Diagnostic Algorithm for Cholesteryl Ester Storage Disease: Clinical Presentation in 19 Polish Patients

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ABSTRACT

Background: Lysosomal acid lipase deficiency (LAL-D) is a rare autosomal recessive lysosomal lipid storage disorder that results in an early-onset, severe, and lethal phenotype, known as Wolman disease, or a late-onset, attenuated phenotype, cholesteryl ester storage disease (CESD). The aim of our study was to describe the clinical presentation of CESD, focusing on the first noted abnormalities in patients. A diagnostic algorithm of CESD was also proposed.

Methods: This is an observational, 1-center study of 19 Polish patients with late-onset LAL-D.

Results: The mean age at which the first symptoms were reported was 4 years and 6 months. A mild hepatomegaly was the most common initial abnormality observed in all (100%) patients. Seven (37%) patients were noted to have mildly to moderately elevated serum transaminases. At the time of first hospitalization all (100%) patients presented with hepatomegaly, 15 (79%) patients presented with elevated serum transaminases and all (100%) patients had dyslipidemia. The mean age at the time of CESD diagnosis was 7 years and 2 months. Diagnoses were based on a deficient LAL activity in leukocytes (in all patients) and the *LIPA* gene mutations (in 47% of them). All the patients were carriers for the mutation c.894G>A in the *LIPA* gene. There was approximately a 3-year delay from initial symptoms to final diagnosis.

Conclusions: Hepatomegaly constitutes the most common presenting clinical sign of CESD. Hepatomegaly and dyslipidemia defined as elevated serum total and LDL cholesterol, elevated triglycerides and normal to low HDL cholesterol, comprises the most characteristic findings at CESD diagnosis.

Key Words: diagnostic algorithm, dyslipidemia, elevated serum transaminases, hepatomegaly, lysosomal acid lipase, splenomegaly

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What Is Known

- Testing for lysosomal acid lipase deficiency is easily available and affordable via a dried blood spot and enzyme replacement therapy is available so that making the diagnosis is key.

What Is New

- In our cohort, there was approximately a 3-year delay from initial symptoms to final diagnosis of cholesteryl ester storage disease.
- The age of cholesteryl ester storage disease diagnosis was about 4 years of age emphasizing the importance of pediatric gastroenterologists to consider this diagnosis in young children.
- All our patients had normal body mass index or overweight with none being obese so when body mass index is normal in this setting a diagnosis of cholesteryl ester storage disease.
- Premature heart disease was not noted in this cohort as there is a high prevalence of a more milder phenotype in Polish population and younger age of patients.

Lysosomal acid lipase deficiency (LAL-D) is a rare autosomal recessive lysosomal lipid storage disorder characterized by the accumulation of cholesteryl esters and triglicerydes. With the exception of erythrocytes, lysosomal acid lipase (LAL) is expressed in all cells, thus its deficiency causes a progressive multiple organ system damage. Depending on the residual enzyme activity, LAL-D results in an early-onset, severe and lethal phenotype, known as Wolman disease, or a late-onset, attenuated phenotype, cholesteryl ester storage disease (CESD) (1–4).

CESD can manifest in childhood, adolescence, or even in adulthood presenting with hepatomegaly or hepatosplenomegaly, elevated serum transaminases, and dyslipidemia (type IIa or IIb hyperlipoproteinemia), including elevated serum total and low-density lipoprotein-cholesterol (LDL-C), high triglycerides and normal to low high-density lipoprotein-cholesterol (HDL-C). Abdominal ultrasound examination reveals normal or increased echogenicity of the liver. The microscopic examination of the liver could reveal features of steatosis, fibrosis or even cirrhosis (1–4).

So far there have been published single case reports and case series on this topic, but the natural history of these children has not been well documented. The aim of our study was to describe the clinical presentation of CESD, focusing on the first noted abnormalities in 19 patients. We also propose, based on our study and the other reported in the literature, a diagnostic algorithm of CESD.

