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LYSOSOMAL ACID LIPASE DEFICIENCY: WOLMAN DISEASE AND CHOLESTERYL ESTER STORAGE DISEASE

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Abstract
Cholesteryl ester storage disease (CESD, OMIM #278000) and Wolman disease (OMIM #278000) are autosomal recessive lysosomal storage disorders caused by a deficient activity of lysosomal acid lipase (cholesteryl ester hydrolase, LAL). Human lysosomal acid lipase is essential for the metabolism of cholesteryl esters and triglycerides. In Wolman disease, LAL activity is usually absent, whereas CESD usually presents some residual LAL activity. In infants, poor weight gain, massive hepatosplenomegaly, calcified adrenal glands (present about 2/3 of the time), vomiting, diarrhea and failure to thrive are indicative of Wolman disease. The clinical picture is more variable in CESD. Hepatomegaly and/or elevation of liver transaminases are almost always present. Hepatic steatosis often leads to fibrosis and cirrhosis. Other signs often include splenomegaly, high total cholesterol and LDL-cholesterol, elevated triglycerides, and low HDL-cholesterol. The diagnosis of LAL deficiency requires clinical experience and specialized laboratory tests. The diagnosis is based on finding deficient activity of acid lipase and/or molecular tests. Pilot screening projects using dried blood spot testing in 1) children with atypical fatty liver disease in the absence of overweight, 2) patients with dyslipidaemia and presence of hepatomegaly and/or elevated transaminases, 3) newborns/neonates with hepatomegaly and abdominal distension/failure to thrive/elevated transaminases are currently underway. Early diagnosis is particularly important for the enzyme replacement therapy. Human trials with recombinant LAL are currently ongoing, raising the prospect for specific correction of LAL deficiency in this progressive and often debilitating disorder.

Key words: lysosomal acid lipase deficiency, Wolman disease, cholesteryl ester storage disorder.

Definition, historic background and incidence

Lysosomal storage disorders (LSD) constitute a group of more than 40 different, genetically conditioned diseases resulting from specific deficiencies in lysosomal functions.

In the case of Wolman disease and cholesteryl ester storage disease (*CESD*, OMIM 278000) lysosomal acid lipase (acid lipase, acid esterase, EC 3.1.1.13) is a deficient enzyme, and this is responsible for the hydrolysis of cholesterol esters and triglycerides at low pH inside lysosomes. The diseases are inherited in an autosomal recessive pattern. The first description of an infant with abdominal distension, massive hepatosplenomegaly and calcification of the adrenal glands was published in 1956 by Wolman [1] who in 1961 reported other siblings with similar symptoms from the same family [2]. Initially he named the disease "generalised

xanthomatosis with calcified adrenals" [1, 2]. Crocker et al. suggested the eponymic name of Wolman disease [3]. Since that time the literature has presented only a few cases of subsequent patients. CESD was first described in 1963 by Fredrickson in a child with hyperlipidaemia and enlarged liver where cholesteryl esters were accumulated. Several years later this disease was reported in adults [4].

The incidence of Wolman disease and cholesteryl ester storage disease is not known precisely. For Wolman disease, it is estimated to be less than 1 per 100,000 live births [5, 6]. The results of screening tests in a group of Iranian Jews in Los Angeles suggest that 1 per 4200 newborns in this population can be affected by Wolman disease. However, the fact that it is an insulated genetic population, not representative e.g. for the European population, has to be taken into account [7]. The analysis of incidence of the most common CESD mutation (p.E8SJM) in the general population has indicated that this disease may be highly underdiagnosed [8]. The incidence of CESD in the German population is estimated at 25/1,000,000 of live births (based on the incidence of the mutation $\Delta 254\text{-}277_1$ in the general population the incidence of homozygotes for this mutation has been estimated at 6/1,000,000). There are no epidemiological data from other countries.

Aetiology and pathogenesis

CESD is caused by a partial deficit of lysosomal acid lipase activity (on the other hand, its deep deficit leads to a severe form known as Wolman disease). This enzyme plays an important role in maintaining cholesterol homeostasis inside cells, as it is necessary for intracellular hydrolysis of cholesteryl esters and triglycerides that have entered cells as a result of lipoprotein endocytosis. In the case of LAL activity deficiency non-hydrolysed cholesteryl esters and triglycerides are accumulated in various organs and the synthesis of endogenous cholesterol and low-density lipoproteins (LDL) is stimulated.

