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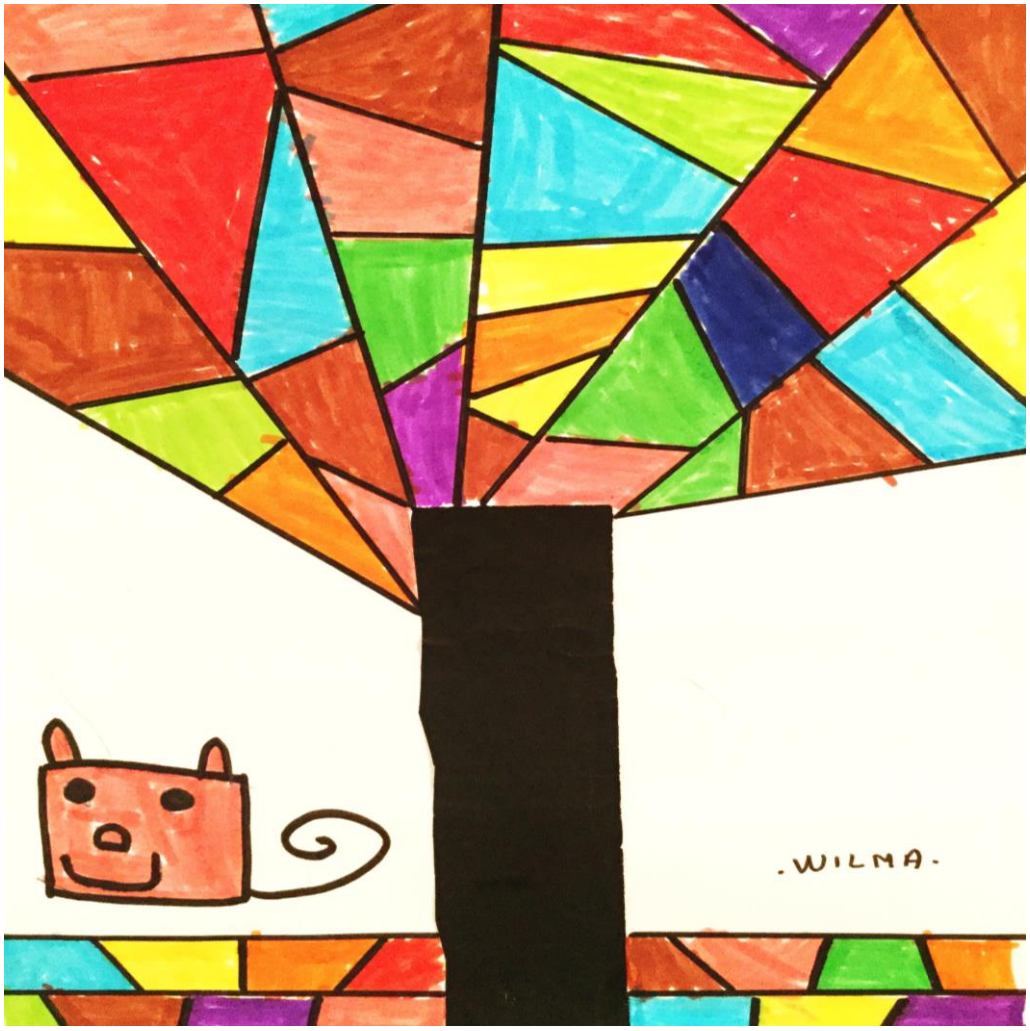

Metabolic changes in acute lymphoblastic leukemia

Ph.D.-thesis by Pernille Rudebeck Mogensen, MHSc

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Original manuscripts

This thesis is based on the following original manuscripts:

Paper 1 Mogensen PR, Grell K, Schmiegelow K, Overgaard UO, Wolthers BO, Mogensen SS, Vaag A, and Frandsen TL. **Dyslipidemia in children diagnosed with acute lymphoblastic leukemia**.

Paper 2 Mogensen PR, Wolthers BO, Grell K, Schmiegelow K, and Frandsen TL. **Association between body mass index and pancreatitis in children with acute lymphoblastic leukemia.** *Pediatr Blood Cancer*. 2018;e27071. https://doi. org/10.1002/pbc.27071

Paper 3 Mogensen PR, Grell K, Vaag A, Schmiegelow K, Kampmann P Overgaard UO, Albertsen BK, Holm M, Brøns C, Grunnet LG, Holm J-C, Juul A, and Frandsen TL. **Insulin resistance and dysmetabolic trajectories in children, adolescents and young adults during 12 months acute lymphoblastic leukaemia therapy.**

Paper 4 Wolthers BO, Mogensen PR, Frandsen TL, et al. **Insulin-dependent diabetes: A chronic complication to acute pancreatitis in childhood acute lymphoblastic leukemia**. *Pediatr. Blood Cancer. 2019;66:e27437.* https://doi.org/10. 1002/pbc.27437

Preface

This thesis was accomplished in collaboration between The Diabetes and Bone-metabolic Research Unit (formerly Department of Diabetes and metabolism) and the Paediatric Oncology Research Laboratory at Copenhagen University Hospital, Rigshospitalet, Denmark. During my fellowship I stayed four weeks with The Children's Obesity Clinic at Copenhagen University Hospital Holbæk.

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Thank you to my *family* and *family in law* for always helping out with daily logistics. Most of all a very special thanks to *Jakob* for being patient with me and making space for my dreams in our dreams, keeping my feet on the ground and still living the dream, and to *Wilma* for making every day so special.

I love you ❤

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Abbrevations

ALL	Acute lymphoblastic leukemia
AYA	Adolescent and young adults
BMI	Body mass index
DI	Disposition index
GC	Glucocorticoid
HbA1c	Glycated haemoglobin
HDL	High density lipoprotein
HOMA-IR	Homeostatic assessment model insulin resistance
HOMA-IS	Homeostatic assessment model insulin secretion
HPA	Hypothalamic-pituitary-adrenal
HR	High risk
IR	Intermediate risk
LDL	Low density lipoprotein
MRD	Minimal residual disease
NOPHO	Nordic Society of Paediatric Haematology and Oncology
SR	Standard risk
TC	Total cholesterol
TG	Triglyceride
VLDL	Very low density lipoprotein
WBC	White blood cell count

Objective

Survival rates of acute lymphoblastic leukemia (ALL) in children/adolescents, and young adults has reached more than 90% and 70%, respectively, through advances and intensification of therapy[1,2]. However, the burden of long-term adverse treatment effects has increased, and today more than 50% of ALL patients experiencing sequelae after treatment[3]. Among the most deleterious and frequent long-term complications are cardiovascular disease and diabetes affecting long-term co-morbidity and mortality[4–9].

The mechanisms accountable for the cardio-metabolic long-term complications in ALL survivors are still unclear, but are likely to be associated to the intensive therapy with high dose glucocorticoids (GCs) exposure[4,10,11] and asparaginase therapy[12,13], and to some extent facilitated through increased insulin resistance[9] and obesity. Changes in body composition, prolonged physical inactivity, unhealthy eating habits and/or genetic predisposition may also contribute to the long term cardio-metabolic complications[4,11].

Since the therapy in the current treatment protocol (NOPHO ALL2008) with GCs and asparaginase has become more intense, we hypothesized that metabolic changes including reversible or irreversible weight gain, dyslipidemia and insulin resistance appearing before diagnosis of ALL or during early ALL therapy are predictive for risk of later cardio-metabolic disease. Furthermore, we hypothesize that combined therapy with asparaginase and GCs may act synergistically and together aggravate metabolic dysfunction compared to GC therapy alone. Accordingly, the overall aim of this thesis was to assess metabolic changes before and during early ALL therapy.

Introduction

Acute lymphoblastic leukemia

Acute lymphoblastic leukemia (ALL) is the most common cancer in children (1−17.9 years of age)[2]. In Denmark 35 children are diagnosed with ALL annually, with the highest incidence among young children 2 to 5 years of age[14]. ALL occurs as an accumulation of progenitor cells (lymphoblasts) in the bone marrow which prevents formation of erythrocytes, leukocytes and thrombocytes[15]. Patients present with symptoms such as anaemia, fatigue, and an increased tendency to bleeding (or bruising) and infections. About 85−90% of ALL patients are diagnosed with pre-B-cell leukemia, 10-15% with T-cell leukemia, and few patients have bilineage- or mature B-cell leukemia[14]. Immunophenotype and cytogenetics are associated with prognosis and thus included as therapy stratifying factors.

Etiology

Besides genetic predisposition, including Downs syndrome and Li-Fraumeni syndrome, risk factors for childhood ALL remains largely unknown[16]. The high incidence observed in very young children points to a potential prenatal disposition. High birth weight has been associated with risk of childhood ALL[17,18] and a diabetic intrauterine environment both in maternal diabetes and gestational diabetes was recently implicated to promote the risk of childhood ALL in offspring[19]. Furthermore, obesity has been indicated as a part of the ALL etiology in adults, but it remains to be further elucidated in children as well in adults[20–22].

Therapy

Therapy of childhood ALL is a success within oncology. Collaboration between international childhood oncology consortia has through research and testing of anti-neoplastic drugs achieved 90% survival rates. Treatment consists of a combination of anti-neoplastic drugs. Patients are stratified into risk groups (varying in anti-neoplastic treatment intensity) based on cytogenetics and therapy response monitored by minimal residual disease (MRD). The induction therapy aims to obtain clinical remission with a three or four-drug regimen. Induction is followed by consolidation and delayed intensification phases which aims to further reduce the burden of disease. The prolonged maintenance phase aims to prevent relapse. In total ALL therapy lasts between two and three years. Patients with a poor treatment response receive allogenic hemopoietic stem-cell transplantation (HSCT).

Recently, adult ALL treatment has been inspired from pediatric ALL protocols. This has led to a significant increase in adult survival rates through the last decades now reaching more than 70%[1,23,24]. The lower survival rates in adults are partly reflected by a different biology in adults that seems less chemo-sensitive to the pediatric therapy[1,23].

Since July 2008 until October 2018 a standardized treatment protocol (NOPHO ALL-2008) was used in the Nordic countries, Estonia and Lithuania[25] for patients (1−45 years of age) diagnosed with ALL. In brief, patients were stratified into high risk (HR) or non-HR treatment groups at time of diagnosis based on white blood cell count (WBC) and immune-phenotype. Following induction (day 29) and consolidation (day 79) patients were stratified into three risk groups; standard risk (SR), intermediate risk (IR), and HR based on cytogenetics as well as treatment response measured through levels of minimal residual disease (MRD). Induction therapy consists of a systemic three-drug regimen including vincristine and doxorubicin, and either prednisolone or dexamethasone for non-HR and HR, respectively. Additionally, intrathecal methotrexate or triple therapy (methrotrexat, cytabine, GCs) was administered depending on CNS involvement (triple for CNS 2/3). Pegylated asparaginase, 6-mercaptopurine and high-dose methotrexate therapy were given from early consolidation phase to patients stratified into SR and IR patients to further

reduce the burden of disease. During delayed intensification and early maintenance 7-day pulses of dexamethasone and pegylated asparaginase were administered in combination with vincristine, methotrexate and 6-mercaptopurine. Late maintenance therapy consisted of methotrexate and 6-mercaptopurine. Patients stratified into HR at day 29 or 79 received high intensity 'block'-therapy or hematopoietic stem cell transplantation. The duration of therapy for all risk group arms was 2.5 years[26,27].