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TABLE 1. Individual cholesteryl ester storage disease patients' characteristics

Sex	First symptoms noted by patient	First signs or symptoms noted by physician (patient's age)	First consultation—laboratory data	Liver biopsy (if performed); Liver in US examination (if performed)	LAL activity in leukocytes [reference values 0.33–1.33 mkat/kg protein]; Chitotriosidase activity in plasma [reference values: <150 nmol/mL/h]	Age at diagnosis	Genotype	Other signs or symptoms not related with CESD; BMI at diagnosis [kg/m^2]
F	Recurrent abdominal pain	Mild hepatomegaly (5 years)	ALT 24; AST n.a.; TC 288-332; HDL-C n.a.; LDL-C n.a.; TG 117; PLT 357	Liver biopsy (10 years)—cirrhosis, lipid storage vacuoles in hepatocytes and Kupffer cells, cholesteryl esters crystals in lysosomes; TLC of total liver lipid extracts revealed massive storage of cholesteryl esters and TGC	LAL—0.047 mkat/kg protein	10 years	n.a.	BMI 17
M	No complain	Mild hepatosplenomegaly (5 years)	ALT 75; AST n.a.; TC 247; HDL-C n.a.; LDL-C n.a.; TG 115; PLT 220	Liver biopsy (6 years)—fibrosis, lipid storage vacuoles in hepatocytes and Kupffer cells, cholesteryl crystals in lysosomes; TLC of total liver lipid extracts revealed massive storage of cholesteryl esters and triglicerydes	LAL—< 0.02 mkat/kg protein	6 years	n.a.	BMI 15
M	No complain	Mild hepatosplenomegaly (5 years)	ALT 87-145; AST n.a.; TC 390; HDL-C n.a.; LDL-C n.a.; TG 155; PLT 200	Liver biopsy (6 years)—cirrhosis, lipid storage vacuoles in hepatocytes and Kupffer cells, cholesteryl crystals in lysosomes	LAL—0.1 mkat/kg protein	6 years	n.a.	Gastric polyp; BMI 16
M	No complain	Hepatosplenomegaly (6 months)	ALT 158; AST n.a.; TC 308; HDL-C n.a.; LDL-C n.a.; TG n.a.; PLT 198	Liver biopsy (2 years)—fibrosis, lipid storage vacuoles in hepatocytes and Kupffer cells, cholesteryl crystals in lysosomes; TLC of total liver lipid extracts revealed massive storage of cholesteryl esters and TGC	LAL—0.094 mkat/kg protein	2 years	n.a.	Pulmonary tuberculosis at the age of 2 years; Agenesis of the left kidney; BMI 15
M	No complain	Mild hepatosplenomegaly (11 years)	ALT; AST n.a.; TC 104; HDL-C n.a.; LDL-C n.a.; TG 74; PLT 199	Liver biopsy (14 years)—hepatocytes swelling; TLC of total liver lipid extracts revealed massive storage of cholesteryl esters and triglicerydes	LAL 0.085 mkat/kg protein	14 years	n.a.	BMI 19
M	No complain	Diagnosed through family screening, mild hepatomegaly at that time (13 years)	ALT 9; AST n.a.; TC 126; HDL-C n.a.; LDL-C n.a.; TG 125	Liver biopsy—not performed	LAL—0.064 mkat/kg protein	13 years	n.a.	BMI 13.5
F	No complain	Mild hepatomegaly and elevated serum transaminases (1.5 years)	ALT 76-107; AST 73-168; TC 283-327; HDL-C 46; LDL-C 183-252; TG 125; PLT 227	Liver biopsy (2.5 years)—fibrosis, lipid storage vacuoles in hepatocytes and Kupffer cells, cholesteryl crystals in lysosomes	LAL 9.2 nmol/mg protein/h [reference range 231.7 ± 94]; Chito (2.5 years) —61	2.5 years	c.894G>A/c.894G>A	Related parents; BMI 15
F	No complain	Diagnosed through family screening; Mild hepatomegaly at that time (2 years)	ALT 55-66; AST 47-53; TC 238-270; HDL-C 55; LDL-C 186; TG 125; PLT 295	Liver biopsy—not performed	LAL 9.8 nmol/mg protein/h [reference range 231.7 ± 94]; Chito 60 (2 years); (101 (14 y)	2 years	c.894G>A/c.894G>A	Diabetes mellitus type 1; BMI 15.5

