. et al.* LP-X in cholestasis. Acta. Hepatogastroenterol. (Stuttg.), 1975, 22(5), p.289-291.
110. *Taramelli R., Pontoglio M., Candiani G. et al.* Lecithin cholesterol acyl transferase deficiency. Molecular analysis of a mutated allele. Hum. Genet., 1990, 85(2), p.195-199.
111. *Tazawa Y., Yamada M., Nakagawa M. et al.* Serum bile acids and their conjugates in breast-fed infants with prolonged jaundice. Eur. J. Pediatr., 1985, 144(1), p.37-40.
112. *Tazawa Y., Yamada M., Nakagawa M. et al.* Significance of serum lipoprotein-X and gammaglutamyltranspeptidase in the diagnosis of biliary atresia. A preliminary study in 27 cholestatic young infants. Eur. J. Pediatr., 1986, 145(1-2), p.54-57.
113. *Torsvik H., Berg K., Magnani HN. et al.* Identification of the abnormal cholestatic lipoprotein (LP-X) in familial lecithin. cholesterol acyltransferase deficiency. FEBS Lett., 1972, 24(2), p.165-168.
114. *Turchin A., Wiebe D.A., Seely E.W. et al.* Severe hypercholesterolemia mediated by lipoprotein X in patients with chronic graft-versus-host disease of the liver. Bone. Marrow. Transplant., 2005, 35(1), p.85-89.
115. *Vaddi K.* Monocyte chemoattractant protein-1. In. Vaddi K., Keller M., Newton R.C. - editors. The Chemokine Fact Book, San Diego, CA. Academic Press., 1997, p.86-98.

116. *Vergani C., Pietrogrande M., Grondona M.C.* Study of the abnormal lipoprotein-X in obstructive and non-obstructive jaundice. Clin. Chim. Acta., 1973b, 48(3), p.243-248.
117. *Vergani C., Pietrogrande M., Grondona M.C., Pizzolato M.* Study of an abnormal lipoprotein (LP-X) associated with cholestasis. Minerva Med., 1973a, 64(28), p.1461-1485.
118. *Vierucci A., Monterisi N., Dettori M. et al.* Cholestasis lipoprotein (LP-X). Characteristics, diagnostic value and distribution in some liver diseases in children. Minerva Pediatr., 1974, 26(3), p.131-143.
119. *Vierucci A., Varone D., Moggi C.* Australia antigen, α-fetoprotein and lipoprotein X in some diseases in children. Quad. Sclavo Diagn., 1971, 7(4), p.829-838.
120. *Vrublovský P., Kubícek R., Seidlová V. et al.* Lipoprotein X in the cholestatic syndrome. Acta Univ. Palacki Olomuc. Fac. Med., 1998, 119, p.423-426.
121. *Watanabe M.* Lipoprotein abnormalities in cholestasis. I. Electrophoretic and ultracentrifugal analyses. Acta Med. Okayama, 1979, 33(4), p.269-285.
122. *Wemmer U., Spelger G.* Diagnosis of cholestasis in acute viral hepatitis in childhood. Klin. Padiatr., 1980, 192(3), p.249-253.
123. *Wieland H., Meissner-Heins H., Heins C., Seidel D.* The significance of LP-X cholesterol in the differential diagnosis of cholestasis. Klin. Wochenschr., 1982, 60(7),p. 343-348.
124. *Williams G.J., Whitington P.F., Weidman S.W. et al.* Correctable plasma lipoprotein abnormalities in infants with choledochal cysts. Pediatr. Res., 1985, 19(2), p.240-247.
125. *Witt I., Ober M.* LP-X in newborns. increased incidence of positive tests without cholestasis. J. Clin. Chem. Clin. Biochem., 1976, 14(4), p.197-202.
126. *Wolf P.* High-molecular-weight alkaline phosphatase and alkaline phosphatase lipoprotein X complex in cholestasis and hepatic malignancy. Arch. Pathol. Lab. Med., 1990, 114(6), 577-579.
127. *Yakabe S., Ikeda K., Ohgami H. et al.* Clinical significance of lipoprotein-X in congenital biliary atresia. *Z Kinderchir*, 1984, 39(3), p.168-170.
128. *Yang C.Y., Manoogian D., Pao Q. et al.* Lecithin.cholesterol acyltransferase. Functional regions and a structural model of the enzyme. J. Biol. Chem., 1987, 262(7), p.3086-3091.
129. *Zhu X., Herzenberg A.M., Eskandarian M. et al.* A novel in vivo lecithin-cholesterol acyltransferase (LCAT)-deficient mouse expressing predominantly LpX is associated with spontaneous glomerulopathy. Am. J. Pathol., 2004, 165(4), p.1269-1278.
130. *Zidan H., Lo S., Wiebe D. et al.* Severe hypercholesterolemia mediated by lipoprotein X in a pediatric patient with chronic graft-versus-host disease of the liver. Pediatr. Blood Cancer, 2008, 50(6), p.1280-1281.