Glucocorticoids

Glucocorticoids (GCs) plays a major role in ALL therapy due to the anti-leukemic (inducing apoptosis of the lymphoblasts), immunosuppressive and anti-inflammatory effects[28,29]. Two types of GCs are used in the ALL therapy; dexamethasone and prednisolone. Dexamethasone has a superior anti-leukemic effect compared to prednisolone and event-free survival and risk of CNS-relapse are improved using dexamethasone[29]. Furthermore, dexamethasone has an improved ability to penetrate the blood-brain-barrier and thereby prevent or kill leukemic cells in CNS.[29] However, adverse effects such as osteonecrosis and steroid psychosis are more often reported from the use of dexamethasone compared to prednisolone[29].

GCs are known to suppress the hypothalamic-pituitary-adrenal (HPA) axis and cause secondary adrenal insufficiency and impaired stress response in nearly all patients undergoing ALL-therapy, often lasting several month after cessation of therapy[30]. Supra-physiological or long-term use of GCs cause acute and persistent adverse metabolic effects including central and peripheral fat accumulation, hypertension, dyslipidemia, insulin resistance, impaired glucose tolerance, hyperglycemia and diabetes[5,10,31–33]. Multiple mechanisms are contributing including a down regulation of the proteins that transport glucose into the cells, increased hepatic gluconeogenesis and inhibition the insulin secretion[31]. Moreover, the mechanisms for weight gain is still poorly understood; however, decreased energy expenditure (in mice) and increased energy intake in children with ALL has been documented[34,35].

GCs influences bone homeostasis which causes osteoporosis and occasionally, osteonecrosis which have been reported in patients undergoing ALL-therapy[36]. Furthermore, a neurocognitive/psychological impact is often observed in patients receiving long-term GC therapy[29].

Asparaginase

Asparaginase hydrolyses the amino acid asparagine into aspartate and ammonia and thus depletes the body of asparagine. Since lymphoblasts have an impaired ability for resynthesizing asparagine, their protein syntheses is compromised and they become target for apoptosis. Most non-malignant cells are able to resynthesize sufficient asparagine for protein synthesis; however, organs producing many proteins are affected by this drug. Accordingly, asparaginase is associated with high risk of pancreatitis and venous

occlusive disease (VOD) of the liver. Furthermore, asparaginase has been shown to induce a decreased insulin secretion in pancreatic β-cells contributing to hyperglycemia during therapy[4,12]. Hyperlipidemia are also induced by asparaginase-therapy[4,13].

Thromboembolism

The risk for symptomatic thromboembolisms is about 5% in ALL children and increasing with age. Our group recently demonstrated a 2.5 year cumulative incidence of thromboembolism in children (1-9.9 years of age), adolescents (10-17.9 years of age) and young adults (18.0- 45.9 years of age) of 3.73% (95% CI, 15.5% and 18.1%), respectively[37]. Including asymptomatic thrombosis the incidence rises to 37−73% in children with ALL[38]. Thromboembolism often coincides with asparaginase and corticosteroid therapy.

Osteonecrosis

Osteonecrosis is a severe adverse event to GC therapy with an incidence between 1.6% and 17.6% in patients treated for ALL. A recent study reported an increased incidence with age and showed a cumulative incidence of 15−20% in patients 10 to 45 years of age[39]. The pathogenesis is not fully understood; however, hyperlipidemia has been suggested as an important risk factor[40]. The clinical symptoms depend on severity and ranges from being asymptomatic to severe with pain from bone and joints as well as impaired joint mobility. Osteonecrosis may lead to joint replacements and persistent pain limiting activity of daily living and life quality significantly throughout the patient's life[39].

Pancreatitis

Asparaginase-associated pancreatitis (AAP) is reported in 2−18% of children treated for ALL and a cumulative incidence of 6.8% has been found in the Nordic countries[41]. The clinical symptoms of AAP are severe upper abdominal pain, nausea and vomiting. The inflammation of pancreas is often accompanied by a systemic inflammatory response syndrome (SIRS) can be mistaken for septicaemia. Acute complications include; hypotension, pseudocysts, need of insulin and potentially death[42]. Persisting complications has been reported such as abdominal pain and pancreatic dysfunction leading to maldigestion and/or diabetes[41]. Besides older age, obesity has been suggested as a predictor of AAP [41,43].

Metabolic dysfunction in ALL

Cardio-metabolic dysfunction including, obesity, dyslipidemia, and (pre-) diabetes is an emergent challenge in survivors of ALL[3,5,6,8,44–49]. The mechanisms responsible for these dysfunctions in ALL survivors are still unclear but are likely related to a multifaceted interaction between ALL disease, exposure to GCs and asparaginase therapy combined with environmental and life style factors (unhealthy diet and physical inactivity). Genetic predisposition and epigenetic changes may also contribute to these adverse long-term cardio-metabolic sequelae in survivors of ALL.

Obesity has become an epidemic in both children and adults during the last decades[50]. Patients with ALL are at increased risk of obesity and the prevalence increases 5- to 10-fold during ALL therapy. Those who do not become obese still obtain substantial weight gain during and after ALL therapy[10,46,51–53]. Furthermore, obesity has been suggested as an important prognostic factor in patients with ALL and have been associated to risk of pancreatitis, end of induction residual disease as well as risk of relapse and event-free survival[43,54–56]. However, the impact of obesity on anti-leukemic therapy remains controversial[55,57,58].

Hypertriglyceridemia and hypercholesterolemia has been observed in up to 72% of children undergoing ALL-treatment[59]. Obesity is known to cause hypertriglyceridemia and hypercholesterolemia; however, patients with ALL are also highly affected by GCs and asparaginase therapy[60].

The risk of corticosteroid-induced hyperglycemia is increased by asparaginase therapy[4,61]. The prevalence of hyperglycemia during ALL therapy has been reported to be 10% to 20% during ALL therapy, most frequently in children above 10 years of age, with resolution after cessation or tapering down of GC and asparaginase[4,9,62–64]. The mechanisms are poorly understood; however , insulin resistance is the most likely cause of hyperglycaemia in children treated for ALL[65]. Drug-induced diabetes in patients undergoing ALL therapy may be a marker for metabolic disease later in life and has also been associated with reduced event-free survival[9,54].

Obesity

Overweight and obesity are defined as 'abnormal or excessive fat accumulation that represents a risk to health'[66]. Overweight and obesity can be defined by body mass index (BMI) which is the body weight in kilograms (kg) divided by the squared height in meters (m^2). The World health organization (WHO) cut-off criteria of being overweight or obese for adults are 25 kg/m^2 and 30 kg/m^2, respectively. In children, adolescents and young adults (until middle age) the BMI changes with sex and age[67] due to changes in growth and body composition. In these populations a BMI standard deviation SD- or z-score can be calculated which defines how much an individual differs from a sex and age matched reference group.

Obesity is a complex disease and the causes reach beyond discrepancy between energy intake and energy expenditure. Besides diet and level of physical activity, neuroendocrine adaptation and regulation play an important role in maintaining the energy homeostasis. It has been suggested that the origins of obesity seen in ALL survivors, actually occurs from early 'obesogenic' behaviors and events which might 'dys-programme' the long-term regulation of energy balance[50]. GC-therapy is known to disturb the lipid metabolism and lead to both peripheral and central fat accumulation. Besides metabolic co-morbidity,

including hypertension, dyslipidemia, fatty liver, insulin resistance[62,68,69] and type 2 diabetes[9]; obesity affects musculoskeletal function and activities of daily living as well as psychological aspects and health[50].

Dyslipidemia

Dyslipidemia is an abnormal amount and/or distribution of lipids in the blood. Most dyslipidemia are hyperlipidemia, but in this thesis it will refer to inexpedient disturbance in one or more of the lipid levels and also represent hypo-lipidemia. Lipoproteins include low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and high-density lipoprotein (HDL). Total cholesterol is often used in risk assessment, and is the sum of LDL, HDL and 0.45 times triglycerides (TG) (if measured in mmol/L)[70]. VLDL and LDL are derived from the liver whereas HDL is derived from the catabolism of TG. TG is primarily derived from dietary fat and elevated VLDL occurs with hypertriglyceridemia. Hypertriglyceridemia and hypercholesterolemia changes blood viscosity and induce atherosclerosis, vascular dysfunction including hypertension in children and adolescents and early cardiovascular events in adults[71].

(Pre-) diabetes

The World Health organisation (WHO) defines diabetes and pre-diabetes as (Figure 1)[72] :

Pre-diabetes

- Impaired fasting glucose (IFG): Fasting plasma glucose between 6.1-6.9 mmol/L
- Impaired glucose tolerance (IGT): fasting plasma glucose< 7.00 mmol/L and 2 hour plasma glucose 7.8-11.0 mmol/L after an oral glucose tolerance test (OGTT).
- Glycated hemaglobin (HbA1c) level of 38-48 mmol/mol*

Diabetes

- Fasting plasma glucose≥ 7.0 mmol/L
- 2 hour plasma glucose ≥ 11.1 mmol/L after (OGTT)

*Defined by the American Diabetes Association[73]

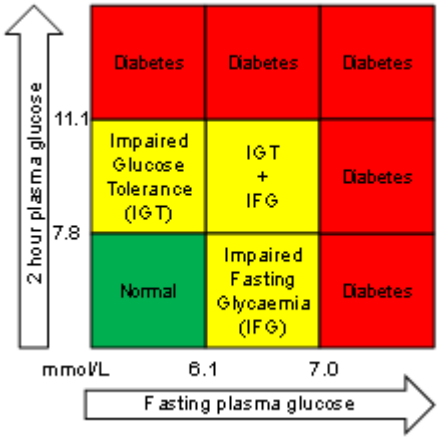


Figure 1. Cut-offs for diabetes and pre-diabetes (impaired glucose tolerance and impaired fasting glycemia) according to WHO guidelines. Figure kindly loaned and made by Anne Cathrine Baun Thuesen.

Type 2 diabetes (T2D) is characterized by hepatic and peripheral insulin resistance and insufficient insulin secretion from the β-cells in the pancreas[69,74]. Additional, the pathophysiology of T2D also involves defects in several other organs including hyperglucagonemia in the pancreatic α-cells, accelerated lipolysis in the adipose tissue, incretin deficiency/resistance in the gastrointestinal tract, increased glucose re-absorption in the kidneys, and insulin resistance in the brain. Type 2 diabetics may have a reduced, normal or even increased release of insulin, combined with reduced insulin sensitivity in the insulin-dependent tissues. Impaired insulin sensitivity primarily affects cellular glucose uptake and metabolism, resulting in IFG[69]. Insulin resistance develops when the cells respond less effectively to the normal amount of insulin needed to transport glucose from the blood [69,75]. In pre-diabetic stages insulin resistance will trigger increased insulin secretion to compensate for an increased plasma glucose level, with hyperinsulinemia and normal glucose levels as a result. Increased insulin levels may lead to decreased insulin sensitivity, and eventually β-cells will be "exhausted" or damaged and not be able to compensate with concomitant impaired insulin secretion[69,76] .

A hyperbolic relationship between insulin sensitivity and insulin secretion[77] has been shown. The hyperbolic curve is shown in Figure 2. This relationship implies that the product of the β-cell function and insulin sensitivity is almost constant. The constant is called the disposition index (DI) and describes the ability of the β cells to compensate for an underlying insulin resistance[78].