TABLE 1. Continued

Sex	First symptoms noted by patient	First signs or symptoms noted by physician (patient's age)	First consultation—laboratory data	Liver biopsy (if performed); Liver in US examination (if performed)	LAL activity in leukocytes [reference values 0.33–1.33 mkat/kg protein]; Chitotriosidase activity in plasma [reference values: <150 nmol/mL/h]	Age at diagnosis	Genotype	Other signs or symptoms not related with CESD; BMI at diagnosis [kg/m^2]
M	No complain	Mild hepatomegaly (2 years)	ALT 70-157; AST 72-107; TC 207-322; HDL-C 35; LDL-C 275; TG 73-189	Liver biopsy (5 years) —fibrosis, lipid droplets in hepatocytes; TLC of total liver lipid extracts revealed massive storage of cholesteryl esters	LAL 11.6 nmol/mg protein/h [reference range 231.7 ± 94]; Chito 215 (4 years), 390 (5 years)	5 years	c.894G>A/c.894G>A	BMI 16
M	Recurrent upper respiratory tract infections, obstructive bronchitis	Mild hepatomegaly (4 months)	ALT 51-85; AST 49-70; TC 329-337; HDL-C 25-37; LDL-C 211-270 TG 151-499; PLT 294-423	Liver biopsy—not performed; Increased echogenicity of liver in US	LAL 5.7 nmol/mg protein/h [reference range 231.7 ± 94]; Chito 225 (5 years)	5 years	c.538+5G>A/c.894G>A	BMI 15.5
M	No complain	Mild hepatomegaly and elevated serum transaminases (10 years)	ALT 74-132; AST 49-87; TC 230-279; HDL-C 38-46; LDL-C 194–206 TG 93-210; PLT 358-374	Liver biopsy (11 years)—porto-portal fibrotic bridges, liver steatosis, macrophages with vacuolised cytoplasm; Normal echogenicity of liver in US	LAL 7.2 nmol/mg protein/h [reference range 231.7 ± 94]; Chito 214 (11 years)	11 years	n.a.	BMI 17.5
F	No complain	Hepatosplenomegaly and elevated serum transaminases (4 years)	ALT 41-690; AST 80-1020; GGT 45–209 TC 288-474; HDL-C 12-24; LDL-C 224–357 TG 197-700; PLT 189-442	Liver biopsy—not performed; Increased echogenicity of liver in US	LAL 6.8 nmol/mg protein/h [reference range 231.7 ± 94]; Chito 1118 (4.5 years)	4.5 years	c.538+5G>A/c.894G>A	BMI 15.5
F	Three-month prolonged neonatal jaundice	Mild hepatomegaly (2 years)	ALT 69-145; AST 47-118; TC 303-365; HDL-C 14-26; LDL-C 236–308 TG 168; PLT 318-442	Liver biopsy (8 years)—macrophages with vacuolised cytoplasm; TLC of liver lipids revealed stain of cholesteryl esters; Normal echogenicity of liver in US	LAL 8.5 nmol/mg protein/h [reference range 231.7 ± 94]; Chito 107 (8 years)	8 years	c.309C>A /c.894G>A	BMI 16
M	Recurrent upper respiratory tract infections	Mild hepatomegaly (2 years)	ALT 54-87; AST 42-67; TC 227-319; HDL-C 33-39; LDL-C 224–276 TG 89-178; PLT 354-370	Liver biopsy (9 years)—hepatocytes with significant storage of fat droplets	LAL 9.8 nmol/mg protein/h [reference range 231.7 ± 94]; Chito 26 (4 years), 99 (9 years)	9 years	c.894G>A/c.894G>A	Gilbert syndrome confirmed genetically at the age of 16 years; BMI 19
M	No complain	Mild hepatomegaly and elevated serum transaminases (7 years)	ALT 77-80; AST 58-80; TC 346-459; HDL-C 29; LDL-C 175–286 TG 150-187; PLT 320	Liver biopsy (7 years) —mild liver steatosis, mild fibrosis	LAL 12.8 nmol/mg protein/h [reference range 231.7 ± 94]; Chito 121 (10 years)	10 years	n.a.	p.G592E mutation of the *LDLR* gene—heterozygote; BMI 17
M	No complain	Mild hepatosplenomegaly and elevated serum transaminases (5 years)	ALT 62-92; AST 68-71; TC 291-364; HDL-C 16-34; LDL-C 217–317 TG 168-197; PLT 230	Liver biopsy (5.5 years)—fatty hepatocytes degeneration, mild fibrosis	LAL 2.3 nmol/mg protein/h [reference range 231.7 ± 94]; Chito 385 (5 years); 590 (8 years)	8 years	c.386A>C/c.894G>A	BMI 20 (overweight)
M	No complain	Diagnosed through family screening, mild hepatosplenomegaly, and elevated serum transaminases noted at that time (7 years)	ALT 52-302; AST 42-219; TC 232-304; HDL-C 28-39; LDL-C 166–249 TG 143-219; PLT 250	Liver biopsy (4 years)—fatty hepatocytes degeneration	LAL 3.7 nmol/mg protein/h [reference range 231.7 ± 94]; Chito 543 (4 years), 876 (7 years)	7 years	c.386A>C/c.894G>A	BMI 19 (overweight)

