Characterization and identification of atypical diabetes in pediatric patients.

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LIST OF ABBREVIATIONS

ACR: Acute cell rejection
ACMG: American College of Medical Genetics and Genomics
ADA: American Diabetes Association
ADP: Adenosine Diphosphate
ALL: Acute lymphoblastic leukemia
AML: Acute Myeloid Leukemia
ATP Adenosine Triphosphate
BDR: Belgian Diabetes Registry
BMI: Body mass index
CGM: Continuous glucose monitor
C-peptide: Connective peptide
CUSL: Cliniques universitaires Saint-Luc
DKA: Diabetes ketoacidosis
FKBP: FK-506 binding protein
FPG: Fasting plasma glucose
GAD65: Glutamate decarboxylase-65
GCC: Glucocorticoid
GCK: Glucokinase
GLUT2: Glucose transporter 2
GLUT4: Glucose transporter 4
G6P: Glucose-6-phosphate
HbA_{1C}: Hemoglobin glycated
HG: Hyperglycemia
HL: Hodgkin-lymphoma
HNF1A: Hepatocyte nuclear factor-1 alpha
HNF4A: Hepatocyte nuclear factor-4 alpha
HNF1B: Hepatocyte nuclear factor-1 beta
HOMA: Homeostatic model assessment
HSCT: Hematopoietic stem cell transplantation
KPD: Ketosis-prone diabetes
LADA: Latent autoimmune diabetes of adults
LT: Liver transplant
IAA: Insulin autoantibodies
IA-2 and IA-2β: Islet antigen-2/2β
IGT: Impaired glucose tolerance
IFG: Impaired fasting glucose
IR: Insulin resistance
IRS: Insulin receptor substrate 1
IL-2/3: interleukin-2/3
ISPAD: International Society for Pediatric and Adolescent Diabetes
MODY: Maturity onset of the young
mTOR protein: mammalian Target Of Rapamycin
NDM: Neonatal diabetes mellitus
NF-AT: Nuclear factor of activated T cells
NHL: non-Hodgkin lymphoma
NODAT: New-onset diabetes after transplantation
PDK: Pyruvate dehydrogenase kinase
PIP2: phosphatidylinositol-4,5-bisphosphate
PIP3: Phosphatidylinositol (3,4,5)-triphosphate
PI3K: phosphoinositide 3-kinase
PTDM: Post transplant diabetes mellitus
OGTT: Oral glucose tolerance test
PG: Plasma glucose
RT: Renal transplant
TBI: Total body irradiation
T1D: Type 1 diabetes
T2D: Type 2 diabetes
WHO: World health organization
ZnT8A: Zinc transporter 8

SUMMARY

Diabetes is defined as a state of chronic hyperglycemia, a criterion that refers to a heterogeneous group of diseases with various etiologies and distinct treatment options. Besides the two main forms of diabetes (type 1 and type 2 diabetes), there are rare subtypes of the disease called atypical diabetes, such as monogenic diabetes and drug-induced diabetes. The former is often misdiagnosed because of its clinical similarities to type 1, and type 2 diabetes and the latter is underdiagnosed because of the limited information and studies conducted about it. Therefore, the objective of my research was to better characterize these fewer common types of diabetes in pediatrics. In this context, three studies were undertaken at the Cliniques universitaires Saint-Luc, in Brussels, two on drug-induced diabetes, including patients treated with anticancer or antirejection treatments, and one on genetic forms of diabetes.

In our first study, we studied the incidence and risk factor of hyperglycemia during treatment of childhood hematologic malignancies by retrospectively collecting 15 years of data from acute lymphoblastic leukemia, Hodgkin lymphoma and non-Hodgkin's lymphoma. Our results showed that approximately a fifth of patients developed hyperglycemia and for the majority within the first month of treatment, corresponding to pre- and induction phases. No hyperglycemia was observed in Hodgkin lymphoma patients. Using multivariate analysis, our study highlights the importance of considering overweight and puberty as potential markers for the onset of hyperglycemia in pediatric patients with acute lymphoblastic leukemia, and the importance of closely monitoring glucose levels when patients have steroid-resistant disease or require hematopoietic stem cell transplantation preceded by total body irradiation.

In our second study, we retrospectively analyzed risk factors of hyperglycemia from 195 children with liver transplant and 20 children with renal transplant. In addition, we prospectively followed height liver (n=4) and renal (n=4) transplant children to evaluate the evolution of their glucose metabolism. Our results showed that diabetes is a major side effect in renal transplant children (20%) and transient hyperglycemia are frequent after a pediatric liver (25%) and renal (35%)

transplant. The onset of hyperglycemia systematically occurred in the post-prandial afternoon period and was associated with the use of glucocorticoids and with the presence of acute events as graft rejection and infection. The biological markers of diabetes were in the normal range for HbA_{1C}, fasting glucose and insulin levels although oral glucose tolerance test showed insulin resistance and impaired glucose tolerance early after transplantation. Our study suggests that random blood glucose monitoring should be reinforced in the afternoon period when children present critical complications such as graft rejection and infections.

In our last study, to identify patients who presented a genetic form of diabetes, the most representative clinical criteria of monogenic diabetes were compiled into a new score. This score was applied to patients with genetically confirmed MODY, and patients diagnosed with T1D. Our study first demonstrates the efficiency and relevance of our internal score by detecting 100% of our MODY patients. Furthermore, the cross-analysis of score criteria between our MODY and T1D cohorts confirms well-documented clinical features such as the absence of islet autoantibodies and the presence of a residual C-peptide secretion and introduces new clinical characteristics: $IDAA_{1C}$ and $GTAA_{1C}$ to help clinicians identify the most common forms of monogenic diabetes. Secondly, our score identified patients with atypical forms of diabetes, which differ from T1D by the absence of islet autoantibodies, high residual C-peptide secretion and $IDAA_{1C}$ score ≤ 9. However, none of these patients had a known pathological gene variant. Finally, our score criteria revealed a clinical polarization between T1D and MODY diabetes, with most patients with atypical diabetes bridging the gap between the two forms of the disease. This discovery confirms the heterogeneity of diabetes, which complicates the precise etiologic classification of diabetes, and recognizes the existence of overlap between different forms of diabetes.

Together, our studies demonstrate the growing interest in the study of atypical forms of diabetes and through our three studies, we have better characterized monogenic diabetes and drug-induced diabetes and provided new clinical criteria and insights to help clinicians better identify atypical forms of diabetes.

OUTLINE

Chapter 1 provides a general introduction to diabetes, its diagnosis and current classification. Common forms of diabetes and diabetes due to other specific causes will be developed. Since pediatrics monogenic diabetes and drug-induced diabetes are the focus of this research work, they will be developed in more detail. **Chapter 2** describes the state of the art regarding the overly general term diabetes and studies that have attempted to stratify the disease into subtypes, which is the first step towards personalized medicine. In parallel, the limitations of the current classification which hinder the current knowledge about atypical forms of diabetes, are developed. **Chapter 3** defines the objective of my research and presents the strategies implemented to study monogenic diabetes and drug-induced diabetes in pediatrics. **Chapter 4** describes the three studies conducted during my thesis to better characterize and diagnose monogenic diabetes and drug-induced diabetes in pediatrics. **Chapter 5** summarizes and concludes the findings of my thesis with future directions.

CHAPTER 1.

INTRODUCTION

DIABETES BACKGROUND

The term “diabetes mellitus” or simply diabetes, represents a heterogeneous group of metabolic disorders characterized by chronic hyperglycemia (high blood glucose) resulting from defects in insulin secretion, insulin action or both[1,2]. According to the IMA-AIM Atlas, in 2020, 6.6% of the Belgian population was diagnosed with diabetes[3]. However, more than one in three Belgian residents are unaware of their diabetes, bringing the true prevalence of diabetes to 10%. According to the INAMI (for Institut National d'Assurance Maladie-Invalidité), diabetes is the most frequent chronic disease in pediatrics and affects just over 3200 Belgian children and adolescents[4].

Several forms of diabetes exist and depend on specific pathogenic processes. In pediatrics, the most common forms of diabetes range from an autoimmune process with absolute deficiency of insulin to a less severe form of diabetes in which insulin production is preserved but inadequately secreted due to tissue resistance to insulin action. Overall, these forms are a combination of autoimmunity, environmental factors, and multiple predisposing polymorphisms with low effect. In contrast, in very rare cases, diabetes can be caused by a strong mutation in a single gene involved in insulin production or secretion, or in β cell loss or dysfunction. This distinction between the different forms of diabetes has important implications for appropriate treatment.

Because there are different forms of diabetes, the disease may develop differently, but the most common symptoms in pediatrics are polydipsia, polyuria, weight loss despite polyphagia, nocturia, extreme fatigue, and visual disturbances[1,2]. In more severe cases, the patient may present ketoacidosis or non-ketotic hyperosmolar syndrome, which may lead to coma or death if left untreated[5]. All forms of diabetes have the same risk of long-term complications related to the presence of hyperglycemia. However, the progression rate of complications depends on the type of diabetes, its management, and associated therapies. Chronic hyperglycemia can lead to long-term complications related to nephropathy, retinopathy, and neuropathy, such as chronic kidney disease, eyes damage, foot ulcers and cardiovascular

disease[6]. To prevent acute complications and reduce the risk of long-term complications, lifelong self-management of blood glucose, exercise and specific diet are essential. In addition to a healthy lifestyle, medical treatment is necessary and consists of insulin therapy and hypoglycemic agents. Insulin therapy is required when endogenous insulin secretion is insufficient or absent and consists of the injection of exogenous insulin. In the absence of absolute secretion of insulin, treatment is required for life and does not cure the disease. Some patients may require antidiabetic agents, also called hypoglycemic agents, when insulin secretory function is not completely diminished[7]. Table 1 lists six major families of oral antidiabetics agents. They all share the common role of lowering blood glucose, but each targets a different cause of impaired glucose homeostasis.

Table 1. Treatment therapy for patients with diabetes.

Type	General function	Example
Biguanides	Increase insulin sensitivity in the liver, muscles and fat and decrease hepatic glucose production	metformin
Sulfonylureas Glinides	Stimulate insulin secretion directly by acting on β cells	glimepiride repaglinide
Alpha-glucosidase inhibitors	Reduce intestinal glucose absorption after meals	acarbose
Incretins (GLP-1 analogs) (DDP-4 inhibitors)	Stimulate insulin secretion after an oral glucose load via the incretin effect. Increase the incretins concentration and therefore the insulin secretion, by inhibiting the dipeptidylpeptidase-4 (DDP-4) responsible of the degradation of glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP)	ozempic saxagliptin
Gliflozins (SGLT2 inhibitors)	Cause an increase in glycosuria to eliminate glucose in the urine blood	empagliflozin
Thiazolidinediones (PPAR-γ agonists)	Increase the sensitivity of the muscles to insulin and reduce the production of glucose in the liver	pioglitazone

GLUCOSE HOMEOSTASIS

The plasma glucose concentration corresponds to blood glucose, or glycemia, and is constantly subject to significant fluctuations, such as after a meal, a period of fasting, or during an episode of stress. Physiologically, in a healthy state, blood glucose is maintained between 70 and 130 mg/dL and never exceeds 126 mg/dL in the fasting state and 199 mg/dL two hours after a glucose challenge[1,8]. This precise and constant regulation of blood glucose throughout the day is largely achieved by insulin, glucagon, epinephrine, growth hormone and cortisol.

Insulin is a hormone produced, stored, and secreted by β cells in the islets of Langerhans located in the pancreas. Insulin plays a hypoglycemic role by stimulating the entry of blood glucose into the cells. In the secretory granules of the β cell, insulin is stored as a single peptide chain called pro-insulin. Before being released into the bloodstream, pro-insulin is cleaved by two proteases into insulin and C-peptide[9]. β cells secrete equimolar amounts of C-peptide as a single peptide chain and insulin as two polypeptide chains (chain A: 21 amino acids and chain B: 30 amino acids) connected by two disulfide bridges[9] (Figure 1).

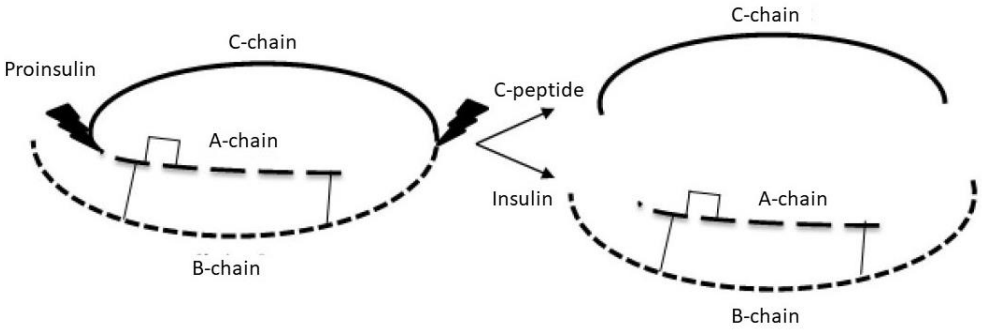

Figure 1. Structure of proinsulin, insulin, and C-peptide. The cleavage of proinsulin by two proteases releases two fragments into the bloodstream in equimolar quantities: insulin and C-peptide. Lightning bolts represent cleavage sites, straight lines represent disulfide bridges.

The secretion of insulin is initiated by the β cell in response to a rise in blood glucose. Glucose enters the β cell via GLUT2, a low affinity (Km 17 mM), high-capacity glucose transporter that allows the import of glucose by facilitated diffusion. Once inside the cell, glucose is immediately phosphorylated by glucokinase (GCK) to glucose-6-phosphate (G6P) and oxidized by glycolysis to pyruvate. Most of the pyruvate enters the mitochondria and, after metabolization and intervention of the oxidative phosphorylation pathway, produces ATP (Adenosine Triphosphate). This increased production of ATP increases the ATP / ADP (Adenosine Diphosphate) ratio and causes the closure of sensitive potassium channels (ATP-sensitive K+ channels: K+-ATP). Channel closure leads to membrane depolarization, which activates voltage-dependent calcium channels (Voltage-dependent Ca2+ channels: VDCC) and calcium entry. This leads to an increase in cytosolic calcium concentration and triggers exocytosis of insulin-filled granules[10,11] (Figure 2).

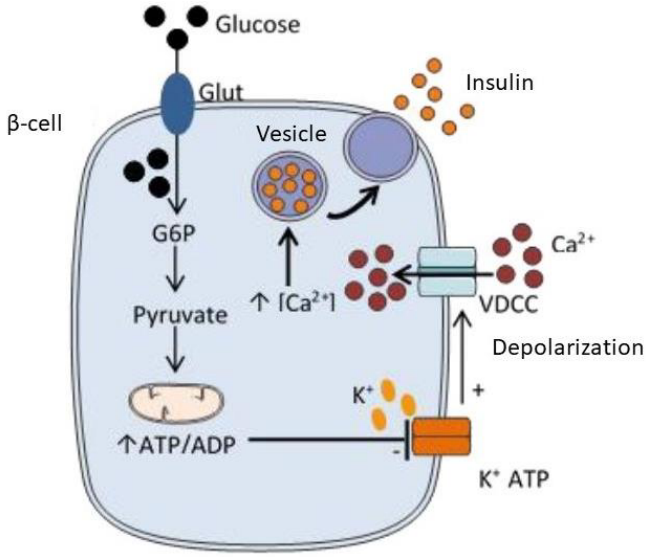

Figure 2. Schematic representation of one of the pathways triggering insulin secretion by the entry of glucose into the β cell. The entry of glucose into the β cell and the increase of ATP/ADP ratio stimulates insulin synthesis and secretion. GLUT2: Glucose transporter 2; G6P: glucose-6-phosphate, ATP: Adenosine Triphosphate, ADP: Adenosine Diphosphate, K+ ATP: channels sensitive potassium channels, VDCC: voltage-dependent calcium channels, Ca2+: calcium. (Figure inspired by Detimary et al., 1996; Henquin, 2000).

The major functions of insulin are to stimulate glucose entry into cells (Figure 3), glucose metabolism, and lipid and protein storage. Insulin binds to specific membrane receptors (insulin receptor) located on muscle, fat and liver cell membranes and activates a signaling pathway that leads to the translocation of glucose transporters to muscle, fat (GLUT4) and liver (GLUT2) cell membranes. The presence of GLUT4 and GLUT2 at the cell membrane allows glucose to enter the cell. Through the entry of glucose into the cell, insulin also participates in the assembly of glucose molecules into glycogen in the liver and muscle to form an available energy store for the organism. In adipocytes, glucose is directed to the synthesis of fatty acids, which constitute another type of energy reserve[12].

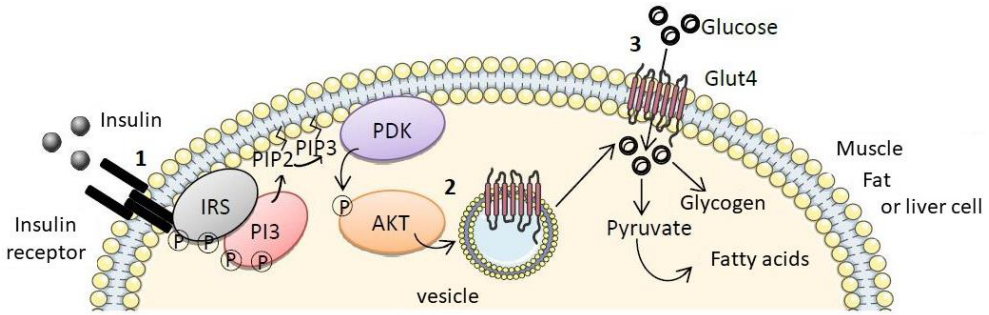

Figure 3. Schematic representation of the translocation of GLUT4 to the surface of the membrane in response to the liaison between insulin and its membrane receptor. Once GLUT4 is on the surface of the cell membrane, glucose enters and is metabolized. IRS: Insulin receptor substrate 1, PI3K: phosphoinositide 3-kinase, PIP2: phosphatidylinositol-4,5-bisphosphate, PIP3: Phosphatidylinositol (3,4,5)-triphosphate, PDK: Pyruvate dehydrogenase kinase, AKT: Protein Kinase B.

CRITERIA FOR THE DIAGNOSIS OF DIABETES IN PEDIATRICS

Diagnostic criteria

Because diabetes is characterized by elevated blood glucose levels, the diagnosis of all types of diabetes is based on blood glucose measurements and the presence of symptoms associated with hyperglycemic crisis. Blood glucose measurements include fasting plasma glucose (FPG), defined as no caloric intake for at least 8 hours, random blood glucose, and glycated hemoglobin (HbA_{1C}). A two-hour blood glucose value obtained from an Oral Glucose Tolerance Test (OGTT) can be used to screen for impaired glucose regulation but is not required if fasting or random blood glucose measurements are available. Therefore, in accordance with the American Diabetes Association (ADA) guidelines[1], criteria for the diagnosis of diabetes are presented in Table 2. Diabetes can be diagnosed after two fasting blood glucose tests ≥126 mg/dL. If a fasting blood test is not possible, another diagnostic option is to have two HbA1c levels ≥ 6.5%. A random blood glucose level ≥ 200 mg/dl with symptoms of hyperglycemia is also a sufficient diagnostic criterion.

Table 2. Criteria for the diagnosis of diabetes.

Fasting plasma glucose ≥ 126mg/dL (≥7.0 mmol/L).
And/or
Two-hour plasma glucose during OGTT ≥ 200mg/dL (≥11.1mmol/L)
And/or
HbA_{1C} ≥ 6.5% (≥48mmol/mol)
And/or
Classic symptoms of hyperglycemic with random glucose ≥200mg/dL

Classical symptoms

With the decline of β cells, insulin is no longer produced in sufficient amounts to regulate blood glucose. Excess glucose is therefore excreted by the kidneys and can lead to significant urine loss (polyuria). This significant loss of water creates a strong desire to drink (polydipsia), and the glucose-deprived body is constantly hungry and

draws energy from the adipose tissue, resulting in weight loss. Extreme fatigue, vomiting, blurred vision, and slow wound healing may also be symptoms of the disease. In severe cases, diabetic ketoacidosis (DKA) may occur as well as a coma. DKA is an acute metabolic complication of diabetes characterized by hyperglycemia, hyperketonemia, and metabolic acidosis[5]. Briefly, in the absence of insulin and glucose, triglycerides are metabolized to glycerol and free fatty acids for energy. Glycerol is used as a substrate for hepatic gluconeogenesis, and free fatty acids are converted to acidic ketone bodies. In addition, in the absence of insulin, ketogenesis is not inhibited and strong organic acids (acetoacetic acid and beta-hydroxybutyric acid) are produced, resulting in metabolic acidosis. Due to insulin deficiency, hyperglycemia causes osmotic diuresis with significant loss of fluid and electrolytes (sodium and potassium). Symptoms and signs of DKA include nausea, vomiting, abdominal pain, polyuria, lethargy, sometimes somnolence, and confusion. DKA can progress to cerebral edema, coma, and death. If DKA is suspected, arterial pH, serum and urine ketones, and anion metabolic gap are measured. According to ISPAD, the diagnosis of DKA is based on the triad of hyperglycemia, ketosis, and metabolic acidosis, and specific criteria are used to establish its presence. DKA is diagnosed by blood glucose >200mg/dL, arterial pH < 7.30, or serum bicarbonate < 15mmol/L (and ketonemia or ketonuria)[2,5].

Measures of glucose metabolism disorders

HbA$_{1C}$

Since 1977, glycated hemoglobin (HbA_{1C}) has been a global indicator of average blood glucose. HbA_{1C} is the fraction of hemoglobin (Hb) exposed to non-enzymatic glycation of the N-terminal part of the Hb A beta chain upon increasing blood sugar levels[13]. Given the life span of erythrocytes (120 days), the HbA_{1C} level is influenced by the blood glucose levels of the last 4 months. HbA_{1C} level is used as a diagnostic tool for diabetes, but also as a marker of diabetes control: its level reflects the average glycemic balance over the past 2 to 3 months. HbA_{1C} results are usually reported as a percentage of total hemoglobin (ideally between 4 and 6%) but can also be reported in International Federation of Clinical Chemistry units (mmol/mol)

using a conversion calculator at http://www.ngsp.org/convert1.asp[13]. However, this measure has some limitations. First, repeated hypoglycemia can decrease the HbA_{1C} level. In addition, abnormalities in hemoglobin can distort HbA_{1C} levels. For example, the presence of hemolysis, acute anemia, or chronic liver disease can artificially underestimate HbA_{1C} levels. These conditions shorten the lifespan of red blood cells. Conversely, a splenectomy, which prolongs the lifespan of red blood cells, artificially increases the HbA_{1C} level. Other causes of structural changes in Hb can also increase or underestimate HbA_{1C} levels, such as respectively thalassemia or sickle cell disease. Therefore, it is important to screen for hemoglobinopathies (e.g., sickle cell disease, an uncorrected iron deficiency, thalassemia) to ensure that the test is not interfered with.

Residual C-peptide secretion

C-peptide (or connective peptide) is a 31 amino acid molecule (3020 Da) derived from the enzymatic cleavage of proinsulin in the β cells of the pancreatic islets. This cleavage simultaneously releases the hormone insulin in equimolar amounts[9]. For this reason, the C-peptide assay can replace the insulin assay by indicating the amount of endogenous insulin produced in an insulin-treated individual[14]. The C-peptide assay is therefore used to estimate the ability of β cells to secrete insulin and can identify a potential β cell defect[15]. Normal fasting C-peptide secretion ranges from 0.26 to 1.29 nmol/L. If it is higher, it indicates hypersecretion of insulin by β cells. If it is lower, β cell function is impaired or destroyed. After oral administration of 75 g of glucose in the oral glucose tolerance test, the peak plasma value is approximately 5 to 6 times the baseline value.

Oral Glucose Tolerance Test (OGTT)

The oral glucose tolerance test is a screening test for diabetes and consists of the oral absorption of a standard amount of glucose with monitoring of the body physiological response by measuring blood glucose and insulinemia. This test is also required to identify glucose regulation disorders such as impaired glucose tolerance and hypersecretion of insulin. The OGTT is performed after an 8-hour overnight fast with a weight-based glucose load (1.75 g/kg for pediatric patients). Glucose and

insulin are measured in the fasting state and at 30, 60 and 120 minutes after glucose ingestion. Glycemia norm is less than 100 mg/dL and the insulin norm is 15 μU/ml at fasting and respectively 140 mg/dL and 80 μU/ml at the end of the test (after 2h). Diabetes is diagnosed when fasting glucose exceeds 126 mg/dL or when it is ≥200 mg/dL at 120'. With fasting measures of insulin and glycemia, the HOMA-IR formula (for homeostasis model assessments of fasting insulin resistance):

$$Ins0(\mu U/mL) \times Gluc0(mmol/L)/22.5$$

is used to identify insulin resistance[16,17]. The normal HOMA index is less than 1.6. A HOMA index between 1.7 and 2.3 defines a moderate form of insulin resistance, and a value ≥2.4 indicates a severe form of insulin resistance.

Glucometer and Flash glucose monitoring system

Diabetes management consists of frequent (at least six times a day) daily self-monitoring of blood glucose levels using a finger-stick blood glucose meter (glucometer) or by scanning a continuous glucose monitor (CGM) (e.g., FreeStyle Libre Flash Glucose Monitoring system). CGM is widely used in many parts of the world to optimize glycemic control, particularly in T1D[13]. Exercise can lower blood glucose levels, so monitoring should be more frequent on days when children exercise or are more active. Blood glucose levels should be checked at least before each meal and before a snack.

Glycemic variability scores

Recently, the $IDAA_{1C}$ and $GTAA_{1C}$ scores have emerged in diabetes research and have begun to be used in clinical practice. The $IDAA_{1C}$ score is used in conjunction with the $GTAA_{1C}$ score to determine levels of glycemic variability in terms of "daily insulin requirement" and "time in normoglycemic range" for adjustment of standard HbA_{1C} levels. The $IDAA_{1C}$ score is a clinical score described by Mortensen[18] whose formula is: HbA_{1C} (%) + (4 × insulin dose (U/kg body weight per 24 h). The $GTAA_{1C}$ score described by our center[19] has the following formula: HbA_{1C} (%) – [3 × % of normoglycemic values (70 – 180 mg/dL)]. The $IDAA_{1C}$ and the $GTAA_{1C}$ scores were calculated at least 18 months after diabetes diagnosis to avoid the influence of partial

remission. A score below 9 for $IDAA_{1C}$ and 4.5 for $GTAA_{1C}$ is considered to have low glycemic variability.

Diabetes diagnostic tools

MODY calculator

The MODY probability calculator (available at www.diabetesgenes.org) provides a probability of having monogenic diabetes to easily and quickly identify atypical diabetes in patients with diabetes and refer them for genetic analysis. This calculator is based on a clinical history suggestive of non-T1D and non-T2D and includes age of diabetes diagnosis, gender, body mass index, HbA_{1C}, insulin vs. oral antidiabetic therapy, duration of insulin therapy, ethnicity (white vs. non-white), presence of diabetes in a first-degree relative, and presence of other manifestations (renal cysts, deafness, partial lipodystrophy, severe insulin resistance in the absence of obesity, severe obesity with other syndromic features)[20,21].

MODY panel gene and whole exome sequencing

In Belgium, all patient suspected of having a genetic form of diabetes are tested for MODY gene sequencing, the so-called “classic MODY panel” which includes the most commonly mutated genes: *GCK, HNF1A, HNF4A, HNF1B, KCNJ11, ABCC8, and INS*. This sequencing is performed by the Centrum Medische Genetica Antwerpen (Antwerp University Hospital – Belgium). If no mutation or variant is found in this initial analysis, whole exome sequencing (WES) may be proposed, but this is not systematic and is mainly associated with research. WES can identify variants in genes known to be involved in a form of monogenic diabetes but can also discover new mutations or variants. WES has accelerated the understanding of the pathophysiology and clinical phenotype of diabetes and has been the first step in reclassifying diabetes and changing the treatment options available to patients with diabetes. When a variant or mutation is found in a gene, its pathogenicity can be assessed using the criteria established by the American College of Medical Genetics and Genomics (ACMG)[22]. This is a list of criteria that support the pathogenic effect of a variant based on epidemiologic, computational, or functional data.