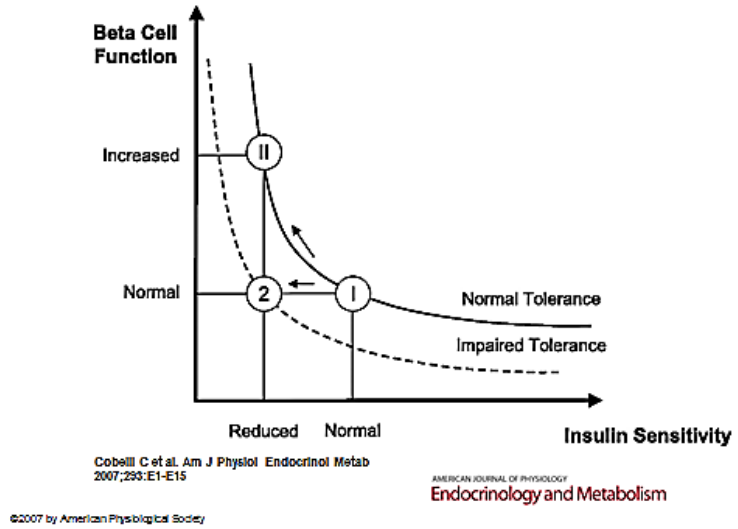


Figure 2. The hyperbolic curve illustrates the β-cell function expressed in relation to the insulin sensitivity (Disposal Index). A person with normal glucose tolerance will respond to decreased insulin sensitivity by increasing the β-cell response (stage II), whereas a person with reduced glucose tolerance is not able to adequately compensate (stage 2). In stage II, the β-cell response is increased, but the dispositions index is normal where in stage 2 is a normal β-cell response, but the disposition index is reduced[78]. The use of figure in this thesis is kindly permitted The American Physiological Society.

Persons who are able to compensate for reduced insulin sensitivity by increasing insulin secretion will be on the normal tolerance curve, whereas persons with a reduced β-cell function who are unable to compensate for decreased sensitivity will be glucose intolerant (IGT)[78].

Insulin resistance

Insulin resistance (IR) in the liver leads to IFG whereas IR in the skeletal muscles results in IGT. Up to 75% of insulin-stimulated glucose uptake occurs in muscles is thus the main site for insulin resistance in type 2 diabetics[69,79]. The defects in the cellular mechanisms of insulin-resistant muscles are impaired insulin signalling, decreased insulin-stimulated glycogen synthesis[80], decreased glucose oxidation[81] and mitochondrial dysfunction[82,83], all leading to hyperglycemia.

Adipose tissue has a minor proportion of glucose uptake, but when insulin resistance occurs in adipocytes, the anti-lipolytic effect of insulin is reduced, resulting in increased cleavage of stored triglycerides into free fatty acid (FFA)[84]. Increased FFA and triglycerides in the blood stream have been shown in several studies to cause insulin resistance by decreasing whole body glucose uptake, glycogen synthesis and glucose oxidation in the skeletal muscle[84,85].

The liver can partly compensate for insulin resistance in the peripheral tissue by decreasing the gluconeogenesis[69]. Fasting plasma glucose concentration correlates directly with hepatic glucose production[86]. The normal inhibition of hepatic glucose production after a meal are decreased by hepatic insulin resistance resulting in elevated postprandial plasma values[75].

Overall, the elevated levels of FFA in plasma, the reduced glucose uptake in the skeletal muscle, and the increased hepatic glucose production together will contribute to hyperglycemia. If compensatory mechanisms resulting in increased insulin secretion are insufficient, as mentioned above, T2D will develop.

Study aims

The aim of this Ph.D.-thesis was to investigate the changes in lipid and glucose metabolism in children, adolescents and to some extend young adults prior to and during therapy of acute lymphoblastic leukemia.

Paper 1

The aim of this retrospective study was to explore pre-therapy lipid profiles in children diagnosed with ALL. And investigate associations between pre-therapy lipid profiles and pre-therapy BMI, early therapy response, on-therapy hyperlipidemia and/or the known steroid- and asparaginase-associated toxicities thrombosis, osteonecrosis and pancreatitis as well as event-free cure rates (EFS).

Paper 2

The aim of this register study was to investigate and potentially reproduce the association between BMI at ALL diagnosis and risk of asparaginase-associated pancreatitis shown in a previous study as well as to improve the understanding of risk factors for pancreatitis in childhood ALL.

Paper 3

The aim of this prospective study was to systematically assess the insulin resistance and insulin secretion as well as metabolic biomarkers during early therapy with glucocorticoids and asparaginase in children, adolescents and young adults (CAYAs) with ALL.

Paper 4

The aim of this descriptive study combined with a survey was to investigate whether diabetes remained a persisting complication to asparaginase-induced pancreatitis in childhood ALL.

Patients and methods

The four studies in this thesis were based on three overlapping cohorts of patients with ALL. All patients were diagnosed with Philadelphia chromosome negative B-cell precursor or T-lineage ALL from July 2008 and enrolled in the Nordic Society of Pediatric Haematology Oncology 2008 (NOPHO ALL2008) protocol. Patients with Down syndrome were excluded from the study cohorts. Study I, II and IV included only children (1−17.9 years of age) and study III included also young adults (18− 45 years of age).

The NOPHO ALL registry

The NOPHO registry is a common database for the seven Nordic and Baltic countries participating in the NOPHO protocol. This database contains information on all protocol patients including demographics, disease characteristics, treatment response as well as toxicities, relapse, and death.

Toxicities

The toxicities thromboembolism, osteonecrosis and pancreatitis were identified and registered in the NOPHO database based on the international Ponte di Legno (PdL) criteria[87]. Venous and/or arterial thromboembolism was registered if confirmed by imaging (ultrasound, CT, or MRI) or by autopsy[87]. Osteonecrosis was indicated by pain in at least one joint and/or limited activities of daily living and confirmed by MRI [87]. Pancreatitis was registered if at least two of the following three features were fulfilled: abdominal pain suggestive of pancreatitis; serum lipase or amylase three or more times above upper normal limit (UNL); and characteristic image findings suggestive of pancreatitis (ultrasound, CT, or MRI)[87].

BMI z-scores

Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared and converted to z-scores according to Danish references based on the LMS method[67]. BMI z-scores at diagnosis were divided in three BMI groups defined as: lean body weight <90th percentile, overweight 90th–99th percentile, and obesity ≥99th percentile[67].

Dyslipidemia

Dyslipidemia was defined as levels above or below the normal range in one or more of the lipids including TC, LDL, HDL, VLDL and/or triglycerides. Hypertriglyceridemia and hypercholesterolemia, were defined and graded according to the PdL consensus criteria[87] based on triglycerides/cholesterol blood concentrations:

- Mild hypertriglyceridemia/hypercholesterolemia 1–10×UNL
- Moderate hypertriglyceridemia/ hypercholesterolemia 10–20×UNL
- Severe hypertriglyceridemia/hypercholesterolemia above 20×UNL

Moreover, we defined the lipid levels as normal if within LNL and UNL and decreased if below LNL.

Insulin resistance, β-cell function and disposition index

Insulin resistance, β-cell function (insulin secretion) and disposition index (DI) were based on fasting plasma glucose and insulin. The insulin resistance was calculated from the homeostasis model assessment (HOMA) of insulin resistance (HOMA-IR) and β-cell function (HOMA-IS) and were calculated as HOMA-IR=[(fasting plasma insulin x 0.144 x fasting plasma glucose/22.5)] and HOMA-IS=[(fasting insulin x 0.144 x20)/ (fasting glucose -3.5)][88]. The relationship between the insulin sensitivity and β-cell function was obtained by calculating the DI. An approximately hyperbolic association between the two measures exists, so that the product is constant for individuals with the same degree of glucose tolerance. DI was calculated as DI=[HOMA-IS x (1/HOMA-IR)][77,89].

Statistics

In general, continuous variables between two groups were compared by Wilcoxon rank sum test (also called Mann-Whitney U-test) and Kruskal-Wallis test were used to compare more than two groups. Spearman's correlation coefficient was used to estimate correlations between continuous variables. Fishers exact test was used to compare categorical variables between groups.

Cumulative incidences were estimated by the Aalen-Johansen estimator taking competing events into account, and the estimates were compared with Gray's test. Time to event-free survival (EFS) was calculated from date of diagnosis or end of induction therapy to date of first treatment failure (relapse, secondary malignancy or death) or most recent follow up. The follow up time was estimated using the reverse Kaplan-Meier method. The Kaplan-Meier method was used to estimate EFS, and the estimates were compared by a 2-sided log rank test.

The Cox proportional hazards model was used to calculate simple or adjusted hazard ratios with the significance evaluated by Wald tests and censoring at the time of competing events if any. The proportional hazard assumption was verified for all Cox models.

Longitudinal measurements (several measurements per patient over time) was dealt with by weighing each patient with the number of measurements for smoothed figures of the data and by mixed effects models taking the covariance between measurements from the same patient into account for analyses of development over time and comparison between groups.

In all analyses two-sided P-values <0.05 were considered statistically significant. All analyses were carried out using the statistical software SAS® version 9.4[90] and R® version 3.5.0[91]

Paper 1

Lipid levels at ALL diagnosis were categorised according to the normal limits (lower normal limit [LNL]=2.5 percentile and UNL=97.5 percentile) in sex and age matched healthy references[92]. Dyslipidemia was defined as levels above or below the normal range in one or more of the lipids and hypertriglyceridemia and hypercholesterolemia, were defined and graded according to the Ponte de Legno criteria[87].

On-therapy mean TG and mean TC were estimated with a cubic smoothing spline taken number of repeated measurements into account for each patient. Approximate 95% percentile confidence intervals were estimated by bootstrapping and mean curves were fitted separately for patients with normal/hypertriglyceridemia and normal/hypocholesterolemia (hypercholesterolemia excluded because of too few patients) at ALL diagnosis, respectively.

The 2.5-year cumulative incidences of thromboembolism, osteonecrosis, and pancreatitis were estimated by the Aalen-Johansen estimator considering, secondary malignancy, relapse and death as competing event. The estimates were compared with Gray's test for all age groups, lipid levels and BMI levels. Age-adjusted hazard ratios of thromboembolism, osteonecrosis, and pancreatitis for the lipid levels at diagnosis were calculated by Cox proportional hazards model with the significance evaluated by Wald tests.

Time to event-free survival (EFS) was calculated from date of diagnosis to the date of first treatment failure (secondary malignancy, relapse or death) or last follow up. EFS was estimated by the Kaplan-Meier method, and estimates were compared by a 2-sided log rank test. The follow-up time was estimated using the reverse Kaplan-Meier.

Paper 2

The patients were followed from onset of consolidation therapy with asparaginase therapy (day 30), excluding those who died before day 30 (N=12), until; pancreatitis, death of any cause, relapse, or day 300 from ALL diagnosis, whichever came first. The 300-day cumulative incidences of asparaginase-associated pancreatitis were estimated by the Aalen-Johansen estimator considering death and relapse as competing events and pancreatitis-specific hazard ratios were estimated by the Cox proportional hazard model.

Paper 3

Differences between children and AYAs at ALL diagnosis were assessed by Wilcoxon two sample test and Fischers' exact test for quantitative and categorical variables, respectively.

Effect of treatment on outcomes were assessed in two longitudinal mixed-effect models: 1) The effect of induction therapy was assessed by comparing visit 2 and visit 3 with time of ALL diagnosis (visit 1), and 2) The effect of adding asparaginase therapy was assessed by comparing visit 4, 5 and 6 with end of induction (visit 4). We did this by using an unstructured covariance model for the longitudinal measures. Right-

skewed outcomes were log-transformed and the relative effect sizes (RES) were reported. For non-skewed outcomes the absolute effect size (AES) were reported. When including age group in the model, the interaction between visits and age group were tested and reported if significant. Reported P-values are from Wald tests with the Satterthwaite approximated degrees of freedom. In all analyses two-sided P-values <0.05 were considered statistically significant. All analyses were carried out using the statistical software SAS® version 9.4[90] and R® version 3.5.0[91].

Ethics

All studies were approved by the ethical committee at the Danish capital Region of Denmark (Protocol no. H-2-2010-002) and the Danish Data Protection Authorities (j.nr. 2012-58-0004) and study 2 and 4 were approved by the ethical review boards in all participating countries. Informed consent was obtained from the participants or if below 18 years of age by their parents or guardian in the clinical study.

