Aagenæs syndrome (LCS1), diet and disease progression

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Summary

It is estimated that the prevalence of persons with rare diseases in Norway is approximately 30 000, a number that has increased due to new technology that makes it possible to describe new diseases. In Norway, a disease is termed rare if the prevalence is less than 1 case in 10 000 people. Overall, more than 80% of rare diseases are caused by genetic defects.

Lymphoedema cholestasis syndrome 1 (LCS1), or Aagenaes syndrome, is one such rare disease, with nearly 100 diagnosed cases worldwide. Oystein Aagenaes, for whom the disease is named, was one of the first to describe this disease in 1968. Most patients originate from south-western Norway. Presently a total of 48 persons in Norway have been diagnosed with LCS1. The large number of cases recorded within our country is believed to be due to a founder effect.

The main challenge in these patients is neonatal cholestasis which causes malabsorption of fat and fat-soluble vitamins. If untreated, the condition leads to rickets, neuropathy and serious growth retardation or death due to haemorrhage. In some patients, cholestasis leads to end-stage liver disease. In adult patients, although periods of recurrent cholestasis might be distressing, primary lymphoedema is typically a greater concern.

The overall aim of this work has been to obtain new evidence-based knowledge about the progression of liver disease in a group of adolescent and adult patients with LCS1, to evaluate the need for the treatment of liver disease outside cholestatic periods or the treatment of lymphoedema. These aims have been supported by the Centre for Rare Disorders (SSD), one of ten nationwide interdisciplinary competence centres working with rare diseases on the behalf of the Norwegian Ministry of Health and Care Services.

In conclusion, although the overall prognosis in LCS1 is highly variable, and is largely dependent on the progression of cholestasis, patients with LCS1 exhibit a relatively good prognosis compared with other types of hereditary neonatal cholestasis. More than 50% of patients can probably expect a normal life span. Data presented in this

thesis show that adults who have survived the initial prolonged period of cholestasis and exhibit stable liver disease may have an even better prognosis. Approximately one-third of patients with LCS1 exhibit severe extremity lymphoedema and require close follow-up; however, lymphoedema is generally well managed with compression of lower limbs and CDT treatment. The diet of the LCS1 patient group is similar to that of healthy controls, with only few deviations from the Norwegian dietary recommendations.

Overall, the care and follow-up of LCS1 patients in this group seems satisfactory, but patients could benefit from following Norwegian dietary guidelines, with specific emphasis on carbohydrate and fat quality, in addition to regular monitoring of the blood levels of vitamins D and E.

The findings presented in this paper may provide potentially valuable information for adult LCS1 patients worldwide.

List of papers

Paper I

Monica Drivdal, Torleif Trydal, Tor-Arne Hagve, Ingunn Bergstad and Øystein Aagenæs. *Prognosis, with evaluation of general biochemistry, of liver disease in lymphoedema cholestasis syndrome 1 (LCS1/ Aagenaes syndrome).* Scandinavian Journal of Gastroenterology, 2006; 41: 469 - 475.

Paper II

Monica Drivdal, Elin Bjørge Løken, Tor-Arne Hagve, Ingunn Bergstad, Øystein Aagenæs. *Do patients with lymphoedema cholestasis syndrome 1/ Aagenaes syndrome need dietary counselling outside cholestatic episodes?* Clinical Nutrition 29 (2010) 525–530.

Paper III

Monica Drivdal, Carl-Erik Slagsvold, Øystein Aagenæs and Bengt Frode Kase. *Hereditary lymphedema, characteristics and variations in 17 adult patients with Lymphedema Cholestasis Syndrome 1 (LCS1)/ Aagenaes syndrome.* Lymphatic Research & Biology. Accepted 29th of June 2014.

Paper IV

Monica Drivdal, Kirsten B Holven, Kjetil Retterstøl, Øystein Aagenæs and Bengt Frode Kase. *A nine year follow-up of patients with lymphedema cholestasis syndrome 1 (LCS1/ Aagenaes syndrome).* Manuscript.

Abbreviations

ABCB11	ATP-binding cassette transporter B11
ABCB4	ATP-binding cassette transporter B4
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
ARC	Arthrogryposis, renal dysfunction and cholestasis (syndrome)
AST	Aspartate Aminotransferase
ATP8B1	ATPase Class 1, Type 8B1
BIA	Bioimpedance Analysis
BMI	Body Mass Index
BMR	Basal Metabolic Rate
BRIC	Benign Recurrent Intrahepatic Cholestasis (BRIC Type 1 and 2)
CCBE1	Collagen and Calcium Binding EGF Domains 1
CDP	Complete Decongestive Physiotherapy
CDT	Complex Decongestive Therapy
CIRH1A	Candidate Gene for (NAICC), Named CIRH1A
CPT	Combined Physical Therapy
CYP7A1	Cytochrome P450 7A1
CYP7B1	Cytochrome P450 7B1
DRV	Dietary Reference Values
DSM-BIA	Direct Segmental Multi-Frequency Bioimpedance Analysis
ECW	Extra Cellular Water
EI	Energy Intake
ESLD	End-Stage Liver Disease
EU	European Union
FGF15	Fibroblast Growth Factor 15
FGF4	Fibroblast Growth Factor 4
FLT4	Fms-Related Tyrosine kinase 4
FOXC2	Forkhead Transcription Factor 2
FXR	Farnesoid X Receptor
GGT	Gamma Glutamyl Transpeptidase
HNF4	Hepatocyte Nuclear Factor 4

HPLC	High-Performance Liquid Chromatography
IBABP	Intestinal Bile Acid Binding Protein
JAG1	Jagged 1 Gene
JNK	c-Jun N-terminal Kinase
kJ	Kilojoules
LCS	Lymphedema Cholestasis Syndrome (general)
LCS1	Lymphedema Cholestasis Syndrome 1 (Aagenaes syndrome)
LXR	Liver X Receptor
MCT	Medium Chain Triglycerides
MDR3	Multidrug Resistant Protein 3
MLD	Manual Lymph Drainage
MRCP	Magnetic Resonance Cholangiopancreatography
NAFLD	Non-Alcoholic-Fatty Liver Disease
NAICC	North American Indian Childhood Cirrhosis
NTCP1	Na+-dependent Taurocholate Cotransporting Peptide 1
OMIM	Online Mendelian Inheritance in Man
OUS	Oslo Universitets Sykehus/ Oslo University Hospital
PBC	Primary Biliary Cirrhosis
PFIC	Progressive Familial Intrahepatic Cholestasis (PFIC type 1, 2 and 3)
PSC	Primary Sclerosing Cholangitis
PXR	Pregnane X Receptor
SHP	Src Homology-2 (SH2) Domain-Containing Phosphatase
SLC10A1	Solute Carrier Protein 10A1
SSD	Senter for Sjeldne Diagnoser/Centre for Rare Disorders
SXR	Steroid and Xenobiotic Sensing Nuclear Receptor
TBW	Total Body Water
TJP2	Tight Junction Protein 2
UK	United Kingdom
US	United States (of America)
VDR	Vitamin D Receptor
VEGFR3	Vascular Endothelial Growth Factor Receptor 3
VPS33B	Vacuolar Protein Sorting 33 Homolog B

1 Introduction

1.1 Rare diseases

Rare diseases are defined differently throughout the world. In Norway, a congenital disease with a prevalence of less than 1 known case in 10 000 inhabitants is termed rare [1, 2] and corresponds to a total less than 500 cases among the Norwegian population. For most rare diseases, far fewer than 500 individuals have been diagnosed with the same condition [3]; in Norway, approximately 30 000 persons are estimated to have been diagnosed with a rare disease [3].

In the European Union (EU) countries, any disease affecting fewer than 5 people in 10 000 is considered rare. In the EU, it is estimated that 6 000 - 8 000 distinct rare diseases affect 6 - 8% of the population, representing between 27 - 36 million affected individuals [4]. This wide range is attributed to variations in how rare diseases are diagnosed within EU countries and the increasing emergence of new technologies to identify new diseases. In the United States (US), a disease is considered rare if it affects less than 200 000 Americans [5]. More than 80% of rare diseases are caused by genetic defects [6].

In Norway, there are ten interdisciplinary nationwide competence centres working with rare diseases on behalf of the Norwegian Ministry of Health and Care Services [1]. These national competence centres are part of the specialist health care services in Norway and currently provide support for more than 300 rare diseases. The centres concentrate on knowledge development, including scientific studies, and spreading competence in cooperation with the specialist health care system and community-based health care services. In addition, the centres provide information and counselling aimed directly at persons with a rare disease and their close relations [1]. Only individuals with rare diseases that are known to require extensive support and services in addition to the medical treatments provided by other parts of the health care system receive services from these centres.

The Centre for Rare Disorders (Senter for sjeldne diagnoser (SSD)) at Oslo University Hospital (OUS) HF is one such centre with a mandate to acquire

specialists with knowledge and experience with more than 70 different rare diseases [7, 8]. One of these diseases is Aagenaes syndrome/Lymphoedema cholestasis syndrome 1 (LCS1).

1.2 A brief overview of LCS1

Aagenaes syndrome was named after the Norwegian professor Dr Oystein Aagenaes, who was one of the first individuals to describe the condition in the 1960s [9]. The syndrome includes liver disease that manifests as idiopathic intrahepatic cholestasis and lymphoedema caused by lymph vessel hypoplasia [10]. Aagenaes syndrome has officially been named lymphoedema cholestasis syndrome 1 (LCS1) by the Human Genome Organisation's (HUGO) Gene Nomenclature Committee (HGNC) [11] and has the Online Mendelian Inheritance in Man (OMIM) number 214900 [12]. In this thesis, the abbreviation LCS1 will be used to refer to this disease. In LCS1, a strong emphasis on dietary and medical follow-up is needed during infancy and childhood because of cholestasis. Adolescent and adult patients have few cholestatic periods. It is unknown whether the liver disease affects individual patients in the absence of cholestasis or whether affected patients require dietary advice outside such periods. The lymphoedema is a daily concern for the patients and an evaluation of the lymphoedema variations within the group of LCS1 has been conducted.

In the following sections, an introduction to both cholestatic liver disease and lymphoedema is presented.

2 Liver disease

Liver disease includes a wide range of conditions (more than 100) that can be inherited or occur in response to viruses or chemicals. Liver diseases can either be acute or chronic and may lead to cirrhosis. Cirrhosis is the ultimate pathological feature of all forms of chronic hepatic injury. Irrespective of the cause of liver disease, destruction of the hepatic architecture and vascular structures, (Figure 1), in conjunction with the deposition of fibrotic tissue leads to the functional decompensation of the liver [13].

Difficulties in accessing data from individual countries hinder the global evaluation of liver disease, but approximately 29 million people in the EU suffer from a chronic liver condition [14]. The four leading causes of cirrhosis and primary liver cancer in Europe include harmful alcohol consumption, viral hepatitis B and C, and metabolic syndromes (non-alcoholic fatty liver disease (NAFLD)) that are related to overweight and obesity [14].

According to the World Health Organization (WHO), available data suggest that approximately 0.1% of the European population is affected by cirrhosis; this estimation corresponds to 1.4 - 2.6 new cases per 10 000 inhabitants per year and an estimated 170 000 deaths annually [15]. An estimated 400 new cases of acute liver failure are diagnosed annually (corresponding to 0.06 per 10 000) in the United Kingdom (UK) [16], and the estimated prevalence rate is 0.05 per 10 000 in Germany [17]. In the US, chronic liver disease has an estimated prevalence of approximately 6.4 cases per 10 000 individuals [18], whereas acute liver failure is estimated to have an annual incidence of 2 300-2 800, corresponding to 0.07 - 0.09 cases per 10 000 individuals [16].

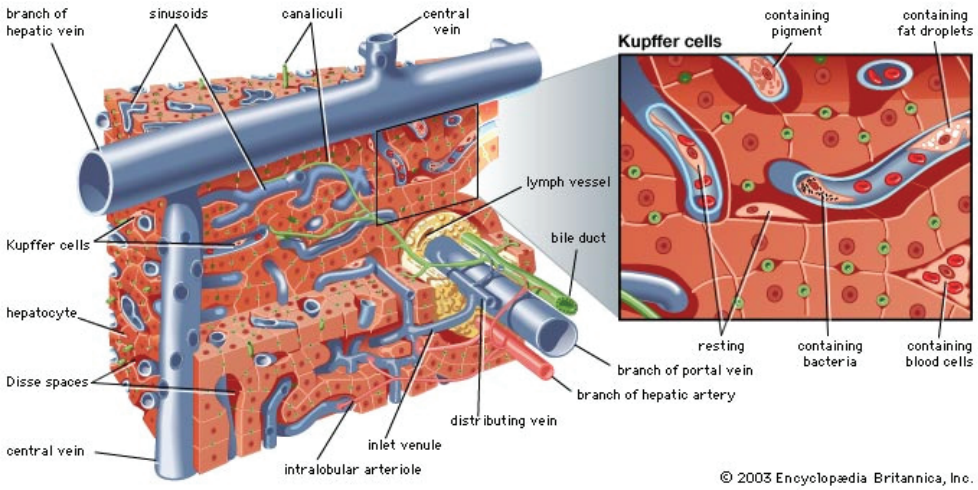
2.1 Cholestatic liver disease

Cholestasis is one of the most common and devastating manifestations of liver disease [19]. Cholestasis is a condition that results from impaired bile acid synthesis or bile flow to the gallbladder and duodenum and clinically presents with fatigue,

pruritus (itching), and jaundice [20]. Cholestasis may be classified as intrahepatic or extrahepatic, and may be of acute or chronic origin. The chronic cholestatic diseases are most often of intrahepatic origin. Cholestasis is considered chronic if it persists more than 6 months [21].