TABLE 1. Continued

Sex	First symptoms noted by patient	First signs or symptoms noted by physician (patient's age)	First consultation—laboratory data	Liver biopsy (if performed); Liver in US examination (if performed)	LAL activity in leukocytes [reference values 0.33–1.33 mkat/kg protein]; Chitotriosidase activity in plasma [reference values: <150 nmol/mL/h]	Age at diagnosis	Genotype	Other signs or symptoms not related with CESD; BMI at diagnosis [kg/m^2]
M	Recurrent upper respiratory tract infections	Mild hepatosplenomegaly and elevated serum transaminases (5 years)	ALT 89-117; AST 63-89; TC 250-314; HDL-C 24; LDL-C 247 TG 209; PLT 213	Liver biopsy (7 years)—cirrhosis, massive microvesicular steatosis; Increased echogenicity of liver in US	LAL 6.4 nmol/mg protein/h [reference range 231.7 ± 94]; Chito 66 (5 years)	11 years	n.a.	BMI 17.5
M	No complain	Mild hepatosplenomegaly (1 year)	ALT 16; AST n.a.; TC 188; HDL-C n.a.; LDL-C n.a. TG 95 l; PLT 125	Liver biopsy (2 years)—lipid storage vacuoles in hepatocytes and Kupffer cells, TLC of total liver lipid extracts revealed storage of cholesteryl esters and TGC	LAL 0.051 mkat/kg protein; [reference values 0.33–1.33 mkat/kg protein]	2 years	n.a.	BMI 15

ALT = alanine aminotransferase, U/L; AST = aspartate aminotransferase, U/L; BMI = body mass index; CESD = cholesteryl ester storage disease; Chito = chitotriosidase; HDL-C = high-density lipoprotein-cholesterol, mg/dL; LAL = lysosomal acid lipase; LDL-C = low-density lipoprotein-cholesterol, mg/dL; n.a. = not analyzed; PLT = platelet count, tys/uL; TC = total serum cholesterol, mg/dL; TG = triglicerydes, mg/dL; THC = thin-layer chromatography; US = ultrasound.

Reference values: ALT, <18 months, <55/60 U/L; 18 months–12 years, boys, <40 U/L, girls, <35 U/L; >12 years, boys, <26 U/L, girls, <22 U/L; AST, <52 U/L; total serum bilirubin, <1.0 mg/dl; total cholesterol, <170 mg/dl; LDL-C, <110 mg/dl; HDL-C, >45 mg/dl; TG, 1–9 years, <75 mg/dl, 10–19 years, <90 mg/dl; PLT, 150–450 K/μL.

METHODS

This is an observational, 1-center study of patients with CESD (5). Over 30 years, there were 19 patients of Polish origin (from 16 families) diagnosed with CESD; medical records from all of them were obtained. The study group encompassed patients aged from 3 months to 13 years (median 5 years).

The diagnoses of all reported patients were based on a deficient LAL activity in leukocytes (in all patients) and the *LIPA* gene mutations (in 9 patients, 47%). Liver biopsy and thin-layer chromatography of total liver lipids were done in 14 (74%) out of 19 cases. One patient was also diagnosed with heterozygous familial hypercholesterolemia.

A retrospective chart review of patients' medical records concerning the first presented signs and symptoms, as well as biochemical (serum transaminases, platelet count, total cholesterol, HDL-C, LDL-C, triglycerides, lysosomal acid lipase activity, serum chitotriosidase activity), histological (microscopic examination of liver biopsies and thin-layer chromatography of total liver lipids), and molecular data (*LIPA* and *LDLR* gene mutations) were collected. Some data have been previously published (5–7). Ethical approval was obtained from the Children's Memorial Health Institute Bioethical Committee, Warsaw, Poland. Individual patients' characteristics are summarized in Table 1.

RESULTS

The mean age at which the first symptoms were reported by a physician (outpatient hospitalizations) was 4 years and 6 months. A mild hepatomegaly was the most common initial abnormality observed in all (100%) patients, while 9 (47%) patients had also a mild splenomegaly. Seven (37%) patients were noted to have mildly to moderately elevated serum transaminases (Tables 1 and 2).