Study-specific methods, results, and conclusions

Paper 1

In this retrospective study we included all children (1−17.9 years of age) diagnosed with ALL and treated at Copenhagen University Hospital Rigshospitalet from July 2008 to December 2016. Patient characteristics, disease-specific characteristics, early treatment response and information of adverse events were collected from the NOPHO registry and medical charts. Fasting blood samples for lipid analysis were collected at time of diagnosis prior to therapy and on-therapy lipid levels were measured during therapy and were collected from medical charts.

Results

Demographic and disease characteristics for the 127 patients included in the study cohort are presented in Table 1 (not shown due to size, please see the manuscript for paper 1). At time of ALL diagnosis 112 patients had lipid measurements available. Of these, on-therapy TG and TC measurements were available for 82 and 71 patients, respectively.

Apart from one patient, all patients (99%) had dyslipidemia at ALL diagnosis presented as decreased HDL levels (98%), hypertriglyceridemia (58%), hypo-/hypercholesterolemia (14%/5%) and/or decreased/ increased LDL levels (13%/1%) (Figure 1.1). Most patients (66%) had combined dyslipidemia with at least two abnormal lipid levels. Overweight and obesity were identified in 13% and 4%, respectively, of the total study cohort (Please see table 1 in the manuscript for paper 1).

Patients with mild hypertriglyceridemia at ALL diagnosis were significantly younger compared to patients with normal TG levels (P=0.045) and patients with hypocholesterolemia were older than patients with normal TC levels (P=0.0017). Immunophenotype and WBC were not associated with mild hypertriglyceridemia (P>0.99 and P=0.56, respectively) (Please see Table 1 in manuscript 1) Mediastinal mass was present in six patients with mild hypertriglyceridemia vs. one patient without hypertriglyceridemia, all seven patients had T-lineage leukemia (P=0.12 and P=0.24 for patients with T lineage ALL and for all patients, respectively).

Six patients were present with mild hypercholesterolemia at ALL diagnosis. Compared to patients with normal levels of TC, they were characterized by all being males (P=0.035), five/six had T lineage ALL (P=0.00017) and thus, stratified into HR at ALL diagnosis (P=0.0026) and six/six stratified into either IR or HR at end of induction and at day 79 (P=0.024 and P=0.029, respectively). Furthermore, hypercholesterolemia tended to be associated with tumor burden since four/six had mediastinal mass at ALL diagnosis (P=0.061) and 3 of six had WBC count above 50 $\times 10^9$/L (P=0.069). Hypercholesterolemia was not statistically associated to BMI group; however, all six patients were lean at ALL diagnosis. Patients with

hypocholesterolemia at ALL diagnosis did not differ in overall characteristics from patients with normal TC levels.

Figure 1.1. Combination of dyslipidemia at ALL diagnosis.

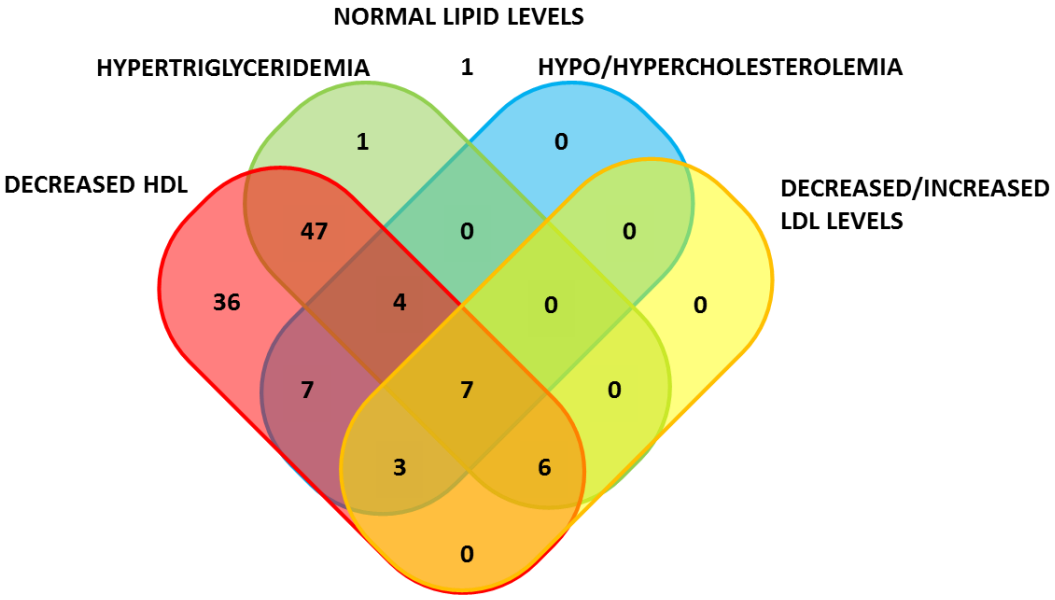


Figure 1.1 shows the combination of dyslipidemia for all the patients at ALL diagnosis. One patient was presented with all lipid levels within the normal range.

Early treatment response

We included MRD measurement from day 29 and day 79. MRD measures were missing for 5 patients at the end of induction (EOI) and 14 additional 14 patients at day 79 (Please see Table 1 in manuscript 1). Three patients died before day 79 and the major part of the missing samples were from patients who stratified into HR due to poor treatment response before end of induction. No association was found between lipid levels or BMI at ALL diagnosis and MRD measures at day 29 and/or at day 79 (P>0.21). Using risk stratification, as a proxy for therapy response) at day 29 and day 79 we showed a significant association between hypercholesterolemia at ALL diagnosis and a poorer risk stratification at day 29 and day 79 (P=0.024 and P=0.025, respectively). Moreover, we found that overweight/obesity at ALL diagnosis was associated with poorer risk group stratification both after induction (P=0.049) and at day 79 (P=0.017) (79 (Please see Table S1 in manuscript 1 supplementary). Hypertriglyceridemia at ALL diagnosis was not associated to risk group stratification at end of induction (P=0.24) though a tendency were seen at day 79 (P=0.098).

On-therapy hypertriglyceridemia and hypercholesterolemia

The on-therapy sampling of TG and TC measurements were very irregular. On-therapy TG and total TC levels within the first 270 days of therapy are demonstrated according to level at ALL diagnosis in Figure 1.2 A-B for 80 patients with median 20 TG measurements (IQR 11–32) and 65 patients with median 23 TC measurements (IQR 14–32), respectively. We could not detect a difference in development in TG or TC over time for the groups defined by at-diagnosis lipid levels illustrated by fitted curves with overlapping confidence intervals in Figure 1.2 A-B. The curve for hypercholesterolemia could not be illustrated due to low numbers. Neither was a difference identified when comparing the fitted curves for TG and TC on-therapy levels for induction therapy groups (prednisolone [non-HR] vs. dexamethasone [HR], respectively) (Figure not shown here).

Toxicities

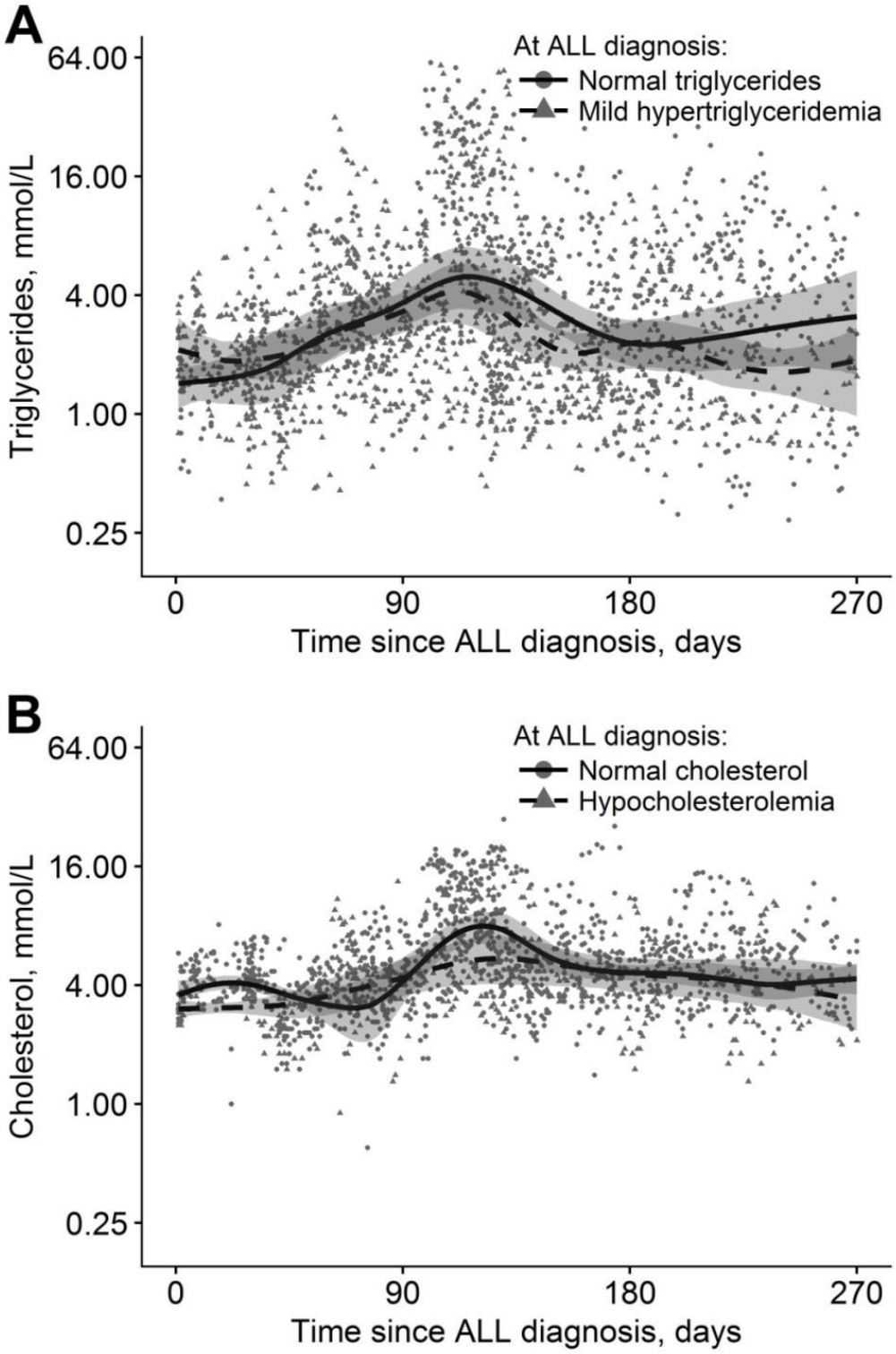
The overall cumulative incidence of any toxicity was 31.1% (22.9–39.4) and the specific cumulative incidences of thromboembolism, osteonecrosis, and pancreatitis were 5.5% (95% CI 2.4–10.5), 7.2% (3.5–12.7), and 18.2% (12.1–25.4), respectively. The cumulative incidence of thromboembolism was significantly higher for patients with hyper- and hypocholesterolemia (16.7% and 20.0%, respectively) at ALL diagnosis compared to patients with normal TC levels (2.2%) (P=0.0074). Consistently, the age-adjusted hazard ratio of thromboembolism was significantly associated with dyscholesterolemia (9.3, 95% CI 1.7–50.8, P=0.011). The other lipid levels and overweight/obesity at ALL diagnosis were not significantly associated to age-adjusted thromboembolism, osteonecrosis or pancreatitis (P≥0.25).

Event-free survival

The median follow-up time was 5.6 years (IQR 3.5–7.5) for the 127 cohort patients and the 5-year EFS was 83.7% (95% CI 77.0–91.0). One patient died during induction therapy, six patients died during first remission (median 151 days [range 52–742] after diagnosis), 12 patients had a relapse (median 1265 days [range 206–2433] after diagnosis), and one patient developed a second malignancy.