A variety of conditions cause cholestasis both in infancy and adulthood [22]. The most common causes of chronic liver disease in children are inherited syndromes of intrahepatic cholestasis, e.g., Alagille syndrome, progressive familial intrahepatic cholestasis (PFIC) [23], and biliary atresia [24]. Even the most common of these conditions is rare. The most accurate figures estimate the rate of biliary atresia to range from 1 in 17 000 to 19 000 live births [25]. Estimates of the incidence of Alagille syndrome range from 1 in 30 000 to 50 000 live births [26]. The true incidence of PFIC is not precisely known, but it is considered a rare disease with an estimated incidence ranging from 1 in 50 000 to 100 000 births [27].

Figure 1. Three-dimensional histological *s*tructure of human liver. Reprinted with permission from Encyclopædia Britannica Online. *Web. 21 Jun. 2014.*



The most common cause of chronic intrahepatic cholestatic liver disease in adults is primary biliary cirrhosis (PBC). The second most common cause is primary

sclerosing cholangitis (PSC), which also damages the extrahepatic bile ducts. Throughout the world, the incidence and prevalence rates of PBC and PSC vary widely [21, 28, 29]. The mean annual incidence rates for PBC and PSC per 100 000 in Norway have been estimated at 1.6 and 1.3, respectively (with a point prevalence per 100 000 of 14.6 and 8.5, respectively) [30]. In PBC, the incidence and prevalence rate in different countries, range from 0.33 - 5.8 and 1.9 - 40.2 per 100 000 inhabitants, respectively [28]. The estimated combined incidence rate of PSC in North America and Europe is 1 per 100 000 inhabitants, and the prevalence rate is 0 - 16.2 per 100 000 inhabitants [28].

2.2 Genetics in cholestatic disease

The dominant pathway for cholesterol elimination from the body is bile acid formation and involves more than 15 enzymes in the hepatocytes. The individual steps are important, and any defects in these enzymes can lead to clinical manifestations of cholestatic liver disease. In the small intestine, bile acids solubilise dietary lipids and fat-soluble vitamins, promoting their absorption. Bile acids are cytotoxic when present in high concentrations [31], and both the synthesis and transport of bile acids are highly regulated processes that occur principally in the liver [19, 32].

Four key nuclear hormone receptors regulate bile acid homeostasis (Figure 2); the farnesoid X receptor (FXR), the vitamin D receptor (VDR), the pregnane X receptor (PXR) also known as the steroid and xenobiotic sensing nuclear receptor (SXR), and the liver X receptor (LXR) [19]. FXR, PXR/SXR and VDR bind bile acids and are therefore sensors of excessive amounts of bile acids [19]. During the last decade, many genetic loci associated with intrahepatic cholestasis have been mapped, and the corresponding genes have been identified; reported examples include mutations in cytochrome enzymes, such as cytochrome P450 7A1 (CYP7A1) or CYP7B1; ATP-binding cassette transporter B11 (ABCB11); ATPase class I, type 8B, and member 1 (ATP8B1) proteins; multi-drug-resistant protein 3 (MDR3); tight junction protein 2 (TJP2); and vacuolar protein sorting 33 homolog B (VPS33B) [19, 33].

The major pathway for bile acid synthesis is the neutral pathway; its first step is the hydroxylation of the carbon atom at C-7 in the cholesterol molecule by CYP7A1. The second step is hydroxylation at C-12 which is mediated by CYP7B1. The CYP27A1 initiates the alternative bile acid biosynthetic pathway. The CYP7A1 is a microsomal enzyme and is considered to be the rate-limiting step in bile acid synthesis [32, 34]. After bile acids have been formed from cholesterol, they undergo amide linkage to glycine and taurine between the carboxyl group of the bile acid and the amino group of glycine and taurine, a process known as conjugation. The conjugation of bile acids has a number of biological and physiochemical consequences, and makes them impermeable to the membranes of hepatocytes, cholangiocytes and enterocytes thereby permitting high bile acid concentrations to accumulate in these compartments. Bile acids are deconjugated when they are exposed to bacteria in the intestine. Deconjugation and dehydroxylation decrease the solubility of bile acids and increase their toxicity [34].

In the enterohepatic circulation bile acids circulate between the liver and the small intestine. Most of the bile acids that return to the liver are in their conjugated form and only a minority are deconjugated. The uptake of bile acids in the enterohepatic circulation is 50-90% and is highly efficient. Bile acid circulation exhibits two modes of regulation. The first is negative feedback of bile acid synthesis from cholesterol in the hepatocytes. The second is negative feedback regulation of bile acid transport in the ileal enterocytes. Both of these regulatory mechanisms are driven by the intracellular concentration of bile acids acting on the nuclear receptor FXR. In the nucleus, bile acids bind to FXR. An FXR-RXR heterodimer acts to stimulate the synthesis of an inhibitory protein SHP, which in turn displaces a promoter factor (HNF4) from the promoter of the CYP7A1 gene. An additional essential negative regulatory factor is FGF15, a peptide secreted by the ileal enterocyte. This peptide acts on a basolateral receptor FGF4 which ultimately suppresses CYP7A1 transcription via a JNK-mediated pathway. The end result of these complex regulatory circuits is the down-regulation of bile acid synthesis through elevated bile acid levels using negative feedback modulated by FXR [32].

Role of nuclear hormone receptors in transcriptional regulation of bile acid synthesis and transport.

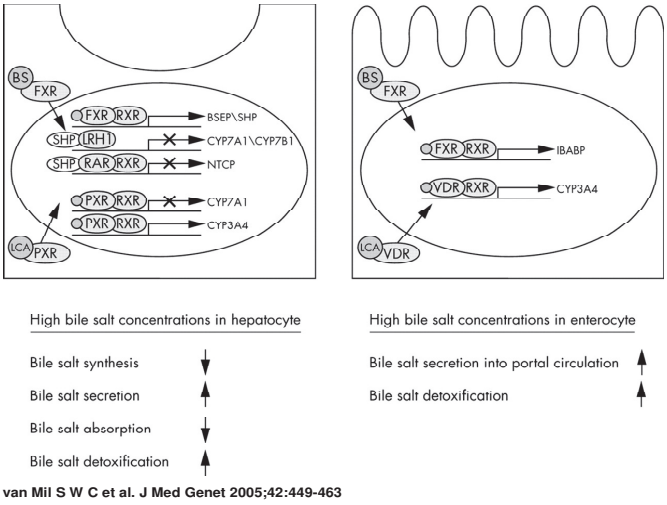


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Figure 2. The role of nuclear hormone receptors in the transcriptional regulation of bile acid synthesis and transport. In response to high concentrations of bile acids, bile acid synthesis and absorption in the hepatocytes decrease, whereas bile salt secretion and detoxification increase due to the action of nuclear hormone receptors. In the enterocyte, detoxification of secondary bile acids is increased and the secretion of bile acids into the portal venous system is eventually enhanced by transcriptional activation of Intestinal Bile Acid Binding Protein (IBABP). (Reprinted with permission from BMJ Publishing Group Ltd. and Journal of Medical Genetics, License Number 3405800367987, license date Jun 11, 2014).

In addition to CYP7A1, other genes regulated by FXR include SLC10A1 and the gene encoding NTCP1, a sodium-dependent co-transporter that is involved in conjugated bile acid uptake. SLC10A1 is down regulated by FXR activation. ABCB11 the gene encoding the bile salt export pump (BSEP), is up-regulated. Therefore, the end result of FXR activation is to decrease the concentration of bile acids in all hepatocytes [32].

Numerous familial and congenital cholestatic disorders are caused by genetic mutations [19, 35-38]. The pathogenesis of biliary atresia is unknown [39]. Alagille

syndrome is an autosomal dominant disorder caused by mutations in the Jagged 1 (JAG1) gene and the NOTCH receptor (a single transmembrane protein that is partially present at the cell surface) [40, 41]. The JAG1 gene encodes a ligand for the NOTCH receptor and thus is part of a signalling pathway that is critical during development [40, 41].

Other common inherited cholestatic diseases caused by mutations in the hepatocellular transport system genes involved in bile synthesis have been described by Hartley at al [38]. Both PFIC1 and benign recurrent intrahepatic cholestasis (BRIC1) are caused by mutations in ATP8B1. PFIC2 and BRIC2 are caused by mutations in ABCB11, also known as BSEP deficiency. PFIC3 known as MDR3 deficiency is caused by mutations in ABCB4

Other familial intrahepatic cholestasis syndromes, such as arthrogryposis, renal dysfunction and cholestasis syndrome (ARC-syndrome) are caused by recessive mutations in VPS33B at chromosome 15q26.1 [42]. North American Indian childhood cirrhosis (NAICC) is caused by mutations in CIRHA1A, which encodes a protein of unknown function [43]. Advances in the understanding of gene defects underlying familial cholestasis syndromes have greatly increased our knowledge regarding the processes of bile flow and regulation [19].

2.3 Diagnosing the cause of cholestasis

There is a broad range of differential diagnosis for cholestasis, especially in infancy and childhood, but also in adults [22, 37].

Early biochemical markers in patients (who are often otherwise asymptomatic) include increases in serum alkaline phosphatase (ALP) and γ-glutamyltranspeptidase (γ-GT), followed by conjugated hyperbilirubinaemia at more advanced stages [22]. Isolated serum ALP elevation is observed in cholestatic liver diseases, including certain rare diseases (e.g., bile acid synthesis defects in PFIC 1 and 2), but may also result from rapid bone growth (e.g., in infants and children), bone disease (e.g., Paget's disease), or pregnancy [22, 44].

In patients who present with cholestatic symptoms such as jaundice, fatigue, pruritus, dark colored urine, and/ or light colored stools, the first critical step is to differentiate intra- from extra hepatic cholestasis. The presence of extra hepatic pathologies such as biliary atresia should be ruled out early in the course of the evaluation as such conditions may be reversible [21, 45]. A careful patient history and physical examination are essential in the diagnostic process [22]. A family history of cholestatic liver disease suggests the possibility of a hereditary disorder.

Instruments and methods used to diagnose cholestasis include abdominal ultrasonography, magnetic resonance cholangiopancreatography (MRCP), testing for serum antimitochondrial antibodies (AMA) in adults and liver biopsy in cases where the diagnosis is still unclear [22].

Rates of dyslipidemia, portal hypertension and malignancies such as hepatocellular carcinoma and cholangiocarcinoma are increased in the setting of cholestatic disease [21].

2.4 Treatment of cholestasis

Medical treatment

The major aim of the treatment for all forms of cholestasis is to reduce the retention of bile acids and other bile constituents in the hepatocytes and improve liver function [46]. In most cholestatic diseases, the cause is unknown, and therapy can only be directed toward the suppression of the pathogenetic process [46]. Nuclear receptors have been recognised as potential targets for the pharmacologic treatment of cholestasis [46].

Cholestyramine, colestipol, and colesevelam bind bile acids and remove them from the circulation. With steady-state administration of such medicines bile acid synthesis (and loss) can increase by a factor of 4. All of these agents decrease pruritus in cholestatic liver disease, provided that bile acid secretion into the intestine is sufficient [32]. The most troublesome symptom of cholestasis is pruritus [21].

Although clinical experience suggests that the degree of itching is closely related to the degree of increase in bile acids, the precise mechanism of cholestatic pruritus remains unclear [47]. Cholestyramine or colestipol can be used to alleviate pruritus; rifampicin, which is a strong agonist of PXR, can also be used as an alternative. Opioid antagonists, such as naltrexone or nalmefene, ameliorate the sensation of pruritus and can be used to treat itching [48]. Sertraline or ondansetron might also be beneficial [21]. Ursodeoxycholic acid (UDCA) has been demonstrated to exert anticholestatic effects in various cholestatic disorders [22]. UDCA acts as an FXR antagonist and increases bile acid secretion [49].

Surgical treatment

At present, there are no definitive medical therapies that can provide curative options to affected patients. Liver transplantation is the only therapy for patients who have progressed to end-stage liver disease (ESLD) [50]. In children, biliary atresia and other cholestatic disorders are the most frequent cause of ESLD [51], but earlier surgical treatment of biliary atresia by the Kasai usually corresponds to later requirements of liver transplantation [45]. The Kasai procedure introduced in Japan in the 1960's, was the first operation for biliary atresia and has saved the lives of many patients with biliary atresia [45]. ESLD manifests as depressed serum proteins, coagulopathy, cholestasis, malabsorption, malnutrition, portal hypertension, renal dysfunction, fluid and electrolyte imbalances, ascites, and encephalopathy from the accumulation of neurotoxins [51, 52]. Sixty percent to 80% of children with ESLD exhibit moderate to severe malnutrition prior to transplant [51].