Diabetes data registry in Belgium

Several countries, including Belgium, have "diabetes registries" whose purpose is to collect scientific data on diabetes. Since 1989, the Belgian Diabetes Registry (BDR) has been collecting epidemiologic information and blood samples from new Belgian cases of diabetes under the age of 40 and their first-degree relatives. The purpose of the BDR is to study all types of diabetes that occur in Belgium before the age of 40 and to contribute to the advancement of knowledge about the heterogeneity, etiology, prediction, and prevention of the disease[23-25].

These criteria, measures and diagnostic tools help to diagnose diabetes and provide evidence for the correct classification of diabetes. For example, if the cause of diabetes is an absolute deficit in insulin secretion due to autoimmune destruction of β cells, C-peptide and serologic tests will show the absence of endogenous insulin secretion and the presence of islet autoantibodies. In contrast, diabetes with preserved insulin production but an inadequate secretory response can be detected by fasting plasma glucose or oral glucose tolerance test (OGTT). These criteria are discussed in more detail in the section “classification of diabetes”.

CLASSIFICATION OF DIABETES

There are several pathogenic processes in the development of diabetes, and therefore there are several types of diabetes. Historically, in the 1950s, more than 95% of individuals presenting with symptoms of diabetes (excluding pregnancy) were classified into two subtypes: “insulin sensitive” for type 1 diabetes and “insulin insensitive” for type 2 diabetes[26,27]. Over time, research into the pathophysiology, genetic and clinical criteria of diabetes have continued to better define the subtypes and improve their classification. Currently, according to the ADA classification, diabetes can be classified into four broad categories (Figure 4): type 1 diabetes, type 2 diabetes, gestational diabetes, and diabetes due to other specific causes[1].

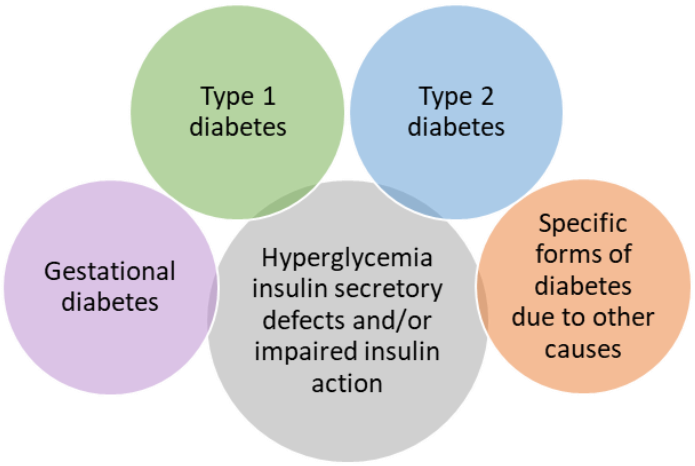

Figure 4. Classification of diabetes mellitus in four categories according to American Diabetes Association (ADA) and International Society for Pediatric and Adolescent Diabetes (ISPAD).

Most cases of diabetes can be classified into two categories: T1D, characterized by a deficit in insulin secretion, or T2D, characterized by insulin resistance and compensatory insulin secretion. Gestational diabetes is diagnosed for the first-time during pregnancy, in the second or third trimester and is associated with insulin resistance leading to a progressive defect in insulin secretion. This type of diabetes is not discussed in this work. In contrast, diabetes due to other specific causes will be the focus of the research in this thesis.

Type 1 diabetes (T1D)

Type 1 diabetes historically called "insulin-dependent diabetes" or "juvenile diabetes", accounts for 5-10% of diabetes in the general population but is the most common form of diabetes in children and adolescents accounting for >90%, with a peak incidence at 10-14 years[28]. T1D is one of the most common chronic diseases of childhood and it is estimated that in 2021, 651.700 children and adolescents are living with T1D worldwide[29,30]. In Belgium, according to the BDR, 42.637 individuals are living with T1D and about 10 new patients per 100,000 Belgian inhabitants under the age of 40 are diagnosed each year, which is comparable to neighboring countries[23].

This form of diabetes is characterized by the progressive and chronic autoimmune destruction of the insulin-secreting β cell[31]. This progressive destruction of β cell mass has a quite variable rate, mainly rapid in children and slow in adults, and leads to absolute insulin deficiency causing hyperglycemia and dependence on exogenous insulin.

Clinical symptoms of the disease (polyuria, polydipsia, weight loss) appear when 90% of the beta cells are destroyed, suggesting the presence of prior stage in the progression of disease[32]. According to the ISPAD guidelines, in the first stage, multiple islet antibodies are present with a normal glucose tolerance blood. The second stage is the onset of abnormal glucose tolerance, and the third stage is the onset of clinical symptoms[33] (**Figure 5**).

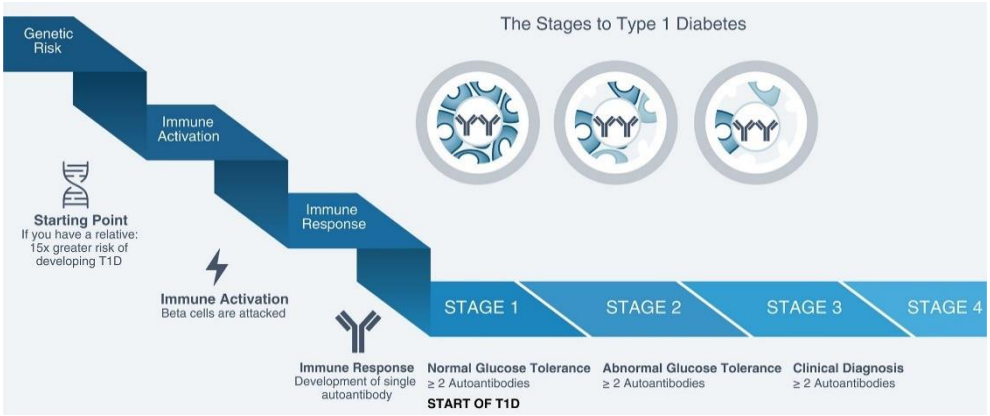

Figure 5. Type 1 Diabetes disease progression. Figure and text provided from ISPAD Clinical Practice Consensus Guidelines 2018. Stages of type 1 diabetes in children and adolescents, page 21. A proportion of individuals who have increased genetic risk of T1D progress at variable rates to immune activation and the development of islet autoimmunity. The development of two or more islet antibodies (stage 1) ultimately progresses to dysglycemia (stage 2) and then to symptomatic T1D (stage 3).

The initial mechanisms of the disease are not yet fully elucidated but given the presence of an autoimmune reaction involved in the destruction of β cells[34], serologic markers of the autoimmune process are present and include autoantibodies against insulin (IAA), glutamate decarboxylase (GAD65), the tyrosine phosphatases islet antigen (IA-2 and IA-2β) and zinc transporter 8 (ZnT8A). It is currently well established that the presence of at least 2 of these 4 types of autoantibodies means a 90% risk of developing T1D[35]. Data on ZnT8A are often lacking in older patients. As another marker of β cells destruction in T1D, C-peptide levels, a marker of endogenous insulin production, are almost undetectable in T1D patients, whereas endogenous insulin production is preserved or partially reduced in most patients with monogenic diabetes or T2D.

The pathological process of T1D begins months or even years before the start of the onset of the auto-immune process and is a complex interaction between predisposing genetic risk and environmental factors. Susceptibility to T1D is determined by strong HLA associations: the heterozygous haplotype DR3 (DRB1*0301-DQA1*0501-DQB1*0201) and DR4-DQ8 (DRB1*04-DQA1*0301-DQB1*0302) alleles have a higher risk of developing islet autoimmunity and T1D (30-fold increased risk)[36]. In this case, 30 to 50% of T1D patients had DR3/DR4 –

DQ8 genotypes[37]. Outside the HLA region, T1D is a polygenic disease, with more than 40 SNPs identified to be associated with T1D[38]. Still to support this strong genetic component in T1D, having a first-degree relative with T1D confers a 15-fold increased risk of developing the disease. However, at least 85% of children diagnosed with T1D do not have a family member with T1D[38-41].

Associated with predisposing genetic risk and still unclear, exposure to environmental factors during pregnancy and childhood has been linked to the autoimmune destruction of β cells in T1D. These risk factors include enterovirus infections such as CMV, rubeola and coxsackie[37,42,43] and certain nutrients such as cow's milk protein[44] and gluten[45,46].

After initiation of insulin therapy, 60% of pediatric T1D[47] patients experience a partial remission of the disease or "honeymoon period", as identified by Mortensen[18]. During this period, the pancreas is able to secrete insulin due to a small fraction of functional residual β cells and improves peripheral insulin sensitivity. As a result, insulin requirements decrease and stable blood glucose levels within the normal range are restored despite fluctuations in diet and exercise. Partial remission is defined by an insulin requirement <0.5 units/kg/day associated with an HbA_{1C} <7%[48]. Because of the transient nature of this phase, all β cell mass is finally destroyed after an average of 9 months and a maximum of 18 months[33,48].

For the diagnosis of T1D, the typical presentation is a child or adolescent, rarely obese, with severe hyperglycemia, islet autoantibody markers, undetectable plasma C-peptide and a classic history of increased polyuria, polydipsia, and weight loss over a period of 2 to 6 weeks. In a more severe form and often with rapid onset of T1D, the presence of DKA is the first manifestation of the disease. It is estimated that approximately 40 to 60% of patients with T1D have developed DKA[49].

Type 2 diabetes (T2D)

Type 2 diabetes, previously referred to as “non-insulin-dependent diabetes” or “adult-onset diabetes,” accounts for at least 90% of all diabetes in the general population. However, in pediatrics, its prevalence is much less common than T1D and accounts for only 5 to 10%, although it has been increasing in recent years[2,7]. This form of diabetes is characterized by a relative deficit in insulin secretion and peripheral insulin resistance. Unlike T1D, individuals with T2D do not have an autoimmune process leading to β cell destruction and therefore have preserved or partially reduced C-peptide levels (endogenous insulin secretion)[7].

The cause of T2D is a complex contribution of many relatively well identified lifestyle (environmental) factors and a genetic susceptibility that is not yet fully elucidated. The main lifestyle factors that increase the risk of insulin resistance and T2D are overweight or obesity, excessive energy intake and insufficient physical activity associated with sedentary behavior[50]. Children and adolescents may also have metabolic syndrome including obesity, insulin resistance, fasting blood glucose >126mg/dL, hypertriglyceridemia, low HDL cholesterol level and high blood pressure[1,7]. Susceptibility to T2D is supported by the finding of some variants identified as associated with T2D (e.g., *TCF7L2, MC4R, CDC123, KCNQ1, IGF2BP2, PHF2, and SLC16A11*)[51] (GWAS study). In addition, certain race/ethnic groups (e.g., Hispanics, African Americans, Indigenous Australians) have been reported to have a higher risk of T2D[52,53] (SEARCH study). The existence of a genetic risk for T2D is also supported by the presence of a high concordance rate of T2D in the same family (in first- and second-degree relatives) and even more so in monozygotic twins[54,55].

The pathophysiological process of T2D proceeds over many years and is characterized by the initial presence of insulin resistance in the liver, peripheral tissues, and adipose tissue, followed by compensatory insulin secretion leading to progressive loss of insulin-secreting β cells and, ultimately impaired insulin secretion with hyperglycemia[7,56]. Part of the pathogenesis of T2D is due to a mild chronic

inflammatory state. Chronic hyperglycemia and hyperlipidemia cause inflammation and stress on β cells, leading to their dysfunction and apoptosis and, at a later stage, to diabetic complications[57,58].

In most cases, at least initially, patients with T2D are not insulin dependent, and some healthy lifestyle measures (weight loss through intensive diet and exercise) are suggested to improve peripheral insulin sensitivity. Hypoglycemic agents (**Table 1**) may be needed to stimulate insulin secretion, increase tissue insulin sensitivity, reduce glucose uptake, or remove excess glucose. In addition, weight loss surgery can lead to diabetes remission in adults[59,60].

For the diagnosis of T2D, children or adolescents with T2D are often obese with insulin resistance, dyslipidemia, and hypertension and, unlike T1D, have an absence of islet autoantibodies and diabetic ketoacidosis and preserved but potentially reduced C-peptide secretion. The presence of a first-degree relative with T2D may facilitate the diagnosis. Acanthosis nigricans and polycystic ovary syndrome may be present, reflecting the presence of insulin resistance[1].

Two asymptomatic stages precede the onset of T2D: Impaired Fasting Glucose (IFG) and Impaired Glucose Tolerance (IGT). Both are used to define “prediabetes” and describe a disorder of glucose metabolism with insulin resistance and a compensatory increase in insulin production[1].

Prediabetes, impaired fasting glucose, and impaired glucose tolerance

Recently, the term “prediabetes” has emerged to categorize people who have an intermediate state between normal glucose homeostasis and diabetes. These individuals have elevated blood glucose levels but are not high enough to be classified as diabetes. People with prediabetes have an IFG and/or IGT and/or HbA_{1C} between 5.7% and 6.4% (39–47 mmol/mol)[61]. IFG and IGT represent abnormalities in glucose regulation with, respectively, fasting plasma glucose between 100–125 mg/dL (5.6–6.9 mmol/L) and plasma glucose 2 hours after a 75-g glucose challenge between 140–199 mg/dL (7.8–11.0 mmol/L) (**Table 3**)[1].

Prediabetes is therefore considered an indicator of the progression of impaired glucose metabolism and represents a high risk of developing diabetes and cardiovascular disease in the future. In 2022, approximately 18% of U.S youth have prediabetes[62] and it is estimated that 70 % of them will subsequently develop T2D[1,63]. In most cases, prediabetes is associated with obesity, dyslipidemia (high triglycerides and low HDL cholesterol) and hypertension. However, intensive lifestyle interventions (weight loss, healthy diet, and physical activity) and metformin can delay or prevent progression to T2D[64]. In contrast, prediabetes can also occur without the typical picture of metabolic syndrome. Indeed, in the context of anti-cancer or anti-organ rejection therapy, drugs can reduce insulin secretion by acting on the sensitivity of tissues to insulin or directly on β cells and can cause IFG and IGT. In these patients, pharmacological, and hygiene-dietary intervention remains necessary to avoid the development of diabetes.

Table 3. Criteria for normal glucose homeostasis, prediabetes and diabetes.

Blood glucose measurements	Normal	Pre-diabetes	Diabetes
Fasting plasma glucose (8h), mg/dL	≤ 99	100 - 125	≥ 126
2-hour post load glucose, mg/dL (During an OGTT)	≤ 139	140 - 199	≥ 200
HbA_{1C}, %	≤ 5,6	5,7 - 6,4	≥ 6,5
Random plasma glucose, mg/mL			≥ 200

Diabetes due to other specific causes

Diabetes due to other specific causes refers to all forms of diabetes other than those listed above. This category includes monogenic diabetes syndromes such as neonatal diabetes and maturity-onset diabetes of the young, exocrine pancreatic diseases such as cystic fibrosis, and diabetes induced by drugs such as anti-cancer or anti-organ rejection treatments[1].

For this research work, monogenic diabetes and drug-induced diabetes will be presented in more detail.

Monogenic diabetes

For many years, patients were classified into two main subgroups of diabetes, T1D or T2D, usually based on clinical criteria such as BMI, presence of islet autoantibodies and the age of onset. However, in 1974, Tattersall reported the first clinical case of a discrete group of familial non-insulin dependent diabetes in children and young adults[65]. Clinically, this group does not fit the classic clinical criteria of T1D or T2D and results from autosomal dominant mutations in a single specific gene involved in β cell function leading to its dysfunction[5]. These types of diabetes are referred to as monogenic diabetes.

Monogenic diabetes refers to diabetes caused by the mutation of a single gene involved in the development or function of the pancreatic β cell and includes maturity-onset diabetes of the young (MODY) and neonatal diabetes mellitus (NDM). MODY is the most common form of monogenic diabetes and typically occurs in adolescence or early adulthood whereas NDM occurs in neonates and young infants[66]. Monogenic diabetes accounts for a small portion of people with diabetes (<5%) and, in pediatrics, 6% of diabetes cases[67-72].

In most cases, the disease is inherited within families (from one of both parents) as a dominant, recessive, or non-Mendelian trait. Sometimes, the disease can occur spontaneously due to a *de novo* mutation[73]. To date, more than 50 genetic subtypes

have been described, each with a well-understood etiology and pathophysiological mechanisms that are better understood than the two common forms of diabetes described previously. In addition, several criteria have been established for the diagnosis of MODY and NDM based on the presence of certain unusual clinical features in T1D or T2D. For example, monogenic diabetes can be distinguished from T1D by the absence of islet autoantibodies and from T2D by the absence of insulin resistance. As each form of monogenic diabetes has a typical phenotype and specific complications, correct diagnosis and appropriate treatment are essential. However, monogenic diabetes remains misdiagnosed with approximately 80%[74] of all forms of monogenic diabetes misdiagnosed as T1D[75,76] or T2D[77,78].

Since the identification of the first clinical monogenic diabetes, advances in molecular genetics have identified several causative mutations for monogenic diabetes. The genetic etiology of all mutations causing β cell dysfunction has been and continues to be studied to describe the clinical phenotypes, features, treatment, extra-pancreatic manifestations, severity of hyperglycemia with its complications and prognosis of each form.

The diagnosis of monogenic diabetes is based on genetic analysis. Due to decreasing costs and increasing capabilities of next-generation sequencing (NGS), genetic analysis is becoming easier and faster. Currently, in Belgium, when monogenic diabetes is suspected, DNA of patient is genetically evaluated for the most common forms of MODY, according to the classic MODY panel (Belgium - Anvers). Whole exome sequencing is possible in case of strong suspicion of uncommon forms of monogenic diabetes or for scientific studies and increases the possibility of diagnosing monogenic diabetes. The ADA suggests that a diagnosis of monogenic diabetes should be considered in children with the following characteristics: 1) diabetes diagnosed in the first 6 months of life, 2) family history of diabetes without type 2 diabetes risk factors (non-obese, low-risk ethnicity), 3) mild fasting hyperglycemia if young and non-obese, and 4) diabetes with negative autoantibodies and without evidence of obesity or insulin resistance[1].

Neonatal diabetes mellitus (NDM)

Neonatal diabetes (or congenital diabetes) is a rare condition (1/90 000 to 1/400 000 infants)[79] that may be temporary (transient neonatal diabetes mellitus; TNDM; 25%) or lifelong (permanent neonatal diabetes mellitus; PNDM; 75%)[80]. This form of monogenic diabetes is caused by a single gene mutation that affects the development, function, or destruction of pancreatic β cell[75] and leads to the onset of hyperglycemia within the first 6 months to 12 months of life[81]. Because of its early onset, NDM is often confused with autoimmune T1D, but most cases of NDM occur before 6 months of age, whereas T1D is extremely rare before the age of 6 months[82-84]. Diabetes may be present isolated or associated with other clinical manifestations. At least 25 different mutations have been described and the most common and characterized forms are *ABCC8* and *KCNJ11* described below.

Most **Transient neonatal diabetes** cases are due to abnormalities in an imprinted region on chromosome 6q24 (~70%, *PLAGL1/HYMAI*)[85] and heterozygous activating mutation of the genes encoding the Kir6.2 subunit (*KCNJ11*; chr.11p15.1) or the SUR1 subunit (*ABCC8*; chr.11p15.1) of the β cell KATP channel (~25%)[86]. Anomalies in the genes *HNF1B* (chr.17q21.3)[87], *INS* (chr.11p15.5)[88] and *ZFP57* (chr.6p22.1)[89] are also causes of PNDM but represent a minority of cases. Except for 50% of 6q24 TNDM cases in which hyperglycemia recurs later in life, TNDM resolves between 6 and 18 months and can be treated with hypoglycemic agents instead of insulin therapy[90-94].

About **anomalies in 6q24 locus**, three different molecular mechanisms of imprinted gene overexpression have been reported to date[95]: complete or partial paternal uniparental disomy of chromosome 6, unbalanced paternal duplication of 6q24 and abnormal methylation of the maternal allele[96]. During the first week of life, infants with 6q24 abnormalities develop severe hyperglycemia without DKA[97] and require insulin, but quickly tapered to finally require any treatment by a median age of 12 weeks[96]. Recurrences of hyperglycemia have been reported in 50% of cases and the presentation is similar to T2D[98].

In **Permanent neonatal diabetes**, most cases are caused by an autosomal dominant mutation in *KCNJ11* (chr.11p15.1) or *ABCC8* (chr.11p15.1)[99]. According to Hattersley and his team, approximately 50% of PNDM cases are caused by these two dominant mutations[67]. At least 20 genetic subtypes have been identified as PNDM by affecting pancreatic β cell development, function, or destruction such as *INS* (chr.11p15.5)[88], *FOXP3* (IPEX syndrome; chr. Xp11.23-p13.3)[100,101], *GCK* (chr.7p15-p13)[102,103], *PDX1* (chr.13q12.1)[104] or *NEUROD1* (chr.2q32)[105]. Mutations in the pre-proinsulin gene (*INS*) are the second most common cause of PNDM and usually result in misfolding of the proinsulin molecule, which is trapped and accumulated in the endoplasmic reticulum, leading to endoplasmic reticulum stress and β cell apoptosis[106-110]. Depending on the mutated gene, PNDM may be isolated or associated with pancreatic aplasia/hypoplasia and other extra pancreatic manifestations (renal cysts, congenital heart, liver dysfunction, brain malformations). Treatment of PNDM depends on the mutated gene. Approximately 90% of patients with *KCNJ11* and ABCC8 mutations can be transferred from insulin therapy into sulfonylurea[111-113]. All other PNDM must be treated with exogenous insulin injections.

The KATP channels are formed by four pores forming Kir6.2 subunits encoded by the *KCNJ11* gene, and four SUR1 regulatory subunits encoded by the *ABCC8* gene[114,115]. In normal physiological situations, any increase in glucose in the β cell causes an increase in its intracellular ATP/ADP ratio, which allows the closure of KATP channels. This leads to a depolarization of the β cell plasma membrane, which opens voltage-dependent calcium channels (VDCC). The rapid entry of extracellular calcium into the β cell leads to an increase in cytosolic calcium concentration and triggers the exocytosis of insulin-filled granules[10,11,115,116]. Normal insulin secretion from pancreatic β cell in a high plasma glucose environment is illustrated in **Figure 6** (chapiter 1 – Glucose Homeostasis). In NDM caused by mutations in *ABCC8* and *KCNJ11*, the KATP channel stays open which maintains the membrane hyperpolarized and therefore prevents the insulin secretion[86,117-119]. NDM caused by *KCNJ11* gene are always heterozygous and in 90% of cases the mutations arise *de novo*. However, the risk of offspring being affected is 50%. For the *ABCC8* gene, mutations are also mostly *de novo*, and because NDM can be recessive, the risk of

future siblings being affected is 25% and the risk of offspring being affected is almost nonexistent[120]. For these two mutated genes, clinical features suggest hyperglycemia, insulin dependence with low or undetectable C-peptide levels, and frequent presentation of DKA[118,121]. For the treatment, high doses of sulfonylurea can overcome these defects, allowing restoration of meal-stimulated insulin secretion with a minimal presence of hypoglycemia[111-113,122]. Even after 10 years of sulfonylurea therapy, patients maintain excellent glycemic control[122].

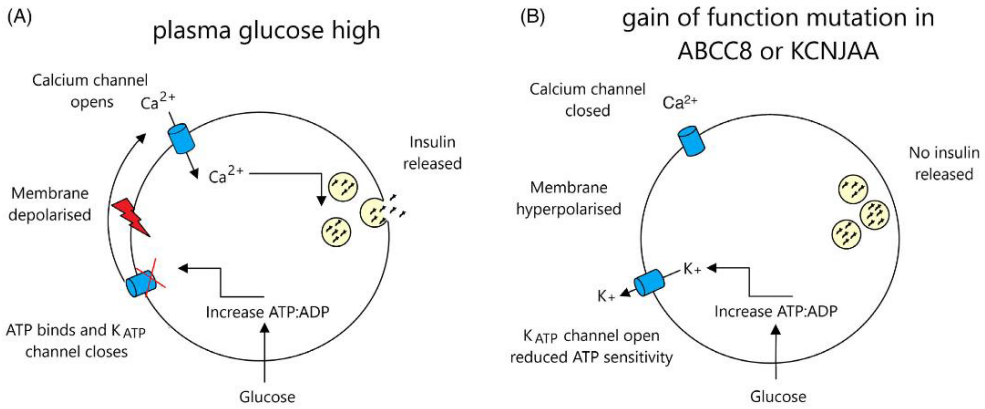

Figure 6. Insulin secretion from the pancreatic beta cell in (A) normal cell in a high plasma glucose environment and (B) in a cell with a K-ATP channel mutation. (A) Glucose enters the cell and is metabolized, causing an increase in ATP, K-ATP channel closure is induced via ATP binding, the membrane is depolarized, and calcium influx is triggered resulting in the release of insulin from its storage vesicles. (B), A gain of function mutation in the K-ATP channel results in the failure of ATP to bind to the channel, causing the channel to remain open, the membrane stays hyperpolarized and no insulin is released. Figure and text provided from the 2018 ISPAD Clinical Practice Consensus Guidelines: 'The diagnosis and management of monogenic diabetes in children and adolescents', page 52. ISPAD adapted its figure from article published by Edghill EL et al. in the journal Reviews in Endocrine and Metabolic Disorders Vol 11 (2010).

In general, signs of frequent urination, low birth weight, rapid breathing, hypovolemia and dehydration are often present in the setting of NDM but are difficult to recognize and attribute to NDM. However, an elevated blood and urine glucose level before 6 months of age should alert to the possibility of neonatal diabetes. Also, a complete absence of insulin may cause DKA and show a reduced or absent C-peptide level. In addition, islet autoantibody testing is negative, and some extra-pancreatic manifestations may be associated depending on the mutated gene. In some cases, mutated genes are related to immune function, such as *FOXP3,* and cause multiple autoimmune disorders. To exclude autoimmune T1D and to confirm the presence of

a mutated gene, islet autoantibody testing (insulin, IA-2, GAD65, and Znt8) and genetic testing should be performed.

Maturity onset diabetes of the young (MODY)

MODY is rare compared to T1D and T2D and is thought to account for 2-4% of children and young adults with diabetes. However, the prevalence of MODY in the general population is difficult to determine because this clinical entity is most frequently (in 80% of cases) misdiagnosed as T1D or T2D due to their similarity[74,77,123,124].

MODY is caused by autosomal dominant mutations (or changes) in a single gene involved in β cell development or function[125], resulting in the onset of hyperglycemia at an early age, usually before the age of 25[83]. Because MODY is characterized by autosomal dominant inheritance, if one parent carries the genetic mutation, the child has a 50% chance of inheriting it. However, sporadic *de novo* mutations have been reported in the *GCK, HNF1A and HNF4A* genes[73]. To date, at least 14 gene mutations have been described to cause MODY, each affecting a well-understood molecular mechanism. Many MODY genes, but not all, are also associated with NDM, such as *ABCC8*[126] and *KCNJ11*[127]. In Europe, four genes account for the majority of MODY cases (**Figure 7**). These genes encode the enzyme glucokinase (GCK-MODY) (Pearson et al., 2001)[128] and transcription factors expressed in pancreatic β cells, including hepatocyte nuclear factor-4α (HNF4A-MODY1) (Yamagata et al., 1996a)[129], hepatocyte nuclear factor-1α (HNF1A-MODY) (Yamagata et al., 1996b)[130] and hepatocyte nuclear factor-1β (HNF1B-MODY) (Kristinsson et al., 2001)[131]. Other cases correspond to rare mutations in *PDX-1* (<1%)[132] and *NeuroD1* (<1%)[131]. The prevalence of MODY subtypes varies between ethnic groups. In Caucasians, *GCK*- and *HNF1A*-MODY are the most common causes of MODY (30-50% and 30-65%, respectively)[74,133]. Sometimes, no genetic etiology is found[134] and these cases of patients are called MODY-X. In Caucasians, MODY-X accounts for 16-45% of MODY cases[134]. Diabetes may be isolated or associated with other clinical manifestations such as renal cysts in cases of mutation or deletion of *HNF1B*[135-137].