Hypertriglyceridemia, hypocholesterolemia and decreased LDL at diagnosis were not associated with EFS (P=0.12, P=0.71 and P=0.10, respectively). The groups with hypercholesterolemia and increased LDL levels could not be included in the analysis due to low numbers. Likewise, EFS could not be compared between decreased and normal HDL or between overweight/obesity and lean patients due to low numbers.

Figure 1.2. On-therapy triglyceride and cholesterol levels according to levels at ALL diagnosis



Discussion of study quality

This study represents nearly all children diagnosed with ALL since July 2008 in the Eastern part of Denmark (corresponding to about 50% of the national cohort) and they have all been treated according to the same protocol. The retrospective design has its limitations since data has been collected prior to the study set up. Accordingly, we failed to collect on-therapy TG and TC lipid measures systematically and HDL and LDL levels were not measured during therapy. Furthermore, sampling frequency was typically more intense in periods with increased lipid levels, making it challenging to classify if a patient overall had high or low levels. Moreover, our cohort was quite small for analyses of time to toxicity/EFS, which can make it difficult to detect significant associations as seen for the analysis of hypertriglyceridemia.

Conclusion

In conclusion, we documented that 99% of children presented had dyslipidemia at ALL diagnosis reflecting a dyslipidemic host-response. Furthermore, cholesterol levels seemed to have an impact on risk for thromboembolism. Further investigation of the interaction between the leukemic pathophysiology, metabolic host-response and therapy has yet to be elucidated.

Paper 2

This paper was a reply to the study by Denton et al who found an association between obesity at ALL diagnosis and the risk of asparaginase associated pancreatitis[43]. In the present study we identified children (1-17.9 years of age) diagnosed with ALL between July 2008 and December 2014 from the NOPHO registry and verified asparaginase-associated pancreatitis cases by questionnaires filled by clinicians. Pancreatitis was registered according to the Ponte di Legno criteria. Overweight and obesity were defined from BMI z-scores.

Results

The cohort (Table 2.1) included 1273 children diagnosed with ALL, receiving consolidation therapy with asparaginase, previously described by Wolthers et al[41]. We identified 85 patients with pancreatitis (300-day cumulative incidence= 6.7%; 95% confidence interval [CI] 5.4–8.1) and 44 patients with relapse or death (300-day cumulative incidence=3.5%; 95% CI 2.6–4.6).

Adolescents ≥10 years had a significantly higher cumulative incidence of asparaginase-associated pancreatitis compared to children <10 years (9.5% vs 5.9%, P=0.037). However, the cumulative incidence of pancreatitis did not differ between BMI groups (Figure 2.1A) or the other baseline characteristics (Table 2.1). Likewise, simple Cox regressions including age and BMI z-scores at ALL diagnosis as continuous variables showed a significant association with age (pancreatitis-specific Hazard Ratio=1.07 per 1-year increase; 95% CI 1.03–1.12; P=0.0013) but not with BMI z-score (pancreatitis-specific Hazard Ratio=1.09 per 1-unit increase; 95% CI 0.92–1.28, P=0.33). Multiple Cox regression with age group and BMI group (obese vs. non-obese) showed no significant interaction between the two, and the adjustment of one variable did not change the (non-) significance of the other ($P_{age\ group}$=0.042, $P_{BMI\ group}$=0.93) (Figure 2.1B).

Figure 2.1 Cumulative incidences of pancreatitis according to A)BMI and B)BMI and age combined.

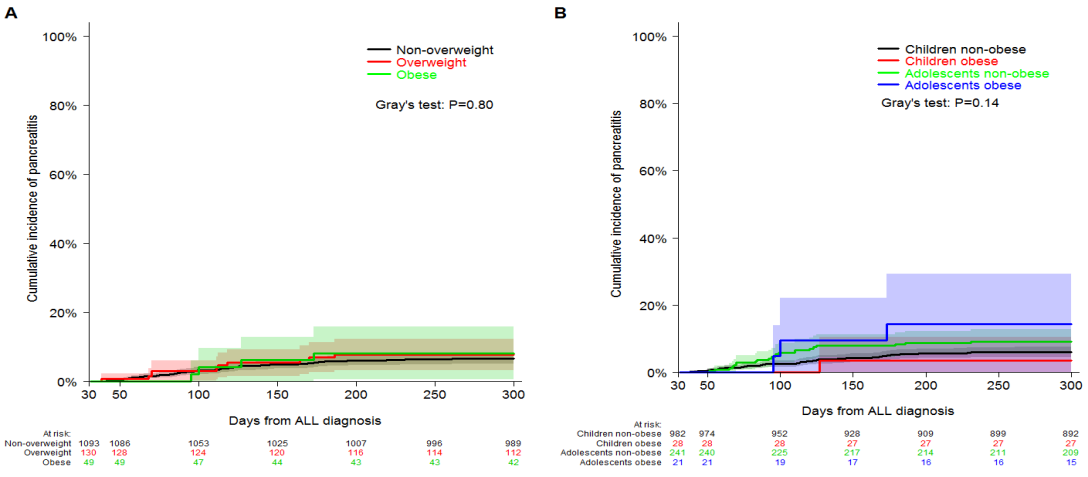


TABLE 2.1 Baseline characteristics and cumulative incidences of pancreatitis

Variable	N (%)	300-day cumulative incidence of pancreatitis, %	95% CI	P-value (Gray's test)
All patients	1273	6.7	5.4–8.1	
Age group				0.037
Children, Age <10 years	1010 (79)	5.9	4.6–7.5	
Adolescents, Age ≥10 years	263 (21)	9.5	6.3–13.4	
Sex				0.46
Male	692 (54)	6.2	4.6–8.2	
Female	581 (46)	7.2	5.3–9.5	
Body mass index (BMI) group^				0.80
Non-overweight (<90 percentile)	1093 (86)	6.5	5.1–8.1	
Overweight (≥90<99 percentile)	130 (10)	7.7	3.9–13.1	
Obese (≥99 percentile)	49 (4)	8.2	2.6–18.0	
End of induction risk group				0.57
Standard risk	631 (50)	6.5	4.8–8.6	
Intermediate risk	453 (36)	7.5	5.3–10.2	
High risk	189 (14)	5.3	2.7–9.1	
Immunophenotype				0.23
Pre B-cell precursor	1116 (88)	6.4	5.0–7.9	
T-cell	157 (12)	8.9	5.1–14.0	

Table 2.1 shows the cumulative incidences of asparaginase associated pancreatitis within 30 to 300 days from ALL diagnosis for selected characteristics. ^One patient did not have BMI data available.*P-value<0.05 are considered significant

Discussion of study quality

This was a large study including more than 1200 patients compared to less than 300 in the study by Denton *et al*[43]. However, the low number of patients with obesity (4%) or overweight (10%) at ALL diagnosis could have attributed to our failure to detect an association between BMI and pancreatitis as seen in Denton *et al*. Our cohort differed considerably from the one of Denton *et al.:* our cohort was younger compared to the cohort in the study by Denton *et al.* (76% < 10 years of age vs. 60%, respectively), and less overweight/obese (14% vs. 35%, respectively). Moreover, our cohort followed the same treatment protocol (NOPHO 2008ALL) in contrast to Denton et als cohort where several protocols were followed. Thus, ALL treatment differed considerably between the two studies with asparaginase used in combination with different drugs. However, it is not clear how much these differences have affected the results regarding an association between BMI and pancreatitis.

Conclusion

The study did not find any association between BMI/obesity at ALL diagnosis and risk of asparaginase associated pancreatitis. However, the study confirmed an increased risk of pancreatitis in older children ≥10 years of age.

Paper 3

This prospective study included children and young adults 1-45 years of age diagnosed at Copenhagen University Hospital Rigshospitalet and Aarhus University Hospital from May 2015 to May 2017. The patients were followed from ALL diagnosis of therapy until approximately one year from diagnosis. Samples were collected at six visits: visit 1) time of ALL diagnosis prior to therapy, visit 2) treatment day 8, visit 3) end of induction therapy, visit 4) treatment day 99, visit 5) week 23(SR)/25(IR) and week 51(SR)/53(IR).

Anthropometric measures (body weight and height) were registered and fasting blood samples were collected and analysed for: glucose, insulin, C-peptide, HbA1c, total cholesterol, LDL, VLDL, HDL and triglycerides. BMI z-scores, HOMA-IR, HOMA-IS and disposition index were calculated.

Results

In the present study patients with ALL presented increased level of HbA1c (median 42 mmol/mol [range 30-50]) and dyslipidemia consisting of increased TG levels (median 2.78 mmol/L [range 0.64−6.78], VLDL levels (median 0.9 mmol/L [range 0.3-3.4] and decreased HDL levels (median 0.37 mmol/l [range 0.09-1.14]) at time of ALL diagnosis. Furthermore, AYAs had an increased BMI (median BMI z-score 1.2 [range -0.9−2.6].

Insulin resistance was detected shortly after the onset of induction therapy with GCs with an almost 9-fold increase in HOMA-IR after just one week of therapy ($RES_{HOMA\text{-}IR}$ 8.8 [95%CI 4.4−17.5], P<0.0001). The patients with ALL remained insulin resistant during induction therapy. The insulin resistance was aggravated by combining asparaginase therapy and GC pulses in the delayed intensification ($RES_{HOMA\text{-}IR}$ 3.2 [95%CI 1.9−5.5], P=0.0002) and did not increase further compared to end of induction levels at visit 5 and 6 (P>0.15).

Insulin secretion tracked with insulin resistance and showed a 5-fold increase ($RES_{HOMA\text{-}IS}$ 4.7 [95%CI 2.7−8.3], P<0.0001) after one week of ALL therapy and an almost 3 fold ($RES_{HOMA\text{-}IS}$ 2.9 [1.7−4.8], P=0.0003) increase at the end of induction therapy. Likewise, a dramatic increase was seen when asparaginase and GC pulses were combined in delayed intensification compared to end of induction levels ($RES_{HOMA\text{-}IS}$ 3.4[2.3−5.1], P<0.0001). The insulin secretion was still increased compared to end of induction levels at visit 5 ($RES_{HOMA\text{-}IS}$ 1.8 [95%CI 1.2−2.5], P=0.0046), but not at visit 6 (P=0.10). However, the disposition index decreased with 40% (RES_{DI} 0.6 [0.4−0.9], P=0.02) after one week of therapy compared to time of diagnosis, but was unchanged at the end of induction therapy (P=0.36), indicating that patients with ALL are able to compensate for the increased insulin resistance and maintain glucose homeostasis during induction therapy. However, AYAs were not able to compensate during induction therapy resulting in a decreased ($RES_{DI\ AYA}$ 0.42 [95%CI 0.28−0.63, P<0.0001] disposition index. No significant changes in disposition index

was observed at visit 4, 5 and 6 in any of the age groups compared to end of induction levels (P>0.06 for all).

The elevated levels of HbA1c at time of ALL diagnosis did not change during induction (P>0.11) but decreased significantly at visit 4, 5 and 6 ending within the normal range (AES_{HbA1c}: -14.2mmol/mol [95%CI -16.7− -11.7], P<0.0001). Furthermore, the dyslipidemia at time of ALL diagnosis resolved during induction therapy (Tabel 3.2), but increased dramatically after induction therapy (Tabel 3.3). Children had higher lipid levels, though still within normal range, compared to AYAs during induction therapy, but no difference between groups were found in later therapy. Increased BMI z-scores were observed at visit 5 and 6 (AES_{BMI} 0.7 [95%CI 0.3−1.2], P=0.0088 and AES_{BMI} 1.1 [0.6−1.6], P<0.0001, respectively). AYAs had a higher BMI than children and AYAs became overweight during already induction therapy whereas children did not have any weight gain during this phase of therapy.