Dietary treatment

The prevalence of malnutrition in liver disease has been reported to be 65 - 90% [52], and cholestatic disorders are the most prevalent liver diseases associated with malnutrition [51]. A direct correlation exists between the progression of liver disease and the severity of malnutrition, and malnourished patients generally have a higher risk than well-nourished patients for adverse clinical outcomes and increased health-care costs [52, 53]. Those at greatest risk of malnutrition from liver disease are children younger than 24 months, those with cholestasis, those awaiting liver

transplantation and patients with hepatic complications such as ascites and bleeding varices [51]. Ongoing individualised nutritional interventions are needed especially in infants and children [54].

Major contributors to malnutrition include inadequate food intake, metabolic disturbances, malabsorption, and decreased capacity of the liver to store nutrients [53]. Decreased plasma concentrations of micronutrients in ESLD may occur secondary to impaired hepatic synthesis of the carrier proteins or from a down-regulatory effect of systemic inflammation [51].

Nutrition screening and assessment are the first steps in nutrition care directed at improving the clinical outcomes of children and adults with chronic liver disease and ESLD [51-53]. The components of nutrition screening include anthropometry, physical assessment, dietary evaluation and biochemical tests [51, 53].

When the flow of bile acids is restricted in patients with cholestasis, fat and fat-soluble vitamins are poorly absorbed, and bile and bile acid components (e.g., bilirubin and cholesterol) accumulate [51-53]. Multiple fat-soluble vitamin deficiencies may be present in 20 - 35% of patients with cholestatic liver diseases [55]. Specific recommendations have been made for calcium, selenium, sodium, and vitamin A, D, E, and K supplementation (Table 1) [22]. Vitamin K deficiencies can cause life-threatening internal bleeding. A lack of vitamin E can cause neurologic damage, and deficiencies of vitamin D can lead to rickets and dental problems [51, 53]. Water-soluble vitamin deficiencies in patients with chronic liver disease can include pyridoxine, thiamine or vitamin B_{12} deficiency and may result in peripheral neuropathy and other neurological disturbances [53]. The zinc deficiency observed in liver disease may be due to diarrhoea, intestinal malabsorption, increased urinary losses or low protein intake [53].

The nutritional goals in infants and children include the support of adequate growth and development; in adults the primary nutritional goal is to improve clinical outcomes. Stable patients exhibit near normal energy requirements, whereas more critically ill patients exhibit higher requirements. To account for higher metabolic rates increased calories must be provided. Infants and children are therefore particularly in need of a diet that is adapted according to individual growth, liver function and the

presence or absence of steatorrhoea. Breastfeeding accompanied by the fortification of the diet with infant formula that is high in medium chain triglycerides (MCT) to optimise fat absorption and to avoid essential fatty acid deficiencies is recommended in infants. Protein powders, glucose polymer powder and liquid fats can be added with vitamins and minerals to oral feeds, if needed [51]. Some patients may need additional enteral nutrition. An increase in sugar intake may result from a lower intake of energy from fat and may need to be addressed in conjunction with dental health care for older children and adults. Many patients with cirrhosis have glucose intolerance or diabetes. Infants and children are at increased risk of developing fasting hypoglycaemia due to reduced gluconeogenesis and a reduced capacity for glycogen storage [54]. Excess calories can contribute to fat synthesis and the accumulation of fat in the liver [53].

Table 1. Overview of some nutrient requirements in childhood liver disease. Modified table from [51] and [54].

Nutrient	Daily requirement	Comments
Energy	< 12 months up to 150% of EER > 12 months up to 120-170% of EER	Check weight, MUAC and growth percentiles to secure energy needs
Protein	2-4 g/kg	Avoid protein restriction
Fat	30-60% of total energy	
MCT	30-70% of total fat	Increase MCT percentage by decreasing bile flow.
EFA	5-11% energy as linoleic acid	
Carbohydrates	40-60% of total energy	
Vitamin A	< 10 kg 1 500 µg/day >10 kg start with 3000 µg/day 300 µg/kg/day up to 7 500 µg of orally available water miscible preparations.	Adjust supplements to keep serum levels within reference ranges. Higher single dose of vitamins A and D may be used in refractory deficiency
Vitamin D, cholecalciferol	<40 kg 3-5 µg/kg >40 kg 75-125 µg/day	
Vitamin E, α-tocopherol	7-134 mg/kg/day	
Vitamin K	2 mg /kg weekly	Monitor INR

Water soluble vitamins	Double nutrient reference values	
Calcium	25-100 mg/kg/day	Supplement as needed
Selenium	1-2 µg/kg/day	Weekly until repletion is achieved
Sodium	Minimum 1 mmol/day	Consider salt restrictions in older children
Zinc	1 mg/kg/day	Weekly until repletion is achieved

Estimated energy requirement (EER), mid-upper arm circumference (MUAC), medium chain triglycerides (MCT), essentially fatty acids (EFA), international normalised ratio (INR).

3 Lymphoedema

The lymphatic system consists of the lymph, lymphatic vessels, lymph nodes, spleen and thymus, as shown in Figure 3. The key functions of the lymphatic system include regulating tissue fluid volume, transporting fats absorbed from the intestines, and priming the immune system against infections by carrying material to local lymph nodes [56].

General peripheral lymphoedema occurs due to a structurally or functionally defective lymphatic system (lymph vessels and/ or lymph nodes) and is a chronic condition [57-59]. Lymphoedema should not be confused with oedema, which has a systemic cause. Oedema is a clinical term in which fluids, particularly interstitial fluids gather and accumulate beneath the skin. Oedema is generally not a permanent issue and can be treated [60].

Interstitial fluid pressure is determined by a complex interplay between the fluid influx (blood capillary filtration), the fluid outflow (venous reabsorption, lymph flow), and the compartment's ability to expand (tissue compliance) [61]. Capillary filtration pressures and interstitial colloid osmotic pressures support transmembrane flux from the capillary into the interstitium, whereas interstitial fluid pressures and intra-capillary colloid osmotic pressures lead to fluid reabsorption. Venous capillaries reabsorb 90% of the fluid that enters the interstitium by normal capillary filtration. Interstitial fluid homeostasis is balanced by lymph fluid formation in the initial lymphatic vessels.

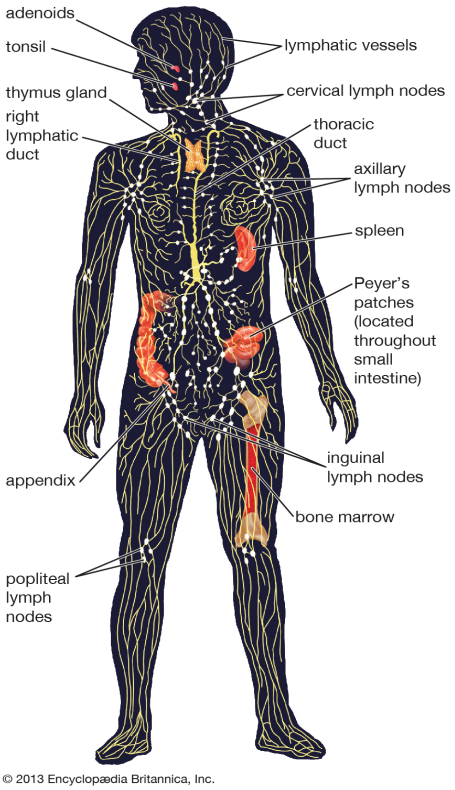

Figure 3. The lymphatic system.
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The following three mechanisms have been suggested for the uptake of interstitial fluid into the initial lymphatics: vesicular transport, osmotic pressure gradients, and fluid pressure gradients. It is generally accepted that interstitial fluid pressure is the main determinant of initial lymph filling and lymph flow [62]. The lymphatic vessels collect lymph (proteins, wastes, water, and fats) from cells in the body and carry the lymph to the lymph nodes. The lymph nodes filter the waste materials and then return the lymph to the blood stream [63]. If dysfunction or obstruction occurs in the lymphatic system, lymphatic stasis occurs, permitting the accumulation of protein-rich fluid within the interstitium. The increased protein concentration leads to increased tissue colloid osmotic pressure, which drives fluid into the interstitium and causes oedema and the clinical manifestation of lymphoedema.

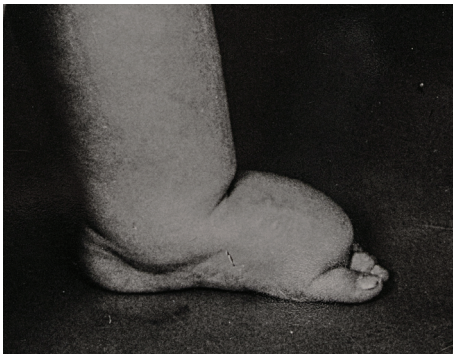


Figure 4. Lymphoedema in the foot of a 4-year-old patient with LCS1.
(Reprinted with permission from O Aagenaes).

Lymphoedema develops only over prolonged periods of time due to the body's capability to compensate. Initially the tissue is soft but will harden if the lymphoedema is not treated. The accumulation of fluid results in the growth of connective tissue. If the swelling persists for an extended period, an inflammatory response develops in the subcutaneous tissues causing tissue fibrosis (scar tissue) in the oedematous area. Fibrosis can be reversed only to a small extent [58, 63]. Lymphoedema is therefore generally progressive and can vary from a mild to a painful disabling and disfiguring condition [64]. When the lymphoedema worsens, the skin becomes less resistant to infections. Swollen skin chaps more easily and

permits the entry of bacteria leading to inflammation, erysipelas or cellulitis [65, 66]. Even a small scratch or an insect bite can cause long-lasting inflammation [67]. However, early diagnosis and treatment may prevent serious progression of the disease e.g. marked disfigurement and physical disability [68]. Lymphoedema is generally not life-threatening, but serious complications may occur especially due to the risk of infections [69-71].

Lymphoedema is divided into a primary and secondary form. Primary lymphoedema results from genetic damage, whereas secondary (acquired) lymphoedema is a consequence of lymphatic failure resulting from trauma, surgery, radiotherapy, or parasitic infection [72, 73]. The clinical manifestations of secondary lymphoedema typically involve a single limb, whereas more widespread involvement of the body may be observed in primary lymphoedema [74].

Studies of the prevalence of lymphoedema are limited. Worldwide secondary lymphoedema is the most prevalent form, representing approximately 80% [75]. One of the most prominent causes of lymphoedema is the lymphatic filariasis which mainly affects people in southeast Asia (65%) and Africa (30%). The WHO estimates that more than 120 million people are currently infected with the parasite, and approximately 40 million are disfigured and incapacitated by the disease [73, 76]. In industrialised countries, cancer therapy is the leading cause of secondary lymphoedema. In the US, it has been estimated that given a 20% incidence of lymphoedema after cancer treatment it yields a potential 1.36 million cases of lymphoedema.It is estimated that 2 - 3 million people suffer from lymphoedema in the US [77]. In the UK, it is estimated that 100 000 persons suffer from lymphoedema [78]. In Norway, the prevalence of lymphoedema has been estimated at approximately 7 000 persons, but this number likely represents an underestimation [79].

3.1 Primary lymphoedema

Primary lymphoedemas are rare disorders and are manifestations of a lymphatic malformation developing during the later stage of lymphangiogenesis. Primary

lymphoedema also includes other types of lymphoedema without an identifiable aetiology, e.g., infection. Primary lymphoedema has also been considered congenital because the great majority of cases are caused by a congenital defect in the lymph transportation system, including the lymphatic channels and the nodes (i.e., aplasia, hypoplasia, or hyperplasia) [80]. Hereditary lymphoedema tends to involve one or both lower extremities but can also involve the upper extremities or even the face [81]. Some hereditary syndromes in which lymphoedema dominates the clinical picture are summarised in the article of Schulte-Merker et al. [82]. The best-known hereditary lymphoedema is Milroy disease (hereditary lymphoedema type I) [83]. The prevalence of primary lymphoedema is approximately 1.15 per 100 000 for individuals younger than 20 years of age [84]. Prevalence estimates for congenital lymphoedema are approximately 1 case per 6000 to 10 000 live births [77].

3.2 Genetics of lymphoedema

Numerous genetic diseases are associated with lymphoedema [85]. Approximately nine known causal mutations for inherited human lymphoedema have been reported [57, 86].

It is unclear whether the variable age of onset for hereditary lymphoedema is caused by locus and allelic heterogeneity or by genetic or environmental modifiers [87, 88]. Furthermore, the mechanisms that regulate lymph vessel remodelling and development are not fully understood [89]. However, two potentially associated genes, fms-related tyrosine kinase 4 (FLT4) and forkhead transcription factor 2 (FOXC2), have been identified. FLT4 encodes for the protein vascular endothelial growth factor receptor-3 (VEGFR-3). VEGFR-3 plays an important role in lymphatic development and regulates the development and maintenance of the lymphatic system [90]. FOXC2 controls the later steps of lymphatic vascular development [89]. The collagen- and calcium-binding EGF domain-containing protein 1 (CCBE1) has recently been described as essential for lymphangiogenesis [91].