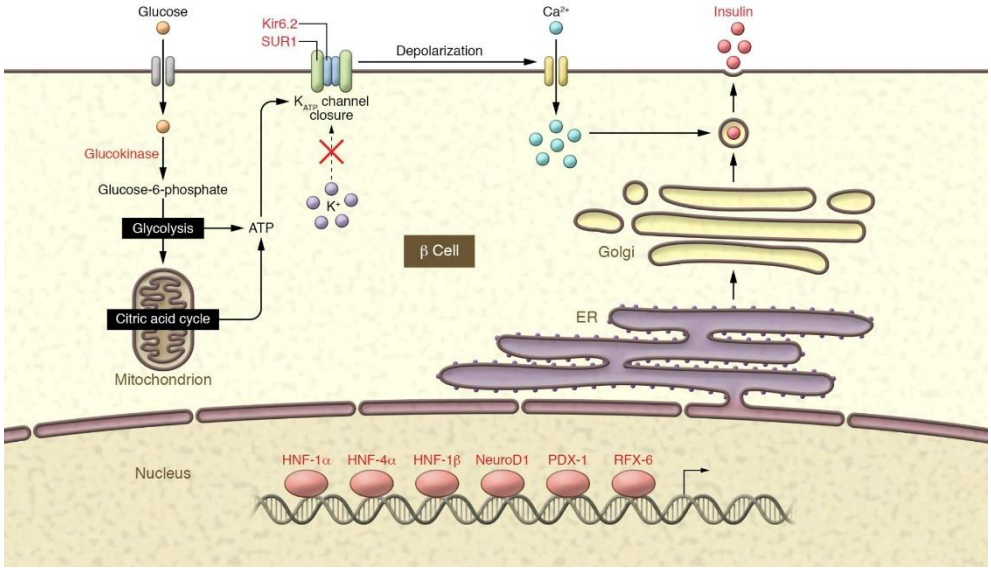

Figure 7. Schematic representation of pancreatic β cell insulin secretion and the genes involved in monogenic diabetes. The genes shown in red are the most common forms of monogenic diabetes. Glucokinase (GCK) phosphorylates glucose to glucose-6-phosphate when glucose enters the β cell. The pancreatic KATP channel, encoded by Kir6.2 and SUR1, regulates insulin secretion. The increase in the ATP/ADP ratio leads to the closure of the KATP channel and causes depolarization of the β cell membrane and activation of voltage-gated calcium channels. Calcium enters the cell and triggers the insulin release from the β cell. Transcription factors (HNF1A, HNF4A, HNF1B, NEUROD1, PDX1, and RFX6) form a network that regulates insulin expression and β cell development and proliferation. Figure published by Haichen Zhang, Kevin Colclough et al. in the Journal of Clinical Investigation (2021).

The clinical manifestations of MODY are variable and depend on the mutated gene. In adolescence or young adulthood, a moderate elevation of fasting blood glucose (110 to 140 mg/dL) may be detected incidentally or during screening in a family with known MODY. Hyperglycemia may also remain silent indefinitely or worsen, leading to the development of characteristic signs of diabetes (polyuria, polydipsia) that are not specific to MODY. In some MODY subtypes, hyperglycemia occurs during pregnancy or after stress (infection) or with weight gain and aging. Several criteria, not all of which are measurable at the time of diabetes diagnosis, may indicate the presence of monogenic diabetes. All MODY subtypes can be distinguished from T1D by the absence of islet autoantibodies[81,138] and most by the absence of ketoacidosis[124]. In addition, outside of the remission phase, MODY patients require lower doses of exogenous insulin to maintain normoglycemia due to the presence of residual C-peptide secretion[139]. Furthermore, HbA_{1C} levels remain stable over time in most subtypes and are often below 7%[139]. Compared to T2D, the MODY patient

is not systematically obese and differs in the absence of insulin resistance and acanthosis nigricans[83].

In conjunction with the criteria described by Shield[21], Hattersley and the Exeter Center developed in 2012 a prediction tool for the common forms of MODY (*GCK*, *HNF1A* and *HNH4A*), called the MODY Probability Calculator (MPC), available on the website http:// www.diabetesgenes.org (accessed in February 2023). This calculator is based on the suggestive clinical history of non-T1D and non-T2D and includes the following criteria: age at diabetes diagnosis, current patient age, gender, body mass index (BMI), HbA_{1C}, insulin vs. oral antidiabetic therapy, duration of insulin therapy, ethnicity (white or non-white), presence of diabetes in a first-degree relative, and presence of other manifestations (renal cysts, deafness, partial lipodystrophy, severe IR without obesity, severe obesity with other syndromic features). Although this calculator includes reliable criteria that suggest the presence of MODY, it remains incomplete and has some weaknesses that will be discussed in Chapter 2 (State of the art).

Unfortunately, some patients with MODY do not consistently meet the criteria mentioned above. Therefore, the only way to establish a correct diagnosis of MODY is through genetic testing. It is important to note that apart from the well-known monogenic forms of diabetes, there is little information to establish the causality of rare variants in uncommon subtypes (MODY-X). For molecular diagnosis, DNA from patients with potential MODY is submitted to the 'classic MODY panel', which includes the most common forms of monogenic diabetes such as *GCK*, *HNF1A*, *HNF4A* and *HNF1B*, accounting for more than 80% of all known MODY cases[140]. Rapid and accurate molecular diagnosis of MODY is essential for therapeutic decisions, prognosis, and family screening[83]. However, each subtype of MODY differs in its clinical features, hyperglycemic profile, and response to treatment.

The *GCK* gene mutation increases the glucose threshold required for insulin release from pancreatic β cells[141]. Individuals with this subtype of MODY have few or no symptoms and may be detected incidentally with mild fasting hyperglycemia. Because this hyperglycemia does not worsen and remains stable over time, MODY-

GCK patients do not benefit from hypoglycemic therapy[142] and do not have the micro- and macro complications of diabetes typically seen in other forms of diabetes (T1D, T2D)[143,144]. Treatment is based on blood glucose and HbA_{1C} monitoring. In some cases, when the HbA_{1C} level exceeds 6.5%, a low-dose oral antidiabetic agent is suggested.

Mutations in hepatic nuclear transcription factors (HNFs) that control pancreatic development include *HNF1A*, *HNF4A* and *HNF1B*. These transcription factors also play a role in regulating β cell function, insulin production and secretion. *HNF1A*- and *HNF4A*- MODY are usually diagnosed with high blood glucose due to progressive β cell dysfunction, which reduces the amount of insulin secreted and causes characteristic signs of diabetes such as polyuria and polydipsia[145]. People with *HNF1A*- or *HNF4A*-MODY usually respond well to low doses of sulfonylureas[146,147]. In some cases, insulin may be needed over time, either alone or in combination with oral antidiabetic agents. Individuals with HNF1B-MODY have a defect in β cell development (pancreatic hypoplasia)[148] resulting in insulin deficiency[149]. They also have hepatic insulin resistance[150] and renal cysts, sometimes associated with genital and urinary tract abnormalities (renal cysts and diabetes [RCAD] syndrome)[135,137,149,151]. These patients require insulin therapy[152] and are followed by a nephrologist for renal disease. For the three HNFs-MODY, the risks of microvascular and macrovascular complications are similar to those observed in people with T1D and T2D[153,154].

In general, a patient presenting with elevated blood glucose before the age of 25 years, without islet autoantibodies and without DKA at diagnosis should raise the possibility of MODY. In addition, some extra-pancreatic manifestations may be associated depending on the mutated gene. As with NDM, islet autoantibody testing (insulin, IA-2, GAD65, and Znt8) and genetic testing should be performed to exclude autoimmune T1D and to confirm the presence of a mutated gene. **Table 4** lists the most common forms of MODY and their characteristics.

Table 4. Characteristics of GCK, HNF1A, HNF4A and HNF1B-MODY.

	***GCK*-MODY**	***HNF1A* / *HNF4A*-MODY**	***HNF1B*-MODY**
Gene function	Phosphorylation of glucose in position 6 Glucose sensor Glucose storage	Transcription of genes that regulate the function of protein. - GLUT2 - Pyruvate kinase, - Insulin Tumor suppressor gene	- Expressed very early in embryonic life. Organogenesis of the urinary and genital tracts, liver, bile ducts and pancreas - Expressed in adulthood. Gene transcription (kidney, pancreas)
Consequence	↑ glycemia threshold ↓ hepatic glycogen synthesis ↑ gluconeogenesis	↓ insulin secretion ↓ glucose reabsorption Benign hepatocellular adenoma leading to haemorrhage or carcinoma	Renal cysts, severe renal failure Atrophy of the pancreas Biological abnormalities of the liver without impaired function Genital abnormality
Presentation of diabetes	Stable β cell function	Progressive β cell dysfunction	Defect on β cell development
Evolution	Moderate, stable hyperglycemia	Strong increase of glycemia	Strong increase of glycemia
Complication	No complication	Angiopathy like T1D/T2D	Angiopathy like T1D/T2D Extra-pancreatic defects
Treatment	No treatment or sulfonylureas	Low dose of sulfonylureas	Insulin therapy and specific medical follow-up (kidney)

Drug-induced diabetes

The ‘drug-induced diabetes’ or ‘diabetes secondary to drugs’ occurs after the use of medication that reduce insulin secretion or insulin action or, can cause permanent β cell damage[155]. The onset of drug-induced diabetes, according to studies mainly conducted in an adult population, depends on the dose of the drug administered, duration of treatment, family predisposition (family history of diabetes, glucose intolerance), advanced age (over 45 – 50 years), body mass index (BMI) and pre-existing glucose intolerance to diabetogenic drugs[156,157]. In pediatrics, few studies have been conducted in children, and adult data and risk factors cannot be extrapolated to children. The majority of diabetogenic drugs are immunosuppressants and are prescribed for the treatment of cancer or transplant rejection. In this context, these two diabetogenic therapies will be developed for this research work.

Diagnostic criteria for drug-induced diabetes are based on the same ADA guidelines as for all forms of diabetes (see “Diagnostic criteria for diabetes in pediatric patients’ diagnosis”)[1]. Patients treated for cancer or after organ transplantation presented hyperglycemia when fasting plasma glucose (FPG) or random plasma glucose (PG) levels exceeded 126 mg/dL (7.0 mmol/L) and 200 mg/dL (11 mmol/L), respectively, for at least two measurements separated by 24 hours, and not under stressful condition such as on the day of transplantation[1]. However, hyperglycemia induced by medication may be transient. In this context, the term “transient hyperglycemia” is used to define patients with hyperglycemia without a diagnosis of overt diabetes. If the patient requires ongoing treatment for hyperglycemia (i.e., insulin or oral antidiabetic agents), the patient has overt diabetes.

In addition to the well-known features of diabetes, patients may have an intermediate state between normal glucose homeostasis and diabetes (prediabetes), including glucose intolerance or impaired fasting glucose. The gold standard for identifying patients with insulin resistance and insulin hypersecretion is the oral glucose tolerance test. According to ADA guidelines, if the patient has an IFG, IGT or HbA_{1C}

between 5.7 and 6.4%, the patient has “prediabetes”. If the patient has an FPG ≥ 126 mg/dL (7.0 mmol/L), HbA_{1C} > 6.5% (48 mmol/mol), a random PG or 2-hour PG during OGTT > 200 mg/dL (11.1 mmol/L), or presents classic symptoms of hyperglycemia, he has diabetes[1].

Diabetes due to hemato-oncology treatment protocols

Pediatrics cancers

Childhood cancer is a rare pathology, accounting for only 1% of all cancers. Cancer develops when one of the other cells suddenly multiplies uncontrollably and lives longer than normal cells. Successive changes in the genes of the diseased cell cause dysfunctions in the regulation of the cell multiplication process. In most childhood cancers, the etiology or triggering factors are not yet understood[158]. According to the Belgian Cancer Registry and the Belgian Society of Paediatric Haematology Oncology (BSPHO), 13 new cases of cancer are diagnosed each year in Belgium per 100.000 children under the age of 15. This means that each year in Belgium, approximately 320 children under the age of 15 and 180 adolescents between the age of 15 and 19 develop cancer[159,160]. The Department of Pediatric Hematology and Oncology at the Saint-Luc University Clinics ‘Institut Roi Albert II’, treats 25% of new cases of childhood cancers, which corresponds to approximately 75 new cases per year[161].

The most common cancers in children are leukemia and lymphoma. They account for 45% of all childhood cancer. Leukemia is a cancer of the white blood cells[160]. In this cancer, the abnormal and uncontrolled production of immature white blood cells fills the bone marrow and prevents the production of normal blood cells. There are two types: acute lymphoblastic leukemia (ALL) and Acute myeloid leukemia (AML). ALL is the most common malignancy in children, with an incidence of approximately 4/100 000 children per year (<16 years of age). Lymphoma is a cancer that starts in the lymphatic system, which includes the bone marrow, thymus, spleen, and lymph nodes. There are two types of lymphoma, depending on the type of cells that become cancerous: Hodgkin’s-lymphoma (HL) or non-Hodgkin’s lymphoma (NHL). HL is characterized by the presence of an abnormal proliferation

of lymphocytes and specific abnormal cells called Sternberg cells, which distinguishes it from NHL[162].

Cancer therapy protocols

Childhood tumors are particularly sensitive to chemotherapy and radiotherapy and regress very quickly with treatment. Chemotherapy includes drugs that block or destroy the division and multiplication of cancer cells. Chemotherapy does not discriminate between cancer cells and normal healthy cells. Radiation therapy uses very high-energy X-rays to damage the tumor or destroy fast-growing cells while causing minimal damage to normal cells. Hematopoietic stem cell transplantation (HSCT) may be useful for some high-risk leukemias and lymphomas. Stem cell transplants allow the production of mature blood cells, which improves survival. Stem cells are harvested from bone marrow or blood, either from the patient himself (autologous transplant) or from a family member or unrelated donor (allogeneic transplant). This is followed by high doses of chemotherapy and total body irradiation (TBI) to destroy the remaining cancer cells and stem cells in the bone marrow. Today, 80% of children with cancer are cured with these treatments[163].

In Belgium, pediatric patients with ALL, HL and NHL are treated with chemotherapy and radiotherapy according to international guidelines. Several protocols have been used for the three pathologies, depending on the era of treatment, the severity of the disease, the age of the patient and the response to treatment. Despite some differences in protocols in the same cohort, the treatment pattern remains unchanged. For ALL patients, the theoretical treatment lasts at least two years and begins with a pre-phase with introduction during seven days of steroids, followed by induction with twenty-one days of steroids, consolidation, interval, reinduction also with twenty-one days of steroids, and finishes with a maintenance phase, which sometimes includes steroids (**Figure 8**). Treatments for NHL and HL are much shorter than for ALL, lasting a maximum of six months. If an ALL patient shows steroid-resistant disease at the end of the pre-phase, the protocol is intensified with an extended consolidation phase with longer doses of steroids and L-asparaginase.

For ALL patients who relapse during treatment or have abnormal cytogenetics, HSCT may be considered, some of them with TBI.

		Pre	Induction	Consolidation	Interval	Reinduction	Maintenance
	Duration (days)	7	28	28	56	49	518
VLR	Steroids (days)	7	21	-	-	21	-
	Aspara (days)	-	2	-	-	1	-
AR	Steroids (days)	7	21	-	-	21	42, for AR and AR-T
	Aspara (days)	-	2	-	-	1	6 for AR-B/T

		Pre	Induction	1°Consolidation	2°Consolidation	Interval	Reinduction	Maintenance
	Duration (days)	7	28	41	13	2x42	2x49	ends at 2 years after day 1
VHR	Steroids (days)	7	21	-	5	-	42	-
	Aspara (days)	-	2	1	1	-	1	-

VLR=Very Low Risk; AR=Average Risk; VHR=Very High Risk; HSCT=Hematopoietic Stem Cell Transplantation; B=immune B cell; T=immune T cell

→ HSCT after 2° consolidation if the patient is qualified for and if HLA compatible donor is available.

Figure 8: Theoretical treatment protocols of steroid and asparaginase for acute lymphoblastic leukaemia patients.

Cancer therapy and diabetes

Endocrine disorders represent the most important long-term sequelae of childhood cancer survivors as previously described in our Departments of Pediatrics and Internal Medicine[164-166]. There is growing evidence that these patients have an increased risk of developing metabolic syndrome, diabetes, and prediabetes. In a recent Australian series of 248 cancer survivors with ALL, AML, lymphoma, or solid tumors, 23% of patients (vs 1% controls) treated during adolescence later developed insulin resistance, impaired glucose tolerance or diabetes[167]. Treatments that present a risk of developing insulin resistance, impaired glucose tolerance, and diabetes during mid- and long-term follow-up are hematopoietic stem cell transplantation with total body irradiation (TBI)[168], cranial irradiation[166], treatment with glucocorticoids[169] and pancreatic irradiation[170]. Of these medications, glucocorticoids are by far the most problematic, with an estimated 10-45% of ALL survivors treated with glucocorticoids as children developing metabolic side effects such as weight gain, fat distribution, hypertension, dyslipidemia, insulin resistance and hyperglycemia[171]. Asparaginase and glucocorticoids are responsible for drug-induced diabetes in up to 42% of ALL patients[172]. In a study of pediatric patients

receiving prednisone and L-asparaginase for ALL, hyperglycemia was observed in 9.7% (41/421)[173].

Diabetes due to anti-rejection treatments

Pediatric liver and renal transplants

Kidney and liver transplantation is the treatment of choice for patients with end-stage renal or hepatic failure. The most common pediatric conditions requiring liver transplantation are biliary atresia, hepatoblastoma, cholestasis and fulminant hepatitis (e.g., viral, toxic)[174]. Thanks to the ability of the liver to regenerate, several transplantation techniques have been developed to perform living donor transplantation (DOVI). This technique, developed at the Cliniques universitaires Saint-Luc (CUSL), in the Pediatrics Department, consists of removing 20% of the liver from the donor, usually one of the parents with ABO system compatibility, and transplanting it into the child. The donor liver regenerates rapidly to regain its initial volume in a few months and the recipient graft quickly assumes normal physiological volume and develops as the child grows[175,176]. Unlike the liver, the kidney does not have the ability to regenerate. Although living donor kidney transplantation is more feasible because the human body can live with only one kidney, it is less common than cadaveric transplantation. According to Eurotransplant data for 2021, there were 417 kidney transplants in Belgium, including 358 cadaveric transplants and 59 living donor transplants[177]. For kidney transplants in children, transplants from living donors are less frequent due to a size mismatch between donor and recipient[178]. While waiting for a kidney transplant, patients can be treated with dialysis (peritoneal or hemodialysis), which replaces kidney function and prevents the accumulation of toxic metabolites and excess water present in the body. Nevertheless, transplantation is the most appropriate treatment for the child's development at the physical and social level[179].

Anti-organ rejection treatment protocols

Every transplant carries the risk of graft destruction by the recipient's immune system[180]. To prevent this rejection, transplant patients benefit from

immunosuppressive therapies that reduce the body's immune response through various mechanisms of action. At CUSL, pediatric liver (LT) and renal (RT) transplant patients receive a standard immunosuppression protocol according to international guidelines[181]. For LT patients, this protocol includes the combination of an anti-CD25 monoclonal antibody (basiliximab, Simulect®) and a calcineurin inhibitor (tacrolimus, Prograft®)[182,183]. For pediatric patients with RT, this protocol is based on the combination of tacrolimus, glucocorticoids, Simulect, and a cell proliferator inhibitor such as mycophenolate mofetil (Cell-Cept®).

Oral tacrolimus administration is high during the first two months of liver transplantation (target blood levels of 8-10 ng/mL) and during the first three weeks of renal transplantation (target blood levels of 10-12 ng/mL) and gradually decreases (LT: 6-8 ng/mL the third month and 4–6 ng/mL between three months and one year; RT: 8-10 ng/mL between D22 and D60 and 5–8 ng/mL after sixty days) to a lifelong maintenance dose depending on the patient's clinical evolution (LT:1-3 ng/mL; RT: 4-6 ng/mL after six months). For LT patients, steroids (Solumedrol®, Medrol®) are administered when acute cellular rejection (ACR) occurs, approximately between the 7th and the 14th day after transplantation. Patients receive high doses of steroids (5 mg/kg/day), which are gradually tapered until the third month after rejection (0.25 mg/kg/day) and space out at the sixth month (0.5 mg/kg/2 days). For RT patients, high doses of Solumedrol (125mg/m²) are administered intravenously during surgery and on the first day. Thereafter, oral prednisone is administered from the second to the fourth day at a high dose (60mg/m²/day) and gradually reduced (10mg/m² in the second month and 5mg/m² in the third month) until the sixth month, when its need is assessed. Glucocorticoid doses are increased when RT patients present ACR.

Anti-organ rejection treatment and diabetes

After solid organ transplantation, immunosuppressive therapies such as calcineurin inhibitors and glucocorticoids are used to prevent graft rejection. Their combined use has enabled organ transplantation and significantly improved graft survival and patient quality of life (morbidity and mortality). However, these anti-rejection drugs are also associated with side effects, including disruption of glucose homeostasis and post-transplant diabetes mellitus (PTDM), which affects a poorly defined

proportion (2-53%) of patients after solid organ transplantation[184-187]. However, diabetes is frequent (approximately 20% of patients) after kidney and liver transplantation[185,188,189]. Pediatric data on the risk of diabetes or prediabetes in children are approximate. The incidence is 4 to 25% in kidney transplant patients and 2.5 to 25% in liver transplant patients, and the risk of diabetes is higher in the first year after transplantation[190].

Molecules of interest involved in drug-induced diabetes.

The molecules described above play an essential role in anticancer or graft preservation. However, one of the important side effects of these treatments is the disruption of carbohydrate homeostasis and, in particular, the onset of hyperglycemia or the development of diabetes. The mechanisms of action of drugs that induce hyperglycemia and diabetes have been extensively studied and are now well understood. In pediatric oncology, treatment protocols include the use of L-asparaginase, high dose glucocorticoids, anticalcineurin inhibitors (cyclosporine, or tacrolimus), and sometimes total body irradiation in combination with HSCT. These molecules are well known to be associated with secondary or transient diabetes. This same is true for treatment after organ transplantation, where high doses of tacrolimus, cyclosporine and glucocorticoids are required to prevent graft rejection. It is important to note that the risk of hyperglycemia is difficult to attribute to a single molecule because drugs are always combined (e.g., glucocorticoids and tacrolimus after organ transplantation or radiation and sirolimus for a hematopoietic stem cell transplantation). In addition to diabetogenic drugs, the stress of surgery or infection and the use of intravenous dextrose can exacerbate hyperglycemia. **Table 5** lists the molecules of interest and their side effects on glucose metabolism.

Table 5. Molecules involved in drug-induced diabetes.

Therapies	Molecules	Side effects
Corticoids	**Prednisone, prednisolone**	**Insulin resistance + Weight gain**[206-209] ↓ uptake of peripheral glucose: GLUT4 is inhibited on muscle and adipose tissues cells ↑ hepatic production of glucose (gluconeogenesis) ↓ lipolysis **Decreased insulin secretion**[206] Inhibition of GLUT2 and potassium channel
Calcineurin inhibitors	**Ciclosporin Tacrolimus**	**Decreased insulin secretion**[212-215] Inhibition of NFAT dephosphorylation induced ↓ transcription of genes implicated in insulin secretion (GCK, GLUT2)
mTOR inhibitors	**Sirolimus**	**Inhibition of insulin signal transduction** Inhibition of mTOR protein which intervenes in the insulin signaling cascade + direct & indirect inhibit° of PI3K/AKT pathway
Asparaginase	**Erwinase L-asparagine Oncaspar**	**Decreased indirectly insulin production** by causing **pancreatitis**[192-194] L-Asparaginase deprive asparagine for insulin molecule
Irradiation	**Abdominal Total body**	**Locally destruction of pancreatic cells**[201]

L-Asparaginase

L-asparaginase is used in combination with other antineoplastic agents to treat pediatric patients with acute lymphoblastic leukemia[191]. L-asparaginase is a protein-based enzyme extracted from Escherichia coli (E. coli) cultures that destroys asparagine by hydrolysis. Asparagine is an essential amino acid for protein synthesis in most leukemic cells. However, they cannot synthesize it themselves and must use the extracellular form. Since L-asparaginase hydrolyzes asparagine, leukemic cells that are unable to synthesize asparagine endogenously are destroyed. There are three types of L-asparaginase: Two from E. Coli (native and pegylated) and one from E. chrysanthemi (Erwinia). The latter is equivalent to Erwinase and is used to treat patients who have developed hypersensitivity to E. coli-derived L-asparaginase. The

second, extracted from E. coli, is combined with polyethylene glycol corresponding to oncaspar (or PEGasparaginase) and is required in case of allergy to both forms of L-asparaginase, native to E. coli and from Erwinia[191].

Regarding its side effects on glucose metabolism, L-asparaginase may directly affect the function of the endocrine pancreas by causing pancreatitis. L-asparaginase-induced pancreatitis is known to affect 2 to 18% of ALL patients, resulting in the rapid development of diabetes[192]. In addition, by depriving insulin molecules of asparagine, it indirectly decreases insulin production. Plasma insulin levels and insulin secretion in response to hyperglycemia are reduced[193]. L-asparagine also appears to decrease the number of insulin receptors[194].

Total Body Irradiation (TBI) followed by Hematopoietic Stem Cell Transplantation (HSCT).

Radiation therapy uses very high-energy X-rays to damage the tumor or destroy fast-growing cells with minimal damage to normal cells. In some high-risk leukemias and lymphomas, TBI may be needed before HSCT to destroy remaining cancer cells. In the context of ALL, studies of childhood cancer survivors have shown that TBI is significantly associated with an increased risk of developing diabetes and impaired glucose metabolism, and that this risk is increased when TBI and HSCT are combined[167,168,195].

The main mechanism involved is directly related to the inflammation caused by exposure to very high-energy X-rays. With site-specific irradiation, such as abdominal irradiation, the inflammation is limited to the irradiated targeted region, but with TBI, the inflammation is systemic and interferes with molecular signaling, cascade pathways, and may also induce hormonal disturbances[196]. This inflammation causes insulin resistance due to prior growth hormone deficiency[197,198] and abnormal adipose tissue redistribution[199,200]. With regard to the pancreas, TBI also reduces pancreatic β cell reserve and directly causes an overall decrease in pancreatic volume[201].

Glucocorticoids

Glucocorticoids are steroidal anti-inflammatory drugs. They modulate the expression of several enzymes and mediators involved in the inflammatory response. By binding to their cytosolic receptor, glucocorticoids (GCs) act as a ligand-dependent transcription factor and enter the nucleus. This complex decreases the expression of molecules of major histocompatibility complex, molecules required for self-marker recognition. In addition, GCs inhibit the synthesis of IL-2, which prevents the proliferation of T-lymphocyte[180,202,203].

The side effects of glucocorticoids on glucose metabolism are well known[204,205] (**Figure 9**). These drugs contribute to diabetes primarily by inducing insulin resistance in muscle and adipose tissue. First, GCs reduce glucose uptake by inhibiting the glucose transporter GLUT4 in adipocytes and muscle. Second, GCs increase lipolysis and promote hepatic gluconeogenesis by stimulating glucagon secretion through transcription of key gluconeogenic enzymes (e.g., G-6-P, PEPCK). The synthesis of glycerol substrates and amino acids is also stimulated[206-209]. Furthermore, by inducing insulin resistance, GCs induce a compensatory process of insulin secretion that progressively leads to the loss of insulin-secreting β cells and ultimately to impaired insulin secretion with hyperglycemia[208,209]. This pathophysiological process is similar to that seen in T2D. The deficit in insulin secretion also results from under-expression of the glucose transporter GLUT2 and ATP-sensitive potassium (KATP) channels on the surface of β cells[206]. Although these effects are well known to clinicians, the risk-benefit ratio of glucocorticoid therapy in the context of solid organ transplantation is still positive, as it greatly influences the risk of rejection[210].