Discussion of study quality

This study has several limitations. The study design and time of visits during consolidation, delayed intensification and maintenance therapy cold have been optimised in order to illucidate the difference in influence from GCs and asparaginase, respectively. Furthermore, not all samples (at visit 4, 5 and 6) were taken as fasting samples, which potentially could affect the HOMA-index, since both glucose and insulin levels would be increased considerably. However, taken the illustrations into account there seems to be a general tendency for the study cohort, so a few non-fasting samples would not change the main findings.

Nearly 75% of the study cohort was below 10 years of age, which gave a skewed age distribution, with a lacking number of AYAs. Furthermore, some of the young adults had a pre-phase with prednisolone before entering the study, which might have influenced their measurements at ALL diagnosis.

The strengths of the study were that all patients followed the same treatment protocol, and samples were taken according to treatment day rather than days from diagnosis. The study was a multicentre study representing the four largest departments treating children and AYAs with ALL in Denmark.

Conclusion

We documented severe metabolic changes in children, adolescents and young adults during the first 12 months of ALL-therapy with glucocorticoids and asparaginase. Furthermore, we observed the combination of GCs and asparaginase to aggravate the metabolic function subsequently compared to GCs alone. These changes may predict later metabolic dysfunction in ALL survivors, but need to be followed up in future studies.

Table 1.1. Demographic and disease characteristics

Variable	Total cohort N (%)	Children N (%)	AYA N (%)
All patients		31 (74)	11(26)
Sex			
Male	29 (69)	21 (68)	8 (73)
Female	13 (31)	10 (32)	3 (27)
Immunophenotype			
Pre B-cell precursor	40 (95)	29 (94)	11 (100)
T-cell	2 (5)	2 (6)	0 (0)
WBC at ALL diagnosis			
<50	38 (90)	28 (90)	10 (91)
≥50 <100	2 (5)	2 (7)	0 (0)
≥100	2 (5)	1 (3)	1 (9)
Risk group at ALL diagnosis			
Non-HR	39 (95)	29 (94)	10 (91)
HR	3 (5)	2 (6)	1 (9)
End of induction risk group			
Standard risk	21 (50)	15 (48)	6 (55)
Intermediate risk	15 (36)	13 (42)	2 (18)
HR	6 (14)	3 (10)	3 (27)
Final risk group			
Standard risk	21 (50)	15 (48)	6 (55)
Intermediate risk	15 (36)	13 (42)	2 (18)
HR	6(14)	3 (10)	3 (27)
Pancreatitis during therapy			
Yes	7 (17)	6 (19)	1 (9)
No	35 (83)	25 (71)	10 (91)

Table 1.2. Primary and secondary outcomes at ALL diagnosis and end of induction therapy.

	Time of ALL diagnosis (visit 1)			End of induction (visit 3)		
Outcome	**Total cohort Median (range)**	**Children Median (range)**	**AYAs Median (range)**	**Total cohort Median (range)**	**Children Median (range)**	**AYAs Median (range)**
HOMA-IR	0.8 (0.0–34.5)	0.7 (0.0–13.5)	2.5 (0.3–34.5)	3.0 (0.7–67.7)	2.3 (0.7–27.1)	4.0 (3.0–68)
HOMA-IS	74 (6–889)	72 (6–889)	76 (16–248)	165 (10–1192)	159 (10–694)	200 (16–1192)
Disposition index	65 (4–608)	72 (22–608)	47 (4–153)	66 (5–288)	69 (7–288)	20 (5–80)
Glucose	4.9 (3.5–13.1)	4.8 (3.5 –6.6)	5.4 (4.2–13.1)	5.0 (3.9–11.3)	4.9 (3.9–9.9)	6.8 (4.7–11.3)
Insulin	32 (2–432)	26 (2 4320)	66 (10–412)	92 (23–1490)	70 (23–642)	126 (44–1490)
C-peptide	278 (27–1520)	264 (27–1520)	650 (133–926)	989 (217–6110)	849 (217–3970)	1660 (370–6110)
HbA1c (mmol/mol)	42 (30–50)	41 (30–50)	45 (40-48)	42 (24–49)	42 (24–49)	40 (35–46)
Total cholesterol (mmol/L)	3.0 (2.2–5.1)	3.2 (2.2–5.1)	2.6 (2.2–4.7)	4.7 (2.5–7.0)	4.8 (3.2–7.0)	3.7 (2.5–6.1)
LDL (mmol/L)	1.6 (0.8–3.5)	1.7 (0.8–3.5)	1.2 (1.0–2.8)	2.8 (0.7–5.1)	2.9 (1.6–5.1)	1.9 (0.7–4.0)
HDL (mmol/L)	0.37 (0.09–1.14)	0.37 (0.09–1.14)	0.61 (0.30–0.68)	1.18 (0.37–2.17)	1.16 (0.37–1.74)	1.44 (0.47–2.17)
VLDL (mmol/L)	0.9 (0.3–3.4)	0.9 (0.3–3.4)	1.0 (0.5–1.2)	0.7 (0.2–1.3)	0.7 (0.3–1.2)	0.4 (0.2–1.3)
Triglycerides (mmol/L)	2.78 (0.64–6.78)	2.99 (0.64–6.78)	2.78 (0.84–3.24)	1.53 (0.54–5.09)	1.64 (0.58–5.09)	1.19 (0.54–2.87)
BMI z-scores	0.5 (-2.5 –2.6)	0.2 (-2.5–1.6)	1.2 (-0.9–2.6)	0.2 (-3.0–2.6)	-0.3 (-2.5–1.8)	1.4 (-3.0–2.6)

Table 1.3. Effect of induction therapy

Induction therapy					
		Visit2 (day 8)		Visit 3 (end of induction, day 29)	
		Relative effect estimate (95%CI)	P-value	Relative effect estimate (95%CI)	P-value
	Primary outcomes				
	HOMA-IR	8.8 (4.4−17.5)	<0.0001	4.0 (2.0−8.1)	0.0004
	HOMA-IS	4.7 (2.7−8.3)	<0.0001	2.9 (1.7−4.8)	0.0003
	Disposition index	0.6 (0.4−0.9)	0.02	0.8 (0.6−1.2)	0.36
	Secondary outcomes				
	Glucose	1.1 (1.0−1.2)	0.0061	1.1 (0.97−1.1)	0.22
	Insulin	8.4 (4.7−15.3)	<0.0001	4.0 (2.1−7.4)	<0.0001
	C-peptide	5.4 (3.8−7.6)	<0.0001	3.4 (2.4−5.0)	<0.0001
	HbA1c (mmol/mol) #	0.7 (-0.3−1.8)	0.17	-1.1 (-2.6−0.2)	0.16
	Lipid levels and BMI				
	Total cholesterol	1.4 (1.3−1.5)	<0.0001	1.5 (1.3−1.5)	<0.0001
	LDL (mmol/L) #	0.9 (0.6−1.2)	<0.0001	1.0 (0.7−1.4)	<0.0001
	HDL (mmol/L) #	0.4 (0.3−0.5)	<0.0001	0.7 (0.6−0.9)	<0.0001
	VLDL	0.8 (0.7−1.1)	0.14	0.7 (0.5−0.8)	0.0006
	Triglycerides	0.9 (0.7−1.1)	0.37	0.6 (0.5−0.8)	0.0012
	BMI z-score#	-0.01 (-0.18−0.15)	0.85	-0.07 (-0.43−0.30)	0.71

Table 1.3 Treatment effect on visit 2 and 3 are compared to visit 1 (time of ALL diagnosis). Relative effect sizes (for right-skeweed outcomes) are shown with corresponding 95% confidence intervals (CI). P-values are reported from Walds test with Satterthwaite approximated degrees of freedom. #Absolute effect sizes (non-skewed outcomes). Abbrevation: HOMA; homestais assessment model, IR; insulin resistance, IS; insulin secretion, HbA1c; glycated hemoglobin, LDL; low-density lipoprotein, HDL; high-densisty lipoprotein, VLDL; very low-density lipoprotein, BMI; body mass index

Table 1.4. Effect of consolidation, delayed intensification and early maintenance therapy

Consolidation/delayed intensification/early maintenance							
		Visit 4 (day 99)		Visit 5		Visit 6	
		Relative effect estimate (95%CI)	P-value	Relative effect estimate (95%CI)	P-value	Relative effect estimate (95%CI)	P-value
	Primary outcomes						
	HOMA-IR	3.2 (1.9−5.5)	0.0002	1.4 (0.7−2.6)	0.29	1.4 (0.88−2.1)	0.15
	HOMA_IS	3.4 (2.3−5.1)	<0.0001	1.8 (1.2−2.5)	0.0046	1.3 (0.9−1.6)	0.10
	Disposition index	1.1 (0.7−1.8)	0.68	1.1 (0.7−1.8)	0.64	1.1 (0.7−1.7)	0.69
	Secondary outcomes						
	Glucose	1.0 (0.9−1,1)	0.79	0.9 (0.8−1.1)	0.27	1.0 (0.9−1.1)	0.87
	Insulin	2.9 (1.7−5.0)	0.0004	1.5 (0.9−2.4)	0.11	1.5 (1.0−2.2)	0.04
	C-peptide	2.6 (1.9−3.6)	<0.0001	1.5 (1.1−2.11)	0.02	1.1 (0.8−1.5)	0.46
	HbA1c (mmol/mol)#	-9.6 (-12.0− -7.2)	<0.0001	-12.4 (-15.1−-9.7)	<0.0001	-14.2 (-16.7− -11.7)	<0.0001
	Lipid levels and BMI						
	Total cholesterol	1.5 (1.3−1.7)	<0.0001	1.9 (0.8−1.6)	0.87	0.8 (0.7−0.9)	0.0082
	LDL (mmol/L) #	0.4 (-0.2−1.1)	0.19	-0.8 (-1.2− -0.4)	0.0009	-0.7 (-1.2− -0.2)	0.0091
	HDL (mmol/L) #	-0.2 (-0.5 – 0.0)	0.10	-0.2 (-0.1−0.2)	0.10	0.0 (-0.1− 0.2)	0.57
	VLDL	3.5 (2.3−5.3)	<0.0001	1.5 (0.8−2.6)	0.20	0.5 (0.3−0.7)	0.0003
	Triglycerides	2.6 (1.9−3.7)	<0.0001	1.2 (0.8−1.8)	0.34	0.7 (0.5−0.9)	0.028
	BMI z-score #	0.1(-1.2−0.5)	0.41	0.6 (0.3−1.2)	0.0058	1.0 (0.6−1.6)	<0.0001

Table 1.4 Treatment effect on visit 2 and 3 are compared to visit 1 (time of ALL diagnosis). Relative effect sizes (for right-skeweed outcomes) are shown with corresponding 95% confidence intervals (CI). P-values are reported from Walds test with Satterthwaite approximated degrees of freedom. #Absolute effect sizes (non-skewed outcomes). Abbrevation: HOMA; homestais assessment model, IR; insulin resistance, IS; insulin secretion, HbA1c; glycated hemoglobin, LDL; low-density lipoprotein, HDL; high-densisty lipoprotein, VLDL; very low-density lipoprotein, BMI; body mass index

Paper 4

In this descriptive study, we included children (1–17.9 years of age) from the NOPHO ALL2008 cohort diagnosed from July 2008 until December 2014. Patients with asparaginase-associated pancreatitis, fulfilling the Ponte di Legno criteria, were registered in the NOPHO database. Out of 1285 patients, 86 patients developed asparaginase-associated pancreatitis during ALL therapy. Among these, seven patients with persisting insulin-dependent diabetes were identified by Wolthers *et al* in a previous study[42]. Questionnaires regarding treatment and pancreatitis characteristics and diabetic status at follow up were sent to the clinicians responsible for treatment.