Mutations in the FLT4 gene cause Milroy disease, and FOXC2 may contribute to lymphatic dysfunction in lymphoedema-distichiasis syndrome [57, 92]. Milroy disease

is transferred as an autosomal dominant trait [93]. Hennekam lymphangiectasia-lymphoedema syndrome is an autosomal recessive disease associated with mutations in the CCBE1 gene [94, 95].

3.3 Diagnosing lymphoedema

The absence of a valid and accepted definition for the presence of lymphoedema and a lack of awareness of the condition decrease the likelihood of early diagnosis [57], and early treatments are therefore often hampered [77]. A recent study by Blome et al. stated that an average of 13.5 years passed from the appearance of the first symptoms to the start of complex decongestive therapy (CDT) treatment in patients with primary leg lymphoedema [68].

Different approaches for the clinical description of lymphoedema have been proposed. In 1957, lymphoedema was described as primary or secondary [96]. Later, primary lymphoedema was divided into three categories classified by age at onset: congenital, praecox, or tarda [75, 97]. Congenital lymphoedemas present within approximately 2 years of birth. Lymphoedema praecox presents between 2 and 35 years of age but typically becomes clinically apparent at or near puberty. The praecox type is the most common form of primary lymphoedema. Primary lymphedoema tarda is considered to be inherent with delayed manifestations and has a late onset (after 35 years of age) [98]. This terminology is imprecise, thereby making classification problematic [99].

The International Society of Lymphology has attempted to unify the broad range of worldwide diagnostic and treatment protocols for peripheral lymphoedema into a Diagnosis and Treatment of Peripheral Lymphoedema Consensus Document that represents a consensus among the international community [100]. However, given the lack of hard data, the fact that no treatment method has undergone any satisfactory meta-analysis, and the current lack of agreement regarding the clinical evaluation of lymphoedema, this document is not commonly used worldwide [100]. In addition, no unified method of lymphoedema classification has yet achieved consensus [100]. The large number of different classifications of lymphoedema and

inadequate or causal descriptions of lymphoedema make it impossible to evaluate and compare therapeutic results among different treatment centres [101, 102]. This inability represents a serious worldwide problem in assessing how best to prevent and treat this disabling disease [101]. Today, as causal genes are identified, there is the possibility of creating a classification based on patient phenotypes and known genetic aetiologies [57].

As mentioned above, the classification systems for diagnosing lymphoedema vary but are generally determined by a patient's clinical history and physical examination [100]. Lymphoedema can be confirmed with lymphoscintigraphy, computed tomography, magnetic resonance imaging, or (duplex) ultrasound [102]. The lymphatic anatomy is demonstrated with lymphoscintigraphy [103].

The common differential diagnoses in Western patients with lower limb swelling include secondary lymphoedema, venous disease, lipoedema, and adverse reactions to limb surgery [103]. Furthermore, comorbid conditions, such as congestive heart failure, hypertension, and cerebrovascular disease, including stroke, might influence the therapeutic approach taken [100]. Complications of chronic lymphoedema include recurrent episodes of erysipelas, superficial lymphangiectases, verrucous lymphoedema (papillomatosis lymphostatica), and angiosarcoma, which is often multifactorial [75]. Erysipelas is commonly observed in conjunction with lymphoedema and complicates or might aggravate the condition [104].

3.4 Treatment

The main aims of treating lymphoedema are to prevent the progression of disease, to achieve mechanical reduction and maintenance of limb size, to alleviate the symptoms that arise from lymphoedema, and to prevent skin infections (erysipelas/ cellulitis/lymphangitis) [105]. The treatment of lymphoedema is divided into conservative (non-operative), pharmacological, and operative methods [100].

Conservative treatment

Lymphoedema is primarily treated with CDT, which is also known as complete decongestive physiotherapy (CDP) or combined physical therapy (CPT) [100, 103, 106]. For mild lymphoedema, elevation of the affected limb(s) and skin care (cleansing and low pH lotions and emollients) are important for successful treatment [100].

CDT involves a two-stage treatment program that can be used in both children and adults. The first phase consists of skin care, a specific light manual massage referred to as manual lymph drainage (MLD), range of motion exercises and compression which is typically applied with a multi-layered bandage-wrapping to enhance lymphatic drainage. Phase 2, initiated promptly after Phase 1, aims to conserve and optimise the results of Phase 1. Phase 2 consists of compression by a low-stretch elastic stocking or sleeve, skin care, continued remedial exercise, and repeated MLD as needed [100, 106, 107]. Such a treatment significantly reduces the extent of lymphoedema and microlymphatic hypertension, paralleled with a considerable decrease in the mean circumference of the limb [103]. In Norway, CDT has been available since the mid-1980s [79].

Pneumatic pumps are another form of compression massage. After pneumo-massage, form-fitting low-stretch elastic stockings or sleeves are used to maintain the reduction in lymphoedema [100, 108]. Typically a combination of the CDT and pneumatic pump methods is used to achieve optimal benefits. Heat therapy with hot water immersion, microwave, or electromagnetic irradiation has also produced some benefit for lymphoedema [100].

Medical treatment

Benzopyrones have demonstrated effectiveness in the treatment of lymphoedema by reducing oedema fluid, increasing the softness of the limbs, and decreasing elevated skin temperature [103]. However, benzopyrones (e.g., coumarin) can be hepatotoxic and therefore are not used in several countries [103]. Benzopyrones are not an alternative for CDT, and the usefulness of such medication has not yet been definitively determined [100].

The use of diuretics specifically for lymphoedema treatment is discouraged [100]. Fungal infections are treated with antimycotic drugs. Prophylactic administration of antibiotics to reduce erysipelas/cellulitis is recommended [100].

Surgical treatment

Surgical treatment can be categorised into physiological methods (flap interposition, lymph node transfers, lymphatic bypass procedures) reductive techniques such as debulking operations (direct excision or liposuction to remove fibro-fatty tissue), and prophylactic surgery [103, 109]. Very few prospective or controlled studies have been performed regarding surgical interventions, making it difficult to draw conclusions about the efficacy of various treatment options, and there is no consensus for surgical intervention, the type of procedures that are performed, or the timing of the intervention [109]. Some authors believe that surgical intervention should be reserved exclusively for patients who fail conservative treatments [103, 109].

Dietary treatment

In chylous reflux syndromes (e.g. intestinal lymphangiectasia), a diet low in triglycerides with long-chain fatty acids and high in triglycerides with medium and short-chain fatty acids is of benefit especially in children. Otherwise, no special diet has proved to be of therapeutic value for uncomplicated peripheral lymphoedema [22]. In obese patients, a reduction in calorie intake combined with an exercise program may decrease limb bulk [100].

4 LCS1

The clinical features of LCS1 include intrahepatic cholestasis in infancy and childhood, with recurrent periods in adolescence and adulthood and the progression of lymphoedema throughout childhood and adulthood. Both the severity of the cholestasis and/ or the frequency of recurrent cholestatic periods can vary considerably among individual patients. No other anomalies than cholestasis and lymphoedema have been described, and mental and motor development proceeds normally [9].

The major concern in LCS1 in infancy and childhood is cholestatic liver disease that may lead to EDSL and the need for liver transplantation. Since 1996, six Norwegian patients have undergone transplantation in early childhood, and the most recent child with LCS1 who died of liver cirrhosis died in 1991 [110].

The mortality in this patient group was high prior to 1970, when adequate nutritional support was not in regular use [111]. The introduction of routine vitamin K injections in approximately 1940 altered the prognosis of this disease by reducing the neonatal mortality [9]. Of 21 patients born before 1970, 11 died in early childhood [111]. The high mortality rates during this period are partly responsible for the young patient population observed in Norway at present. Severe growth retardation, rickets and peripheral neuropathy were frequent until adequate infant formula and vitamin supplementation were provided [111]. If cholestasis during infancy and childhood goes into remission, the liver disease generally remains quite stable, despite recurrent cholestatic periods. Only one patient has died of cirrhosis and hepatocellular carcinoma in adulthood [111], (Figure 5).

Patients with LCS1 may require different actions from kindergarten, school or work, especially in connection with cholestatic periods. Patients' abilities to concentrate in these periods may decline because of itching or sleepiness, hence reducing the capacity to learn or work. As lymphoedema is chronic and requires daily follow-up, adjusted physical activity may be considered. Vocational guidance is advised.

As of June 2014 the total number of patients diagnosed with this syndrome worldwide is approximately 100, of whom 48 were diagnosed in Norway. Many of the Norwegian

patients can be traced back to a common ancestor in the western part of Vest-Agder county in Norway. The founder effect likely explains the high prevalence of LCS1 in Norway compared with other countries [9, 112]. The incidence of LCS1 in Norway has been fixed at approximately 5 new cases per decade, and the disease affects both genders equally.

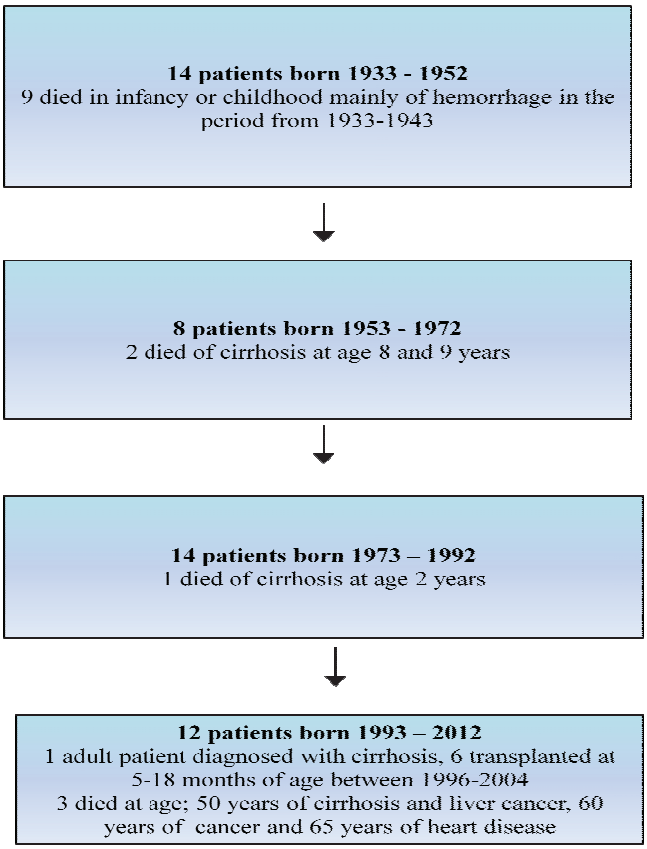


Figure 5. A total of 48 LCS1 patients diagnosed in Norway in 2014 of whom 33 are alive.

To our knowledge, the number of patients (in parenthesis) who have been, diagnosed in other parts of the world are as follows: Denmark (2), Great Britain (6), Sweden (5),

Poland (4), Czech Republic (1), Bulgaria (3), Austria (1), Greece (4), Italy (2), Egypt (2), Iraq (3), the US (15) (Minnesota (11) and Chicago (4)), and Australia (1) [110]. Some of these patients have died.

Several articles have been published, but knowledge of the adult patients as a group is scarce due to the small number of patients and the short time period which adequate treatment has been available to permit a normal lifespan. The oldest patient in Norway currently is 70 years old, and is the longest-surviving member of this patient group. The published articles on LCS1 in Norway are largely those written by Aagenaes (et al.) [10, 111, 113-116] or researchers from other regions of the world [117-123].

4.1 Cholestasis and lymphoedema in LCS1

Cholestasis

Cholestasis is recognised shortly after birth and persists until 1 year or even 6 - 7 years or more with varying severity in different patients [9, 113]. The histological picture of liver biopsies in infants is giant cell-transformation with intracytoplasmic pigment retention, and a slight increase in connective tissue in the periportal tracts and around the central veins [113]. In the adult cases, intracanalicular cholestasis with few multinucleated or giant cells has been identified [9] with variable degrees of increased connective tissue [113]. Liver histology findings do not demonstrate progression to fibrosis, and signs of portal hypertension are absent [9]. Laboratory examinations during cholestatic periods demonstrated hyperbilirubinaemia (primarily conjugated bilirubin), increased transaminases and ALP, hyperlipaemia, increased pre-β (VLDL) and β-lipoproteins (LDL), a slight decrease in albumin, and an increase in α_2-globulin [9]. Examinations of serum lipids during the cholestatic periods have demonstrated hyperlipaemia with elevated triglycerides, cholesterol and phospholipids [9]. In cholestatic periods, ALP is always increased [9]. Serum copper increases during cholestatic periods [9]. During cholestatic periods, all patients exhibit hepatomegaly, abnormal liver function, and severe malabsorption.

In infancy, jaundice is normally observed within the first 2 - 4 weeks after birth [111]. The serum bilirubin concentration is markedly increased in the first months, with a decrease into the normal range at age 3 - 4 years. At high levels of bile acids (> 40 µmol/L), itching starts and often persists as a problem from approximately 6 months of age. The increased bile acids in infancy decrease slowly until the age of about 6 years when they remain moderately elevated [111]. The liver enzymes ALT and AST increase, but only moderately increased at 2 - 3 years of age. Moderate elevations of γ-GT, approximately twice the upper normal level, are observed in this syndrome, and the use of Fenemal (phenobarbital) increases this enzyme substantially in LCS1 patients [111].
Typically cholestasis eases during the first 1 - 2 years of life but may reoccur over irregular intervals. A long cholestatic period in the first 2 - 3 years of life can cause liver failure leading to death or the need for liver transplantation [111]. Most patients have a benign course of liver disease in adulthood [110], although some patients may suffer slowly progressive cirrhosis throughout adulthood [111].