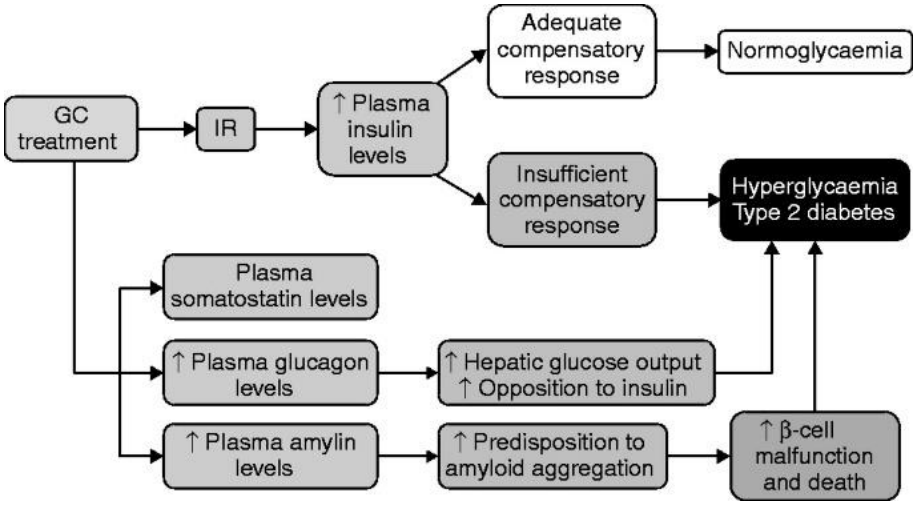

Figure 9. Effects of glucocorticoids (GCs) on glucose homeostasis. GCs can induce insulin resistance (IR) in peripheral tissues and lead to a compensatory adaptive process with increased insulin release. Hyperinsulinemia may develop to maintain normoglycemia. In the case of an insufficient β cell response, impaired glucose tolerance may progress to hyperglycemia and T2D. GCs can also induce hyperglucagonemia with increased hepatic glucose output that exacerbates hyperglycemia and glucose intolerance. Elevated amylin levels have been associated with GCs use and increase predisposition to amyloid aggregation related to increased β cell malfunction and death. Figure published by Alex Rafacho and al. in Journal of Endocrinology (Volume 223: Issue 3; R49–R62; Dec 2014) 'Glucocorticoid treatment and endocrine pancreas function: implications for glucose homeostasis, insulin resistance and diabetes'.

Calcineurin inhibitors

When calcineurin is not inhibited, this molecule induces the transcription of survival factors and stimulates the growth and expansion of the pancreatic beta cell mass. Calcineurin inhibitors (CNIs), including cyclosporine A (CsA) and tacrolimus (FK506), are widely used as immunosuppressive drugs in the post-transplant and sometimes prior to HSCT for cancer. Cyclosporine forms a complex with cyclophilin. This complex blocks the calcineurin-dependent phosphatase that normally dephosphorylates the NF-AT transcription factors (nuclear factor of activated T lymphocytes), which stimulates interleukin-2 (IL-2) synthesis. Thus, cyclosporin inhibits IL-2 gene transcription by blocking NF-AT activation. It also inhibits IL-3 and interferon-gamma transcription. Tacrolimus has the same mechanism of action as cyclosporine, but it binds to FK-506 binding protein (FKBP) instead of cyclophilin[211].

When calcineurin is inhibited by cyclosporine or tacrolimus, the diabetogenic effects observed are decreased insulin secretion through its inhibitory effect on NF-AT dephosphorylation, which is involved in insulin secretion (GLUT2, glucokinase).

Cyclosporine decreases insulin synthesis by interfering with the synthesis of insulin and proinsulin mRNA[212-215] (Figure 10). Tacrolimus has the same diabetogenic effects as cyclosporine, but the incidence of diabetes is higher. A patient treated with tacrolimus has a two- to fivefold increased risk of developing diabetes[216-220]. However, tacrolimus has a greater immunosuppressive (anti-rejection efficacy) effect than cyclosporine and is therefore more commonly used in the clinic[203,221].

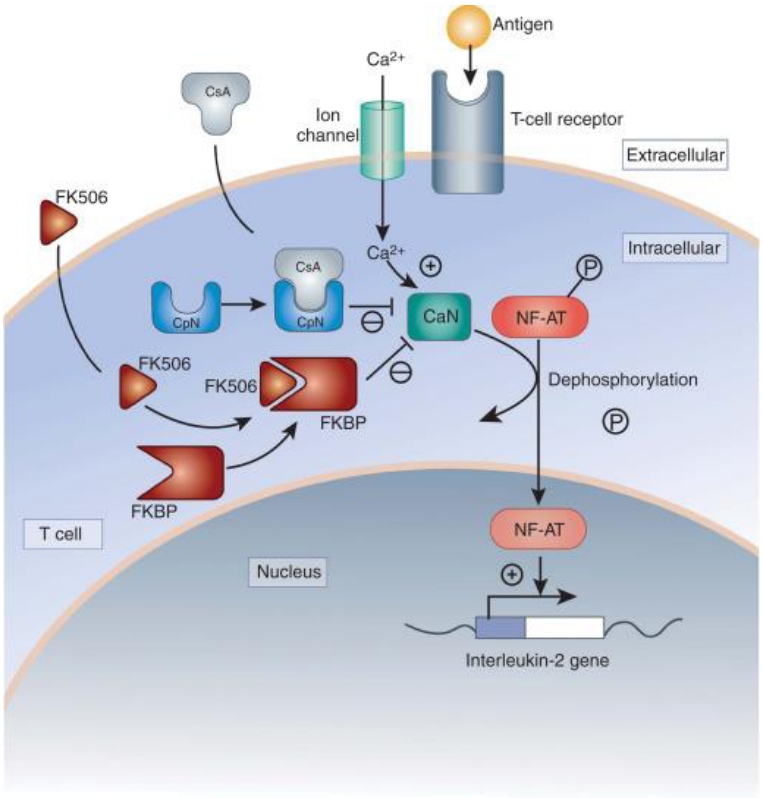

Figure 10. Mechanism of Cyclosporin A (CsA) and tacrolimus (FK506). CsA binds to cyclophilin (CpN) in the cytoplasm. The CsA–CpN complex blocks calcineurin (CaN) activity depriving the dephosphorylation of the nuclear factor of activated T cells (NF-AT). NF-AT is retained in the cytoplasm and is unable to activate transcriptional targets such as interleukin-2, which is necessary for T-cell activation. The mechanism of action of tacrolimus is the same as cyclosporine, but it binds to FK-506 binding protein (FKBP) instead of cyclophilin. Figure published by Brian Becknell and al, in Kidney International journal (Volume 82, Issue 10, 2012, Pages 1049-1051), 'A new 'tac' for childhood nephrotic syndrome'.

mTOR inhibitor

Sirolimus inhibits the mammalian Target Of Rapamycin protein (mTOR protein), which is involved in cell proliferation and the insulin signaling cascade[222]. By binding to the same protein as tacrolimus (FKBP), the complex inhibits the cellular signaling pathway of IL-2 synthesis and blocks T cell proliferation[180,203]. With regard to the disruption of glucose metabolism, sirolimus may induce insulin resistance and, ultimately, diabetes through the development of dyslipidemia, resulting from increased total cholesterol and apolipoprotein C-III[223,224].

CHAPTER 2. STATE OF THE ART

DIABETES, THIS OVERARCHING TERM

The overarching term ‘diabetes mellitus’ refers to a heterogeneous group of metabolic disorders characterized by the presence of hyperglycemia. All of these metabolic disorders are related to a defect in insulin secretion, insulin action or both and are associated with disturbances in carbohydrate, fat and protein metabolism [1,2]. The diagnosis of diabetes is based on two repeated measurements of blood glucose measurements to detect hyperglycemia and the diagnostic criteria are the same for all forms of diabetes: HbA_{1C} ≥6.5%, fasting blood glucose ≥126 mg/dL, 2-hour glucose after an oral glucose tolerance test ≥200 mg/dL, or a random glucose ≥200 mg/dL in a patient with classic symptoms of hyperglycemia (polyuria, polydipsia, and polyphagia)[1,2]. In combination with blood measurements, the presence of symptoms related to the hyperglycemic crisis, such as polyuria, polydipsia, and polyphagia, may contribute to the diagnosis[1,2]. From this perspective, the diagnosis of diabetes may seem relatively straightforward, but there are many forms of diabetes and determining the correct subtype of diabetes is more complex. Some forms of diabetes are a combination of autoimmunity, environmental factors, and multiple small effect predisposing polymorphisms, while other forms of diabetes may be caused by a strong mutation in a single gene involved in β cell function or induced by drugs that affect glucose homeostasis. Regardless, all forms of diabetes have the same risk of long-term complications related to the presence of hyperglycemia, including retinopathy, nephropathy, and neuropathy, as well as an increased risk of cardiovascular, peripheral arterial and cerebrovascular disease[1,2]. However, the rate of progression of complications depends on the type of diabetes, its management, and associated therapies. Therefore, distinguishing between the different types of diabetes has important implications for individual health (mental and social), diabetes management, prognosis of complications, and selection of appropriate treatment. In this case, research on the etiology, pathophysiology and clinical criteria of diabetes continues over time to better define the subtypes and improve their classification. According to the American Diabetes Association (ADA) guidelines, diabetes can be classified into four broad categories: type 1 diabetes (T1D), type 2 diabetes (T2D), gestational diabetes and diabetes due to other specific

causes. Most individuals with symptoms of diabetes (excluding pregnancy) can be classified as T1D (5-10% of all cases) and T2D (85-90%). Most research on diabetes is devoted to these two forms. A small percentage of diabetes cases (<5%) do not fit into T1D, T2D or gestational diabetes and are classified as diabetes due to other specific causes category, a category that remains poorly defined. This subtype is defined as a 'catch-all' category and includes all atypical forms of diabetes such as, but not limited to, monogenic diabetes and drug-induced diabetes.

In pediatrics, most individuals with diabetes can be classified into two categories: T1D (90%), characterized by autoimmune destruction of β cells resulting in absolute insulin deficiency, and T2D (5-10%) characterized by an inadequate insulin response resulting from prior insulin resistance. Both diseases are caused by multiple genes and environmental factors (i.e., viruses for T1D and obesity for T2D) and are the subject of intense research to better characterize their etiology, physiopathology, and treatment. Thus, the distinction between the two forms is based on age at onset, body mass index (BMI), severity of diabetes (i.e., degree of loss of β cell function, ketoacidosis), degree of insulin resistance, presence of islet autoantibodies and the need for therapy for survival (i.e., insulin injection, oral antidiabetic or exercise and diet recommendations)[225]. Based on these parameters, the typical presentation of T1D for healthcare professionals is that of a child or adolescent with normal BMI, severe hyperglycemia, islet autoantibody markers, low or undetectable C-peptide levels, symptoms of hyperglycemic crisis (polydipsia, polyphagia, loss of weight) associated with ketoacidosis in half of the cases. In contrast, the typical phenotype proposed for T2D is a combination of obesity/overweight, metabolic syndrome (e.g., dyslipidemia, hypertension) in the post-pubertal period, absence of islet autoantibodies, insulin resistance with elevated or normal C-peptide levels, and presence of acanthosis nigricans. The distinction between these two forms is important for therapeutic management, because if T1D is diagnosed, the patient requires lifelong exogenous insulin, whereas in T2D, treatment is based on lifestyle interventions and oral medications (i.e., metformin).

However, occasionally, some patients cannot be clearly classified as having T1D or T2D at the time of diagnosis and present with an overlapping phenotype

between the two forms. A patient may have diabetes with the fulminant features of T1D such as absolute insulin deficiency and ketoacidosis but be negative for islet autoantibody markers. In contrast, a patient may have the phenotype of T2D with obesity and preserved C-peptide secretion but not have insulin resistance. These patients, whose criteria do not fit to the classic description of T1D or T2D, are often considered to have an atypical form of diabetes. Considering these forms of diabetes as non-T1D or non-T2D does not allow their characterization and the study of their etiology and pathogenicity. Furthermore, misdiagnosis or lack of diagnosis deprives the patient of therapeutic decisions and educational approaches. The current classification of diabetes based on islet-directed antibodies and certain undefined clinical characteristics such as age of onset and BMI are not sufficient to differentiate individuals with diabetes. Therefore, various strategies have been proposed to further subdivide T1D and T2D and to revise the classification of diabetes by including more forms of diabetes.

EVOLUTION OF THE DIABETES CHARACTERIZATION TOWARDS PERSONALIZED AND PRECISION MEDICINE

Historically, in the 1950s, more than 95% of individuals with symptoms of diabetes (excluding pregnancy) were classified into two subtypes: “insulin sensitive” for T1D and “insulin insensitive” for T2D[26,27]. Currently, the ADA guidelines classify diabetes into four categories: T1D, T2D, gestational diabetes and diabetes due to other specific causes. In pediatrics, the majority of patients have T1D (>90%) or T2D (5-10%). Research to understand the etiology and pathophysiology of diabetes has therefore focused on these two main types of diabetes. Over time, however, the general understanding of diabetes and its classification has become less clear since the discovery of ketosis-prone diabetes (KPD)[226], latent autoimmune diabetes of adults (LADA)[227] and monogenic forms of diabetes. In fact, some patients with diabetes do not fit with the T1D or T2D phenotype or have an overlap between the two forms. A patient with T2D may have de novo ketoacidosis, autoimmune diabetes may develop in adulthood, and an entire family may have a mild form of diabetes. In addition, the increasing prevalence of T1D in adulthood and T2D in youth increases

diagnostic confusion[77]. Moreover, rapid advances in molecular genetics and its increased use have highlighted the heterogeneity within and between diabetes subtypes.

The recognition of overlapping features, diagnostic imprecision and the overly broad term diabetes mellitus has led to the introduction of new approaches to objectively regroup forms of diabetes into subtypes based on the phenotypic picture of the patient, associated complications, and comorbidities to assist the clinician in selecting the best therapeutic interventions. The best known, replicated, and validated reclassification study approach was proposed by the ANDIS study[228]. Leif Groop and his team in 2018 proposed a data-driven approach to define diabetes into five clusters (the Ahlqvist classification) shown in **Figure 11**. Individuals with newly diagnosed diabetes in the 'All New Diabetics In Scania' (ANDIS) study were grouped by phenotypic similarity based on six clinical biological parameters that are commonly used clinically to diagnose diabetes and reflect the major risk factors and pathogenesis of diabetes. These clinical variables include the presence of GAD65 autoantibodies, age at diabetes diagnosis, BMI, HbA_{1C} at diagnosis, and homeostatic model assessment estimates of insulin secretion capacity (HOMA-B index) and insulin resistance (HOMA-IR index). The ANDIS clusters were named according to their most characteristic feature. The 'severe autoimmune diabetes' (SAID) cluster is defined by GAD65 positivity, low insulin secretion, relatively low BMI and high HbA1c, and includes all individuals with T1D and LADA. The other four clusters include different forms of T2D and are all GAD65 negative. The 'severe insulin-deficient diabetes' (SIDD) cluster is similar to the SAID accepted for GAD65 positivity. The 'Severe insulin-resistant diabetes' (SIRD) cluster is characterized by obesity, severe insulin resistance, high insulin secretion, but relatively low HbA_{1C}. The last two clusters are both characterized by obesity and no insulin resistance and differ in the time of onset. The 'mild obesity-related diabetes' (MOD) cluster is characterized by an early onset and the 'mild age-related diabetes' (MARD) cluster by a late onset. The distinction between the four T2D clusters is primarily based on the risk of complications. The SIDD cluster has the highest risk of early diabetic retinopathy and neuropathy, while the SIRD cluster has a higher risk of renal

complications, such as chronic renal failure, albuminuria, and end-stage renal disease, as well as non-alcoholic fatty liver disease.

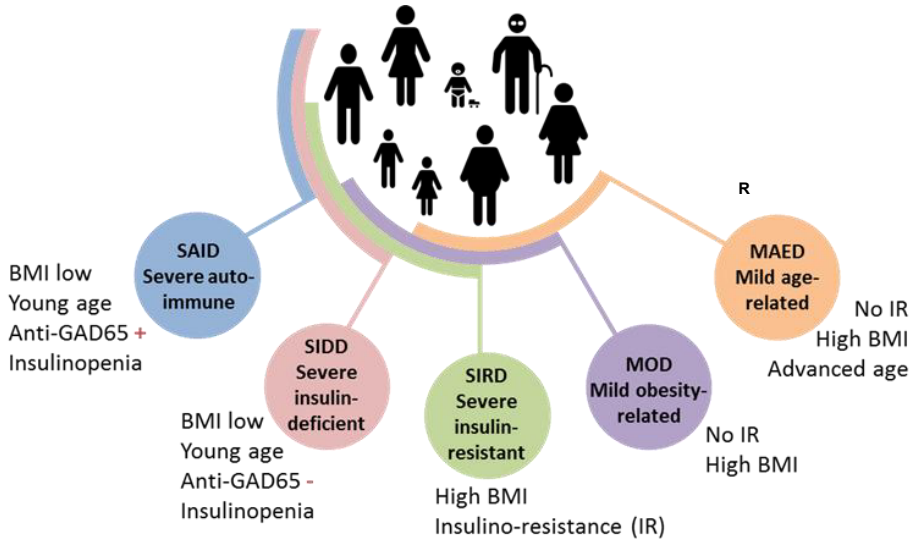

Figure 11. Subclassification of adult-onset diabetes and their association with outcomes. This figure has been inspired by article 'Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables' written by Emma Ahlqvist et al and published in Lancet Diabetes Endocrinology (May 2018).

However, although this new clustering is reproducible in several different populations, this first step in the study of diabetes heterogeneity does not include genetic data or diabetes progression or resolution. Only T1D and four subtypes of T2D are proposed, and important parameters that could aid in diagnosis, such as the presence of other islet autoantibodies or a family history of diabetes, are missing. Other studies have proposed clustering algorithms to reclassify individuals with diabetes, such as the German Diabetes Study (GDS)[229] or the Verona Newly Diagnosed Type 2 Diabetes Study (VNDS)[230], but they are essentially based on the subclassifying T2D according to its severity and course by identifying complications. Nevertheless, any further reclassification of diabetes is part of a general progression towards precision medicine and demonstrates the need to deconstruct the generic term 'diabetes' and to accurately characterize patients, currently based on clinical phenotypes, but increasingly complemented by laboratory tests, including genetic analysis. Indeed, in addition to phenotypic data, genetic data can be used as a

complementary approach to characterize and classify diabetes. In contrast to phenotypic information, genotypic data have the advantage of being stable and not evolving over time. Moreover, genetic data are more likely to indicate the cause of the dysregulation of glucose homeostasis. The integration of genetic data emerged with the discovery of genetic forms of diabetes. In fact, the discovery of diabetes caused by single gene mutations has completely changed the vision of diabetes classification and treatment towards a personalized medicine. The identification of mutations in the insulin receptor (*INSR*) gene in 1988 and in the *GCK* gene in 1992 was the first step in recognizing the heterogeneity of diabetes and the need for individualized treatment. Over time, advances in molecular genetics, accelerated by next-generation sequencing, have led to the discovery of other monogenic forms of diabetes and the introduction of several distinct treatment options based on the mutated genes. Because monogenic diabetes results from a single mutation in a gene involved in β cell function or development, each mutation causes a different outcome in β cell dysfunction and is treated differently depending on the severity of diabetes. Treatment is therefore individualized and defines personalized diabetes management, which is a further step towards precision medicine. As shown in figure 12, precision medicine starts from a global medicine where subtypes are identified, allowing stratification and ultimately personalized management of the patient according to their symptoms, complications, and responses to treatment options.

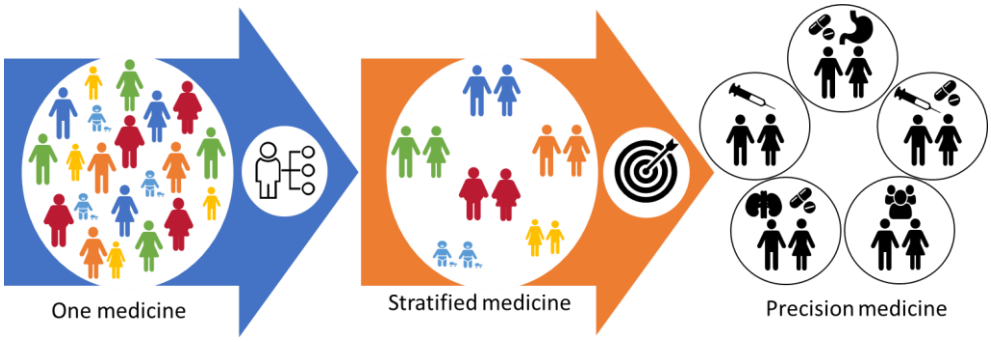

Figure 12: Stratified and precision medicine. This figure has been inspired by article 'Diabetes precision medecine: plenty of potential, pitfalls and perils but not yet ready for prime time' written by Simon griffin, and published in Diabetologia (July 2022).

Beyond monogenic diabetes, genetic analysis has also improved the characterization of subtypes of T1D and T2D, given the presence of genetic predisposition in these two types. However, to date, these advances have not led to significant changes in the reclassification of diabetes. Further work is needed to refine the diagnosis in patients with forms of diabetes other than the two most common forms.

ATYPICAL FORMS OF DIABETES.

As mentioned earlier, most people are familiar with the two most common forms of pediatric diabetes (T1D and T2D), but rare and atypical forms of diabetes can also affect children and adolescents. These atypical forms of diabetes may share similarities with T1D and T2D but differ greatly in etiology and pathogenesis. These forms of diabetes can lead to different health issues than the common forms and therefore require a completely different therapeutic approach. It is therefore important to recognize individuals with atypical diabetes to provide appropriate treatment and to assess potential complications. In general, atypical diabetes is suspected when clinical criteria differ from the typical features of T1D and T2D or when criteria overlap between the two phenotypes. However, a significant number of patients with atypical diabetes are often misclassified as T1D or T2D, such as monogenic diabetes because of their similarities, or underdiagnosed, such as drug-induced diabetes, because of the limited information and studies conducted on them.

The case of monogenic diabetes

In contrast to T1D and T2D, monogenic diabetes, a rare condition estimated to account for 1–4% of diabetes in Europe, results from mutations in a single gene with autosomal dominant inheritance that alter either insulin production or insulin action[2,67]. Monogenic diabetes, which includes MODY and neonatal diabetes, is considered as an atypical form of diabetes and is included by the ADA in the very large and non-specific category of diabetes due to other specific causes[1]. While the current diagnosis of T1D is essentially based on the presence of islet autoantibodies[138], the diagnosis of monogenic diabetes is based on a genetic

analysis and is characterized by early neonatal diagnosis (for PND and TND), onset of hyperglycemic symptoms before 25 years of age (for MODY), and a strong familial component[142,231]. In addition, in contrast to T1D, monogenic diabetes is often described as a more controllable diabetes with better glycemic parameters, absence of anti-islet antibodies, and relatively preserved C-peptide secretion[232]. In the case of neonatal diabetes, the diagnosis is quickly suspected because the onset of diabetes before 6 months of age is rarely associated with T1D[82], and genetic analysis is then rapidly proposed. However, for MODY, a significant number of patients share many features with T1D or T2D and are misclassified[75,77,78]. Genetic analysis is less suggested for MODY forms, and many cases remain underdiagnosed. Lack of awareness of monogenic diabetes among healthcare providers is a major barrier to correct diagnosis, especially in the context of the overlapping clinical features of T1D and T2D and the clinical heterogeneity of patients diagnosed with monogenic diabetes. In this context, it is accepted that within a convention of care for patients with diabetes 2 to 3% of active patients suffer from undetected genetic forms[233]. However, the diagnosis of a genetic origin of diabetes has multiple consequences, first of all at the therapeutic level. Indeed, some forms of monogenic diabetes do not require treatment (e.g., *GCK*-MODY), others respond to oral agents (e.g., *HNF1A*-MODY) or require specific medical follow-up due to the presence of extra pancreatic manifestations (e.g., *HNF1B*-MODY). In addition, in certain genetic forms of diabetes, the risk of microvascular and macrovascular complications of diabetes may be negligible, which can make life easier for the patient[234]. Finally, the discovery of a dominant or recessive mutation may imply the transmission of the disease to offspring and affect the health of other members of the patient's family. In conclusion, the differentiation of MODY from other forms of diabetes represents an opportunity for personalized or precision medicine. Given that genotyping of patients with diabetes is proposed in the approach of a precision medicine for diabetes treatment. If a patient is suspected of having monogenic diabetes, a classic genetic test is proposed, which only includes the most common form of monogenic diabetes. If no mutation or variant is found in this initial analysis, whole exome sequencing may be proposed for unresolved cases, but this is not systematic and is mainly associated with research. In fact, genotyping is a relatively new concept that has not yet entered

clinical routine and may be under-requested due to its high cost. Therefore, before proceeding with an expensive genetic test, the patient's likelihood of having a genetic form of diabetes is assessed using the 'MODY calculator'[21], based on clinical features, treatment, and the presence of other diabetes cases in the family. However, this score has some limitations: it was constructed and evaluated only on the three most common forms of MODY (*GCK*, *HNF1A* and *HNF4A* genes) and does not include important criteria such as the absence of an auto-immune process involved in T1D and the presence of residual C-peptide, an endogenous marker of insulin secretion functionality. Thus, at present, the lack of clinical criteria for genetic forms of diabetes persists, depriving patients of a correct diagnosis and appropriate treatment. The identification of additional criteria for genetic forms of diabetes and the characterization of their phenotype has become essential to help clinicians identify appropriate patients for genetic testing.

The case of drug-induced diabetes

Like monogenic diabetes, drug-induced diabetes is classified by the ADA as an atypical form of diabetes within the broad, non-specific category of diabetes due to other specific causes[1]. This catch-all category includes all forms of atypical diabetes that do not correspond to T1D, T2D or gestational diabetes with different etiologies, ranging from diabetes caused by mutations to diabetes induced by drugs. This situation of grouping all forms of atypical diabetes under a single subtype, results in a poorly defined category with a lack of clinical criterion, even though we know that individualized therapies for diabetes will be improved by better characterization of the many pathways leading to β cell dysfunction or destruction. Because of that, drug-induced diabetes is an understudied and misdiagnosed form of diabetes. Although the risk of developing a disorder of glucose metabolism after the use of immunosuppressive therapy is well known, pediatric data are insufficient, preventing the use of diagnostic tools for early detection of metabolic abnormalities. In addition, most studies of the incidence and risk factors for hyperglycemia and diabetes have been conducted in adult populations, whereas pediatric studies are scarce and adult data and risk factors cannot be extrapolated to children. In addition, there is a need

to diagnose the onset of hyperglycemia and diabetes in children and adolescents treated with anticancer and antirejection therapies because of their association with unfavorable prognosis, increased mortality and cardiovascular events, and graft dysfunction and rejection[189,235-237].

Finally, the real incidence and impact of diabetes after the use of anticancer and antirejection therapies is poorly known because the definition has changed over time, preventing a global consensus on previous clinical studies. Indeed, for transplant patients, several terms are used in the literature to describe the onset of diabetes after organ transplantation such as “New-onset diabetes after transplantation” (NODAT) or Post-transplant diabetes mellitus (PTDM). NODAT refers to individuals who develop diabetes after organ transplantation with exclusion of people with pre-transplant undiagnosed diabetes and post-transplant resolved hyperglycemia (148). Some studies have excluded from the NODAT definition all patients who developed hyperglycemia rapidly after transplantation, whether the hyperglycemia was resolved late or spontaneously[238]. The lack of consensus on the NODAT definition has led to some confusion. Therefore, the notion PTDM is currently more commonly used to describe the presence of diabetes after organ transplantation. PTDM describes the presence of diabetes in the post-transplant period, regardless of the time of onset of hyperglycemia, and includes persistent hyperglycemia, transient post-transplant hyperglycemia with resolution in the postoperative year[1]. Therefore, reliable early markers of dysregulated glucose homeostasis are needed in clinical practice to identify children with cancer or liver or kidney transplantation who are at risk of developing diabetes before significant β cell loss. This early identification of risk is important for therapeutic efficacy: glucose lowering is most effective in reducing diabetes and cardiovascular risk in dysglycemic patients with preserved β cell function[239,240].