Results

All seven patients remained in need of insulin therapy after a median of 4.2 years (range 1.7 9.2. The seven patients with insulin-dependent type 3C (pancreatogenic) diabetes are presented in Table 4.1. They were 9−17 years of age at diagnosis of pancreatitis and received a median of 4 pegylated-asparaginase injections (range 1−8) prior to diagnosis of pancreatitis. Six of seven patients had pancreatic pseudocysts at time of pancreatitis diagnosis and all patients presented abdominal pain, nausea, and vomiting. Four of seven patients had plasma glucose measured at pancreatitis diagnosis and all were hyperglycemic with plasma glucose levels ≥11.9mmol/L. The patients had median HbA1c levels of 64 mmol/mol (range 42-86). Three of the children presented a poor glycaemic control at their last clinical follow up.

Table 4.1 Characteristics of patients with persistent insulin-dependent diabetes

ID no	Immuno-pheno type	Sex	Age at pancreatitis diagnosis (years)	WBC at ALL Diagnosis (10^9/L)	Risk group	Asparaginase administrations prior to pancreatitis (N)	Amylase level at pancreatitis diagnosis (U/L)	Lipase level at pancreatitis diagnosis (U/L)	Plasma glucose at pancreatitis diagnosis (mmol/L)	Pseudocysts/drainage required	FU time (years)	Symptoms of pancreatitis at last FU	HbA1c at last FU (mmol/mol)
1	B-cell precursor	F	9·2	1.7	Non-high-risk	4	845	3900	NA	No/-	9.2	No	86
2	T-cell	F	13.2	153.8	High-risk	3	798	1273	NA	Yes/Yes	8.2	No	47
3	B-cell precursor	M	10.5	8.4	Non-high-risk	8	657	1780	NA	Yes/Yes	1.7	NA*	NA*
4	B-cell precursor	F	16.7	2.6	Non-high-risk	5	512	NA	15,8	Yes/No	5.3	No	42
5	B-cell precursor	F	9.2	3.2	Non-high-risk	1	NA	420	17,2	Yes/No	4.2	No	63
6	B-cell precursor	M	14.1	14.5	Non-high-risk	4	90	NA	24.8	Yes/No	4.0	No	46
7	B-cell precursor	M	15.3	34.3	Non-high-risk	5	744	NA	11,9	Yes/Yes	4.0	No	52

Baseline data on the seven study patients. Asparaginase administrations prior to pancreatitis represents the number om pegylated asparaginase administrations prior to pancreatitis. These injections were given intramuscularly at 1000 units/m^2.
*Data not available the patient died. Abbreviations: F=Female, M=male, ALL = acute lymphoblastic leukemia, FU = follow-up, WBC = white blood cell count.

Discussion of study quality

This study illuminates important associations in the understanding of long-term sequelae after asparaginase associated pancreatitis. Assessment of the glucose tolerance would provide additional knowledge of those at increased risk of developing diabetes. Despite limited number of cases with persistent insulin-dependent diabetes, they represent the entire cohort from seven different countries. It would have been a strength to include data from age-matched children with ALL who did not develop asparaginase-associated pancreatitis as a control group, since we do not know the prevalence of insulin-dependent diabetes in this group.

Conclusion

This study is the first study to document that persistent insulin-dependent diabetes is a common (8%) long-term complication to asparaginase-associated pancreatitis in ALL therapy in children. Inexpensive monitoring of plasma glucose and/or HbA1c is recommended for all children developing asparaginase-associated pancreatitis.

Summary of main findings

In summary, this thesis documented changes in lipid metabolism in children and adolescents at time of ALL diagnosis prior to therapy. We did not find any significant associations between these lipid alterations and on-therapy lipid levels, therapy outcome or event-free survival. However, we did find an association between dyscholesterolemia at ALL diagnosis and risk of thromboembolisms during therapy. Moreover, we rejected the hypothesis on the association between obesity at ALL diagnosis and risk of asparaginase-associated pancreatitis but underlined that asparaginase-associted pancreatitis has long-term implications for patients. Furthermore, we have described significant changes in glucose and lipid metabolism during the first year of treatment, aggravated by additional asparaginase therapy compared to GC therapy alone, which potentially re-program inappropriate cardio-metabolic function leading to increased risk of cardio-metabolic disease later in life.

Overall discussion of results

This thesis contributes findings about the metabolic changes in children, adolescents and young adults with ALL.

Lipid metabolism

We found significantly dyslipidemic changes in children and adolescents at ALL diagnosis prior to any therapy which indicates a link between pathogenesis of ALL and lipid metabolism. Similar findings have been indicated in small studies of ALL and in other cancer types, but there seems to be paucity in ALL literature on this topic. The pathogenesis of ALL is not well understood and it is not known if metabolic changes potentially play a role in development of ALL or perhaps, how ALL induces these dyslipidemic changes. An explanation could be early stages of cachexia syndrome which induces loss of muscle and adipose tissue and increases lipolysis, but since ALL often develops over a short time period significant weight loss before ALL diagnosis might be unusual. A recent study on mice found increased lipolysis in the adipocytes few days after injection of leukemic inhibitor factor, normally secreted by tumour cells,[93] which indicates ALL to directly affect lipid homeostasis, and could support our results. We did not find any association between BMI at time of ALL diagnosis and TC, LDL and TG levels. Thus, BMI does not seem to affect the dyslipidemia found at ALL diagnosis. However, we did not have information on BMI prior to ALL-diagnosis; so we cannot know whether the patients had a weight loss during the disease period before ALL diagnosis. Interestingly we found an increased BMI in AYAs at time of ALL diagnosis (paper 1, 2, 3), compared to healthy peers. Obesity has previously been indicated to be a risk factor for ALL in adults[20,54]. However, if increased BMI reflecting fat mass is the causal risk factor or if it is a surrogate for other factors, that potentially increase risk of both leukemia and increased BMI is not known[94].

Obesity at ALL diagnosis has been associated with a poorer EFS and higher risk of relapse[54,56]. We could not reproduce this result or find an association with MRD (paper 1). However, overweight/obese patients had an increased risk of being stratified into the high risk group (reflecting lower efficacy of ALL therapy), compared to non-overweight/obese patients (Paper 1). It has been debated whether obese patients with ALL have a worse outcome due to under treatment[95,96] . Therapy dosage is often adjusted by the body surface area (BSA) based on height and bodyweight calculated from Du Bois & Du Bois[97], but this method might not be sensitive enough for children or individuals with an abnormal physique[98]. In contrast, it has also been shown that obese patients have reduced anti-neoplastic drug clearance compared to lean individuals, which suggest over treatment[95]. BSA is not sensitive to body composition, where the relative distribution between active and passive absorbent tissues are not taken into account and thereby the drug metabolism and elimination[95]. Poorer treatment response in heavier patients with ALL may be caused by a

protective mechanism of adipocytes towards leukemic cells leading to drug resistance [99–102]or adipocytes ability to attract and fuel the leukemic cells[103].

Furthermore, obesity at ALL diagnosis has been indicated to be associated with risk of pancreatitis during therapy in a study with 262 patients with ALL[43]. We were not able to reproduce this association in our study including 1273 children and adolescents with ALL (paper 2). Our results were supported by other studies failing to show an association between hypertriglyceridemia and risk of pancreatitits[104,105], which could be the plausible explanation for obesity to be a risk factor[106]. We found both hyper- and hypocholesterolemia compared to normal levels of total cholesterol at diagnosis to be significantly associated to risk of thromboembolism during therapy (paper 1). Thromboembolism in patients with ALL are usually related to complications with the central venous line[107]. Our results suggests that there might be some influence from plasma lipid concentration and/or persisting changes in the vascular wall induced by the ALL[108], but the mechanism need to be further elucidated. Furthermore, the results are in line with a large randomized controlled trial showing an association between the formation of blood cloths and cholesterol levels, and could indicate a need for statin therapy for patients being at high risk of thromboembolism without having dyslipidemia[109].

Our results indicate that dyslipidemia at ALL diagnosis is, primarily consists of low levels of HDL and hypertriglyceridemia (paper 1). The dyslipidemia seemed to resolve during induction remission therapy, which have been supported by Moschovi et al.[110], where after a dramatically lipid peak is observed when GC and asparaginase therapy is combined during delayed intensification (paper 1 and 3). Increased TC and TG levels at diagnosis did not seem to be associated to, or predictive of, worse on-therapy lipid levels (paper 1). Actually, Increased TC and TG levels at ALL diagnosis rather seemed to predict less increase in on-therapy lipid levels, though still elevated, compared to those without normal TC and TG levels at ALL diagnosis. These observations suggest that the observed dyslipidemia is a result of two different processes, one at the development of ALL, and one as a result of therapy. As already discussed, the lipid levels at diagnosis could be associated to cachexia syndrome, resolving with remission of disease during induction therapy; while the lipid peak seemed to be induced by intensive asparaginase therapy on top of high dose dexamethasone therapy. Other studies have also found asparaginase and GC therapy to induce hypertriglyceridemia and hypercholesterolemia in patients with ALL[13,105,111,112]. Older age has been suggested as risk factor for hyperlipidemia during ALL therapy which contrasts our results suggesting worse lipid levels in children, compared to adolescents and young adults, respectively (paper 1 and 3). This finding could be cause due to a low number in the AYA group in our study.

Glucose metabolism

Therapy-induced hyperglycemia is a known adverse effect to ALL therapy with GCs and asparaginase[7,112]. It often resolves after GC and asparaginase therapy[61,63], but it may not be transient since survivors of ALL have an increased risk of cardio-metabolic dysfunction including a 3-fold increased risk of type 2 diabetes[8,9]. Asparaginase has been suggested to cause impaired insulin secretion and GCs is known to cause insulin resistance [112]. Likewise, our results points to GC induced insulin resistance during induction therapy (paper 3). However, when asparaginase was added to GC pulses in the delayed intensification therapy, both insulin resistance and insulin secretion were dramatically increased, leading to maintenance of a near normal glucose homeostasis. Adolescents and young adults couldn't compensate as well as children (<15 years of age), leading to an increase in plasma glucose and decreased disposition index. Older age has been identified as a risk factor of medication-induced hyperglycemia/diabetes in other studies[4,7,9,64,113–115]. Furthermore, adolescents had a significant higher BMI at diagnosis and became overweight during induction therapy, which also might influence their insulin resistance[69]. Studies have shown sarcopenia in adolescents during ALL therapy[116], which also will lead to decreased peripheral insulin sensitivity in this age group.

It is impossible to determine if and when overt type 2 diabetes will develop in these patients. However, we have now shown that this first year of intensive therapy with GCs and asparaginase causes severe metabolic dysfunction, which is likely to be take part in the persistent dysmetabolic changes seen in survivors of ALL[3,47,117]. The mechanisms behind these drug-induced metabolic alterations is not known; but may be influenced by epigenetic changes in DNA methylation and/or histone acetylations which influences the gene expressions[118].

Our results showed that AYAs could not compensate for their insulin resistance, indicating impaired insulin secretion. We also found impaired insulin secretion in a sub-group (7-8%) of patients diagnosed with asparaginse-associated pancreatitis during ALL-therapy (Paper 3 and 4). In paper 1 we found a cumulative incidence of risk of pancreatitis of more than 18% which is significantly higher than the average cumulative incidence in the NOPHO-countries. At University Hospital Rigshospitalet a lot of research has focused on pancreatitis, why there might has been increased clinical awareness on this toxicity (the Hawthone effect). It could indicate that many patients with mild pancreatitis are not identified other places, since pancreatitis often is taken for a sepsis and treated with antibiotics, which also will resolve a mild pancreatitis. However, these mild or early pancreatitis changes may cause transient or persistent impaired insulin secretion that over time will cause hyperglycemia and diabetes.