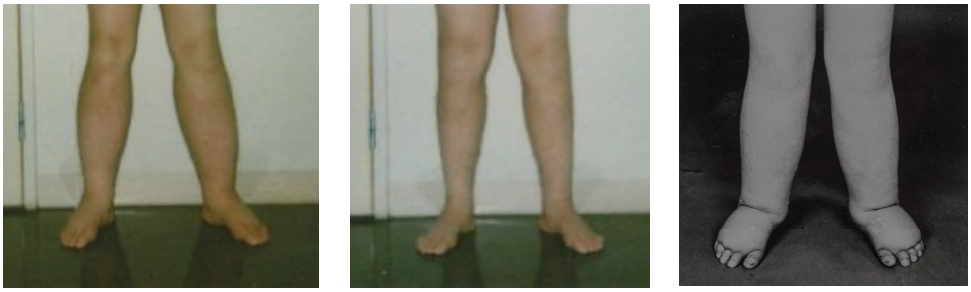
The mean birth weight in patients with LCS1 is normal [9, 111]; the infants exhibit a normal appetite, but are characterised by having large amounts of pale, fatty stools and dark urine [9]. When cholestasis is diagnosed dietary treatment should begin with a high-energy and low-fat diet and supplemented with MCT-fat, fat-soluble vitamins, vitamins in general, and medication.
Bleeding tendencies, rickets, anaemia, and growth retardation have been ascribed to malabsorption caused by the cholestasis. The bleeding tendency observed in the first patients described, was likely due to malabsorption rather than the liver disease per se [9]. No deaths from haemorrhage have occurred since vitamin K substitution was regularly instituted [9].

The severity and length of recurrent periods vary among individuals, but most last for 2 - 6 months (range 2 weeks to approximately 1 year) [111]. One or more cholestatic periods are usually observed in puberty and often in pregnancy [111]. Cholestasis may therefore be triggered by hormonal changes [124, 125]. Infections, trauma or operations may also be followed by a cholestatic period [111]. During cholestatic

periods in adolescence and adulthood patients may need medical and nutritional care, resembling what they received as children and what is often needed for cholestasis. The need for medical and nutritional follow-up in these periods depends upon the severity of the cholestasis and its duration. Some of the patients miss up to 6 months to a year of school due to cholestatic periods. Three men have experienced no cholestatic periods after reaching adulthood [111].

Lymphoedema

Lymphangiography has demonstrated hypoplasia of the lymphatics, collateral filling with dermal backflow, and retarded emptying of contrast from the lymphatics [114]. Approximately half of patients develop lymphoedema before 6 - 7 years of age, and the other half do so before puberty [111].



Before CDT treatment | After CDT treatment | Before CDT treatment was available

Figure 6. Lymphoedema in an adult patient with LCS1 before and after 2 weeks of CDT treatment and in a 4-year-old patient before CDT treatment was available. (Reprinted with permission from K Vik and O Aagenaes).

The lymphoedema primarily affects the lower extremities, but the hands, arms, periorbital soft tissue, thorax, and small intestine can also be affected [110, 111]. Lymphoedema can have quite severe social implications, especially for women with LCS1, and recurrent erysipelas has been a problem for many of the patients [111].

Lymphoedema was the reason for obtaining disability pension in four of the nine adults evaluated in 1998 [111].

4.2 Genetics in LCS1

LCS1 exhibits an autosomal recessive inheritance pattern, and the genetic cause is assumed to have originated in the long arm of chromosome 15q [112, 126]. The exact genetic mutation in LCS1 remains unknown [110, 127]. There is a considerable interfamilial and intrafamilial variation within LCS1, as is often observed in rare diseases [93, 126, 128, 129].

Some have suggested a biological connection between lymphatic and hepatic function in lymphoedema cholestasis syndromes (LCS) [111, 114, 127]. The hypothesis is that mutations causing LCSs act primarily upon the lymphatic system, and in some patients the liver is especially vulnerable to deleterious consequences of deranged lymphatic function, thus resulting in cholestasis. The lymphoedema and cholestasis manifested in LCSs are therefore expected by some to share a single underlying aetiology; however given that the CCBE1 mutation has been found in only one LCS patient to date, a mutation in another, unidentified gene cannot be excluded as the underlying cause of the cholestasis [110, 127].

4.3 Diagnosing LCS1

Neonatal cholestasis has many different causes [22]. If the child does not have any relatives with LCS1 or ancestors from south-western Norway, it can be difficult to reach a correct diagnosis before the lymphoedema develops. Regardless, to establish the diagnosis of LCS1, cholestasis must be present in the first year of life, and lymphoedema must be identified at that time or later. Ancestors from south-western Norway, relatives diagnosed with LCS1, blood tests, or biopsies of the liver are not sufficient to make the correct diagnosis.

Lymphoedema is diagnosed clinically. Diagnosing the early signs of lymphoedema is difficult and often causes late initiation of CDT in patients with LCS1. The last patient

diagnosed with LCS1 in Norway was nearly 40 years old before finally receiving the correct diagnosis in 2012; by that time, the patient had developed extensive lymphoedema. Five patients with LCS1 have been mistakenly diagnosed with biliary atresia, but when surgically explored, the biliary tree was normal [111]. The correct diagnosis was then not established until lymphoedema appeared. Today, advanced techniques for exploring the biliary tree have rendered such operations unnecessary. There are several differential diagnoses for isolated cholestasis or lymphoedema as described in sections 2.3 and 3.3, respectively. However, LCS1 is the only known hereditary lymphoedema that is associated with cholestasis [118].

4.3.1 Treatment of LCS1

The same treatments for patients with cholestasis or lymphoedema, due to other causes (as described in section 2.4 and 3.4) can be used in patients with LCS1.

It should be specifically noted that LCS1 patients born after 1970 have clearly superior outcomes to those born between 1945 and 1970. The dietary treatment for LCS1 patients after 1970 was infant formula including MCT (Pregistimil) after weaning until malabsorption was no longer present, supplementation with vitamins A, B, C, D, E and K, and medication with cholestyramine (Questran) (8 - 16 g/day), and phenobarbital (Fenemal) (3 - 5 mg/kg/day) [111]. The improvement observed in the infants and children was primarily due to the introduction of infant formula with MCT and the use and monitoring of fat-soluble vitamin supplements [111]. This nutritional improvement has prevented severe rickets, neuropathy and bleeding and improved growth in children [111]. Itching was kept relatively moderate by the use of cholestyramine and/ or phenobarbital.

Today the physicians and dieticians at OUS, Rikshospitalet recommend the following regimen for children with cholestasis (including patients with LCS1): supplementation with fat-soluble vitamins, including vitamins K_1 (Ka-vit liquid (20 mg/ml) 2 mg p.o.), A and D (Ketovite liquid 5 ml x 2 p.o.); multivitamins (Ketovite tbl, 1 x 3 p.o.); and a vitamin E mixture ((50 mg/ml) 100 mg x 1) [130]. In addition a reduced-fat diet is recommended in which 50% of the fat intake is from MCT; however, the quantity of MCT used depends upon the nutritional status of the infant. If the infants are breastfed the mothers are advised to nurse the baby at each mealtime with the first

milk that contains less fat and thereafter substitute with infant formulas such as Caprilon (75% MCT), Pregestimil (54% MCT) or Nutramigen (55% MCT). Medication may include ursodeoxycholic acid (Ursofalk), rifampicin (Rimactan) and cholestyramine (Questran).

Some of the patients have undergone plastic surgery for elephantiasis due to unsatisfactory treatment of lymphoedema [9, 114]; therefore, lymphoedema must be treated as soon as it is identified. The severity of lymphoedema has improved substantially, especially after 1985, when CDT became available in Norway [111]. As in other lymphoedematous diseases, antibiotics may be used to prevent erysipelas.

5 Diet and health

It is generally accepted that nutrition has major effects on health throughout life [131-133]. The role of dietary factors in the development of chronic disease are particularly evident [134]. Dietary recommendations or dietary reference values (DRV) are therefore provided by health authorities to promote health and prevent lifestyle-related diseases, and reduce the risk factors for such diseases [135]. The recommendations form the basis of the national nutrition policy, education, and consumer materials for the general public and for specific groups, industry, nutrition educators and health professionals treating specific diseases. Nutritional recommendations must be based on current scientific and medical knowledge and are therefore regularly updated [136].

To assess and evaluate the complex relationships between diet and specific health outcomes accurate and consistent measurements of dietary intake and patterns of eating are crucial [137, 138]. Several measurement methods have been developed to quantify the amount of certain substances eaten and to compare dietary intake with current recommendations [139, 140].

5.1 Dietary assessment challenges

Several challenges are involved in choosing the best method of dietary assessment, depending on the objectives of the study, the type of information required, the available resources and the population of interest [141]. The primary goal of nutritional assessment is to identify nutritional risk factors that influence morbidity or mortality and that may be modifiable.

The data required both in larger epidemiological health surveys and for individuals are potentially subject to many sources of both random and systematic error [142]. The different methods that can be used have both advantages and limitations, and none of the methods are entirely satisfactory [142, 143].

The data also rely on the accuracy of reports of current or habitual food intake by the study participants; physical and psychological characteristics of the study participants therefore play a significant role in acquiring data [144]. One of the most common errors is under-reporting. In instances in which the subjects themselves report their energy intake (EI), the EI is generally under-reported compared with energy expenditure [145]. This discrepancy makes it more difficult to identify individuals or populations who suffer nutritional deficits.

5.2 Dietary assessment methods

Dietary assessment methods allow for a determination of the macronutrient (energy, proteins, fats, carbohydrates) and/ or micronutrient (vitamins, minerals) intake of a given individual or population. Measurements of body composition and energy expenditure add supplemental information to support the nutritional advice given for the individual or population of concern.

Dietary assessment methods used for evaluating individual food consumption can be classified as prospective or retrospective methods. The three most common methods used to assess dietary intake are food frequency questionnaires (FFQ), diet records (weighted or estimated), and 24-hour recall [146]. Diet records and weighed food records are prospective methods, whereas food frequency questionnaires and 24-hour recall are retrospective methods.

The advantages of prospective methods are as follows; they are not affected by recall bias; intake is quantified; they provide information about typical meal and food patterns; and portion size estimates are more accurate than retrospective methods. Disadvantages include a high subject burden and a high drop-out rate, generally no more than a week of data recording is possible. Furthermore monitoring can alter eating behaviours (the manner of eating or eating different type of foods), and multiple data collections are required over several months to capture habitual intake [147].

Retrospective methods are simple and inexpensive, and do not affect food habits. These methods can be used on a large scale and can last up to one year. The disadvantages of retrospective methods are the reliance on the subject's memory and his or her ability to estimate portion sizes, recall food preparation methods, and remember food composition.

6 Aims

The overall aim of this research project was to obtain new evidence-based knowledge about the progression of liver disease in a group of adolescent and adult patients with LCS1 over a period of nine years, to evaluate the need for the improved treatment and follow-up of liver disease in the absence of cholestasis and/or in relation to lymphoedema.

The specific aims were as follows:

- To investigate the prognosis of patients with LCS1 and to assess biochemical liver markers compared with healthy controls (**paper I**).
- To evaluate the diet of patients with LCS1 compared with healthy controls and the general Norwegian population (**paper II**).
- To describe lymphoedema in adult patients with LCS1 (**paper III**).
- To evaluate dietary intake, nutritional status and the progression of liver disease over nine years of follow-up (**paper IV**).

7 Subjects and methods

This thesis was based on observational data acquired from two case-control studies performed cross-sectionally, nine years apart. Patients with LCS1 and their respective controls were evaluated from late autumn (October/ November) of the starting year until early spring (February) of the following year, both in 2000/2001 and 2009/2010. The first study (papers I and II) was performed as part of a Master's Degree. The second study in 2009/2010 (papers III and IV) was planned as a follow-up of the first study to evaluate the progression of liver disease and to explore other aspects of LCS1. To keep the data measurement methods as consistent as possible between the two studies, the same methodology for dietary intake and biochemical evaluation that was used in the first study was also used in the follow-up study.

7.1 Subjects

7.1.1 Subjects 2000/2001

All known patients in Norway who have been diagnosed with LCS1 were invited to participate in the study (two patients were not asked; one was intentionally excluded because of geographical distance, and the second was forgotten due to misunderstandings). Names and addresses for the written invitations were provided by the "Interest group for Aagenaes syndrome" and by O Aagenaes. One informational meeting about the study was held in the summer of 2000 by M Drivdal and O Aagenaes near Lyngdal, close to where most of the patients lived.

Of 23 available patients, sixteen (seven females and nine males) provided written consent. Parents of three participants between 10 and 17 years of age gave consent on behalf of their children to participate in the study; consent was also given by the patients themselves. Patients or parents who declined participation include three children who were under ten years of age and two adolescents who cited personal reasons and the large volume of blood required.