Clinical characteristics

The majority of patients with Wolman disease have a very similar clinical manifestation. The disease usually develops within the first weeks of life with symptoms of increased vomiting and diarrhoea, hepatosplenomegaly and rapidly progressive cachexia. Cholestasis and elevated body temperature are almost always observed [1, 3]. Death usually occurs between 3 and 6 months of life [9].

Anaemia usually develops at around the 6^{th} week and progresses along with the disease. The results of other haematological tests indicate lymphocyte vacuolization and an increased number of foam cells in the bone marrow that at later stages of the disease are also present in the peripheral blood. The cholesterol and triglyceride blood levels are usually normal [9].

Hepatosplenomegaly, which has been reported even as early as on the 4^{th} day of life, is a constant feature of this disease and can be extensive, leading to breathing limitations due to mechanical chest compression.

A symptom pathognomic, though not always present, of Wolman disease is calcified and symmetrically enlarged adrenal glands. Enlarged liver and calcified adrenals are visible on plain X-ray images of the abdominal cavity, and on ultrasound and CT scans as well. Symptoms related to the central nervous system are rare; however, the psychomotor development is delayed.

Contrary to Wolman disease, CESD can have a varied clinical manifestation (Table 1). This disease usually starts during the first decade of life, with lipid metabolism disturbances accompanied by liver enlargement and elevated serum levels of hepatic transaminases. Hepatomegaly, elevated cholesterol levels and transaminase activity may be observed as early as during the first months of life. In all patients liver damage is progressive, and with time it finally leads to liver fibrosis [9]. The presence of chronic liver failure and fibrosis may occur as early as during late childhood or early adolescence. In approximately 1/3 of patients splenomegaly is also observed. Other symptoms reported in literature include jaundice, recurrent abdominal pain, gastrointestinal bleeding, delayed puberty [9]. In CESD there are no changes in the structure (calcification) or functions of the adrenal glands, as there is in the case of Wolman disease.

Diagnostics

Wolman disease is suspected based on a clinical picture (Figure 1, Table 2).

Table 1

Differentiation between Wolman disease and cholesteryl ester storage disease

	Cholesteryl ester storage disease	Wolman disease
Life span	– to adulthood	– till 3–14 months of age
Onset of disease symptoms	– mild, first symptoms during childhood or later	– acute, during the neonatal/ infant period
Hepatomegaly, in most cases with splenomegaly	– often the only symptom, the beginning in the I/II decade of life	– always beginning in the first weeks of life
Enlargement and calcification of the adrenal glands	– extremely rare, several cases described	– often, beginning in the first weeks of life small adrenal insufficiency
Other gastrological symptoms (steatorrhea, abdominal distension, vomiting, diarrhea)	– may not be present	– often, beginning in the first weeks of life
Premature atherosclerosis	– increased risk	– not present
Activity of lysosomal acid lipase	– residual	– lack
Hypercholesterolaemia	– always	– rare
Hypertriglyceridaemia	– often	– rare
Elevated levels of transaminases	– always	– rare
Changes in blood counts (anemia, thrombocytopenia)	– not present	– often