CHAPTER 3. OBJECTIVES & STRATEGIES

PEDI LABORATORY RESEARCH CONTEXT

The PEDI laboratory is part of the institute for the Experimental and Clinical Research Institute (IREC for “Institut de Recherche Experimentale et Clinique”) of UCLouvain and studies pediatric diseases of the liver and endocrine pancreas. For its research projects, the PEDI laboratory collaborates with the pediatric services of the ‘Cliniques universitaires Saint-Luc’. For several years, the pancreas team has been interested in the most common pediatric endocrine disease, type 1 diabetes. Studies of our laboratory focus on understanding the development of the disease and on the search for markers. In this context, prior to my thesis work (biomedical master), I was involved in part in understanding the autoimmune destruction of β cells at the time of disease diagnosis. It is well accepted that in the pathogenesis of T1D, β cell destruction occurs via an inflammatory process related to cytokine secretion and activation of specific receptors and lymphocytes. We hypothesized that reducing inflammation or modulating the expression of these immune mediators within β cells may represent a method to preserve endogenous insulin secretory capacity. We evaluated in a mouse model, the efficacy of two molecules: empagliflozin (SGLT2 inhibitor) and γ-aminobutyric acid (GABA) (inducer of α cell proliferation and their transdifferentiation into β cells, according to the work of Ben-Othman and his team[241]) on the reduction of inflammation and destruction of β cells present during the onset of T1D. Our results showed that empagliflozin had a protective effect on β cells by reducing blood glucose, inflammatory responses, fatty acid synthesis, and endoplasmic reticulum stress, and that GABA had a stimulatory effect on α cell proliferation[242]. This initial research contributed to my first publication and several conference presentations during my thesis.

Recently, other causes of diabetes in pediatric patients, such as drug-induced diabetes or genetic forms of diabetes, have attracted the interest of our laboratory due to their increasing presence and concern in clinics. In this perspective, the objectives of my thesis were to study the atypical side of diabetes, whether it is represented in a cohort of patients with a genetic form of diabetes or present secondary to a pathology.

OBJECTIVES OF MY THESIS AND BRIEF STRATEGIES

The objective of my research was to better characterize atypical forms of diabetes, particularly monogenic diabetes, and drug-induced diabetes in pediatrics, as both are currently understudied and underdiagnosed or misdiagnosed. Although diabetes is broadly defined as a state of chronic hyperglycemia, this criterion refers to a heterogeneous group of diseases with different etiologies and distinct treatment options. Five to 10% of patients with diabetes have insulin-dependent T1D, which is attributed to autoimmune destruction of insulin-producing β cells, while 90 to 95% of patients have T2D, which is treated with oral antidiabetic agents, among others. Although the diagnosis of T1D is confirmed by the detection of specific antibodies, T1D and T2D are clinically characterized mainly by the phenotypic picture and the evolution of the patient, without recourse to specific etiological criteria. Monogenic diabetes is less frequent (up to 5% of cases), of recent discovery, and is often confused with T1D or T2D depriving the patient of an adapted treatment, which can be simplified (i.e., mutation affecting *HNF1A*: oral antidiabetics in replacement of insulin) or multidisciplinary (i.e., mutation affecting the *HNF1B* gene associated to renal diseases). It is recognized that in a care agreement of patients with diabetes, 2 to 3% of active patients suffer from undetected genetic forms[233]. *It is therefore important to improve the diagnosis of atypical forms of diabetes in children and adolescents by using clinical markers to help clinicians choose the best therapeutic interventions for the patient.* As for drug-induced diabetes, it is often understudied and underdiagnosed. Indeed, despite all the evidence suggesting that immunosuppressive drugs increase the risk of developing diabetes, its incidence, associated risk factors, and predictive biological markers remain unknown in the pediatric population. Furthermore, it is particularly important to assess the risk of diabetes in the context of pediatric transplantation and under chemo- and radiotherapy because its occurrence is associated with an unfavorable prognosis (e.g., rejection, increased duration of hospitalization and an increase in cardiovascular events). *It is therefore necessary to characterize and analyze the evolution of hyperglycemia in children with cancer and under anti-rejection therapy.*

Aim I: Characterization and evolution of hyperglycemia in a cohort of pediatric patients on immunosuppressive therapy: DIABONCO (study I) and DIABGRAFT (study II) studies.

To study the drug-induced diabetes, the DIABONCO and DIABGRAFT studies were initiated and included pediatric patients treated for **cancer** (acute lymphoblastic leukemia, Hodgkin's lymphoma or non-Hodgkin's lymphoma), in collaboration with the team of Pr. Bénédicte Brichard, and those treated for a **liver transplant**, in collaboration with the team of Pr. Etienne Sokal or for **kidney transplant**, in collaboration with the team of Dr. Nathalie Godefroid.

To define the incidence and identify the risk factors associated with the development of transient hyperglycemia or overt diabetes in these patients, their personal and family history, anthropometric and glycemic data, and those related to their treatment protocols were collected and constitute the retrospective part of the study. For transplanted children, a prospective protocol was implemented to characterize hyperglycemia. In this case, 14 days after liver or kidney transplantation, C-peptide and proinsulin were tested, and a blood glucose sensor was placed in any consenting patient. On approximately day 28, patients were subjected to an oral glucose tolerance test (OGTT). For kidney recipients only, samples and tests were repeated at 3, 6 and 9 months.

Criteria for the diagnosing of drug-induced diabetes are based on the same ADA guidelines as for all forms of diabetes (chapter “Criteria for the diagnosis of diabetes in pediatric patients”). Patients treated for cancer or after organ transplantation were considered to have hyperglycemia if fasting plasma glucose (FPG) or random plasma glucose levels exceeded 126 mg/dL (7.0 mmol/L) and 200 mg/dL (11 mmol/L), respectively, on at least two occasions separated by 24 hours, and not under a stressful condition such as the day of the transplantation. For the retrospective part of our research work, the term “transient hyperglycemia” was used to define patients with hyperglycemia without a diagnosis of overt diabetes, and diabetes was reported if the patient required ongoing treatment (i.e., insulin or oral antidiabetic agents). For the prospective part of our research work, the OGTT was used, and patients were classified according to the ADA guidelines. If a patient had impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) or HbA_{1C} from

5.7 to 6.4% (39–47 mmol/mol), he was considered to have “prediabetes” and if he had FPG ≥ 126 mg/dL (7.0 mmol/L) or a random PG or 2-h PG levels during OGTT > 200 mg/dL (11.1 mmol/L) or hemoglobin A_{1C} (HbA_{1C}) > 6.5% (48 mmol/mol) or if the patient had classic symptoms of HG, he had diabetes.

Aim II: Detection of atypical forms of diabetes in pediatric patients treated for T1D using an in-house score (DIAMODIA score): GENEPEDIAB study (study III).

To identify monogenic forms of diabetes, the GENEPEDIAB consortium was initiated, bringing together the CUSL and four French-speaking hospitals. To select patients with atypical diabetes, a DIAMODIA score (for "DIAgnose MOnogenic DIAbetes") was created based on the MODY probability calculator, which seemed incomplete to us (available at www.diabetesgenes.org), to which we added the lack of islet autoantibodies, the narrow range of glycemic variability, the persistence of C-peptide secretion and the absence of ketoacidosis at diagnosis. This score referred to weak and strong criteria and was graded from “2” to “5” depending on the number of positive criteria encountered by the patients. Patients with at least one strong criterion and one weak criterion were considered to have atypical diabetes and formed the ADia (ADia for Atypical Diabetes) cohort. ADia patients were then genotyped using a classic MODY panel (*GCK*, *HNF1A*, *HNF4A*, *HNF1B*, *KCNJ11*, *ABCC8* and *INS* genes). Patients with a negative result on the first genetic screening were then subjected to a whole-exome sequencing to identify variants associated with dysregulation of glucose metabolism. In parallel the DIAMODIA score was internally validated in a cohort of patients who tested for monogenic diabetes.

Ethics, consents, confidentiality in relation to clinical studies.

For the three studies, the Ethics Committee (Ethics Committee Hôpitaux-Facultés Saint-Luc) approved the study protocols and the studies were conducted in accordance with the Declaration of Helsinki. All children and their parents signed an assent and an informed consent form to participate in the study. With the signed consent form, the parents authorized the use of their child's data in compliance with the Belgian law of July 30, 2018, on the protection of privacy and European regulations (General European Regulation on the Protection of Personal Data RGPD of May 25, 2018) in application of the law of August 22, 2002, on patient's rights and the law of May 7, 2004, on experiments on the human beings. The scientific managers of this study and the people who process the data are committed to respecting this confidentiality. To ensure confidentiality, each patient was assigned a number that did not correspond to the patient's administrative number or date of birth. The patient was not identifiable by name or otherwise recognizable in any of the records, results or publications relating to the study. Data was anonymous and restricted to those who participated in the study.

CHAPTER 4.

RESULTS

RESULTS – DIABONCO STUDY (PUBLICATION 1)

In this section, results of the DIABONCO study published in DIABETIC MEDICINE journal (DOI: 10.1111/DME.14720) were exposed. We describe in this publication the incidence, risk factors and evolution of hyperglycemia in a cohort of pediatric patients treated for cancer.

KEY MESSAGES

- It is well known that hyperglycemia in childhood cancer is caused by using glucocorticoids, asparaginase and total body irradiation.
- Two new risk factors of hyperglycemia were identified in pediatric patients with acute lymphoblastic leukemia: puberty and steroid-resistant disease.

This work will help clinicians to identify patients with acute lymphoblastic leukemia at risk of early onset of hyperglycemia, by considering BMI and pubertal stage as potential markers and by monitoring blood glucose levels closely during treatment intensification for steroids-resistant disease or relapse, especially when total body irradiation and stem cell transplantation are required.

DIABETIC Medicine

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CHARACTERIZATION AND RISK FACTORS OF HYPERGLYCAEMIA DURING TREATMENT OF CHILDHOOD HEMATOLOGIC MALIGNANCIES.

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Keywords: Leukaemia, lymphoma, diabetes mellitus, steroids, stem cell transplantation, radiotherapy, overweight.

ABSTRACT

Background: Secondary forms of diabetes are often understudied and underdiagnosed in children and adolescents with cancer. The objectives of our cohort study were to study the incidence and risk factors for hyperglycaemia in leukaemia and lymphoma patients.

Methods: We retrospectively collected 15 years of data from paediatric patients treated for acute lymphoblastic leukaemia (ALL), Hodgkin's lymphoma (HL), and non-Hodgkin's lymphoma (NHL) immediately at cancer diagnosis. We studied risk factors for hyperglycaemia in univariate and multivariate analyses.

Results: Our study cohort included 267 patients corresponding to 179 patients with ALL, 48 with NHL and 40 with HL. Eighteen percent of ALL patients (32/179) and 17% of NHL patients (8/48) developed hyperglycaemia, with more than 61% developing hyperglycaemia within the first month of treatment. No hyperglycaemia was observed in HL patients. Multivariate analysis showed the following hyperglycaemia risk factors for ALL patients: overweight or obesity (OR 3.79) and pubertal onset (OR 4.27) at cancer diagnosis, steroid-resistant disease (OR 3.44) and hematopoietic stem cell transplant (HSCT) (OR 4.75).

Conclusion: In our cohort, 18% of patients with ALL or NHL developed early-onset hyperglycaemia after chemotherapy/radiotherapy. Patients with ALL with increased hyperglycaemia risk can be readily identified by measuring BMI and puberty stage at cancer diagnosis. Also, glucose monitoring should be reinforced when patients show steroid-resistant disease and/or require HSCT.

INTRODUCTION

Children and adolescents diagnosed with acute lymphoblastic leukaemia (ALL), Hodgkin's lymphoma (HL), and non-Hodgkin's lymphoma (NHL) are treated with specific and individual chemotherapy protocols sometimes combined with radiotherapy and/or hematopoietic stem cell transplant (HSCT). Thanks to research initiatives allowing constant re-evaluation of these protocols, survival rate of childhood cancer exceeds 83%[1]. However, the effectiveness of these treatments is not without consequences: 50% of childhood cancer survivors (CCS) develop endocrine sequelae including metabolic syndrome and glucose metabolism disorders such as diabetes, insulin resistance and impaired glucose tolerance (IGT)[2-4]. In the general population, diabetes confers a 2 to 3 times increased risk of cardiovascular disease and corresponds to 12-55% of cases of end-stage renal disease worldwide[5], being as such the 7th expected leading cause of death by 2030[6].

In CCS, the incidence of hyperglycaemia is still ill-defined and might range between 11 and 35% of cases[7-13]. Moreover, despite the whole body of evidence that asparaginase[12], steroids[14] and total body irradiation[15] increase the risk of developing hyperglycaemia and diabetes, risk factors are missing and – asides from treatments – understudied (e.g., pre-existing obesity, sex, age, ethnicity, family history of diabetes, etc.). The purpose of our study was to assess the incidence and associated risk factors of developing hyperglycaemia in children and adolescents diagnosed with ALL, HL and NHL. Deciphering the factors associated with the onset of hyperglycaemia in paediatric patients treated for cancer will provide leverage for lifestyle or therapeutic intervention from a prevention perspective in newly diagnosed patients.

MATERIALS AND METHODS

Study design

The DIABONCO retrospective study is being carried out in collaboration with the Paediatric Haematology and Oncology (Institut Roi Albert II) of Cliniques universitaires Saint-Luc in Belgium (Brussels). Our investigations included patients receiving treatment protocols conferring a diabetogenic risk. These included total body, cranial and abdominal irradiation (respectively TBI, CI and AI), steroids and L-asparaginase. Our cohort was therefore composed of patients treated for acute lymphoblastic leukaemia (ALL), Hodgkin's lymphoma (HL) and non-Hodgkin's lymphoma (NHL). The local ethical committee (Saint-Luc and UCL Hospital-Faculty Ethics Committee) approved this study protocol (approval number 2018/20MAR/122) and the study was conducted in accordance with the Declaration of Helsinki.

Inclusion and exclusion criteria

We included all children and adolescents aged 0 to 18 years treated with the aforementioned diabetogenic treatment protocols and diagnosed at Cliniques universitaires Saint-Luc with ALL, NHL or HL between January 2004 and December 2019. We excluded patients with an incomplete file or a history of the following conditions: previous diabetes (i.e., type 1, type 2, neonatal or monogenic diabetes), pancreatitis, steatosis, Down syndrome, pancreas and liver surgery, kidney disease and previous cancer other than leukaemia and lymphoma. The patients were stratified according to the presence or absence of hyperglycaemia during the treatment protocol and during clinical follow-up, which ended in August 2020. The groups were called the " hyperglycaemia-positive ALL, NHL or HL " and the "hyperglycaemia-free ALL, NHL or HL".

Treatments protocols

In Belgium, ALL, HL and NHL paediatric patients are treated with chemotherapy and radiotherapy according to international guidelines. Several protocols were used for the three pathologies depending on the treatment era, severity of the disease, the age of patients and the response to treatment. Despite some differences in protocols in the same cohort, treatment pattern remains unchanged. For ALL patients, the theoretical treatment lasts at least two years and begins with pre-phase with the introduction during seven days of steroids and followed by induction with twenty-one days of steroids, consolidation, interval, re-induction with also twenty-one days of steroids and finishes with maintenance phase, which sometimes includes steroids (Table S1 and Figure S1). Treatments for NHL and HL are much shorter than ALL treatment and last a maximum of six months. If an ALL patient presents steroid-resistance disease at the end of the pre-phase, the protocol will be intensified with an extended consolidation phase with longer doses of steroids and L-asparaginase. When ALL patients present a relapse during treatment or an abnormal cytogenetics, HSCT may be considered, some of them with TBI.

Diagnosis of hyperglycaemia

According to guidelines of the international consensus for diabetes of the American Diabetes Association (ADA), we considered that patients developed hyperglycaemia when random capillary blood or plasma glucose levels exceeded 11 mmol/L (200 mg/dL), for at least two measurements separated by 24 hours. Hyperglycaemia was identified based on glycaemic measurements during treatment protocols and clinical follow-up. Inpatients are subjected to daily blood analyses, which periodically include the measurement of plasma glucose levels. When hyperglycaemia occurs, the theoretical protocol implemented in clinics requires the confirmation of this hyperglycaemia by plasma glucose measurement and capillary glucose monitoring until resolution of hyperglycaemia.

Variables of interests

For all patients, the following data were collected and managed using REDCap (Research Electronic Data Capture) tools[16,17] provided by the Vanderbilt University

(Nashville, USA) and hosted at Cliniques universitaires Saint-Luc. We collected personal patient data such as sex, date of birth, country of origin, weight, height and gestation at birth, complications during pregnancy (pre- or post-term, events, foetal macrosomia), dysmaturity, hypoglycaemia and hyperglycaemia in the neonatal period, the presence or absence of previous overweight (BMI > 85th centile)/obesity (BMI > 95th centile)[18], endocrine disease, autoimmune disease, acanthosis nigricans, sickle cell anaemia, any chronic treatment, date of death if patient died. Regarding the patient's family history, we registered the presence or absence of previous gestational diabetes, polycystic ovarian syndrome, infertility, dystocia, consanguinity, diabetes, metabolic syndrome, sickle cell anaemia, pancreatic or liver surgery. We also gathered information about the primary diagnosis and its treatment: type of cancer, diagnosis date, stage and localization of the tumour, anthropometric data on diagnosis, tanner stage, blood pressure (systolic and diastolic), treatments protocols (presence or absence of steroids, asparaginase, radiotherapy and HSCT) and the presence of treatment side effects such as steroid-resistant disease, allergy to asparaginase, pancreatitis and steatosis induced by treatment protocol.

When the patient developed hyperglycaemia more than twice, we reported the date, the anthropometric data of onset, the blood pressure, the treatment for the hyperglycaemia (e.g., insulin therapy, metformin), its doses per day, and its duration. To obtain the number of blood glucose levels recorded, we counted all blood glucose measurements from the start of treatment protocol until the end of our study (August 2020). The duration of blood glucose monitoring was evaluated by counting blood glucose measurements performed without an interruption of more than six months and deceased patients were excluded. To evaluate the percentage of patients having been tested for blood glucose after the maintenance phase, we included only ALL patients treated before 2015 and HL patients treated before 2017 to have a sufficient delay between the end of maintenance phase and the end of our study for the metabolic outcome monitoring. Standard deviation score (SDS) for height, weight and BMI were assessed using respectively Belgian Flemish reference charts and Cole's Corpulence Curve[19,20].

Statistical Analysis

Descriptive statistics were used to summarize the results considering numbers and percentages for discrete variables, means with standard-deviations (SD) and medians with interquartile range (IQR) for continuous variables. The clinical characteristics of patients were compared according to the occurrence or not of hyperglycaemia using Student t test or Mann-Whitney test (as appropriate) for continuous variables and Fisher exact test for discrete variables. Kaplan-Meier estimates of the probability of remaining free of hyperglycaemia were plotted. A binary logistic regression analysis was performed to predict hyperglycaemia occurrence from all potential predictors available by estimating odds ratios and their 95% confidence intervals. All covariates with a p-value less than 0.10 in univariate analysis were introduced into a multivariate model (Wald Chi-Square). Variance inflation factor analysis was performed to detect a potential multicollinearity problem. A backward elimination strategy was used to estimate the best prediction model. Analyses were performed using SAS V9.4 software (SAS Institute Inc., Cary, NC, USA). All p-values were two-sided and values less than 0.05 were considered statistically significant.

RESULTS

Patient characteristics

We included 267 children and adolescents out of 303 patients (Figure 1) treated in the Cliniques universitaires Saint-Luc from January 2004 and December 2019, divided as such: 179 (67.0%) patients were diagnosed with ALL, 48 (18.0%) with NHL and 40 (15.0%) with HL. We excluded 36 patients because of an incomplete file (nALL =5; nNHL=2; nHL=2), Down syndrome (nALL=2), death soon after cancer diagnosis (nALL=5), tumour removal with no chemotherapy (nNHL=2), previous transplantation of liver (nNHL=8), kidney (nNHL=2) or cardiac (nNHL=1) and cancer other than leukaemia and lymphoma (nALL=2; nNHL=5). Clinical characteristics of the three cohorts are summarized in Table 1.

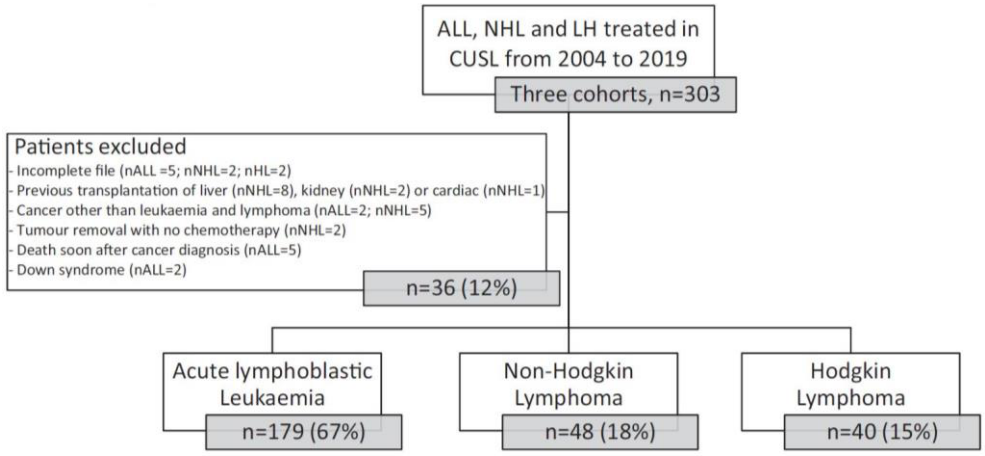


Figure 1. Flow chart of the study. Out of 303 patients treated in the Cliniques universitaires Saint Luc (CUSL) from January 2004 and December 2019, 179 (67%) patients were diagnosed with Acute lymphoblastic leukaemia (ALL), 48 (18%) with non-Hodgkin lymphoma (NHL) and 40 (15%) with Hodgkin lymphoma (HL). n: number of patients.

Table 1. Patients characteristics

	Acute lymphoblastic leukemia	**Non-Hodgkin lymphoma**	**Hodgkin lymphoma**
N	179	48	40
Age at cancer diagnosis, median (P25-P75)	4.8 (3.1; 10.8)	9.7 (7.1; 13.8)	13.2 (10.4; 15.5)
[0-8] years [n (%)]	120 (67.0)	16 (33.3)	6 (15.0)
[9-18] years [n (%)]	59 (33.0)	32 (66.7)	34 (85.0)
Gender, male [n (%)]	107 (59.8)	34 (70.8)	29 (72.5)
Weight SDS, median (P25-P75)	-0.1 (-0.8; 0.5)	-0.2 (-0.8; 0.8)	-0.1 (-0.7; 0.5)
Height SDS, median (P25-P75)	0.1 (-0.5; 0.6)	0.0 (-0.3; 0.5)	-0.2 (-0.7; 0.5)
Body Mass Index SDS, median (P25-P75)	-0.3 (-1; 0.6)	-0.4 (-1.1; 1.1)	0.0 (-0.8; 0.8)
Tanner staging P < 2 [n (%)]	138 (77.1)	28 (58.3)	16 (40.0)
Tanner staging M/G < 2 [n (%)]	138 (77.1)	27 (56.2)	17 (42.5)
Death [n (%)]	17 (9.5)	5 (10.4)	0 (0.0)

SDS= Age- and gender Standardized Scores, using Belgian references; P= Pubic; M=Mammary; G= Genital

Treatments characteristics

Treatment characteristics are presented in Table 2. The median duration of cancer treatment was 32.9 (25.6; 33.7) months for ALL patients, 3.5 (2.6; 13.1) months and 3.8 (2.8; 6.2) months for NHL and HL patients, respectively. All three cohorts received steroids whereas asparaginase was prescribed to ALL (100,0%) and NHL cohorts (33.3%) but not to HL patients. The proportion of patients receiving radiotherapy was 9.5%, 6.3% and 37.5% in the ALL, NHL and HL cohorts. Patients from the ALL cohort required cranial (64.7%) and total body (41.2%) radiotherapy, while HL patients received abdominal (66.7%) and cervical (33.3%) irradiation. Of the three irradiated patients of the NHL cohort, each received radiation at a different site (AI, CI, TBI). The frequency of patients requiring HSCT was 9.5% (17/179), 10.4 % (5/48) and 7.5 % (3/40) for the ALL, NHL and HL cohorts respectively.

Table 2. Treatments characteristics

	Acute lymphoblastic leukemia	**Non-Hodgkin lymphoma**	**Hodgkin lymphoma**
N	179	48	40
Duration of cancer treatment, month, median (P25; P75)	32.9 (25.6; 33.7)	3.5 (2.6; 13.1)	3.8 (2.8; 6.2)
Cancer treatment lower risk [n (%)]	151 (84.4)	38 (79.2)	40 (100.0)
Cancer treatment higher risk [n (%)]	28 (15.6)	10 (20.8)	-
Treatment with steroids [n (%)]	179 (100.0)	48 (100.0)	40 (100.0)
Treatment with asparaginase [n (%)]	179 (100.0)	16 (33.3)	-
Treatment with radiotherapy [n (%)]	17 (9.5)	3 (6.3)	15 (37.5)
Cranial irradiation [n (%)]	11 (6.1)	1 (2.1)	-
Total body irradiation [n (%)]	7 (3.9)	1 (2.1)	-
Abdominal irradiation, [n (%)]	-	1 (2.1)	10 (25.0)
Cervical irradiation [n (%)]	-	-	5 (12.5)
Total irradiation doses, Grays, median (P25; P75)	18 (12; 18)	10 (8; 18)	40 (20; 40)
Treatment with HSCT [n (%)]	17 (9.5)	5 (10.4)	3 (7.5)
- Allogenic transplantation [n (%)]	15 (8.4)	2 (4.2)	-
- Autologous transplantation [n (%)]	2 (1.1)	3 (6.2)	3 (7.5)
Asparaginase-induced pancreatitis [n (%)]	2 (1.1)	1 (2.1)	-

HSCT=Hematopoietic Stem Cell Transplantation

Incidence and evolution of hyperglycaemia during the treatment

Of the 267 children and adolescents, 17.9% (32/179) of the ALL patients and 16.7% (8/48) of NHL patients developed hyperglycaemia (Table 3). No hyperglycaemia was observed in the HL cohort. Hyperglycaemia developed rapidly after initiation of chemotherapy protocols: approximatively 61.0% (19/32) of ALL patients and all NHL patients except one (7/8) developed hyperglycaemia within the first month of treatment, corresponding to pre- and induction phases (Figure 2).

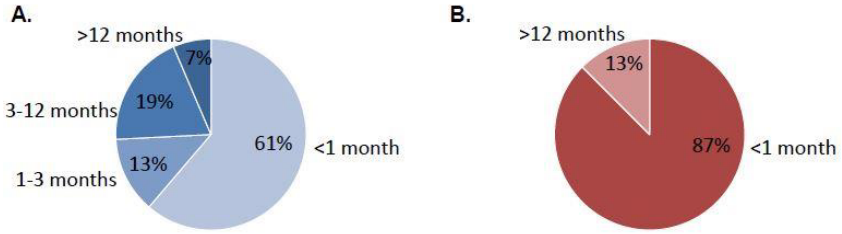


Figure 2. Distribution of hyperglycaemia onset over time in acute lymphoblastic leukaemia (ALL) and non-Hodgkin lymphoma (NHL) paediatric cohorts. Most of (A) ALL patients (61%) and (B) NHL patients (87%) developed hyperglycaemia within the first month of treatment.