At the time of first hospitalization (first consultation), all (100%) patients presented with hepatomegaly and 9 (47%) of them also with splenomegaly, 15 (79%) patients presented with elevated serum transaminases and all (100%) patients had dyslipidemia (in the form of elevated serum total and LDL cholesterol, high triglycerides and low HDL cholesterol) (Table 2). In 7 patients data concerning the aspartate aminotransferase (AST) activity were unavailable.

Liver biopsy was performed in 15 out of all 19 patients at the mean age of 6 years and 6 months. Lipid storage process (on the basis of the presence of lipid storage vacuoles in hepatocytes, lipid-laden macropgahes, and thin-layer chromatography of liver lipids) was reported in all (100%) of them. The evidence of fibrosis was reported in 7 (47%) patients and cirrhosis in 3 (20%) patients. No correlation of age and features of fibrosis/cirrhosis was observed.

The CESD diagnosis of all reported patients was based on a deficient LAL activity in leukocytes. The mean age at the time of

TABLE 2. Clinical and biochemical cholesteryl ester storage disease presentation—summary

	N	%
Initial presentation		
Hepatomegaly	19	100
Hepatosplenomegaly	9	47
Elevated serum transaminases	7	37
Presentation at first consultation		
Hepatomegaly	19	100
Hepatosplenomegaly	9	47
Elevated serum transaminases	15	79
Dyslipidemia	19	100

CESD diagnosis was 7 years and 2 months. In our cohort, there was approximately a 3-year delay from initial signs or symptoms to final diagnosis. Seventeen (89%) out of 19 patients had normal body mass index (BMI) at diagnosis, 2 other was overweight.

The molecular data were available in 9 (47%) patients. All patients from our cohort were carriers for the mutation c.894G>A in the *LIPA* gene. Among them, 4 (44%) patients were homozygous for this mutation. The other variants were c.386A>C (2 patients were heterozygotes), c.538+5G>A (2 patients were heterozygotes), and c.309C>A (heterozygous in 1 patient). Serum chitotriosidase activity was measured in 12 patients; in 6 (50%) of them it was slightly elevated.

DISCUSSION

Cholesteryl ester storage disease is characterized by a varied and often slightly expressed set of clinical and biochemical manifestations (2). The average age of the apparent disease onset in our study (4 years and 6 months) was similar to other reported cases in the literature (8–10). The features that all our patients presented at diagnosis were hepatomegaly and dyslipidemia (when screened), while other features, that is, splenomegaly or elevated serum transaminases were inconstant. Almost all patients had normal BMI at diagnosis.

Hepatomegaly was the most common presenting sign, and it is reported in the literature that hepatomegaly could persist as the only CESD sign for years (8–10). Splenomegaly is less commonly reported than hepatomegaly. Patients from our cohort did not present with features of hypersplenism (assessed by a normal platelet count). Mildly elevated serum transaminases are also frequently noted in CESD (because of hepatocyte damage) but less commonly than organomegaly (2,4,9).

Cholesteryl esters and triglicerydes accumulation in lysosomes constitutes a trigger of the liver fibrosis, micronodular cirrhosis and ultimately liver failure (2,10,11). In the natural course of CESD, liver involvement manifests as early fibrosis progression (10,11). Bernstein et al (10) reviewed 135 cases of CESD reported in the literature and noted that all patients had a significant liver disease progressing to cirrhosis and liver failure.

More than a half of our studied patients, however, had no complain. This means that CESD may develop insidiously or have a relatively mild phenotype. The latter could be related to the presence of c.894G>A mutation on at least 1 allele of the *LIPA* gene. The presence of this mutation alleviates the clinical phenotype. It is noteworthy that in the Polish population there is a high frequency of this mutation, what could be explained by the founder effect. The liver fibrosis and cirrhosis could progress slowly, remaining asymptomatic. Thus, the disease may be overlooked leading to delayed diagnosis or misdiagnosis.

Lipid abnormalities are characteristic for CESD phenotype featured with the disturbance of hepatic lipid metabolism and an abnormal lipid profile in the serum (1–4). While the hypercholesterolemia may be explained by the increased synthesis of apolipoprotein B-containing lipoproteins, the exact mechanism of decreased plasma HDL-C is not yet fully understood. The percentage of our patients presenting with dyslipidemia (100%) was higher than in the series of 135 patients reviewed by Bernstein et al (87%) (10).