Besides the influence from ALL and ALL-therapy; BMI, lipidemia and insulin sensitivity are also influenced by genetic predisposition and life style behaviours. Lack of physical activity during and after ALL treatment is a well-known risk factor for developing metabolic disorders including obesity and insulin resistence[68,119]. Physical exercise improves body composition and is inversely related to the amount of subcutaneous and visceral adipose tissue and decreases BMI in children during ALL-treatment compared to controls[119]. Physical exercise is associated with an increase in glucose uptake into insulin sensitive tissue and is therefore associated with increased glucose tolerance and insulin action, and thereby a decreased risk for insulin resistance and T2D[68]. There is still a lack of interventional studies with physical exercise to patients with ALL and the existing ones are often characterized by small samples and poor quality of the intervention[120]. Many factors have to be taken into account as for example the motivation for physical exercise. These factors have been included in ongoing Rehabilitation including Social and Physical activity and Education in Children and Teenagers with Cancer (RESPECT) study, which has succeseded in feasibility, but we are still waiting exited for the results[121,122].

Link between cancer and diabetes

Birthweight and gestational diabetes as well as maternal diabetes are known to influence the risk for diabetes in the child. Furthermore, it has been indicated that the same parameters increase risk for ALL in the child[17,19]. A link between diabetes and cancer has been known and assessed through epidemiologic research for a long time, usually as patients with diabetes developing cancer[123,124]. It is interesting to take this into account with the abovementioned discussion on obesity, drug dosage and increased risk of ALL and increased risk of relapse during ALL therapy. Whether some of the underlying mechanisms are the same remain unknown. However, genetics as well as epigenetic markers could influence both development of ALL and diabetes and be a part of the explanation for the increased risk diabetes in survivors of ALL. This hypothesis needs to be assessed in future studies.

Strengths and limitations

A major limitation in paper 1 and 3 is the lack of a healthy control group. Due to ethical and logistic causes it was not possible to match a healthy control group, which is a general challenge in pediatric research. Little is known about the metabolism in pre-school children, who represents the majority of the study population in this thesis. Accordingly, we have not been able quantify how much the study cohort actually deviates from a healthy population.

Controversy exists whether BMI z-score should be based on national or international references and how cut offs for normal body weight, overweight and obesity should be defined in a pediatric population[125]. BMI

z-score refers to an individual deviance from the median of sex and age matched references, disregarding if this median is unhealthy biased by the worldwide obesity epidemic[125]. The references used for calculating the BMI z-scores in this thesis are 30-40 years old, and the median BMI have probably increased since then. However, this was a time before the obesity epidemic, so the references can be considered as healthy references. Calculating BMI z-score based on International Obesity Task Forces (IOTF) references, WHO or using cut off values according to *Cole et al.* would give a slightly different distribution of the patients in the different BMI-groups, but will unlikely change the results[126].

BMI is of limited value in distinguishing between if excessive body weight is caused by excess fat or lean mass. However, the advantages (simplicity and inexpensiveness) of BMI as a screening tool for defining obesity for research outweigh these limitations. BMI z-score at ALL diagnosis has been shown to reflect body fat percentage in adolescents with ALL[116], but it is unknown to which extend the BMI z-score represents the body fat percentage in young children and adults with ALL. The use of Dual-energy x-ray absorptiometry scan (DEXA) and magnetic resonance imaging scans could provide information on body composition and exactly location of adipose tissue. Furthermore, this information could potentially contribute to the abovementioned discussion on drug dosing.

The homeostasis assessment model (HOMA) was used to determine insulin resistance and β cell function, which has been validated against golden standards[88]. Assessments as hyperinsulinemic euglycemic clamp, intravenous glucose tolerance test (IVGTT) or oral glucose tolerance test (OGTT) are preferable to assess insulin sensitivity (hepatic and peripheral), insulin secretion and glucose tolerance, but these are more expensive and less feasible to apply for this cohort than the HOMA-index. We tried to perform OGTT in the clinical study, but it was rather difficult to complete systematically. The time from ALL diagnosis to onset of therapy is very short and hectic, and even during therapy it was challenging to get the OGTT fitted into the clinical setting. Furthermore, we used HOMA-IS and the inverse HOMA-IR to estimate the disposition index, fully aware of that it is normally the first phase insulin response (from a dynamic test) that are being used to determine the β-cell function[78]. However, we found a perfect hyperbolic association between the insulin secretion and sensitivity.

Challenges in clinical research and ethical considerations

Doing clinical research in children with cancer is a major challenge and many considerations need to be taken into account. To include patients in a study an informed consent is necessary. However, the ethical perspective in obtaining informed consent from parents who just had their child diagnosed with cancer can be discussed. Are the parents in a position where they can understand the information about the study? Who should give this information? Will the parents agree to participate out of fear that the rest of the

treatment will receive lower priority if the say no? Should the participation in a study just be a part of the treatment as long as there is no danger or excessive overload for the child? And how can we improve treatment and reduce adverse effects if the research is not prioritised? This discussion is very important not only for clinical oncology research in children, but in principle overall for clinical research. The participation rate in this thesis has been very high thanks to the patients, their parents and the clinicians in the oncologic/hematological wards, all contributing to this thesis.

Concluding remarks and perspectives

In conclusion, this thesis shows that dyslipidemic changes were present already prior to therapy in patients diagnosed with ALL. The lipidemic status at diagnosis did not seem to influence the dysmetabolic traits during therapy. Patients undergoing ALL therapy become insulin resistant shortly after onset of GC therapy and this insulin resistance is aggravated by additional asparaginase therapy. Likewise, moderate to severe dyslipidemia was observed during therapy with a combination of the two drugs. These metabolic changes prior and during therapy are likely associated to cardio-metabolic late effects in survivors of ALL. We recommend a systematically screening for dyslipidemia and prediabetes in children, adolescents and young adults with ALL, both during and after end of therapy. In addition, the data from this thesis will be followed up in the (Acute Lymphoblastic Leukemia Survival, Toxicity And Rehabilitation (ALL-STAR) study) which can contribute to a better understanding of the metabolic mechanisms both prior, during and after ALL therapy in order to prevent metabolic late effects in survivors of ALL. These mechanisms may also contribute to a better understanding of the pathogenesis of ALL.

Even though we know that asparaginase and GCs are the 'bad guys' regarding deleterious effects on the metabolic function, ALL therapy depends on these drugs in order to cure patients with ALL. Essentially the right drug dosages must be clarified and the ultimate future target is to understand the interpatient variation of pharmacokinetics in anti-leukemic drug therapy. Furthermore, studies are needed in order to assess how a reversion of the cardio-metabolic complications is obtained. Interventions with physical exercise and/or healthy diet could potentially contribute to not only an improved metabolic function in patients with ALL, but also better health-related quality of life[127]. These studies should be supported by psychological perspective focusing on how to secure motivation for a healthy lifestyle in this population (and their parents). Diet supplementary such as fish oil may also induce decreased lipid levels during ALL therapy as suggested in a pilot study (*unpublished data*). Additionally, pharmaceutical interventions with metformin or statins could be beneficial in order to normalize lipid and glucose metabolism in patients with ALL[94,109]. Metformin has also shown anti-cancer effects; yet it has to be tested in patients with ALL.

Summary in English

Survival rates of acute lymphoblastic leukemia (ALL) in children/adolescents, and young adults have reached more than 90% and 70%, respectively, through advances and intensification of therapy. However, the burden of long-term adverse treatment-related effects has increased, and today more than 50% of ALL patients experience sequelae after treatment. Among the most deleterious and frequent long-term complications are cardiovascular disease and diabetes affecting long-term co-morbidity and mortality.

The mechanisms accountable for the cardio-metabolic long-term complications in ALL survivors are still unclear. Accordingly, the aim of this thesis was to investigate the changes in lipid- and glucose metabolism in children, adolescents and to some extend young adults prior to and during therapy of acute lymphoblastic leukemia.

Retrospectively we determined the lipid profile in 127 children diagnosed with ALL and assessed the associations to on-therapy lipid levels, early therapy outcomes, toxicities and event-free survival. Prospectively we followed 42 children, adolescents and young adults through the first year of therapy with glucocorticoids and asparaginase therapy. Furthermore, we tried to reproduce a study on the association between obesity and risk of asparaginase-associated pancreatitis and descripted the long-term sequelae of pancreatitis.

The results of the studies in this thesis showed changes in lipid metabolism in children and adolescents at time of ALL diagnosis prior to therapy. We did not find any significant associations between these lipid alterations and on-therapy lipid levels, therapy outcome or event-free survival. However, we did find an association between dyscholesterolemia at ALL diagnosis and risk of thromboembolisms during therapy. Furthermore, we confirmed significant changes in glucose and lipid metabolism during the first year of treatment, aggravated by additional asparaginase therapy compared to GC therapy alone. Furthermore we documented that asparaginase-associated pancreatitis may lead to persistent insulin-dependent diabetes.

In conclusion, metabolic changes are already observed at time of ALL diagnosis in children and young adults. ALL therapy including glucocorticoids and asparaginase therapy induces further severe metabolic dysfunction which potentially leads to increased risk of cardio-metabolic disease later in life.

Summary in Danish (Dansk resumé)

Overlevelsen for akut lymfoblastisk leukæmi (ALL) hos børn og unge voksne har nået henholdsvis 90% og 70% grundet intensivering af behandlingen. Risikoen for langvarige bivirkninger er steget i takt med den intensiverede behandling og i dag oplever over 50% af ALL-patienter senfølger efter behandling. Blandt de hyppigste og mest skadelige senfølger er kardio-vaskulær sygdom og diabetes, der medfører øget risiko for følgesygdom og dødelighed.

Årsagerne bag de kardio-metaboliske senfølger hos ALL overlevere, er stadig uklare. Formålet med denne afhandling var at undersøge ændringer i lipid- og glukosemetabolismen hos børn, og unge voksne før og under behandling af ALL.

Retrospektivt undersøgte vi lipid-profilen i 127 børn diagnosticeret med ALL og vurderede sammenhængen med lipid niveauer under behandling, behandlingsrespons, bivirkninger og event-fri overlevelse. Prospektivt fulgte vi 42 børn, unge og unge voksne gennem det første år af ALL behandlingen med glucocorticoider og asparaginase. Desuden forsøgte vi at reproducere sammenhængen mellem fedme og risiko for asparaginase-associeret pankreatitis samt beskrive de langsigtede følgevirkninger af pancreatitis.

Resultaterne af studierne i denne afhandling viste ændringer i lipid-metabolisme hos børn og unge ved ALL diagnose inden opstart af behandling. Vi fandt ingen betydelige sammenhænge mellem disse lipid-ændringer og lipid-niveauer under behandling, resultat af behandlingen eller event-fri overlevelse. Imidlertid fandt vi en forbindelse mellem både lave og høje niveauer af kolesterol ved ALL diagnose og risiko for blodpropper under behandlingen. Derudover har vi bekræftet signifikante ændringer i glukose og lipid-metabolisme i løbet af det første år med behandling, forværring af yderligere asparaginase terapi sammenlignet med glucocorticoid-behandling alene. Endvidere dokumenterede vi at asaparginase-associeret pancreatitis kan føre til vedvarende insulin-afhængig diabetes.

Konklusionen på afhandlingen er, at metaboliske ændringer allerede er observeret på tidspunktet for ALL-diagnose hos børn og unge voksne. ALL-behandling, herunder glucocorticoider og asparaginase terapi, inducerer yderligere alvorlig metabolisk dysfunktion, som potentielt fører til øget risiko for kardio-metabolisk sygdom senere i livet.

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