The median number of blood glucose measurements recorded per patient was 24 (19; 36) for ALL patients, 26 (18; 40) for NHL patients, and 5 (3; 7) for HL patients (Table 3). Median duration of follow-up of blood glucose levels recorded during treatment protocols was 8.6 months (6.2; 12.7) and 3.6 months (2.4; 6.1) for ALL and NHL patients respectively and covered the four first phases of cancer treatment for ALL patients and all the treatment protocol period for NHL patients (Table 3, Figures S1 and S2). Blood glucose measurements are constantly performed during treatment protocols for ALL and NHL patients, with a peak during the induction phase and a decrease during maintenance and remission phases (Figures S1 and S2). For HL patients, median blood glucose monitoring lasted 4 days (1; 68) and was close to diagnosis (Table 3). The percentage of patients with blood glucose recorded after maintenance phase for the metabolic outcome monitoring was 77.8% (91/117), 76.7% (33/43) and 88.9% (32/36) for ALL, NHL and HL patients, respectively (Table 3).

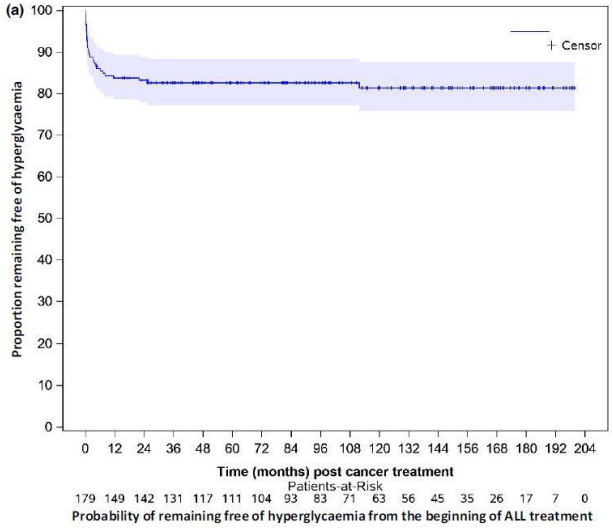
At 12 months post ALL treatment, the probability of remaining free of hyperglycaemia was 83.8% and remained relatively unchanged thereafter (end in

August 2020). In the NHL group this probability remained unchanged at 85.4% after one month of cancer treatment (Figure 3). Half (16/32) of hyperglycaemia-positive ALL cohort and three out of eight hyperglycaemia-positive NHL patients were treated with insulin and all required insulin therapy only during treatment protocol, except one ALL patient who remained insulin dependent (Table 3). Besides this only known case from our cohort with persistent diabetes, median duration of insulin therapy for the sixteen patients with ALL and the three patients with NHL was 15 days (3; 30) and 13 days (12;14), respectively.

Table 3. Incidence of hyperglycaemia

	Acute lymphoblastic leukemia	**Non-Hodgkin lymphoma**	**Hodgkin lymphoma**
N	179	48	40
Hyperglycaemia [n (%)]	32 (17.9)	8 (16.7)	0
Insulin treatment [n (%)]	16 (8.9)	3 (6.3)	0
Number of blood glucose levels, median (P25; P75)	24 (19; 36)	26 (18; 40)	5 (3; 7)
N*	162	43	40
Duration of blood glucose monitoring, month, median (P25; P75)	8.6 (6.2; 12.7)	3.6 (2.4; 6.1)	0.13 (0.03; 2.2)
N**	117	43	36
Patient with blood glucose recorded after maintenance phase [n (%)]	91 (77.8)	33 (76.7)	32 (88.9)

*Due to interrupted follow-up, deceased patients (n=17) were excluded.
** Dead patients and ALL patients treated after 2015 and HL patients treated after 2017 were excluded.



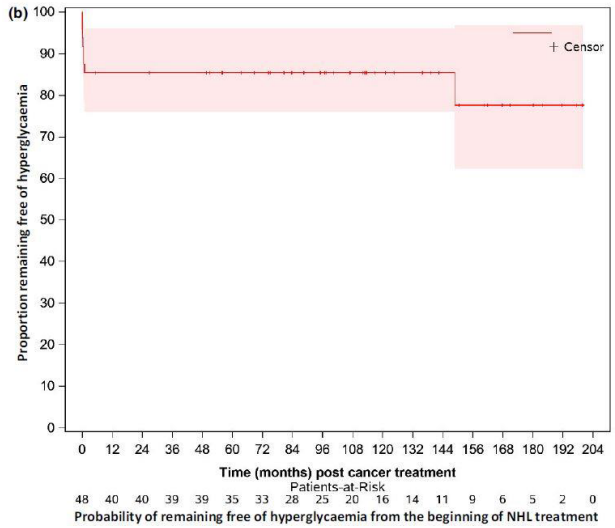


Figure 3. Kaplan-Meier estimates the probability of remaining free of hyperglycaemia in (a) acute lymphoblastic leukaemia (ALL) and (b) non-Hodgkin lymphoma (NHL) paediatric cohorts.

(a) At 12 months post ALL treatment, the probability of remaining free of hyperglycaemia was 83.8% and remained relatively unchanged thereafter.

(b) In the NHL group this probability remained unchanged at 85.4% after one month of cancer treatment.

Risk factors for hyperglycaemia

In univariate analysis, age older than 8 years and greater BMI SDS were significantly associated with the onset of hyperglycaemia (OR 1.01; p=0.002 and OR 20.80; p=0.008, respectively) as shown in Table 4 and illustrated in Figure 4.

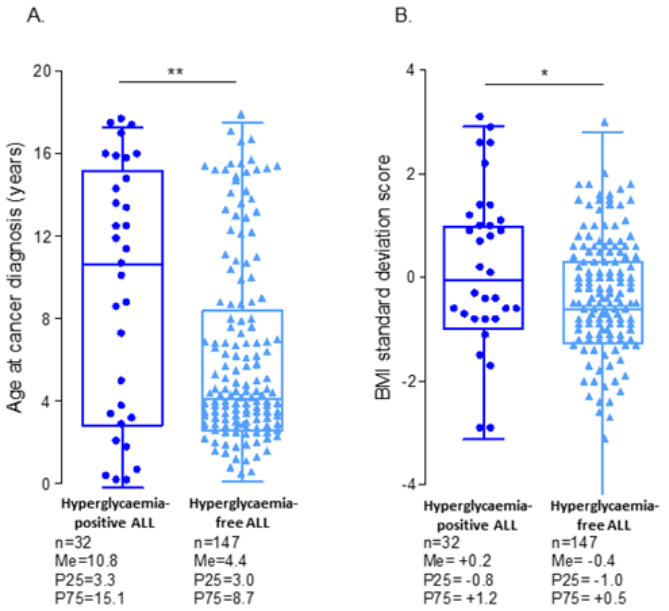


Figure 4. (A) Age and (B) BMI as risk factors of hyperglycaemia in acute lymphoblastic leukaemia (ALL) paediatric cohort.

ALL patients with older age and higher BMI are more at risk of developing hyperglycaemia. Each point represents a patient. The box plot represents the median, the minimum and maximum values.

Asterisks (*, **) show a significant difference between hyperglycaemia-positive ALL and hyperglycaemia-free ALL cohorts (*=P<0.05, **=P<0.01, Mann Whitney test).

Median age at cancer diagnosis was 10.8 (3.3; 15.1) years for the hyperglycaemia-positive ALL cohort and 4.4 (3.0; 8.7) years for the hyperglycaemia-free ALL cohort and median BMI SDS at cancer diagnosis was 0.2 (-0.8; 1.2) and -0.4 (-1.0; 0.5) respectively (Table 5). Furthermore, the unadjusted odds ratio of hyperglycaemia for a patient over 8 years old was higher (OR 4.62) compared to patients younger than 8 years, and this difference was significant (p<0.001). Other covariates were also significantly associated with the onset of hyperglycaemia such as a Tanner stage at cancer diagnosis equal to or greater than 2 (OR 4.88; p<0.001), a positive history of obesity/overweight (OR 4.29; p=0.008), a steroid-resistant disease (OR 3.20; p=0.014), or HSCT (OR 5.11; p=0.002). Furthermore, high-risk treatment was associated with hyperglycaemia development compared to low-risk treatment (OR 4.01; p=0.002) (Table 4).

After adjustment in the multivariate analysis, the best model to predict hyperglycaemia occurrence included two individual factors and two factors related to treatment. ALL patients with history of obesity/overweight (OR 3.793, 95% CI 1.026 – 14.022), a pubertal stage equal to or greater than 2 (OR 4.269, 95% CI 1.676 – 10.875) at cancer diagnosis, the presence of steroid-resistant disease (OR 3.445, 95% CI 1.114 – 10.657) and the use of HSCT (OR 4.754, 95% CI 1.099 – 20.554) were associated with a higher risk of developing hyperglycaemia (Tables 4 and 5).

Table 4. Univariate (Likelihood Ratio) and multivariate (Wald Chi-Square) logistic regression analyzes of factors leading to hyperglycaemia occurrence for ALL and NHL cohorts.

		Univariate analysis		Multivariate analysis	
	N	p-value	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)
ALL predictors					
Age at cancer diagnosis	179	0.002	1.010 (1.004-1.017)		
Age: [0-8] vs [9-18]	179	<0.001	4.615 (2.066-10.312)		
BMI SDS	179	0.017	1.486 (1.072-2.059)		
BMI SDS Overweight vs Normal weight	179	0.008	20.800 (2.231-193.96)		
Cancer treatment risk higher vs lower	179	0.002	4.006 (1.649-9.730)		
History of overweight at cancer diagnosis	179	0.008	4.293 (1.464-12.588)	0.046	3.793 (1.026-14.022)
Tanner staging ≥2	179	<0.001	4.880 (2.159-11.032)	0.002	4.269 (1.676-10.875)
Steroid-resistant disease	179	0.014	3.204 (1.265-8.113)	0.032	3.445 (1.114-10.657)
HSCT	179	0.002	5.111 (1.795-14.553)	0.037	4.754 (1.099-20.554)
NHL predictors					
Cancer treatment risk higher vs lower	48	0.038	5.667 (1.104-29.073)		

ALL= Acute Lymphoblastic Leukemia; NHL= Non-Hodgkin Lymphoma; CI= Confidence Interval; BMI= Body Mass Index; SDS= Age- and gender Standardized Scores, using Belgian references; HSCT= Hematopoietic Stem Cell Transplantation.

Due to insufficient statistical power, no association between TBI and hyperglycaemia onset could be demonstrated but five out of the eight patients (seven ALL and one NHL) who received TBI, developed hyperglycaemia (Table 5). The same observation applied for asparaginase-induced pancreatitis as a risk factor since logistic regression was not possible because no patients in hyperglycaemia-free ALL cohort developed an asparaginase-induced pancreatitis during cancer treatment. However, all three patients (two ALL and one NHL) who developed asparaginase-induced pancreatitis, subsequently developed hyperglycaemia (p=0.031) (Table 5). In contrast, there was no association between cranial irradiation and hyperglycaemia since in a total of eleven ALL patients receiving cranial irradiation, only one developed hyperglycaemia (Table 5).

Due to the low number of NHL patients and hyperglycaemia-positive NHL patients, univariate analysis only allowed us to identify that high-risk treatment was significantly associated with hyperglycaemia onset compared to low-risk treatment in the NHL cohort (OR 5.67; p=0.038) (Table 4).

There was no difference in the gender, family history of diabetes or metabolic syndrome, type T or B cancer (nature of the disease), type of transplant and between the anthropometric data reported at cancer and hyperglycaemia diagnosis (weight, height, BMI).

Table 5. Comparison of the clinical characteristics of hyperglycaemia-positive ALL and hyperglycaemia-free ALL cohorts

	Hyperglycaemia-positive ALL, n=32	**Hyperglycaemia-free ALL, n=147**	**p-value**
Age at cancer diagnosis, median (P25; P75)	10.8 (3.3; 15.1)	4.4 (3,0; 8.7)	0.016
[0-8] [n (%)]	13 (40.5)	108 (73.5)	<0.001
[9-18] [n (%)]	19 (59.5)	39 (26.5)	
BMI SDS, median (P25; P75)	0.2 (-0.8; 1.2)	-0.4 (-1.0; 0.5)	0.017
History of overweight [n (%)]	7 (21.9)	9 (6.1)	0.011
Tanner staging ≥2 [n (%)]	16 (50.0)	25 (17.0)	<0.001
Steroid-resistant disease [n (%)]	9 (28.1)	16 (10.9)	0.021
Cancer treatment lower risk [n (%)]	21 (65.6)	130 (88.4)	0.003
Cancer treatment higher risk [n (%)]	11 (34.4)	17 (11.6)	
Radiotherapy treatment [n (%)]	5 (15.6)	12 (8.2)	0.193
Cranial irradiation [n (%)]	1 (3.1)	10 (6.8)	NA
Total body irradiation [n (%)]	4 (12.5)	3 (2.0)	NA
Treatment with HSCT [n (%)]	8 (25)	9 (6.1)	0.003
Asparaginase-induced pancreatitis [n (%)]	2 (6.3)	0 (0.0)	0.031

ALL=Acute Lymphoblastic Leukemia; BMI=Body Mass Index; SDS=Standard Deviation Score; HSCT=Hematopoietic Stem Cell Transplantation. Student t test or Mann-Whitney test for continuous variables and Fisher exact test for discrete variables were used to obtain the p-values.

DISCUSSION

Our study describes the incidence and risk factors of hyperglycaemia onset, immediately at treatment initiation, in a cohort of paediatric patients treated for ALL, NHL or HL. We showed that 18% of ALL patients and 17% of NHL patients developed hyperglycaemia described as a random capillary blood or plasma glucose level exceeding 11 mmol/L (200 mg/dL) for at least two measurements separated by 24 hours. The incidence of hyperglycaemia observed in our ALL cohort is similar to a previous study carried out in 2008 by the team of Howard where 16% out of 871 paediatric patients with ALL presented hyperglycaemia during treatment[8]. More recently, three studies described 16.5% (22/133) and 15.7% (16/102 and 57/363) of ALL paediatric patients with hyperglycaemia (in more than two consecutive measurements)[9,11,21]. The impact of NHL treatment protocols on hyperglycaemia onset is less studied, however the study by Neville et al. showed in a smaller cohort of 20 NHL patients a high incidence of glycaemic dysregulation: 5 patients (25%) developed either hyperinsulinemia, IGT or diabetes[22].

In our study, the majority of ALL (61%) and NHL (87%) patients developed hyperglycaemia within the first month of chemotherapy, corresponding to pre- and induction phases that are the most aggressive in terms of steroid doses. Only half of hyperglycaemia-positive ALL and NHL patients were given insulin therapy and one hyperglycaemia-positive ALL patient presented persistent non-type 1 diabetes. Also, we observed that the majority of the three cohorts (ALL: 77.8%, NHL: 76.7% HL: 88.9%) benefited from blood glucose control during monitoring of side effects but this monitoring did not include a dynamic test such as the oral glucose tolerance test.

Since all patients treated for leukaemia or lymphoma required steroid treatment but not all developed hyperglycaemia, we sought to identify hyperglycaemia predisposing risk factors in our paediatric cohort. Our multivariate analysis revealed that a history of obesity/overweight at cancer diagnosis is associated with a higher risk of developing hyperglycaemia in ALL patients, as described elsewhere[7,12,13,23]. Also similar to other studies presented by Gregoriou in a recent review[24], being older than 10 years (Gregoriou) or 8 years (this paper) was identified as a strong risk factor of hyperglycaemia in our univariate analysis ($p < 0.001$), but was not an independent risk factor in our multivariate analyses.

Associated to the age factor, we identified a strong correlation between pubertal entry (Tanner stage ≥2) and hyperglycaemia risk. In normal puberty, rising sex steroid and growth hormone levels are associated with reduced insulin sensitivity, which may predispose to the development of the metabolic syndrome in overweight/obese children. Indeed, reduced insulin sensitivity is not uncommon (8%) in extremely obese children[25].

The stronger treatment-related risk factor for hyperglycaemia that emerged from our study is steroid-resistant disease for ALL patients. Patients with steroid-resistant disease receive a more aggressive and risky treatment protocol and "high-risk treatment" was associated with hyperglycaemia onset for ALL patients in our univariate analysis (OR 4.01; p=0.002). Although we do not know if patients received "high risk" treatment due to the more aggressive nature of the cancer or because of steroid-resistant disease.

HSCT is also a composite risk factor of hyperglycaemia for ALL patients in our study. Indeed, HSCT is often preceded by TBI and may require the use of steroids in case of graft versus host disease symptoms. Studies carried out on CCS showed that TBI and HSCT together increase the risk of IGT and diabetes[15,22,26]. In our study, we also observed that ALL patients who received TBI followed by HSCT tended to develop hyperglycaemia (5/8), though the number of patients with TBI was insufficient to reach significance. Moreover, in our NHL cohort, HSCT did not emerge as a risk factor of hyperglycaemia, yet two out of five patients with HSCT developed hyperglycaemia.

L-asparaginase induces hyperglycaemia as a result of reduced insulin synthesis due to depletion of the available pool of asparagine concurrent with hyperglucagonemia and probably by a reduction of the number of insulin receptors[27]. In agreement with the results of the study by Irga et al. describing paediatric patients with ALL, NHL and severe aplastic anaemia, we did not find a correlation between L-asparaginase treatment and hyperglycaemia onset[28]. However, as emphasized in a review paper by Hijiya, L-asparaginase-induced pancreatitis is known to affect 2 to 18% of ALL patients and consequently causes rapid development of diabetes[29]. In our study, it is noticeable that all patients who developed pancreatitis induced by L-

asparaginase had subsequently developed hyperglycaemia, although it concerns only 3 patients.

The absence of hyperglycaemia observed in HL patients can be explained by several hypotheses. Potentially less blood glucose monitoring is done linked to their outpatient status, compared to inpatient treatment for ALL and NHL patients. In addition to less blood glucose testing, there is no exposure to potential diabetogenic treatment protocols such as L-asparaginase and TBI, and there is a discontinuous prescription of steroids with a lower theoretical cumulative monthly dose of steroids for HL patients compared to ALL patients. Also, HL patients required abdominal and cervical irradiation, yet we retrieved only TBI as a risk of hyperglycaemia development in our ALL cohort. Abdominal, cervical and cranial radiation did not appear to induce hyperglycaemia in our study, although this is contrary to findings in other studies which suggested effect of abdominal radiation[30-32].

Strengths of our study include the large sample size of ALL patients, inclusion of risk factors for hyperglycaemia, complete patient records and numerous harmonized blood glucose data for ALL and NHL. Furthermore, we studied the incidence of hyperglycaemia from the initiation of cancer treatment and not only during remission (after 2 years). One major limitation of our study was the inclusion of patients who received different ALL, NHL and HL treatment protocols from different treatment eras. The retrospective nature of the study was a limitation although patient records were mostly complete. Moreover, the availability of blood glucose data varied and decreased in maintenance and remission phases for the three cohorts, preventing a potential diagnosis of persistent diabetes or late diabetes (i.e., irradiation treatment) and was limited for HL patients by the ambulatory follow-up.

In conclusion, in our paediatric study, hyperglycaemia was diagnosed in 18% of ALL patients, 17% of NHL patients but not in HL patients. Puberty and overweight at the time of cancer diagnosis as well as steroid-resistant disease, HSCT preceded by TBI, and asparaginase-induced pancreatitis were identified as risk factors for hyperglycaemia in paediatric patients with ALL. We believe our study may help clinicians to identify ALL patients at risk of early onset of hyperglycaemia, since our study highlights the importance of considering BMI and pubertal stage as

potential markers for the onset of hyperglycaemia in children and adolescents receiving diabetogenic cancer treatments. In addition, our study shows the importance of closely monitoring blood glucose levels during treatment intensification when patients present steroid-resistant disease or relapse, especially when TBI and HSCT are required. Recognising the reduction of available blood glucose levels in remission phase and their absence in Hodgkin lymphoma cohort, we also point out the need to monitor blood glucose levels at each follow-up visit to enable the diagnosis of transient, persistent, or late-onset diabetes. The DIABONCO study includes a prospective part with characterization of survivors inside hyperglycaemia-positive ALL and NHL cohorts and will be able to evaluate the presence of persistent subclinical diabetes or IGT.

SUPPLEMENTARY MATERIAL

Table S1. Theoretical treatment protocols of steroid and asparaginase for acute lymphoblastic leukaemia patients.

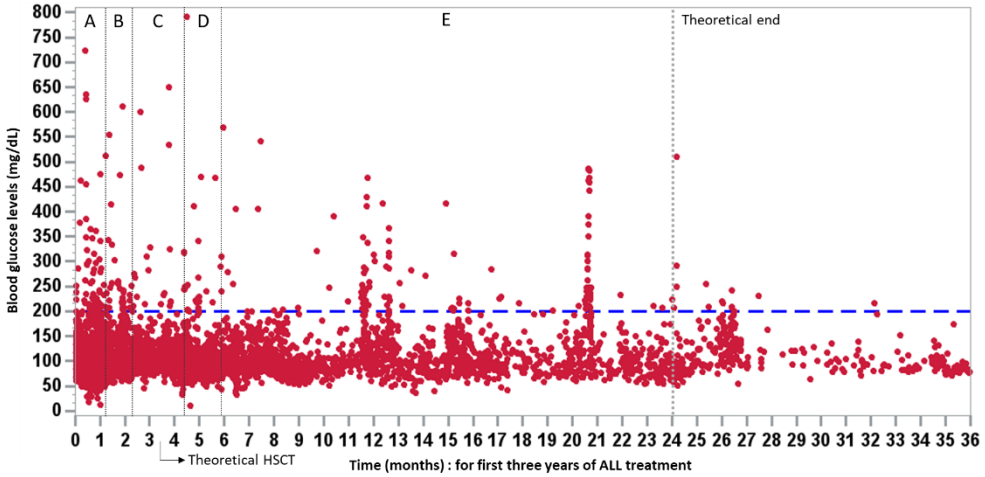
		Pre	Induction	Consolidation	Interval	Reinduction	Maintenance
	Duration (days)	7	28	28	56	49	518
VLR	Steroids (days)	7	21	-	-	21	-
	Aspara (days)	-	2	-	-	1	-
AR	Steroids (days)	7	21	-	-	21	42, for AR and AR-T
	Aspara (days)	-	2	-	-	1	6 for AR-B/T

		Pre	Induction	1°Consolidation	2°Consolidation	Interval	Reinduction	Maintenance
	Duration (days)	7	28	41	13	2x42	2x49	ends at 2 years after day 1
VHR	Steroids (days)	7	21	-	5	-	42	-
	Aspara (days)	-	2	1	1	-	1	-

VLR=Very Low Risk; AR=Average Risk; VHR=Very High Risk; HSCT=Hematopoietic Stem Cell Transplantation; B=immune B cell; T=immune T cell

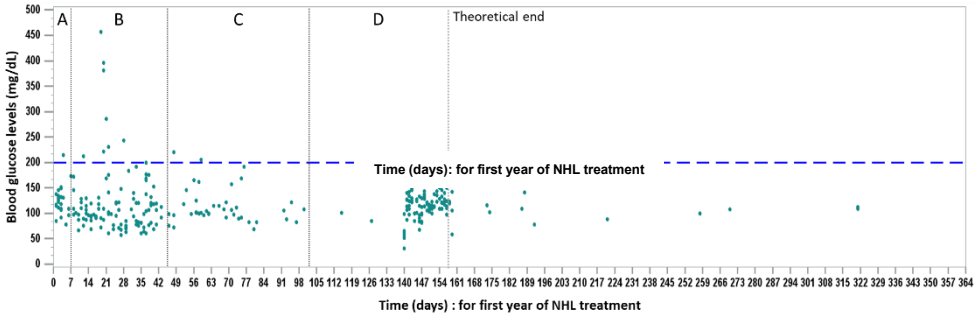
→ HSCT after 2° consolidation if the patient is qualified for and if HLA compatible donor is available.

Figure S1: Blood glucose levels performed during acute lymphoblastic leukaemia (ALL) treatment protocol. Graph shows blood glucose measurements in relation to acute lymphoblastic leukaemia treatment protocol.



	A	B	C	D	E
Phases	Pre and induction	Consolidation	Interval	Reinduction	Maintenance
Duration (days)	35	28	56	49	518 (74 weeks)

Figure S2: Blood glucose levels performed during non-Hodgkin lymphoma (NHL) treatment protocol. Graph shows blood glucose measurements in relation to non-Hodgkin lymphoma treatment protocol.



i.e.: Inter-B-NHL	A	B		C		D	
Phases	Pre	Induction		Consolidation		Maintenance	
Course		n°1	n°2	n°3	n°4	n°5	n°6
Duration (days)	7	17-20	17-20	24-27	24-27	27	27

AUTHOR CONTRIBUTIONS

All authors have read and approved the final version of the manuscript. PA.L. had the idea for the study, designed the study, wrote and reviewed the manuscript. S.W. designed and performed the study, collected and analysed the data, decided on the statistical method, wrote and reviewed the manuscript. K.S. decided on the statistical method, performed and wrote the statistical analysis. B.B. and M.dV. contributed to the reflection on the results. B.B., M.dV., A.VD. and C.B. reviewed the manuscript.

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CONFLICTS OF INTEREST

The authors declare no potential conflicts of interest.

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RESULTS – DIABGRAFT STUDY (PUBLICATION 2)

In this section, results of the DIABGRAFT study published in Frontiers Pediatric journal, Section Pediatric Endocrinology were exposed. We describe in this publication the incidence and risk factors of hyperglycemia in a cohort of liver and renal pediatric transplant patients and analyze their glycemic profile.

KEY MESSAGES

- To our knowledge, DIABGRAFT study is the only one that combines retro- and prospective parts which include glucose screening test rarely performed in pediatric patients who benefited from a liver or renal transplant.
- Children with liver and renal transplants were more at risk of developing HG when glucocorticoids were required.
- HbA_{1C} and fasting glucose lack sensitivity for early detection of glucose intolerance.
- Oral glucose tolerance test and glucose sensors showed insulin resistance, impaired glucose tolerance and HG in the post-prandial afternoon period.

Our results may help clinicians to identify liver and renal transplant children at risk of early hyperglycemia by considering the importance of random blood glucose monitoring in the afternoon period when children present critical complications such as graft rejection and infections.

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CHARACTERIZATION, EVOLUTION AND RISK FACTORS OF DIABETES AND PREDIABETES IN A PEDIATRIC COHORT OF RENAL AND LIVER TRANSPLANT RECIPIENTS.

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ClinicalTrials.gov ID: NCT05464043

Keywords: Diabetes, Hyperglycemia, Impaired glucose tolerance, Insulin resistance, Liver transplantation, Renal transplantation, Glucocorticoids

ABSTRACT

Background: Hyperglycemia (HG) and prediabetes are rarely sought in pediatric liver (LT) and renal (RT) transplantation, yet their presence indicates a high risk of diabetes and cardiovascular disease. The objectives of our DIABGRAFT study were to retrospectively (rDIABGRAFT) and longitudinally (pDIABGRAFT) characterize HG and (pre)diabetes in a cohort of children with LT or/and RT.

Methods: We retrospectively analyzed risk factors of HG from 195 children with LT from 2012 and 2019 and twenty children with RT from 2005 to 2019 at Cliniques universitaires Saint-Luc. In addition, we prospectively followed four LT and four RT children to evaluate the evolution of their glucose metabolism.

Results: Our rDIABGRAFT study showed that 25% and 35% of LT and RT children respectively presented transient HG and 20% of RT developed diabetes. The occurrence of HG was associated with the use of glucocorticoids and with acute events as graft rejection and infection. In our pDIABGRAFT cohort, biological markers of diabetes were in the normal range for HbA_{1C}, fasting glucose and insulin levels. However, oral glucose tolerance test and glucose sensors showed insulin resistance, impaired glucose tolerance and HG in the post-prandial afternoon period.

Conclusion: Our study shows that children with LT and RT were more at risk of developing HG when glucocorticoids were required and that HbA_{1C} and fasting glucose lack sensitivity for early detection of glucose intolerance. Also, measurement of glycemia immediately after the transplantation and in postprandial period is key to detect dysglycemia since insulin resistance prevailed in our cohort.