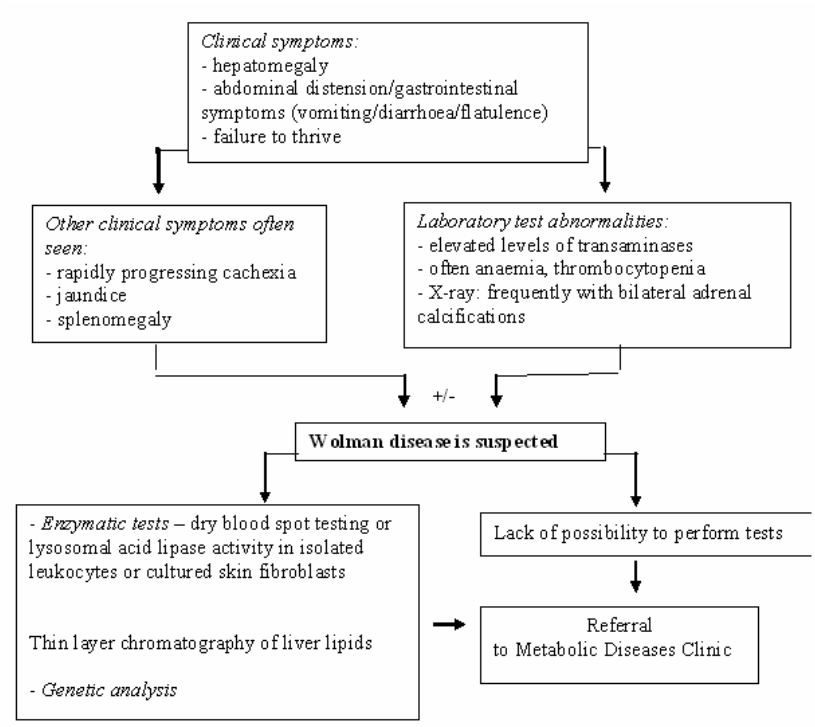

Figure 1 – A diagnostic algorithm for Wolman disease

Table 2

Indications to consider diagnostic tests for Wolman disease in newborns/infants

Disease symptoms	Laboratory/additional test results
Hepatomegaly/hepatosplenomegaly Jaundice	Elevated levels of cholesterol and/or triglycerides Elevated activity of transaminases, elevated bilirubin levels Bone marrow biopsy: lipid-loaded histiocytes, foam cells
Adrenal enlargement with calcifications	X-ray/CT/ultrasound: significantly and symmetrically enlarged adrenal glands with calcifications
Steatorrhea	Hypoalbuminaemia, hypofibrynogenaemia
Vomiting/diarrhoea/flatulence	Anaemia, thrombocytopoenia
Growth failure, rapidly progressing cachexia	Prolonged prothrombin time
Elevated body temperature/fever	

Basic laboratory tests

There are no specific routine laboratory tests to help suspect Wolman disease. Hepatic transaminase levels are often elevated. With the disease's progress anaemia becomes more severe, and thrombocytopenia may develop. Slightly elevated levels of cholesterol and triglycerides (upper limits of reference ranges) are observed, and at advanced stages coagulation disturbances may develop.

In CESD routine laboratory tests reveal hyperlipidaemia (elevated cholesterol and triglyceride levels) with significantly decreased levels of HDL and slightly elevated levels of hepatic transaminases and LDL.

Enzyme tests (Table 3)

The basis for diagnosing this condition is to confirm a lack of LAL activity in dry blood spot testing [10], isolated leukocytes of the peripheral blood or cultured skin fibroblasts. The activity of lysosomal acid lipase in patients with cholesteryl ester storage disease is usually 1–10% of the reference range.

Table 3

Laboratory tests used in lysosomal acid lipase deficiency diagnostics

Test type	Sample type	Assay advantages	Assay limitations
Lysosomal acid lipase enzyme activity tests			
Leucocytes	Heparinized whole blood EDTA whole blood	Concentrated, homogenous sample Multiple enzymes can be analyzed from one sample.	Whole blood shipping challenges.
Cultured fibroblasts	Skin punch biopsy	Single cell type. Multiple enzymes can be analyzed from one sample.	Invasive sample type. Biopsy handling and shipping challenges. Weeks to grow cells, longest time to result.
Dried blood spots	Whole blood Heparinized whole blood spotted onto filter paper	Easy sample collection and shipment.	Not offered in all region of the world. Considered a screening assay (positive result should be confirmed in leukocytes by enzyme activity assay and/or DNA analysis).
DNA analysis			
Whole *LIPA* gene sequencing		Can provide confirmation of enzyme activity findings. Knowledge of mutational background in a proband allows quick diagnostics	Potentially more expensive. New, undescribed mutations require confirmation of their pathogenic character.

Microscopic presentation

Under a light microscope, the presence of foam cells (lipid-loaded macrophages) in a bone marrow smear or vacuolated lymphocytes in a blood smear may be present in patients afflicted with Wolman disease. Using an electron microscope, typical free cholesterol needle-like crystals and so-called lipid droplets can also be observed in the hepatocytes for Wolman.

Microscopic tests of the liver in patients with CESD usually reveal a great number of lesions similar to Wolman disease, with some differences attributed to the fact that this pathological process has been developing for a significantly longer time. The following lesions can be observed: (1) in the parenchymal hepatocytes there are droplets of fat similar to the ones observed in typical liver steatosis; (2) Kupffer cells are enlarged due to smaller vacuoles and periodically acid-Schiff-positive granules; (3) a varied level of fibrosis in the septa that in some patients leads to micronodular cirrhosis with oesophageal varicose veins; (4) focal periportal accumulation of lymphocytes, plasma cells and foamy macrophages with small or lack of birefringence; and (5) vacuolisation and (6) massive accumulation of birefringent material in hepatocytes [11, 12].