Five healthy sex-, age- and residential area (council)-matched controls for each study subject were randomly drawn from the National Registry of Norway. Each of these control persons were telephoned individually, and the first person who agreed to participate in the study was included as the control person. For one patient, none of the five controls were willing to participate; therefore, one control person from another patient was included.

7.1.2 Subjects 2009/2010

In 2009/2010, all of the patients who had taken part in the first study were over 18 years of age; therefore, only patients above 18 years of age with LCS1 were invited to participate in this study. A total of 20 patients were invited.

Eighteen patients provided written consent, and 17 (eight females and nine males) were finally included. Both the patient who first gave consent as well as the two other patients who did not participate, refused due to geographical distance or personal reasons. To recruit controls, information about the study was displayed on notice boards in hospitals and at the University of Oslo. One control person for each patient, matched by birth year (+/- 1 year) and sex was recruited among the employees of Lovisenberg Deaconess Hospital, Oslo University Hospital, students from the University of Oslo, or friends of students and employees.

7.2 Methods

7.2.1 Case-control design

Due to the heterogeneity of the patient group with respect to age and gender, it was considered important to establish a control group that was similar in some aspects (age, gender) to compare different biochemical and nutritional parameters. Additionally case-control studies are commonly used for initial, inexpensive evaluation of risk factors and are particularly useful for rare conditions [148]. Furthermore, the case-control design was chosen because such studies can

determine if there is an association between the condition (e.g., nutritional status) and the risk factor (e.g., diagnosis of LCS1).

7.2.2 Methods 2000/2001

All of the data were obtained in or near the participants' home living area, and all contact during the study period between the study participants and the project manager (M Drivdal) was conducted through phone and postal mail. All participants received a package that contained information about the proceedings of the study, a scale, a log book for dietary records, information about the blood samples, and a prepaid address sticker for the return of the scale and log book.

Food records

A 4-day weighed record was chosen to estimate the group means of micro- and macronutrient intake and current individual intake of macronutrients and energy. Dietary fat intake was of particular interest due to the cholestasis and recurrent cholestatic periods. A weighed record was chosen because it is considered a more accurate measure of dietary fat intake than a 24 h recall or an FFQ [142]. The single weighed record was also chosen given limited financial resources. The participants recorded their food intake for 4 consecutive days (3 week days and one weekend day). Phillips digital scales with a precision of +/- 1 gram and a maximum capacity of 2500 grams were used. Written information, scales and a logbook were sent to the participants by post. All participants were contacted by phone (by M Drivdal) on the second day of registration to encourage completion of the registration and to answer questions from participants regarding the dietary recording.

All food records were returned by the participants and checked and coded by the same person (M Drivdal) before statistical calculations were performed. The registered intake of food and supplements was computed using the food database AKF96, which is based on the 1995 Norwegian food composition table, and the food calculating system BEREGN [149]. The contributions of vitamins A, D and E from medical supplements (Ketovite, Kanavit, Multibionta) were not included in the database; these were therefore calculated manually and added to the registered

intake before the statistical analysis. As no food database existed for vitamin K at the time of the study, the intake of this vitamin could not be evaluated from the diet registration. In the AKF96 food database energy from fibre was not included.

A telephone interview about food habits and general food consumption was also conducted. This interview also included questions about other topics, such as medications and smoking.

The basal metabolic rate (BMR) was calculated for each participant based on equations for weight, height, sex and age [150]. Body weight (to the nearest kilogram) and height (to the nearest centimetre) were measured at home by the participants themselves and registered in the food logbook. To evaluate whether energy intake met energy requirements, the Goldberg cut-off was used to define under- or over-reporting [151, 152]. Cut-off value 1 evaluated whether the reported energy intake was representative of the usual intake, and cut-off value 2 evaluated whether the reported intake was a probable measure of the food consumed during the actual dietary registration of concern.

Biochemical analyses

Fasting venous blood samples from the participants were drawn at the nearest local hospital laboratory located in Stavanger, Flekkefjord, Kristiansand or Oslo. The laboratory personnel at different sites were provided written and oral instructions about how to collect and store the samples. This information was prepared by a biochemist at OUS, Rikshospitalet and made available by M Drivdal. Haematologic indices were determined at these hospitals using standard methods due to the limited stability of the samples. The other samples were stored at –20°C for a maximum of 10 weeks before transportation on dry ice and were subsequently stored at –70°C for four months before analysis at OUS, Rikshospitalet. Standard methods and equipment were used for general clinical biochemistry work (Hitachi 917; Roche, Basel, Switzerland; AutoDELFIA; Perkin Elmer, Boston, Mass., USA; Axsym; Abbott, Chicago, Ill., USA; and BN II; Dade Behring, Liederbach, Germany). Specific fatty acids in serum total lipids were analysed by gas chromatography using SP-2340 capillary columns (Supelco Inc., Bellefonte, PA, USA) using a Hewlett-Packard

5880A gas chromatograph (Avondale, PA, USA) [153]. Fat-soluble vitamins were protected from light and analysed at Vitas AS with an EDTA-reversed phase HPLC from electrochemical reduction followed by fluorometric detection with HP 1100 liquid chromatography (Agilent Technologies, Palo Alta, CA, USA).

7.2.3 Methods 2009/2010

The follow-up study was performed at the Centre for Rare Disorders in cooperation with the Resource Centre for Oral Health in rare medical conditions (TAKO-Centre) at the Lovisenberg Deaconess Hospital and in cooperation with the Department of Vascular Investigations, OUS, Aker. Except for the dietary records, all data acquired were obtained in Oslo at the Lovisenberg Deaconess Hospital and OUS, Aker. For patients who lived too far from Oslo for a day visit, overnight accommodations in Lovisenberg Deaconess Hospital´s guesthouse were provided.

Additional evaluations not included in the 2000/2001 study were included in the follow-up study to evaluate the patients lymphoedema, oral health status, bone health and quality of life. The methods of assessing oral health status, bone health, and quality of life are not described in further detail here given that these data are not included in the thesis.

Food records

All patients received a scale and instructions for the dietary records by postal mail, and they were asked to complete this portion of the study before they met at Lovisenberg Deaconess Hospital for blood samples and other evaluations. The controls received the scales and the information about the food registration directly from M Drivdal before the other evaluations were performed. The dietary data were acquired and handled as described in section 7.2.2 with the exception of a few differences, which are described below.

Energy and nutrient intake data from the food records were computed using KBS, a software system developed at the University of Oslo, Norway. Energy and nutrient values of the food items were based on the official Norwegian food composition database published in 2006 [154]. For the evaluation of dietary intake in the

2009/2010 study, the energy from fibre was included in the calculation of total consumed energy intake. This energy from fibre adds an 8 kJ higher energy level per gram of fibre, and was therefore corrected for in the dataset from 2000/2001 before the statistical analyses from baseline to follow-up were performed (in paper IV). The Goldberg cut-off was not used in this study because of the small sample size, and the lack of information about individual activity levels [155].

No interview about general food habits was performed, as in 2000/2001, instead each participant had a consultation with M Drivdal during which the dietary records were checked to eliminate possible misunderstandings regarding food registration and to include other relevant data that seemed to be missing e.g., information about dietary supplements, beverages, smoking or medication.

Biochemical analyses

All blood samples were drawn at the Lovisenberg Deaconess Hospital. Haematologic indices were determined at the hospital using standard methods due to the limited stability of the samples. Serum 25(OH)D levels were measured by radioimmunoassay (DiaSorin, Stillwater, MN, USA) at the Hormone Laboratory, OUS, Aker. The other samples were stored at –70°C for a maximum of 26 weeks before analysis at OUS, Rikshospitalet. Standard methods and equipment were used for general clinical and biochemical studies (Modular analytics SWA; Roche, Basel, Switzerland; AutoDELFIA; Perkin Elmer, Boston, Mass., USA; Axsym; Abbott, Chicago, Ill., USA; and BN II; Dade Behring, Liederbach, Germany).

Evaluation of the lymphoedema

An experienced physician at Department of Vascular Investigations, OUS, Aker, obtained data about lymphoedema in the LCS1 patients. A clinical history was obtained, including symptoms such as heaviness, asymmetric swelling and reductions in limb function caused by swelling, episodes of exudation and erysipelas, and the use of compression garments. These data were supplemented by information from a self-administered questionnaire with response categories that was designed to obtain information about patients' experiences and their subjective

thoughts on living with lymphoedema. The questionnaire was designed for the LCS1 patients in this study by M Drivdal and approved by O Aagenaes and CE Slagsvold (Appendix 1).

The patients were asked to stop compression therapy (compression garment, CDT and pulsator use) four days prior to the clinical examination. The clinical examination included inspection, palpation, the estimation of dystrophic changes, and the evaluation of Stemmer's sign (inability to pinch a skin fold at the base of the second toe or finger).

In addition, all patients underwent air plethysmography and colour Duplex ultrasound scanning, because lymphoedema may cause changes in the subcutis, that manifest as subcutaneous hypoechoic layers visible on ultrasound. These hypoechoic layers or subcutaneous tissue changes, which are related to the distribution and extent of lymphoedema, were recorded and incorporated into the individual patient lymphoedema classification (paper III). The examinations for subcutaneous tissue oedema were performed using B-mode ultrasonography (General Electric Logiq E9 colour duplex scanner with a 7 MHz linear array probe).

Bio-Impedance Analyses

A direct segmental multi-frequency bioimpedance analysis (DSM-BIA) was included to describe the extent of lymphoedema more thoroughly in each patient [156, 157]. The BIA measurements of participants were obtained after overnight fasting and were performed with patients in an upright position wearing light clothing. In BIA, an electric current is conducted along the path of least resistance in the body. This path represents the tissue with the highest water content. Most of the low-frequency current passes through extracellular water (ECW), including lymph. At higher frequencies, the current passes through both extra- and intracellular fluids. These data allow the evaluation of the oedema content of different body segments among LCS1 patients. The relevant body composition measurements for oedema evaluation include total body water (TBW) and ECW for the entire body as well as for five separate segments (four limbs and the trunk). The InBody 720 manufacturer defines an ECW:TBW ratio of > 0.40 as the presence of oedema. An oedema index > 0.40

was defined as lymphoedema in this work. The DSM-BIA measurements were performed using an InBody 720 Body Composition Analyzer, Biospace Co. Ltd, Seoul, Korea.

7.2.4 Statistics

Descriptive statistics are presented as median values (due to the vulnerability to extreme values in small samples), standard deviations, quartiles and ranges. Non-parametric tests (the Wilcoxon sign rank sum test for related samples and the Mann-Whitney U test for independent samples) were used to evaluate differences in dietary, biochemical and impedance measurements between patients and controls. Fisher's exact test was used to evaluate categorical data. All of the reported p-values are based on two-sided tests with a significance level of 5%. The statistical analyses were performed using the Statistical Package for Social Sciences (SPSS, version 18.0, SPSS, Inc., Chicago, USA). Confidence intervals were calculated using the Wilcoxon signed rank sum test for significant data (Minitab Statistical Software, Release 15 for Windows, State College, Pennsylvania, USA).

8 Summary of papers

8.1 Paper I

Prognosis, with evaluation of general biochemistry, of liver disease in lymphoedema cholestasis syndrome 1 (LCS1/Aagenaes syndrome)

Introduction: In this paper, a clinical description of liver disease in Norwegian patients with LCS1 is presented.

Results: Cirrhosis occurred in four patients born before 1990, and three subjects died in infancy or early childhood. One subject exhibited slow progression of cirrhosis and died in adulthood. Slowly progressing cirrhosis occurred in one additional patient who is currently alive. Two other patients were considered to be at moderate risk of developing cirrhosis. Three patients had undergone liver transplant due to cirrhosis in childhood.

The biochemical values for aspartate amino transferase (AST), (P=0.055), alkaline phosphatase (ALP), (P=0.001), γ-glutamyl transferase (γ-GT), (P=0.002), and bile acids (P=0.045) were significantly elevated in the patient group compared with healthy controls, despite being near normal reference ranges, whereas total protein (P=0.008) and serum albumin levels (P=0.005) were significantly lower than in healthy controls.

Conclusions: Compared with other types of hereditary neonatal cholestasis, patients with LCS1 exhibit a relatively good prognosis. More than 50% of patients with LCS1 can expect a normal life span.

8.2 Paper II

Do patients with lymphoedema cholestasis syndrome 1/Aagenaes syndrome need dietary counselling outside cholestatic episodes?

Introduction: In this paper, the need for dietary counselling outside cholestatic periods in fifteen patients with LCS1 was assessed. The patients' diets were compared with healthy matched controls and the general Norwegian dietary recommendations.

Results: The patients (n=15) exhibited diets similar to the diets of the healthy controls, except for a significantly lower intake of energy from total fat (*P*=0.040) and saturated fat (*P*=0.020). The patients met most of the dietary recommendations for macronutrients, except for saturated fat, monounsaturated fat, refined sugar and fibre. Supplements were needed to meet the micronutrient recommendations. The patient group exhibited a significantly lower serum level of α-tocopherol (*P*=0.010) compared with the control group, and the serum 25-OH D level was below reference ranges.