INTRODUCTION

Solid organ transplantation (SOT) is the therapeutic choice for patients in end-stage renal or liver disease. After transplant, immunosuppression is required to ensure graft survival but is associated with side effects, including glycemic disorders. One of the most frequent complications observed with immunosuppressants is hyperglycemia (HG), which increases the probability of developing prediabetes and overt diabetes. Prediabetes, an intermediate state between normal glucose homeostasis and overt diabetes, represents a major health problem because in 2012 it was estimated that 70% of the prediabetic American citizens (33,5%) will develop diabetes within their lifetime[1-3]. Diabetes affects an ill-defined proportion of transplant patients (2 to 53%)[4-7] and is common in the context of adult liver and renal transplantation[5,8,9]. Yet the incidence of transient HG, and the progression to overt diabetes in pediatric liver and renal transplantation remain unknown. However, it is known that both are associated with an unfavorable acute prognosis (i.e., mortality, graft rejection, increased hospital stays) and an increased cardiovascular risk in the long term in adult patients, this risk being correlated to the presence of metabolic syndrome[9-12]. In addition, the use of fasting blood glucose and HbA1C levels might not allow early detection of impaired glucose tolerance (IGT) as a preamble to prediabetes. It is therefore essential to gather knowledge on the evolution of glucose in pediatric patients after SOT. The objectives of our DIABGRAFT study were to assess the incidence and associated risk factors of developing hyperglycemia in liver and renal transplant children and longitudinally analyze the evolution of glycemic profile (i.e., HG, IGT and diabetes) in these patients during the post-transplant period.

MATERIALS AND METHODS

Study design

The DIABGRAFT study was conducted in collaboration with the Pediatric Hepatology and Gastroenterology and Specialized Pediatrics (Endocrinology and Nephrology Units) Services of Cliniques universitaires Saint-Luc (CUSL) in Belgium (Brussels). This study was approved by the local ethical committee (CUSL and UCLouvain Hospital-Faculty Ethics Committee; approval number 2019/12MAR/118) and was conducted in accordance with the Declaration of Helsinki. Our study included liver and renal transplant pediatric patients (<18 years of age) at CUSL. Were excluded patients with a history of diabetes (i.e., type 1, type 2, neonatal or monogenic), pancreatitis, Down Syndrome, cystic fibrosis (n=1), a second organ transplantation for our LT cohort (n=4; cardiac, renal), patients deceased shortly after transplantation (< one year, n=14), and patients with incomplete medical record (n=8).

DIABGRAFT was constituted of two parts. Its retrospective part (rDIABGRAFT) consisted of collecting data of pediatric patients who benefited from a liver transplant performed at CUSL between April 2012 and April 2019, or that benefited from a renal transplant in our center between September 2005 and April 2019. The prospective part (pDIABGRAFT) of the study consisted of a longitudinal glycemic evaluation of liver and renal transplant children in CUSL between 2020 and 2022 with the use of dynamic endocrine testing (Fig. 1). Informed consents were collected from parents and from all children over six years of age.

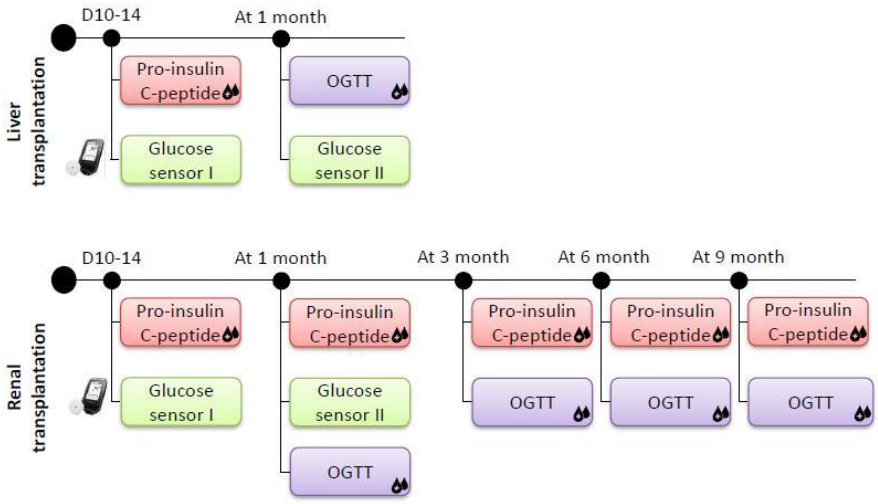


Figure 1: Protocol of prospective DIABGRAFT study. For LT and RT cohorts, pro-insulin and C-peptide secretion was measured after two weeks of transplant and measures had continued at one, three, six and nine months for RT patients. A glucose sensor was placed on the patient two weeks post-transplant for one month to detect the presence of early dysglycemia. An OGTT was performed after one month of transplantation and, for RT patients also at three, six and nine months.

Treatments protocols for pediatric liver and renal transplant patients

At CUSL, liver and renal transplant children receive standard immunosuppression protocol as per international guidelines[13]. For LT patients, this protocol includes the association of a monoclonal anti-CD 25 antibody (basiliximab, Simulect®) and a calcineurin inhibitor (tacrolimus, Prograft®)[14,15]. For RT patients, this protocol is based on a combination of Tacrolimus, glucocorticoids, monoclonal anti-CD 25 antibody and a cell proliferator inhibitor as mycophenolate mofetil (Cell-Cept®). Doses of glucocorticoids are introduced or increased when a LT/RT patient presents an acute cellular rejection (ACR). Complete treatment protocol is available in supplementary data (Text S1).

About glucose monitoring, after a liver or renal transplantation at CUSL, glycemia is measured daily during hospitalization (between two weeks and one month) and for LT patients, glucose monitoring is regularly performed during a month until the patient returns to his home (after three months), after what yearly glycemic control is performed. For RT patients, the measure of fasting glycemia continues once

weekly until the 6th month post-transplantation, when the control becomes once a month.

Classification of glucose status

We defined hyperglycemia based on guidelines of the international consensus for diabetes of the American Diabetes Association (ADA): patients presented HG when fasting plasma glucose (FPG) or random plasma glucose (PG) levels exceeded respectively 126 mg/dL (7.0 mmol/L) and 200 mg/dL (11 mmol/L) for at least two measurements separated by 24 hours, and not under a condition of stress such as the day of the transplant[16].

For our rDIABGRAFT study, the term “transient hyperglycemia” was used to define patients with HG (as described above) without overt diabetes diagnosed and diabetes was notified when patient required a persistent treatment (i.e., insulin or oral antidiabetics). For our pDIABGRAFT study, as we used dynamic testing, we classified our patient based on ADA guidelines: when a patient presented impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) and/or HbA1C from 5.7 to 6.4% (39–47 mmol/mol), we defined a “prediabetes” state. IFG was defined as FPG between 100 and 125 mg/dL (5.6 and 6.9 mmol/L) and IGT as 2h-PG levels during an oral glucose tolerance test (OGTT) from 140 to 199 mg/dL (7.8 and 11.0 mmol/L)16. Diabetes was defined when a patient presented FPG ≥ 126 mg/dL (7.0 mmol/L) or a random PG or 2-h PG levels during OGTT > 200 mg/dL (11.1 mmol/L) or hemoglobin A1C (HbA1C) > 6.5% (48 mmol/mol) and/or when the patient presents classic symptoms of HG[16].

Dynamic testing of glucose homeostasis

After obtaining the consent of pediatric patients and their parents, a glucose sensor (The FreeStyle Libre Flash Glucose Monitoring system, Abbott) was placed on the patient two weeks post-transplant for one month to detect the presence of early dysglycemia. Pro-insulin and C-peptide secretion by enzyme-linked immunosorbent assays (ELISA) was measured after two weeks of transplant to analyze the insulin secretion function of beta-cell, and measures had continued at one, three, six and

nine months for RT patients. The enzyme-linked immunosorbent assays used for our analyses were performed as per manufacturer's instructions (Proinsulin 10-1118-01 and C-peptide 10-1136-01 kits, Mercodia). To analyze the insulin sensitivity and secretion over time, an OGTT was performed after one month of transplantation and, for RT patients also at three, six and nine months (Fig. 1). Patients were not treated with insulin during tests. The OGTT was performed after 8hours of overnight fasting with a weight-based glucose load (1.75 g/kg for pediatric patient)[17]. Glucose and insulin were measured at fasting and at 30, 60 and 120 minutes after the ingestion of glucose. Insulin resistance (IR) was evaluated with HOMA-IR (for homeostasis model assessments of fasting insulin resistance; Ins0(μU/mL) × Gluc0(mmol/L)/22.5)[18]. If the HOMA index is less than 1.6, the result is normal. When the HOMA index is between 1.7 and 2.3, the patient presents a moderate form of IR and if the value is greater than 2.4, he suffers from a severe form of IR.

Data collection

Patient history data included sex, date of birth, height, weight and gestation at birth (i.e., term, pre- or post-term), country of origin, date of death if patient deceased, the presence of hypo- and hyperglycemia in the neonatal period, dysmaturity, any chronic and hormonal treatment before the transplant, presence of dialysis for RT, its duration and type (e.g., hemodialysis, hemodiafiltration, peritoneal dialysis), endocrine or autoimmune diseases, acanthosis nigricans and sickle cell anemia. Also, we collected data about familial history such as the presence or absence of consanguinity, metabolic syndrome, diabetes (type 1, type 2, gestational and monogenic), polycystic ovarian syndrome, fetal dystocia, and sickle cell anemia.

We included information about the liver or renal transplant such as disease etiology, transplant date, the type of immunosuppressants administrated (tacrolimus, cyclosporine A, sirolimus, glucocorticoids), the use and duration of glucocorticoids in pre- and post-transplant period, the presence and date of liver or renal rejection, the type of transplant (living or cadaveric) and the link with the donor. We also collected anthropometric data in pre- and post-transplant period (at one, three, six and nine months after RT): weight, heigh, body max index (BMI) in standard

deviation score (SDS) and Tanner stage. To obtain values in SDS we used Belgian Flemish reference charts and Cole's Corpulence Curve[19,20].

We collected glycemia and HbA_{1C} data before and after transplantation. When a patient presented HG after the first day of the transplantation, we reported the number of its occurrence, the date of its first and last observation and if a treatment was received (e.g., insulin therapy, antidiabetic oral), its doses per day, and its duration. The number of glycemia recorded was obtained by counting all measurements performed from the first consultation at CUSL (pre-transplantation evaluation) until the end of our data collection (in November 2021 for LT and in April 2022 for RT). The duration between the day of the transplantation and the last glycemia recorded was calculated to obtain the glycemia follow-up. We used REDCap (Research Electronic Data Capture) tool to collect and manage study data[21,22].

Statistical Analysis

Discrete variables are described as numbers and percentages, and continuous variables were presented as medians with interquartile range (IQR). The characteristics of children were compared according to the occurrence or not of HG using Fisher exact test for discrete variables and Student t test or Mann-Whitney test for continuous variables. A binary logistic regression analysis was performed to predict HG occurrence from all potential predictors described in data collection section and results were expressed by estimating odds ratios (OR) with their 95% confidence intervals. Due to the low number of RT patients, only univariate analysis was performed. For our LT patients, covariates with a p-value less than 0.10 in univariate analysis were introduced into a multivariate model (Wald Chi-Square). The potential predictors "graft rejection" and "CMV (cytomegalovirus)" were not introduced in multivariate model due to their interaction with "glucocorticoids" and "infection" respectively. All p-values were two-sided/2-tailed and values less than 0.05 were considered statistically significant. All statistical analyses were performed with Stata® V17 software (Statacorp, Texas, USA).

RESULTS

Patients and treatment characteristics of our rDIABGRAFT study.

Characteristics of our rDIABGRAFT LT and RT cohorts are summarized in Tables 1 and 2 (Tables S1: LT countries and S2: LT pathologies), respectively. We collected data from 195 pediatric patients treated at CUSL with liver transplantation (LT) (Fig.2). The median age of liver recipients was 18 months (10; 36) and the majority (179/195) received a liver from a living donor. All patients were treated lifelong with tacrolimus and 65% (126/195) were temporally treated with glucocorticoids. Regarding acute complications, 44% (86/195) were diagnosed with a viral infection (nCMV=55/195; nEBV=42/195) and approximatively half (104/195) of our total LT cohort presented graft rejection, the majority of which was treated with glucocorticoids (91/104) whereas five patients (2.6%) required a second transplantation.

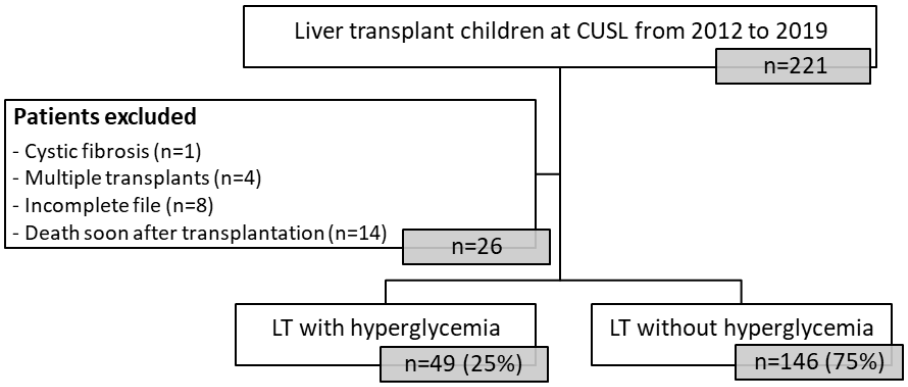


Figure 2: Flowchart of rDIABGRAFT pediatric LT cohort. Out of 195 pediatric patients who benefited from a liver transplant in the Cliniques universitaires Saint Luc (CUSL) between April 2012 and April 2019, 25% (49/195) patients presented hyperglycemia. n: number of patients, LT: liver transplant.

About our renal transplantation (RT) cohort, we collected data about 20 pediatric patients (Fig.3). The median age of renal recipients was 12 years (9; 15), seven patients (35%) received a renal transplant from a living donor, all patients were treated with tacrolimus and glucocorticoids after the transplantation, and fourteen (70%) were still under both treatment at the end of data collection. For acute complications, fourteen (70%) presented infection: nine (45%) were diagnosed with a viral infection (nCMV=4/20 and nEBV=7/20) and seven (35%) presented bacterial infection. Seven (35%) patients presented a confirmed or borderline graft rejection for which they received shots and/or increased doses of glucocorticoids. Two patients (10%) were re-transplanted.

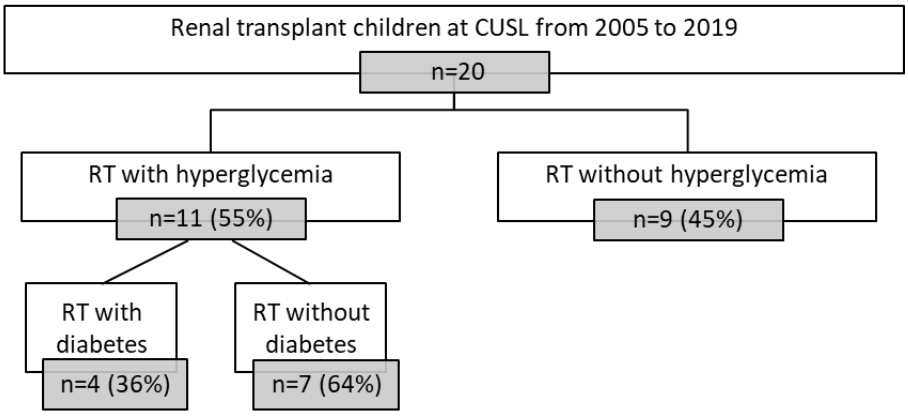


Figure 3: Flowchart of rDIABGRAFT pediatric RT cohort. Out of 20 pediatric patients who benefited from a renal transplant in the Cliniques universitaires Saint Luc (CUSL) between April 2004 and December 2019, eleven (55%) presented hyperglycemia. Out of them, four (20%) developed overt diabetes and the remaining seven (35%) patients presented HG without overt diabetes. n: number of patients, RT: renal transplant.

Table 1. Characteristic and treatment of pediatric liver transplant patients (rDIABGRAFT).

	LT total Cohort, n=195	LT HG positive n=49	LT HG negative n=146
CHARACTERISTIC			
Gender, man, n (%)	98 (50,3)	24 (49.0)	74 (50.7)
Alive, n (%)	193 (99.0)	48 (98.0)	145 (99.3)
Age of liver transplant, months, median (p25; p75)	18.1 (10.1; 36.2)	11.9 (9.2; 22.4)	20.2 (10.5; 44,3)
Age≤1 years (%)	72 (36.9)	25 (51.0)	47 (32.2)
Age≤2 years (%)	122 (62.6)	38 (77.5)	84 (57.5)
Weight SDS, median (p25; p75)	-1.3 (-2.3; -0.4)	-1.5 (-2.7; -0.8)	-1.3 (-2.2; -0.3)
Height SDS, median (p25; p75)	-1.6 (-2.6; -0.6)	-1.8 (-2.6; -0.7)	-1.6 (-2.6; -0.6)
BMI SDS, median (p25; p75)	-0.9 (-1.7; +0.3)	-1.2 (-1.8; +0.1)	-0.8 (-1.7; +0.4)
TRANSPLANTATION AND TREATMENTS			
Treatment before liver transplantation	21 (10.7)	6 (12.2)	15 (10.3)
- Glucocorticoids, n (%)	8 (4.1)	2 (4.1)	6 (4.1)
- Immunosuppressors, n (%)			
Living donor for liver transplant, n (%)	179 (91.7)	44 (89.8)	135 (92.5)
- Father	66 (33.8)	18 (36.7)	48 (32.9)
- Mother	79 (40.5)	20 (40.8)	59 (40.4)
- Aunt/Uncle	23 (11.8)	6 (12.2)	17 (11.6)
- Siblings	2 (1.0)	-	2 (1.4)
- Cousin	7 (3.6)	-	7 (4.8)
- Grandparents	2 (1.0)	-	2 (1.4)
Immunosuppressive treatments post-transplant			
- Tacrolimus, n (%)	195 (100.0)	49 (100.0)	146 (100.0)
- Glucocorticoids, n (%)	126 (64.6)	39 (79.6)	87 (59.6)
- Resumption of glucocorticoids	22 (11.3)	9 (18.4)	13 (8.9)
COMPLICATIONS			
Acute graft rejection or suspicion	104 (53.3)	36 (73.5)	68 (46.6)
Glucocorticoids doses elevation or treatment	91 (46.7)	33 (67.3)	58 (39.7)
Viral infection post-transplant, n (%)	86 (44.1)	30 (61.2)	56 (38.4)
- CMV, n (%)	55 (28.2)	22 (44.9)	33 (22.6)
- EBV, n (%)	42 (21.5)	10 (20.4)	32 (21.9)
- Hepatitis C	4 (2.1)	1 (2.0)	3 (2.0)
Second liver transplantation	5 (2.6)	3 (6.1)	2 (1.4)

LT: Liver transplant; SDS: Age- and gender Standardized Scores, using Belgian references; BMI: Body mass index; PTLD: Post-transplant lymphoproliferative disease; CMV: Cytomegalovirus; EBV: Epstein-Barr Virus

Table 2. Characteristic and treatment of pediatric renal transplant patients (rDIABGRAFT).

	RT n=20	RT HG positive n=11	RT HG negative n=9
CHARACTERISTIC			
Gender, man, n (%)	13 (65.0)	7 (63.6)	6 (66.7)
Alive, n (%)	19 (95.0)	10 (90.9)	9 (100.0)
Age of renal transplant, year, median (p25; p75)	12.3 (9.3; 15.6)	11.5 (10.4; 14.3)	13.1 (5.1; 16.5)
[0-8], n (%)	5 (25.0)	2 (18.2)	3 (33.3)
[9-18], n (%)	15 (75.0)	9 (81.8)	6 (66.7)
Overweight/Obesity before transplant, n (%)	4 (20.0)	3 (27.3)	1 (11.1)
Weight, SDS, median (p25; p75)	-0.9 (-2.0; -0.1)	-0.6 (-2.2; +0.0)	-1.1 (-1.9; -0.3)
Height, SDS, median (p25; p75)	-1.3 (-2.2; -0.6)	-2.1 (-2.5; -1.5)	-0.6 (-1.2; -0.4)
BMI, SDS, median (p25; p75)	-0.1 (-1.4; +0.7)	+0.3 (-1.0; +1.1)	-0.3 (-1.6; +0.2)
PERSONAL HISTORY			
Other transplantats (liver), n (%) - LT before RT, n (%) - LT the same day as RT, n (%)	3 (15.0) 2 (10.0) 3 (15.0)	3 (27.3) 2 (18.2) 3 (27.3)	- - -
Treatment before renal transplantation - Glucocorticoids, n (%) - Immunosuppressors, n (%)	 5 (25.0) 4 (20.0)	 4 (36.4) 3 (27.3)	 1 (11.1) 1 (11.1)
Dialysis treatment, n (%) - Hemodialysis, n (%) - Peritoneal dialysis, n (%)	13 (65.0) 10 (50.0) 9 (45.0)	6 (54.5) 6 (54.5) 5 (45.5)	7 (77.8) 4 (44.4) 4 (44.4)
TRANSPLANTATION AND TREATMENTS			
Living donor for renal transplant, n (%) - Father, n (%) - Mother, n (%) - Friend, n (%) - Sister, n (%)	7 (35.0) 2 (10.0) 3 (15.0) 1 (5.0) 1 (5.0)	3 (27.3) - 1 (9.1) 1 (9.1) 1 (9.1)	4 (44.4) 2 (22.2) 2 (22.2) - -
Immunosuppressive treatments post-transplant - Tacrolimus, n (%) - Glucocorticoids, n (%) - Patient treated until the end of the study, n (%)	 20 (100.0) 20 (100.0) 14 (70.0)	 11 (100.0) 11 (100.0) 7 (63.6)	 9 (100.0) 9 (100.0) 7 (77.8)
COMPLICATIONS			
Graft rejection or suspicion - Graft rejection - Suspicion with immunosuppressors treatment - Glucocorticoids doses elevation or treatment - Tacrolimus doses elevated	7 (35.0) 3 (15.0) 4 (20.0) 7 (35.0) 1 (5.0)	6 (54.5) 3 (27.3) 3 (27.3) 6 (54.5) 1 (9.1)	1 (11.1) - 1 (11.1) 1 (11.1) -
Infection post-transplant, n (%) - Bacterial infection, n (%) - Virus infections, n (%) CMV, n (%) EBV, n (%)	14 (70.0) 7 (35.0) 9 (45.0) 4 (20.0) 7 (35.0)	10 (90.9) 5 (45.5) 6 (54.5) 3 (27.2) 5 (45.5)	4 (44.4) 2 (22.2) 3 (33.3) 1 (11.1) 2 (22.2)
Weigh gain post-transplant, n (%)	9 (45.0)	5 (45.5)	4 (44.4)
Second renal transplant	2 (10.0)	2 (18.2)	-

RT: Renal transplant; SDS: Age- and gender Standardized Scores, using Belgian references; BMI: Body mass index; LT: Liver transplant; PTLD: Post-transplant lymphoproliferative disease; CMV: Cytomegalovirus; EBV: Epstein-Barr Virus

Early transient HG in pediatric LT patients is associated with glucocorticoid use, graft rejection and viral infection.

Out of 195 LT pediatric patients, 25.1% (49/195) developed transient HG (Fig.2; Table 3) and for most of them (92%) HG appeared during the first two weeks after transplantation (Fig.4; Fig.S1). No overt diabetes was observed but a third (16/49) of our HG-positive LT cohort was treated with insulin.

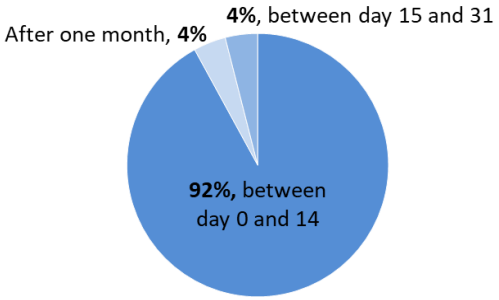


Figure 4: Onset of hyperglycemia in pediatric LT cohort (rDIABGRAFT). For most of our pediatric liver transplant patients (92%), hyperglycemia occurred during the first two weeks after transplantation.

In univariate analysis, the use of glucocorticoids (OR 2.64 95% CI 1.23-5.71) and the presence of critical condition such as graft rejection (OR 3.18 95% CI 1.56-6.48) and viral infection (OR 2.54 95% CI 1.31-4.93), in particularly Cytomegalovirus (OR 2.79 95% CI 1.41-5.53) were significantly associated with the onset of HG as shown in Table 4. After adjustment with multivariate logistic regression analysis (Wald Chi-Square tests), incidence of transient HG after LT was higher in children who received glucocorticoids (2.96, 95% CI 1.32-6.61) and presented a viral infection (OR 2.20, 95% CI 1.09-4.44) (Table 4).

Table 3. Incidence of hyperglycemia in LT cohort (rDIABGRAFT).

	Pediatric liver transplant patients, n=195
Hyperglycemia the day of the transplantation, n (%)	115 (59.0)
Hyperglycemia, more than two days with glycemia > 200mg/dL, n (%)	49 (25.1)
Days in hyperglycemia, median (P25; P75)	4 (3; 8)
Transient insulin treatment, n (%)	16 (8.2)
Transient insulin treatment duration, days, median (P25; P75)	8 (2; 16)
Number of blood glucose levels recorded, median (P25; P75)	48 (37; 66)
Duration of blood glucose monitoring, days, median (P25; P75)	675 (325; 1355)

Table 4. Uni and multivariate analysis for LT cohort (rDIABGRAFT).

	LT HG positive	LT HG negative	Univariate analysis		Multivariate analysis	
	n=49	n=146	p-value	OR (95% CI)	p-value	OR (95% CI)
Glucocorticoids post-transplantation	39 (79.6)	87 (59.6)	0.01	2.64 (1.23-5.71)	0.01	2.96 (1.32-6.61)
Graft rejection	36 (73.5)	68 (46.6)	0.001	3.18 (1.56-6.48)	-	-
Virus infection	30 (61.2)	56 (38.4)	0.01	2.54 (1.31-4.93)	0.03	2.20 (1.09-4.44)
CMV	22 (44.9)	33 (22.6)	0.003	2.79 (1.41-5.53)	-	-

LT HG: Liver transplant hyperglycemia; OR: Odds ration; CI: Confidence interval; CMV: Cytomegalovirus

Pediatric LT patients present HG in afternoon, IR and diabetes at one-month.

As we observed with rDIABGRAFT that our LT cohort presented transient HG early after transplantation (i.e., 0-14 days), we performed dynamic testing close to transplantation (day 14 and day 30) in four LT children. Table 5 presents the patients, treatments, and characteristics of the pDIABGRAFT LT cohort.

Table 5. Pathology and glycemic profile data of LT cohort (pDIABGRAFT)

		LT1	LT2	LT3	LT4
Medical record	Gender	Woman	Woman	Woman	Woman
	Country origin	Algeria	Algeria	Romania	Russia
	Pathology	Alagille Syndrome	Biliary cirrhosis, Progressive familial intrahepatic cholestasis	Budd-Chiari syndrome	Alagille Syndrome
	Donor	Living	Living	Living	Living
	Age of transplant, years	11.5	8.0	5.3	5.2
	Graft rejection	Yes	Yes	Yes	Yes
	Glucocorticoids	Yes	Yes	Yes	Yes
Secretion	[C-peptide], pmol/L	665.9	752.9	3142.0	1285
	[Pro-insulin], pmol/L	10.1	5.2	18.8	19.4
OGTT	Fasting glycemia, 0’	107	50	70	98
	Glycemia at 120’	122	212	-	250
	HOMA-IR	6.3	2.6	-	2.3

All patients presented fasting glucose and c-peptide level in normal range (Table 5) whereas glucose sensor placed at day 14 post-LT for one month showed chronic HG occurring in postprandial afternoon period (Fig.5). Parallelly, all children received high doses of glucocorticoids for graft rejection and required insulin. Moreover, during the OGTT performed at one-month post-LT (n=3), all presented IR (HOMA-IR > 1.7) while in two of them, glycemia peaked respectively at 212 and 250 mg/dL at 120' (Fig.6). We thus observed in our LT cohort two patients with diabetes at one-month post-LT.