Other biochemistry tests

Until recently in order to diagnose LAL deficiency a thin layer chromatography of lipids isolated from a liver biopsy specimen was used. Using this test it is possible to observe a profile of lipid storage with massive amounts of cholesteryl esters, triglycerides and free cholesterol which is typical of Wolman disease/CESD. Due to its invasiveness the procedure to obtain a liver biopsy specimen is currently performed very rarely.

Molecular tests (Table 3)

LAL deficiency is related to different mutations of the *LIPA* gene located on the 10q23.2-q23.3 chromosome coding a protein of lysosomal acid lipase. In the *LIPA* gene 47 mutations causing LAL deficiency have been described. Among them, 18 mutations are responsible for the phenotype of Wolman disease, and the rest for CESD.

In the event of Wolman disease gene rearrangements, including deletions, insertions or nonsense mutations, lead to the lack of or extremely low activity of acid lipase. *LIPA* gene mutations leading mainly to the inhibition of protein synthesis, exon deletion, defective splicing of transcripts are causes of the lack of or very low LAL activity.

Mutations causing CESD lead to some residual LAL activity. In patients with CESD the mutation $\Delta 254\text{-}277_1$ in exon 8 is identified most frequently.

Genetic counselling

Genetic counselling is indicated for parents and siblings of patients with LAL deficiency in order to determine the risk of a child being sick or further mutation transmission.

Differential diagnosis for CESD (Figure 2)

The presence of elevated cholesterol and triglyceride blood levels in paediatric patients is usually attributed to familial combined hyperlipidaemia (FCH) or, more frequently, to metabolic syndrome, also known as insulin resistance syndrome [13, 14]. The first disease is inherited in an autosomal dominant pattern and is characterised by a lipid profile that varies with time (from mixed hyperlipidaemia to hypercholesterolaemia or only hypertriglyceridaemia), the other is characterised by insulin resistance, central obesity, mixed dyslipidaemia with low HDL level and hypertension. In general, it is also associated with liver steatosis (so-called non-alcoholic fatty liver disease, NAFLD).

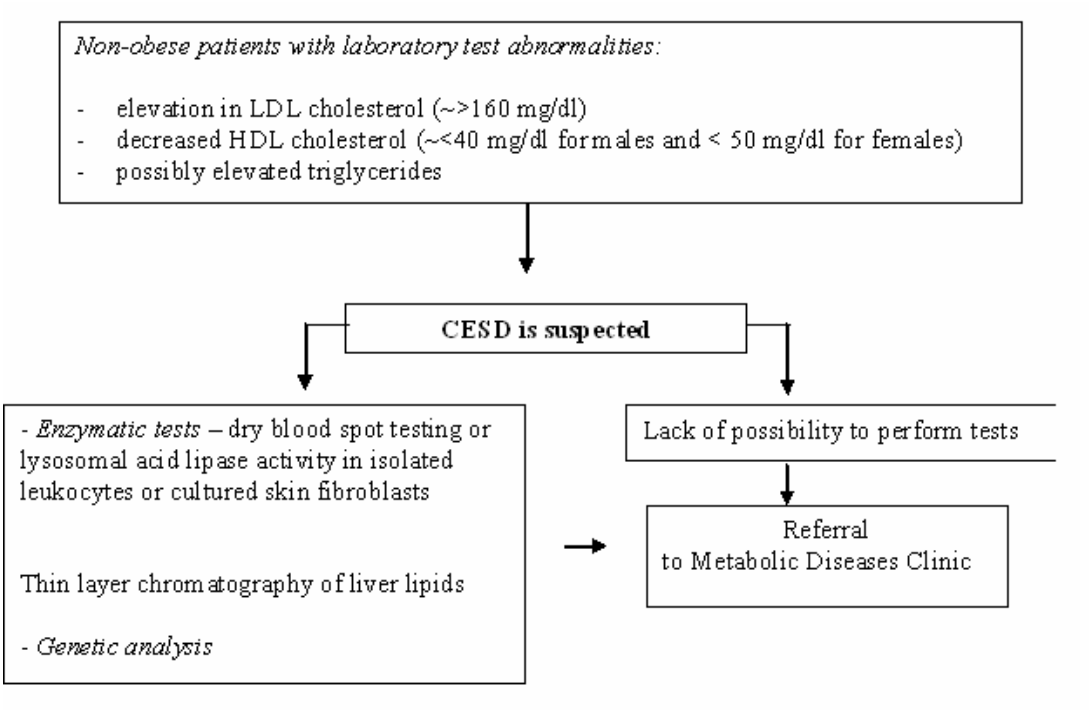

Figure 2 – Differential diagnosis for CESD

Therapeutic management

Wolman disease

So far only palliative treatment has been available and has included blood transfusion to alleviate anaemia and compensation of adrenal insufficiency.