Conclusions: Patients with LCS1 could benefit from dietary counselling regarding fat quality, carbohydrate intake (including fibre), and individual needs for vitamins D and E. To ensure normal serum 25-OH D and α-tocopherol levels, regular examinations to determine the need for supplementation with vitamins D and E are recommended.

8.3 Paper III

Hereditary lymphedema: characteristics and variations in 17 adult patients with Lymphedema Cholestasis Syndrome 1 (LCS1)/Aagenaes syndrome

Introduction: In this study, lymphoedema in 17 out of 20 Norwegian adult patients with LCS1 was examined. Individual clinical variations in the group are described.

Results: The lymphoedema in patients with LCS1 is similar to other hereditary lymphoedemas. However, fluid retention in the lower extremities is more pronounced, and lymphoedema is generally more extensive, as lymphoedema findings in the arms, face and trunk may be observed. Limited tissue fibrosis was observed, despite longstanding lymphoedema.

Conclusion: Approximately one-third of patients with LCS1 exhibit severe extremity lymphoedema (grades III and IV), and their condition requires close follow-up. More frequent use of compression in the upper extremities is advised.

8.4 Paper IV

A nine-year follow-up of diet and liver disease in patients with LCS1/Aagenaes syndrome

Introduction: The aim of this follow-up study was to evaluate the progression of liver disease and nutritional status in patients with LCS1 over a period of nine years.

Results: Dietary and biochemical data demonstrate few differences from 2000/2001 until 2009/2010 compared to dietary recommendations and normal reference ranges for biochemical markers. Compared with the controls, the patients exhibited significantly higher levels of alkaline phosphatase (P=0.015 and P=0.002), γ-glutamyltransferase (P=0.001 and P<0.001), bile acids (P=0.037 and P=0.016), and fibrinogen (P= 0.046 and P<0.001) and lower albumin (P=0.033 and P<0.001) and α-tocopherol levels (P=0.011 and P=0.003) at baseline and follow-up, respectively. Our findings suggest the presence of low-grade hepatobiliary dysfunction in LCS1 patients despite stable liver disease.

Conclusion: The present study demonstrates that patients with LCS1 have a diet that is similar to that of healthy controls with no clinical worsening of liver disease over a nine-year period.

9 Discussion

9.1 Methodological considerations

9.1.1 Study design

The study design included a combination of different methods or aspects of methods within the observational category (case-control, cross-sectional, cohort). Compared with randomised control trials, observational studies may not establish a cause-and-effect relationship because the two groups may differ in ways other than the variable under study. This phenomenon is caused by larger confounding biases in observational studies; therefore, these studies often serve as descriptions of events rather than explanations for events [148] as can also be seen in this thesis.

Other weaknesses include the limited power of the statistical calculations, which is a consequence of the small sample sizes. Control subjects were included in both studies as a reference group for nutritional and biochemical parameters. However, a limitation is that the control group was not similar in the two studies. A fundamental step in the design of clinical research is the computation of power and sample size [158]. Power calculations are performed to ensure that the sample size drawn from a population is sufficiently large to reflect the true value in the source population for a given probability, e.g., 95%. Due to the rarity of LCS1, power calculations were not performed. All patients with LCS1 in Norway were invited to participate in the first study. Various numbers of patients were evaluated as a group in the different papers; however, in each paper, at least 50% of currently living patients with LCS1 were included. An even higher proportion of subjects with LCS1 were included when only the age groups of interest are considered. This fact is believed to increase the strength of our conclusions despite the small sample size.

The aims (presented in section 6) were primarily evaluated using a case-control study design. In addition, the study design can be viewed as cross-sectional given that data were obtained only once in both studies, as well as longitudinal (cohort) given that the two studies were performed nine years apart. Generally, case-control studies provide relatively weak empirical evidence, even when properly executed,

and case-control studies are less reliable than cohort studies [148]. We believe that combining the two case-control studies (performed nine years apart in a cohort fashion) strengthened the ability of the study to identify valid associations (paper IV). The inferences made are also strengthened by the fact that both case-control studies included a representative sample of Norwegian LCS1 patients.

9.1.2 Validity

Valid, precise and generalisable estimates of results can be obtained using the best suited study design as well as the best possible study conduct and data analysis [148]. If correctly used, these techniques would reduce bias or errors and lead to increased objectivity and accuracy in the estimates. The accuracy and precision of scientific work is typically challenged by limited resources (e.g., financial resources, staff, time, available study subjects) as was also observed in this project. Regardless, errors in estimation are traditionally classified as either systematic or random. Studies with few systematic errors are referred to as valid and exhibit a low grade of bias, whereas little random error will produce more precise estimates [148]. The validity of a study is often separated into internal validity and external validity/generalisability. Violations of internal validity can be classified into confounding, selection bias and information bias. Internal validity is considered a prerequisite for external validity [148].

9.1.3 Internal validity

Confounding

Confounding is a considerable issue in observational studies, and the use of a case-control design can give rise to several possible confounding variables. To reduce the confounding effects of gender and age, the subjects were matched according to these variables. In the first study, subjects were also matched by residential area, in particular to reduce the confounding effects of different local dietary habits and the availability of foods. Matching for residential area was not performed in the follow-up study due to a lack of financial resources and inconvenience, as all of the data were obtained in Oslo. The healthy controls in the follow-up study are likely not

representative of the general population, and these controls exhibited higher educational levels than the patients. Despite this fact, the results obtained from the control groups in the two studies were quite similar, strengthening their use as acceptable control groups to some extent.

The patients in the follow-up study completed a weighed diet registration nine years ago. The patients may have had an increased understanding of and interest in the information derived from their dietary information than the controls and therefore may have completed the dietary records more thoroughly. Several patients reported low alcohol intake due to their knowledge of the adverse effects of alcohol consumption on the liver. This low intake may confound the liver biomarkers relative to healthy controls.

Selection bias

Selection biases result from the procedures used for selecting subjects to participate in the study as well as from factors that influence study participation. The biases occur due to differences between those who participate and those who decline to participate or are considered to be ineligible for the study. The observed associations will therefore be a mixture of forces that determine disease occurrence and other findings [148].

Selection bias is of high concern in case-control studies and plays a major role in reducing validity. This project included a high percentage of Norwegian LCS1 patients and is probably representative of the majority of the LCS1 patients in Norway. Regardless, both self-selection into the study and the exclusion of patients for the prognostic evaluation and follow-up are potential sources of selection bias. In this respect, O Aagenaes's knowledge of the individual patients has been valuable for comparing non-participants against patients who were included in the studies. No patients appeared to be distinctly different from those included in the two studies with the exception of one patient with cirrhosis who was omitted in these evaluations [110]. Another weakness is that the project included a small sample size due to the rarity of the condition, making the estimates more vulnerable to extreme

observations. Patients who were not included could potentially skew the associations observed in the presented publications.

The control group in the first study was randomly selected from the Norwegian population and matched to the patients with respect to age, sex, and residential area. The first of the five possible controls who consented to participate may have been more interested in health or differed in other ways from the general Norwegian population (with whom the patients were intended to be compared). After all, the matching of the control group of randomly selected persons from the general Norwegian population reduces bias. The control group in the follow-up study was more subject to selection bias, probably further weakening the results or comparisons between patients and controls in this study (compared with the first study). Furthermore, the inclusion of friends and colleagues may have influenced the usefulness of the follow-up study's control group. Biases introduced with the control groups may have been particularly high in this project, and high levels of self-selection bias, especially in the follow-up study, could threaten the validity of the conclusions.

None of the patients or the controls were excluded in the data sampling process, but self-exclusion by patients who were not interested in participation or self-inclusion by control persons are forms of selection bias. Longitudinal studies are susceptible to bias by differential loss to follow-up and the lack of control over risk assignment, leading to confounder symmetry. Two of the patients were lost to follow-up in 2009/2010.

Information bias

Inaccurate measurement of study variables or errors in the information obtained can occur to some extent in any study. In the papers presented here, the dietary evaluations are especially prone to information bias. In dietary surveys, under-reporting is well known and includes both under-recording and under-eating [142]. The latter is due to less dietary intake than usual and could cause weight loss if it were persistent over a longer period. Under-recording leads to a discrepancy between energy intake and energy expenditure during the weight recording period.

The self-registration of weight and height by the participants in the first study could lead to differential misclassification of the BMR, the Goldberg cut-off or the BMI. In addition, individual weights were probably not correctly or equally adjusted for lymphoedema in either of the two studies and may have worsened the differential misclassification of these variables. This measurement error may also have arisen because some subjects underwent CDT close to the data sampling period, whereas others did not received such therapy. A better classification of these variables could have provided more representative energy intake data for the patient group than the data presented in the papers, possibly allowing the intake of vitamins and minerals in the patient group to reach recommendations (papers II and IV). The higher BMI observed in patients compared with controls was probably caused by excess weight related to lymphoedema.

The nutrient intake evaluations for the patients group compared with healthy controls were based on the measured median intake from one single 4-day weighed record. A single 4-day registration cannot capture the habitual intake and may produce information bias. To determine the proportion of individuals at risk of inadequate intake of a particular nutrient, multiple records must be collected (e.g., 3 - 4 days four times a year) [159]. In particular, dietary evaluations require a longer collection period to draw valid conclusions.

Because of the prospective design of the study, recall bias is less of a problem in this study. Other information biases may be due to differing equipment used for the biochemical analyses, especially in the comparisons from patient baseline to patient follow-up (paper IV).

9.1.4 External validity/ generalisability

When selecting a subgroup from a target population, it is usually important to ensure that the subjects selected are as representative as possible to allow assumptions to be made regarding the source population and populations outside Norway. Due to the high percentage of LCS1 patients included, inferences of the results may be generalised beyond those subjects who were studied. The study results should only be generalised to populations that closely resemble those we studied and should most likely not be generalised to patients with non-specific forms of LCS. It is

unknown whether the factors that distinguish other groups from the studied group can modify the effect in question. Because of the heterogeneity of individuals with LCS1 as well as the possible heterogeneity of LCS1 patients outside Norway, one should be cautious in generalising the findings presented here. In the first study (paper I), it was important for the findings to be as generalisable as possible for patients in Norway; therefore, the patient with cirrhosis was omitted from the evalutions.

9.2 Discussion of the main results

A problem arises due to a lack of other comparable publications, as this is the first time the majority of LCS1 patients have been evaluated outside cholestatic periods. The similar biochemical and dietary findings in the two studies, which were performed nine years apart, strengthen the reliability of the conclusions.

9.2.1 Diet

The nutritional findings in the group of LCS1 patients who had recovered from the prolonged period of childhood cholestasis were similar to those of healthy control persons and general Norwegian nutritional recommendations (papers II and IV). The final height was normal, and the growth retardation that had been observed in previous years due to cholestasis was not present in our study subjects. Normal adult height in these patients has also been reported by Aagenaes [111].

To provide further details, the patients reported a lower dietary fat intake and exhibited lower serum α-tocopherol levels than did healthy controls, both in 2000/2001 and 2009/2010. The serum 25-OH D level was below normal reference ranges as also observed in the general population [160, 161]. The possible lower fat intake referred to in (papers II and IV) in this thesis may be due to habits and preferences that were acquired during cholestatic periods, especially at a young age. Patients may have experienced discomfort when eating greasy foods, possibly due to underlying cholestasis. The patients met most of the dietary recommendations for macronutrients, except for saturated fat, monounsaturated fat, refined sugar and

fibre. Supplements were needed to meet the micronutrient recommendations. Dietary intake of nutrients that is below the recommended levels has also been reported for the general Norwegian population [162].

However, the findings may be subject to measurement errors, e.g., under-reporting or under-eating as indicated by the low BMR:energy intake ratios. If the reported energy intake had been higher, recommendations could have been reached. Additionally, a tendency to ingest less fish and vegetables was observed in patients compared with the healthy controls and the general Norwegian population [162, 163]. In addition, the general Norwegian population exhibits fibre and fish consumption levels that are below recommendations [162]. These studies therefore suggest that patients with LCS1 may derive benefit from dietary counselling with respect to fat quality and carbohydrate (including fibre) intake, similar to the recommendations provided by health authorities for the general population.

9.2.2 Liver disease

Clinical signs of progression of liver disease were not observed during the nine-year follow-up period although some of the biochemical markers were significantly different than those of the healthy controls. Additionally, some of the biochemical markers in LCS1 patients were outside the normal reference ranges. The differences observed in biochemical markers between patients and controls may have been caused by a slow ongoing progression of liver disease. The significantly lower serum α-tocopherol in LCS1 patients supports the possible presence of subclinical cholestasis [164, 165]. Both the lower fat intake and the recurrent cholestatic periods within the patient group are probably too few to have caused the lower α-tocopherol levels observed, and our sample size was likely too small to detect such a relationship. The numbers of cholestatic periods are also subject to recall bias by the patients in addition to their difficulty in recalling subtle itching periods as cholestasis. This bias makes it difficult to draw any firm conclusions relating the presence of cholestatic periods and the lower plasma α-tocopherol levels. Furthermore, hypoplasia of lymph vessels along the gut in LCS1 patients might exist and could potentially be a contributing factor to the lower α-tocopherol levels observed [166].