The lipid phenotype is similar to other familial diseases presenting with hypercholesterolemia, such as familial combined hypercholesterolemia (FH) (12–14). Interestingly, similarly to our study (1 case), there is a possibility of the coexistence of both, CESD and FH. The first association of CESD with FH was reported by Stitziel et al in 2013 (15). Currently, there is a rising number of studies screening the patients with clinical FH phenotype with no revealed FH genotype for pathogenic *LIPA* mutations (13,14).

Taking into consideration our results and review of the literature we propose an algorithm of CESD diagnosis (Fig. 1). In general, in a case of coexisting hepatomegaly or hepatosplenomegaly and dyslipidemia defined as elevated serum total and LDL cholesterol, elevated triglycerides, and normal to low HDL cholesterol, possible CESD diagnosis should be considered. In a case of hepatomegaly or hepatosplenomegaly we should always perform

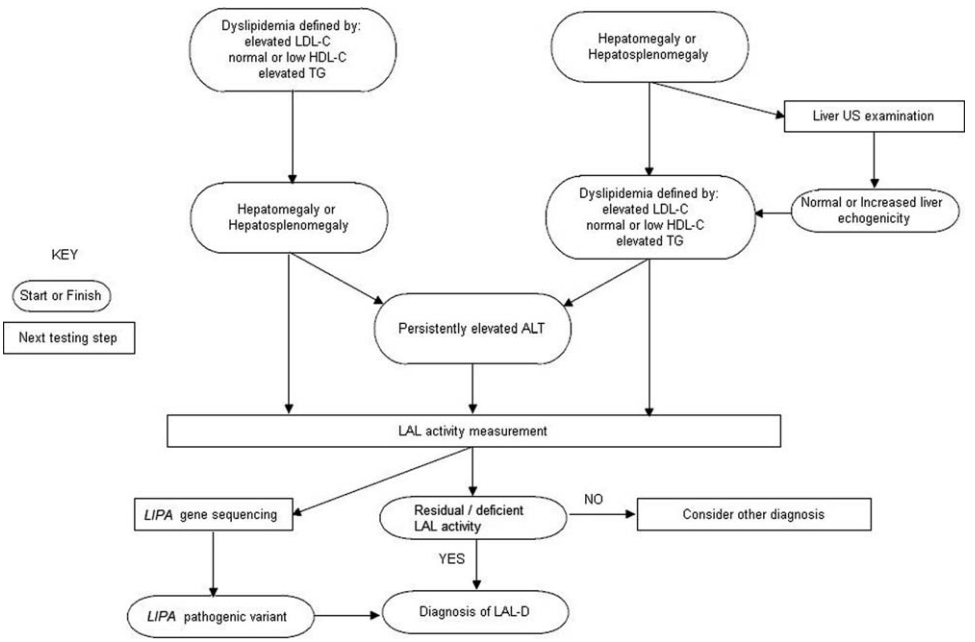

FIGURE 1. Algorithm of cholesteryl ester storage disease diagnosis.

the lipid serum profile and the serum aminotransferases activity. The siblings should also be screened.

LAL activity measurement in dried blood spot should be the first diagnostic step; if confirmed or doubtful, a measurement of enzyme activity in patients' leukocytes or skin fibroblasts is imperative for definite confirmation of LAL-D. Dry blood spot method, introduced by Hamilton in 2012, is very useful in clinical practice and helps to effectively discriminate between individuals with LAL-D and unaffected controls (16–18).

Recently, Baratta et al (19,20) and Shteyer et al (21) reported that serum LAL activity could be reduced in patients with nonalcoholic fatty liver disease or with secondary causes of dyslipidemia. In these patients, in vitro LAL activity is, however, never as low as measured in CESD patients. It strengthens the fact that CESD diagnosis should be confirmed by molecular analysis.

Microscopic examination of the liver biopsy specimens of CESD patients typically reveals the lipid-laden hepatocytes and macrophages and the microvesicular steatosis which progresses to fibrosis and even cirrhosis (1–4,10). The lipid-laden macrophages are pathognomonic of the storage process and absent in nonalcoholic fatty liver disease. There are also some unique histopathologic or immunohistochemical findings helpful in CESD diagnosis (22). On examination under polarized light, the cholesteryl ester crystals within lysosomes were detected is some of our studied patients, what constitutes a pathognomonic finding for LAL-D (see Table 1 and (6,23)). Additionally, needles of free cholesterol can be seen on electron microscope examination of liver biopsy specimen (7).

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