Bone marrow stem cell transplantation

The literature reports several descriptions of patients with Wolman disease treated with bone marrow transplant. Four of them died due to complications associated with the procedure, and improvement was observed in one patient [15].

Cholesteryl ester storage disease

In patients with CESD the treatment has so far been focused on reducing the blood plasma levels of cholesterol and lipids as a result of a low-fat diet and the inhibition of the synthesis of endogenous cholesterol with HMG-CoA reductase inhibitors. It has been demonstrated that treatment with lovastatin and simvastatin reduces the levels of cholesterol, triglycerides and LDL. Unfortunately, despite modification of biochemical parameters it is not known whether such treatment affects in any way the clinical manifestation and course of the disease [16–18].

In one patient, liver transplantation was also performed; however, despite initial improvement the patient developed serious hypertension and renal failure leading to death [19, 20].

Enzyme replacement therapy (ERT)

Studies with recombinant human lysosomal acid lipase in patients with LAL deficiency are currently under way (www.clinicaltrials.gov identifier: NCT01371825 [21].

Conclusions

1. Wolman disease and cholesteryl ester storage disease are rare genetically conditioned metabolic diseases inherited in an autosomal recessive pattern.
2. These diseases are caused by a deficiency of lysosomal acid lipase activity.
3. In the case of Wolman disease, symptoms develop during infancy and almost always lead to death by the age of one year. Hepatosplenomegaly, fatty stools, increased waist circumference, other gastrointestinal symptoms, adrenal calcifications visible on X-ray images and inhibited development are observed as early as from the first weeks of life.
4. In the case of CSED, the disease usually manifests clinically in the first decade of life, and the main symptoms include liver and spleen enlargement. With time, liver fibrosis, chronic failure and cirrhosis are observed.

5. An initial diagnosis is based on a clinical manifestation, and in order to diagnose this disease it is necessary to confirm a lack of lysosomal acid lipase activity and/or identification of pathogenic mutations in the *LIPA* gene.
6. Studies with recombinant lysosomal acid lipase in patients with Wolman disease and CESD are currently under way, giving a chance for treatment of this disease.

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Резиме

ДЕФИЦИЕНЦИЈА НА ЛИЗОЗОМАЛНА КИСЕЛА ЛИПАЗА: ВОЛМАНОВА БОЛЕСТ И CHOLESTERYL ESTER STORAGE DISEASE

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Cholesteryl ester storage disease (CESD, OMIM #278000) и Wolman disease (OMIM #278000) се автозомно рецесивни лизозомални болести предизвикани поради дефициентна активност на лизозомалната кисела липаза (cholesteryl ester

hydrolase, LAL). Хуманата лизозомална кисела липаза е есенцијална за метаболизмот на холестерол естерите и триглицеридите. Кај Волмановата болест, LAL активноста е обично отсутна, додека кај CESD обично се среќава извесна резидуелна активност. Кај доенчиња, мало зголемување во тежина, масивна хепатоспленомегалија и калцифицирани адренални жлезди (присутни кај 2/3), повраќање, дијареја и ненапредување се индикативни за болеста на Волман. Клиничката слика е повеќе варијабилна кај CESD. Хепатомегалија и/или зголемување на трансаминазите се речиси секогаш присутни. Хепаталната стејатоза често води до фиброза и цироза. Други знаци вклучуваат спленомегалија, висок тотален и LDL холестерол, зголемени триглицериди и низок HDL холестерол. Дијагнозата на LAL дефициенција бара клиничко искуство и специјализирани лабораториски тестови. Дијагнозата се базира на наодот на дефициентна активност на киселата липаза или на молекуларните тестови. Пилот скрининг-тестови со користење на исушена дамка крв кај: 1) деца со атипично замрсен црн дроб, а во отсуство на дебелина; 2) пациенти со дислипидемија и присуство на хепатомегалија и/или зголемени трансаминази; 3) новородени со хепатомегалија и абдоминална дистензија/ненапредување, зголемени трансаминази се во тек. Раната дијагноза е особено значајна за ензимската заменска терапија. Испитувањата на рекомбинантниот LAL се изведуваат моментално кај луѓето и даваат надеж за специфична корекција на LAL дефициенција кај ова прогресивно и често дебилитирачко заболување.

Клучни зборови: дефициенција на лизозомална кисела липаза, Волманова болест, cholesteryl ester storage disorder (CESD).