All patients included in these two studies who survived the first prolonged period of cholestasis, the biochemical findings in the baseline and follow-up studies were similar and reflect stable liver disease. The stability of the liver disease after puberty are also seen in the cirrhotic patient who was excluded from the group evaluation as well as in the two patients (aged 21 and 23 years) described in paper I in whom clinical-biochemistry indices had been persistently abnormal over the preceding few years, indicating the possible development of cirrhosis. Only minor changes in the biochemical markers were observed during the nine-year follow-up period in these three patients. The γ-GT levels and ALP levels declined in all three patients during the nine-year follow-up period. The ALT levels increased slightly in two of the patients and are likely only a reflection of common variability. Additionally, the different analytic equipment used for biochemical marker testing may have caused some of the differences observed in the LCS1 patients between baseline and follow-up. The findings of similar dietary intake and nutritional status in the LCS1 patients compared with healthy controls are reasonable when viewed in the light of the benign character of the liver disease observed in this patient group.

The recommendations that adult patients with LCS1 should regularly monitor serum levels of vitamins D and E to ensure that levels of these vitamins are within reference ranges is based on findings from both 2000/2001 and 2009/2010. The Directorate of Health in Norway has recommended that the majority of the population should have 25-OH D levels above 50 nmol/l to ensure adequate vitamin D status [167]. Given the lower α-tocopherol levels in LCS1 patients as well as the age-related decline that was not observed in healthy controls (paper I), attention should also be paid to α-tocopherol levels [168, 169]. In addition, given the findings of possible low-grade chronic inflammation (papers I and IV), avoiding low α-tocopherol values may be beneficial for LCS1 patients [170-172].

9.2.3 Lymphoedema

An important aim of this work was also to describe lymphoedema in adult patients with LCS1 (paper III). Approximately one-third of the patients exhibited severe lymphoedema in the lower limbs (grades III and IV) that required close follow-up. The lymphoedema was similar to that observed in other hereditary lymphoedemas;

however, in LCS1, the lymphoedema was generally more extensive and often included lymphoedema in the arms, face and trunk. It is difficult to compare the lymphoedema grade in the LCS1 patients with other lymphoedematous diseases because of the worldwide lack of a uniform method of diagnosing lymphoedema [100]. The results are therefore heavily dependent upon the physicians who perform such evaluations and their experience with lymphoedema diseases. In this study, an experienced physician with knowledge of this field collaborated in acquiring these data, and the patient-related knowledge gained by O Aagenaes over several years also contributed to this evaluation. Limited tissue fibrosis was observed, despite longstanding lymphoedema, most likely due to patient awareness of the importance of lymphoedema treatment as well as close follow-up by physiotherapists trained in CDT. The importance of lymphoedema treatment and follow-up are well documented [173-175]. Some patients were advised to use compression garments on the upper extremities in addition to the compression applied to the lower extremities. Although LCS1 patients often exhibit extensive lymphoedema, good care may reduce some of the extra burden that it causes [176, 177]. A few patients also had albumin levels below normal reference ranges, but oedema formation resulting from these levels do not seem likely due to the fact that albumin concentrations typically must be below 20 g/l before oedema forms. Moreover, the combination of liver disease and lymphoedema might make the LCS1 patients more vulnerable to adverse effects caused by low albumin levels.

9.2.4 Prognosis

More than 50% of the LCS1 patients can expect a fairly normal lifespan (paper I), and the evaluation of the same patients over a nine-year period suggests that currently, patients may exhibit an even better prognosis (paper IV). The average age of the Norwegian LCS1 patients in this study project at follow-up was 31 years. The oldest patient in the Norwegian LCS1 cohort is presently 70 years old. It is therefore difficult to project the true lifetime prognosis because too few patients have yet had the possibility reaching old age. All of the liver transplant patients have survived to date, but these patients are still very young, and it is still too early to provide any estimates of their prognosis.

Some prognostic calculations have been performed here to provide a comparison to other neonatal cholestatic disorders. If the LCS1 patients who died of haemorrhage are either omitted 1) (48 – 9 = 39, n = 39) or included, 2) (n = 48) in the prognostic evaluation (see below) due to the routine use of vitamin K in newborn patients, the estimates would be as follows:

1) Nine children out of 39 patients have died of cirrhosis (3 patients) or been liver transplanted (6 patients) producing a probable shortened life expectancy of 9/39 = 0.23 i.e., 23%). Additionally, it is likely that 2 adults died due to liver disease before reaching old (> 70 years) age (11/ 39 = 0.28 i.e. 28%). Summarised this yields a survival rate of about 72%.

2) When the nine patients who died of haemorrhage before the 1940ies are included, and when three of these are expected to have died due to cirrhosis before old age (with a rate of cirrhosis before old age assumed to be 11/ 39 = 0.28; the estimate become 14/ 48 = 0.29 i.e. 29%).

The estimates given in 1) and 2) provide a lifetime prognosis of approximately 70% given that their liver diseases are stable and no one dies before the age of 70. At present, we do not know whether patients without proven cirrhosis (who were included in this study) have a longer life expectancy than was estimated for the entire group (paper I). However, this estimate seems probable; hence, the conclusion provided in paper IV.

The overall prognosis of LCS1 is highly variable and is largely dependent on the progression of cholestasis as also observed in other genetic syndromes associated with severe neonatal cholestasis. In α-1-antitrypsin deficiency, a relatively good prognosis has been reported, and only approximately 8 - 11% of patients will develop clinically significant liver disease during their lifetime [178]. These data suggest that α-1-antitrypsin deficiency patients have a better outcome than LCS1 patients.

In Alagille syndrome, the prognosis varies among the various published reports, but overall survival has been reported to average 70%; up to 50% of patients require liver transplantation [179, 180]. Other clinical features in addition to liver disease complicate the prognosis of these patients [180], and comparisons with LCS1 should

be made with caution. Emerick et al reported a 20-year predicted life expectancy of 75% for all patients, 80% for those not requiring liver transplantation, and 60% for those who required liver transplantation [181]. In Alagille syndrome 33% of patients with neonatal cholestasis are reported to require liver transplant [179]. In LCS1, three out of 36 patients died of cirrhosis before liver transplantation was available, and additionally 6 out of then 39 has been transplanted indicating that approximately 8% - 23% of patients need liver transplantation. This rate is lower than the rates that have been reported for Alagille syndrome. Moreover, infants with Alagille syndrome who present with cholestasis have a 50% probability of long-term survival without liver transplantation [182]. In LCS1, all patients present with neonatal cholestasis, but most seem to have long-term survival without liver transplantation (30/ 39 = 77%). Three patients were diagnosed with LCS1 after undergoing transplantation. Given these data, LCS1 patients seem to have a better prognosis than patients with Alagille syndrome.

Patients with NAICC and most PFIC patients, including Greenland familial cholestasis, are candidates for liver transplantation, and have poorer outcomes than patients with the other types of neonatal cholestasis mentioned above. Some of these patients develop rapid liver failure in the first six months of life, some develop severe cirrhosis in childhood with death before 10 years of age, and a few develop slowly progressive cirrhosis with death in adulthood [27, 183-186].

Concluding remarks

The reliability of our dietary and biochemical findings is strengthened by the similarities observed at the baseline and during follow-up. Our findings are also strengthened when compared to the findings by Aagenaes et al. in 1968 and 1998 (paper IV).

The validity of the major dietary findings (fat and fibre) as well as the findings of the subclinical, slowly progressive liver disease (based on our evaluations of biochemical liver markers) appears to be acceptable. The external validity of these findings outside the Norwegian cohort or in spontaneous cases may be problematic due to modifier genes and environmental influences [187] but may be considered good for Norwegian patients without proven cirrhosis.

The theoretical implications of the results of this thesis include the following: increasing awareness of the possibility of subclinical cholestasis in these patients, increasing patient awareness of diet; ensuring careful lymphoedema care and ensuring serum vitamin D and E monitoring. An information sheet provided by SSD about LCS1 could include these findings.

10 Conclusions

This work indicates that greater than 50% of LCS1 patients without proven cirrhosis probably can expect a normal life span (paper I), and the prognosis may be even better in patients who survive the first prolonged period of cholestasis in childhood (paper IV). The liver disease appears to be clinically stable despite significant different biochemical liver markers compared to healthy controls both in baseline and follow-up. The dietary intake and nutritional status in this patient group is similar to healthy controls and the general Norwegian population (papers II and IV). Furthermore, in the LCS1 patient group, only minor deviations from the Norwegian dietary recommendations are observed (papers II and IV). The lymphoedema that develops in one-third of LCS1 patients is serious and requires close follow-up, but the lymphoedema is generally well managed with compression and CDT (paper III).

Overall, the care and follow-up of the LCS1 patients in this group seems satisfactory, but patients may benefit from following the Norwegian dietary guidelines, with a specific emphasis on carbohydrate and fat quality, in addition to regular monitoring of serum vitamin D and E levels.

The findings presented in the thesis may be applicable to adult LCS1 patients worldwide.

11 Future perspectives

Continuing efforts are needed to reveal the exact genetic location of the LCS1 mutation and the underlying reason for the cholestasis that is observed in LCS1. Whether the cholestasis and lymphoedema are caused by the same aetiology is beyond the scope of this thesis and requires further exploration. Identifying the location of the gene could also be important to establish the carrier frequency in the population of the most affected areas in southwestern Norway.

Other future goals may also include investigating the possibility of providing more specific dietary interventions as well as performing other therapeutic interventions to reduce the number of erysipelas episodes or the fibrosis observed in lymphoedema.

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List of errata

In section 9.2.4 at page 54, line 8 it is written ”Additionally, it is likely that 2 adults died due to liver disease before reaching old (> 70 years) age 11/39 = 0.28 i.e. 28%. Summarised this yields a survival rate of about 72%.”

The correct sentence is ”Additionally, one adult patient died due to liver disease before reaching old (> 70 years) age 10/39 = 0.26 i.e. 26%. Summarised this yields a survival rate of about 74%.”

Appendices

Appendix 1: Lymphoedema questionnaire

Appendix 1

"Oppfølgingsundersøkelse av personer med Aagenæs syndrom"
Senter for sjeldne diagnoser, Oslo universitetssykehus, Rikshospitalet
Monica Drivdal, Tlf: 95 97 56 16 E-post: uxildr@rikshospitalet.no

Lymfødemanamnese

Navn (blokkbokstaver)_____

Kryss av i den boksen, og noter ned det som stemmer mest for deg. Ved noen spørsmål er det nødvendig og/eller mulig å krysse av flere.

1. Har du lymfødem? Ja □ Nei □ Av og til □
2. Hvor mange dager siden er det du brukte bandasjer/kompresjonsstrømper/ behandling for lymfødemet?
   4 dager □ 5-10 dager □ Mer enn 10 dager □ Annet _____
3. Opplever du ditt lymfødem som plagsomt? Ja □ Nei □
4. Når ble lymfødemet påvist hos deg? Noter din alder _____ eller årstall _____
5. Når startet du behandling? Noter din alder _____ eller årstall _____
6. Bruker du å bandasjere beina dine? Ja alltid □ Nei □ Av og til □
7. Bruker du elastiske kompresjonsstrømper? Ja alltid □ Nei □ Av og til □
8. Rekker kompresjonstrømper/ bandasje opp til: Kneet □ Lysken □ Bukse □
9. Sover du med beina hevet? Ja alltid □ Nei □ Av og til □
10. Bruker du pulsator? Ja regelmessig □ Nei □ Av og til □
11. Får du lymfedrenasje hos fysioterapeut? Ja □ Hvor ofte _____ Nei □
12. Har du hatt behov for intensiv lymfedrenasje behandling? Ja □ Hvor ofte ____
    Nei □
13. Hva bestod lymfødembehandlingen av i puberteten? Bandasjering □
    Kompresjonsstrømper □ Pulsator □ Lymfedrenasje □ Ikke noe □
14. Har du noen form for fysisk aktivitet utover vanlige dagligdagse gjøremål, eller går du i skogen/ terrenget/ på ujevnt underlag? Ja □ Nei □
15. Opplever du at du er mindre mobil/ bevegelig pga lymfødemet? Ja □ Nei □

16. Opplever du sideforskjeller, som f.eks det ene beinet har mer lymfødem enn det andre? Ja □ Nei □

17. Vet du at du har □, eller opplever du □ å ha lymfødem andre steder på kroppen som hender □, armer □, ansikt □, mage □, brysthule □ ?

18. Opplever du tyngdefølelse □, (spreng)smerte □ eller andre tilsvarende symptomer fra ditt lymfødem? Ja □ Nei □
Hvis ja, Daglig □ Ukentlig □ Av og til □ Sjeldent □ Aldri □

19. Har du hatt infeksjon (rosen/ erysipelas) i huden? Ja □ Nei □
Hvis ja, hvor mange ganger? _____

20. Hvor ofte smører du huden din på lymfødemutsatte områder med salve/ lotion/ krem?
Daglig □ Ukentlig □ Sjeldent □ Aldri □

21. Har du ortopedisk tilpasset skotøy? Ja □ Nei □

22. Hva slags behandling har du fått for hevelsen du har i beina dine? Kryss av det som passer:
    a. □ Ingen
    b. □ Lymfødebehandling
    c. □ Bandasjering
    d. □ Jeg bruker kompresjonsstrømper
    e. □ Pulsator
    f. □ Spesielle øvelser