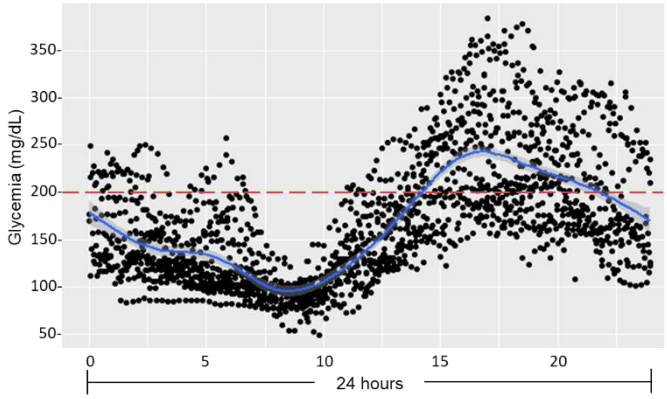
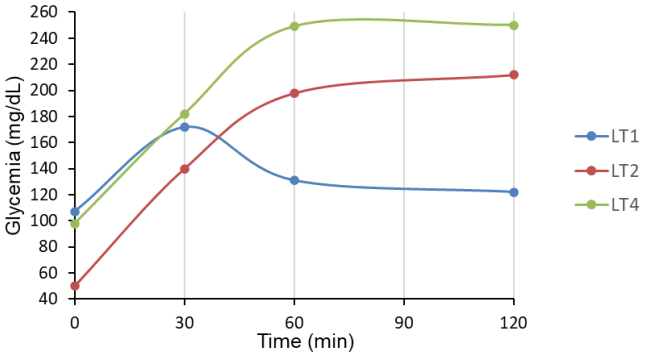


Figure 5: Continuous glucose monitoring after pediatric liver transplantation (pDIABGRAFT). Data of the continuous glucose monitor placed at day 14 post-LT for one month were regrouped on 24 hours and showed chronic hyperglycemia occurring in postprandial afternoon period.

Figure 6: OGTT at one-month post liver transplant children (pDIABGRAFT). Color legend: blue: LT1, red: LT2, green: LT4. The oral glucose tolerance test (OGTT) performed at one-month post LT showed that fasting glucose were in the normal range whereas for two of them glycemia peaked respectively at 212 (LT2) and 250 (LT4) mg/dL at the end of the test (120'), corresponding to overt diabetes.



Chronic HG is associated with graft rejection and infection in pediatric RT patients.

Out of our 20 pediatric patients with renal transplantation, 55% (11/20) presented HG (Table 6). Out of eleven patients with HG, four of them developed overt diabetes (20% of total cohort, 36% of HG cohort), still treated at the end of data collection with antidiabetic medication (oral antidiabetics in 2/4 and a combination of oral antidiabetics and insulin in 2/4). The remaining seven patients (35% of total cohort, 64% of HG cohort) presented HG without overt diabetes, during a median duration of seven days (6; 12) and four of them (57%) required insulin during a median duration of four days (2; 8).

Table 6. Incidence of hyperglycemia and overt diabetes in RT cohort (rDIABGRAFT).

	Pediatric renal transplant patients, n=20
Glycemia >200mg/dL the day of the transplantation, n (%)	10 (50.0)
Hyperglycemia: more than two days with glycemia > 200mg/dL - Transient hyperglycemia, n (%) - Days in hyperglycemia, median (P25; P75) - Overt diabetes, n (%)	11 (50.0) 7 (35.0) 7 (6; 12) 4 (20.0)
Insulin and antidiabetic treatments, n (%) - Insulin treatment for transient HG, n (%) - Duration of transient insulin treatment, day, median (P25; P75) - Current antidiabetics treatment, n (%) - Current insulin treatment, n (%) - Total dose of insulin, Unit/kg/j, median (P25; P75)	7 (35.0) 4 (20.0) 4 (2; 8) 4 (20.0) 2 (10.0) 0.30 (0.30; 0.32)
Number of blood glucose levels recorded, median (P25; P75)	136.5 (110; 229)
Years of blood glucose monitoring, median (P25; P75)	8.8 (6.3; 10.9)

No precise timing for developing HG was observed with our RT pediatric patients (Fig.S2), but a concomitance with the occurrence of critical events such as graft rejection and infection has been observed. Indeed, univariate analysis (Likelihood Ratio) was performed to evaluate the association between risk factors and HG, and our analysis showed that graft rejection (OR 14.0, 95% CI 1.25-156.61) and infections post RT (OR 12.5 95% CI 1.09-143.43) were significantly associated with a higher occurrence of HG (Table 7).

Table 7. Univariate analysis for RT cohort (rDIABGRAFT).

	RT HG positive	RT HG negative	Univariate analysis	
	n=11	n=9	p-value	OR (95% CI)
Graft rejection	7 (63.6)	1 (11.1)	0.03	14.0 (1.25 - 156.61)
Infection post-transplantation	10 (90.9)	4 (44.4)	0.02	12.5 (1.09 - 143.43)

RT HG: Renal transplant hyperglycemia; OR: Odds ration; CI: Confidence interval

All our patients with a re-transplantation or bi-organ transplantation (4/20; two second RT and two previous LT) presented chronic HG but logistic regression was not possible because no patient in the HG-free RT cohort required another transplant.

For our LT and RT pediatric patients, there was no difference between occurrence of HG and gender of patient, history of overweight/obesity, BMI (pre- and post-transplant) and the use of glucocorticoids before the transplantation, donor status (cadaveric or living), pathology requiring the transplant, weight gain or family history of diabetes.

Insulin resistance and diabetes occur early after pediatric renal transplantation.

For our RT cohort, as we did not observe a specific moment of HG occurrence but more a concomitance with the presence of critical events, we analyzed the evolution of glucose over time (at one-, three-, six- and nine-months post RT). Then, we followed four RT pediatric patients (Table 8). One RT patient (TR1) disagreed to use glucose sensor at one month post RT.

Our analyses showed that all our RT pediatric patients presented normal fasting glycemia and HbA_{1C} levels from all the post-transplant follow-up period (up to 9 months) (Table 8). Moreover, glucose sensor (placed after 2 weeks post-RT) data showed HG in the afternoon as illustrated in Figure 7, with data regrouped on 24 hours. OGTT performed at one-month post RT showed that two patients presented IGT with glycemia above 140 mg/dL at the end of the test, suggesting prediabetes, and one presented glycemia above 200mg/dL (TR1: 221 mg/dL) at 120', corresponding to overt diabetes (Fig. 8). Dosage of pro-insulin and C-peptide showed that no patient presented a β-cell dysfunction whereas HOMA-IR showed severe IR (HOMA-IR >2.4) for all our RT patients (Table 8). At three-, six- and nine-months post-RT, all patients had normalized their glycemia at the end of the test (<140 mg/dL) (Fig. 8), but they had continued to present moderate and severe IR except one at nine-month (Table 8).

Table 8. Glycemic profile data of RT cohort (pDIABGRAFT). 1

		RT1	RT2	RT3	RT4
Medical record	Gender	Man	Man	Man	Woman
	Country origin	Belgium	Romania	Romania	Belgium
	Donor	Cadaveric	Cadaveric	Cadaveric	Cadaveric
	Age of transplant, year	5.1	6.8	16.8	14.9

	Patients	14 days	1-month	3-month	6-month	9-month
C-peptide (pmol/L)	RT1	899	608	317	392	368
	RT2	748	798	344	1887	444
	RT3	1891	1918	1075	1101	848
	RT4	1122	3832	725	541	571
Pro-insulin (pmol/L)	RT1	11.7	4.5	3.9	2.5	2.1
	RT2	9.4	13.0	4.6	27.8	5.2
	RT3	19.0	19.5	9.5	12.2	7.2
	RT4	11.8	48.0	4.3	4.0	4.2
HbA1c	RT1		5.5	4.9	6.0	5.3
	RT2		-	5.8	5.6	5.4
	RT3		5.2	5.1	5.7	5.9
	RT4		5.1	5.0	4.9	5.4
Fasting glucose (OGTT 0')	RT1		84	91	-	82
	RT2		88	87	84	93
	RT3		98	100	96	84
	RT4		76	80	88	85
OGTT 120'	RT1		221	120	-	81
	RT2		141	126	105	122
	RT3		120	130	113	109
	RT4		172	97	103	125
HOMA INDEX	RT1		1.7	2.1	-	1.2
	RT2		2.8	3.0	2.7	2.8
	RT3		3.7	3.6	5.1	1.7
	RT4		3.0	3.5	3.6	4.3

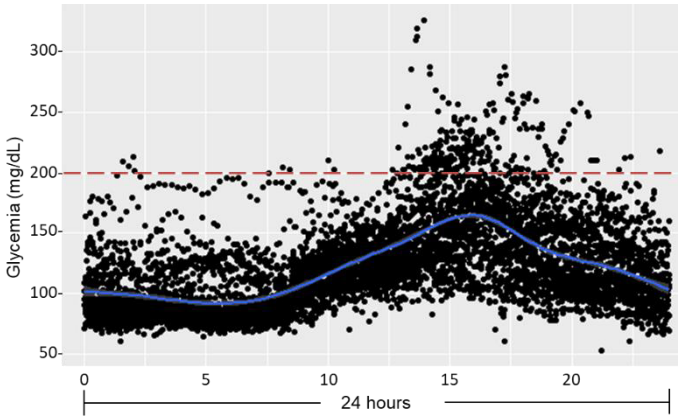


Figure 7: Continuous glucose monitoring after pediatric renal transplantation (pDIABGRAFT). Data of the continuous glucose monitor (CGM) placed at day 14 post-RT for one month were regrouped on 24 hours and showed hyperglycemia in postprandial afternoon period.

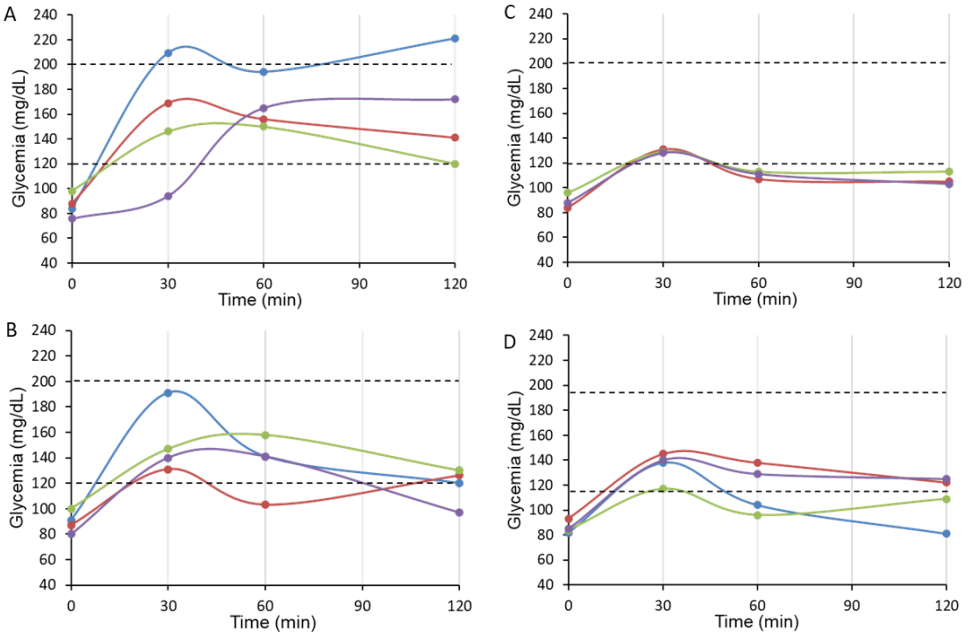


Figure 8: OGTT at one, three, six and nine-month post-renal transplant children (pDIABGRAFT). Color legend: blue: RT1, red: RT2, green: RT3, violet: RT4. The oral glucose tolerance test (OGTT) performed at (A) one-month post RT showed that fasting glucose were in the normal range whereas two patients (RT2, RT4) presented impaired glucose tolerance (>140 mg/dL) at the end of the test, suggesting prediabetes and one presented glycemia above 200mg/dL (RT1) at 120', corresponding to overt diabetes. At (B) three-, (C) six- and (D) nine-months post-RT, all patients had normalized their glycemia at the end of the test.

DISCUSSION

Our study describes the incidence and risk factors of hyperglycemia and analyzes glycemic profile in a cohort of liver and/or renal pediatric transplant patients. To our knowledge, our study is the only one that combines retro- and prospective parts which include glucose screening test rarely performed in pediatric patients who benefited from a liver or kidney transplant.

In our LT cohort, 25% (49/195) of pediatric patients presented early HG with no overt diabetes afterwards. For our pediatric RT cohort, 55% (11/20) of pediatric patients presented HG. For 35% of them (7/20), HG were transient and the remaining 20% (4/20) developed overt diabetes, currently treated with antidiabetic treatment (insulin and/or oral antidiabetics). Studies performed before 2014 were based on variable definitions of diabetes, but the introduction of recommendation in 2014 by the American Journal of Transplantation and guidelines in 2017 by ADA for “post-transplantation diabetes” induced the observation of rates of diabetes closer to our results[23,24]. Indeed, the recent study by Calani et al. reported 13 % (17/127) of diabetes in RT pediatric patients[25].

In a prevention perspective, we sought to identify relevant risk factors of HG onset after a pediatric LT and RT. The first finding of our DIABGRAFT study was, as expected, the association between HG and the use of glucocorticoids for LT cohort. The negative effect of glucocorticoids on glucose metabolism is well documented in transplant children[26-28]. Associated to the use of glucocorticoids, graft rejection was also correlated to the risk of HG in our univariate analysis for our both cohorts. According to the immunosuppressive treatment protocol, high doses of glucocorticoids are introduced for LT and increased for RT when a patient presents ACR[14]. The other risk factor of HG observed for our both cohorts was the presence of infections and can be explained by two hypotheses. Various studies described that following a metabolic stress such as infection in this case, various hormones such as cortisol, glucagon, catecholamines and pro-inflammatory cytokines are secreted and may provoke HG onset[29-32]. In parallel, HG concomitant to an infection also may be related to an intensive prior immunosuppressive treatment[33]. Our study

suggests that these three risk factors of HG indicated a specific moment when a LT and RT patient has a higher risk of developing HG, when glucocorticoids were required and when a graft rejection and an infection occur.

We did not observe risk factors as older age at the time of the transplant and history of overweight/obesity usually seen in adults[5,7,12,34], potentially because our cohorts were principally composed by liver transplant patients under the age of two years and underweighted. Also, overweight/obese patients waiting for a kidney transplant were on a specific diet to lose weight before transplantation.

In addition, the high proportion of transient HG and overt diabetes observed in our RT cohort compared to our LT cohort can be explained by several hypotheses. RT patients were directly administrated glucocorticoids for at least six months after transplantation, although LT patients received this treatment only in some specific cases, as graft rejection[15,35,36]. In addition, our RT patients were pubertal (12 years, Tanner stage ≥2), whereas the majority of LT cohort was under the age of two (Tanner stage=1) and in agreement with our previous study, with pediatric patients treated with glucocorticoids for a leukemia, Tanner stage ≥ 2 is associated with a higher risk of developing HG[37].

The other main finding of our DIABGRAFT study was that pediatric LT and RT patients developed early IGT and IR after the transplant. In our study, the normal C-peptide levels secretion showed that there was no effect of glucocorticoids or tacrolimus on β cell function, but the globally abnormal values of OGTTs showed that all our transplant patients developed IGT by the installation of IR already at one-month post-transplant, until 9-month for our RT cohort. In addition, our glucose sensor and OGTT data confirmed that non-fasting glucose monitoring (i.e., random) should be widely recommended for early detection of glucose abnormalities and that fasting plasma glucose and HbA_{1C} measurements lack power/sensibility to identify post-prandial hyperglycemia. Indeed, in our both cohorts, all pediatric transplant patients had fasting blood glucose and HbA_{1C} in the normal range whereas glucose sensor confirmed the presence of HG in post-prandial afternoon period and values of OGTT indicated the presence of prediabetes and the onset of diabetes. Our findings are similar to a recent study carried out on Egyptian pediatric kidney

transplant recipients where OGTT was able to detect a high proportion of abnormalities in glucose metabolism (23.3%)[38]. The increase of glycemia in post-prandial afternoon period is widely described and related to the use of glucocorticoids. Studies characterizing the circadian glycemic pattern by Burt et al. showed that the glucose peak after 8h of the prednisolone administration corresponds to the action peak of prednisolone[39,40].

Our study presented some limitations. First, the retrospective nature was a limitation although we excluded patients with an incomplete medical record. In addition, like for any surgical intervention, clinical parameters, including glycemia, are frequently recorded close to the surgery and less afterwards. Moreover, it may be expected that patients with a critical condition such as graft rejection and infection had benefited from a closer control of glycemia included in the global clinical parameters compared to patients without complication. Also, we highlighted with our prospective study that HG appeared in the post-prandial afternoon period whereas in our retrospective study, glycemia collected in patient medical record was often carried out in the fasting stage due to the tacrolimus dosing protocol. Thus, we obtained a potential underestimation of the occurrence of HG. Finally, since CUSL is an international center for pediatric liver transplantation, our patients and their parents were mostly foreigners and recruitment could be less effective even with the intervention of a translator. In parallel, since patients were returning home after surgery, the monitoring of glycemia by our center was performed every six months then annually.

In conclusion, diabetes is a major side effect in RT children (20%) and transient HG are frequent after a pediatric liver (25%) and renal (35%) transplant yet underestimated due to fasting glycemic measures and HbA_{1C}. The onset of HG systematically occurred in the post-prandial afternoon period and was associated to the use of glucocorticoids and with acute events as graft rejection and infection. HG was characterized by IGT and IR early after transplantation, and only detected by OGTT. Our study suggests that random blood glucose monitoring should be reinforced in the afternoon period when children present critical complications such as graft rejection and infections.

SUPPLEMENTARY MATERIAL

Text S1. Treatment protocols for pediatric liver and renal transplant patients.

At CUSL, liver and renal transplanted children receive standard immunosuppression protocol as per international guidelines1. For LT patients, this protocol includes the association of a monoclonal anti-CD 25 antibody (basiliximab, Simulect®) and a calcineurin inhibitor (tacrolimus, Prograft®)2,3. For RT patients, this protocol is based on a combination of Tacrolimus, glucocorticoids, Simulect and a cell proliferator inhibitor as mycophenolate mofetil (Cell-Cept®).

Oral administration of tacrolimus is high during the first two months after the transplantation for LT cohort (blood levels target at 8-10 ng/mL) and during the first three weeks for RT cohort (blood levels target at 10-12 ng/mL) and gradually decreases (LT: 6-8 ng/mL the third month and 4–6 ng/mL between three months and one year; RT: 8-10 ng/mL between D22 and D60 and 5–8 ng/mL after sixty days) until a lifelong maintenance dose depending on the patient clinical evolution (LT: 1-3 ng/mL; RT: 4-6 ng/mL after six months). For LT patients, steroids (Solumedrol®, Medrol®) are administrated when they present an acute cellular rejection (ACR), approximately between the 7th and the 14th day post-transplant. Patients receive high doses of steroids (5 mg/kg/day) which progressively decrease until the third month after rejection (0.25 mg/kg/day) and space out in the sixth month (0.5 mg/kg/2 days)2. For RT patients, high doses of Solumedrol (125mg/m²) are administrated intravenously during surgery and on day one. Then, oral administration of prednisone begins at day two until day four at a high dose (60mg/m²/j) and gradually decreases (10mg/m² in the second month and 5mg/m² in the third month) until the sixth month where its necessity is evaluated. Doses of glucocorticoids are increased when a LT and RT patients presents ACR.

1.Agency EM. Guideline on clinical investigation of immunosuppressants for solid organ transplantation. Committee for medicinal products for human use (CHMP) 2008; Doc. Ref. CHMP/EWP/263148/06.

2.de Magnée C, Brunée L, Tambucci R, et al. Is ABO-Incompatible Living Donor Liver Transplantation Really a Good Alternative for Pediatric Recipients? Children (Basel). 2021;8(7).

3.Gras JM, Gerkens S, Beguin C, et al. Steroid-free, tacrolimus-basiliximab immunosuppression in pediatric liver transplantation: clinical and pharmacoeconomic study in 50 children. Liver Transpl. 2008;14(4):469-477.

Table S1. Countries of pediatric liver transplant patients (rDIABGRAFT).

Country	**Number of patients, n=195**
Africa, n=88	
Algeria	58
Israel	19
Morocco	7
Tunisia	2
Guinea	1
Syria	1
Eastern Europe, n=63	
Ukraine	28
Russia	26
Romania	5
Moldova	2
Poland	2
Western Europe, n=32	
Belgium	28
France	2
Luxemburg	1
Netherlands	1
United Kingdom	1
Southern Europa, n=7	
Greece	4
Italia	2
Portugal	1
Asia, n=4	
Uzbekistan	2
India	1
Kazakhstan	1

Table S2. Pathologies of pediatric liver transplanted patients (rDIABGRAFT)

Country	**Number of patients, n=195**
Cholestatic disease, n=144	
Bile duct atresia	118
Alagille syndrome	12
Familial progressive intrahepatic cholestasis	10
Sclerosing cholangitis	3
Cholestasis	1
Metabolic and genetic liver disease, n=25	
Tyrosinemia	10
Crigler-Najjar	5
Alpha 1 antitrypsin deficiency	1
Carbamoyl phosphate synthetase deficiency	1
Ornithine carbamoyl transferase deficiency	1
Glucuronyl transferase deficiency	1
Familial hypercholesterolemia	1
Glycogenosis	1
Maple syrup urine, leucinose	1
Wilson disease	1
Nephronophthisis	1
Zellweger syndrome	1
Malignant liver disease, n=12	
Hepatoblastoma	10
Hepatocarcinoma	2
Chronic liver disease, n=10	
Cirrhosis of unknown origin	6
Autoimmune liver disease	3
Congenital hepatic fibrosis	1
Acute liver failure, n=4	
Toxic-allergic hepatitis	2
Neonatal herpic hepatitis	1
Budd Chiari	1

Figure S1: Visualization of blood glucose levels in pediatric liver transplant patients. Graph shows glycemia data of liver transplant children over a period of one year.

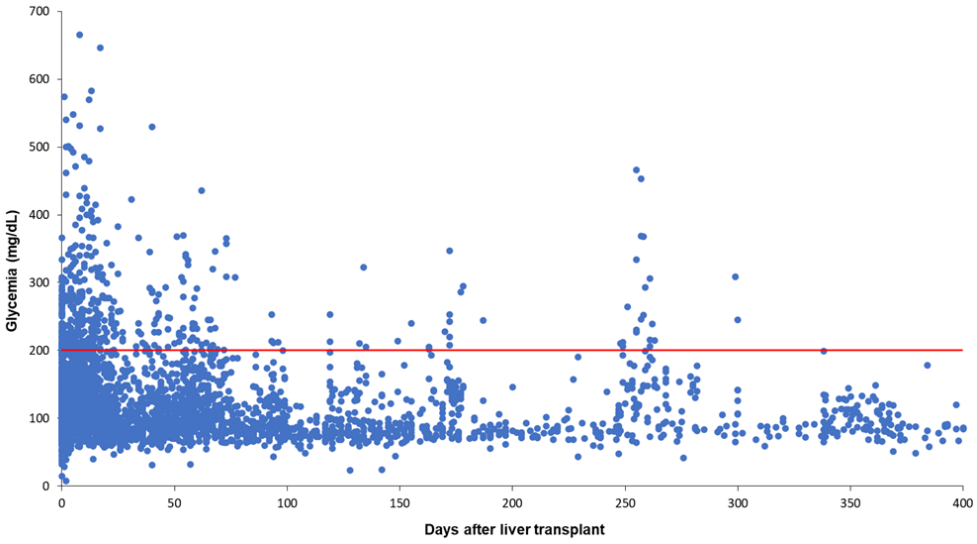
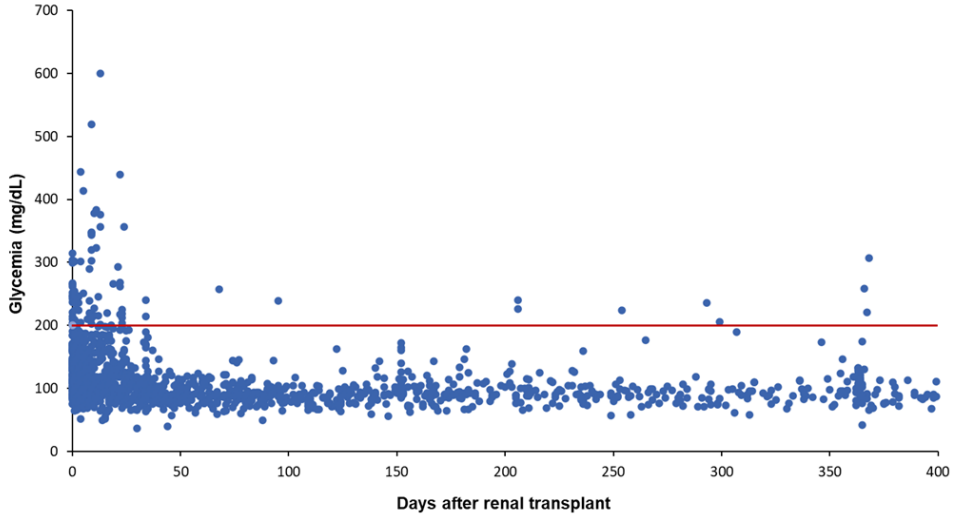


Figure S2: Visualization of blood glucose levels of pediatric renal transplant patients. Graph shows glycemia data of renal transplant children over a period of one year.



AUTHOR CONTRIBUTIONS

PA.L. had the idea for the study, designed it and reviewed the manuscript. S.W. designed and performed the study and assays, collected, and analyzed data, followed patients, decided and performed statistical methods and wrote and reviewed the manuscript. V.M. collected data and with A.R performed statistical methods. P.lHdB. performed dynamic tests and blood sample. N.R. and N.G. recruited patients who had undergone renal transplantation. N.R., N.G., X.S., I.S., R.R. and E.S. were doctors of pediatric patients and reviewed the manuscript. All the authors have read and approved the final version of the manuscript.

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DISCLOSURES: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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RESULTS – GENEPEDIAB STUDY (MANUSCRIPT 3)

In this section, results of the GENEPEDIAB study are outlined. We describe the results obtained after the use of an internal score based on clinical features differ from T1D and T2D phenotypes to identify patient with atypical diabetes.

KEY MESSAGES

- To our knowledge, screening for atypical forms of diabetes in a T1D population is rarely performed in pediatric patients.
- The GENEPEDIAB study shows the efficiency and relevance of the DIAMODIA score by detecting 100% of our MODY patients.
- The study confirms well-documented clinical characteristics such as the absence of islet autoantibodies and the presence of a residual C-peptide secretion, and introduces new clinical characteristics to help clinician in the identification of the most common forms of monogenic diabetes (*GCK, HNF1A, HNF1B, KCNJ11 and ABCC8*)
- Absence of islet autoantibodies, IDAA$_{1C} \leq 9$ and residual C-peptide are effective criteria for detection of atypical diabetes.
- Our DIAMODIA score criteria demonstrate a clinical polarization of T1D and MODY diabetes, and patients with atypical diabetes bridge the gap between the two typical forms of the disease by being clinically different from T1D and from monogenic diabetes.

A NEW SCORE TO IDENTIFY PEDIATRIC PATIENTS WITH ATYPICAL DIABETES ASSOCIATED WITH GENE POLYMORPHISMS: INSIGHTS FROM THE GENEPEDIAB CONSORTIUM STUDY

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INTRODUCTION

Diabetes mellitus is globally defined by a state of chronic hyperglycemia and refers to a heterogeneous group of diseases in terms of etiologies and therapeutic options[1]. Although the diagnosis of type 1 diabetes (T1D) is confirmed by the determination of specific antibodies[2], T1D and type 2 diabetes (T2D) are clinically characterized essentially by the phenotypic picture and the evolution of the patient, without recourse to precise etiological and pathognomonic criteria. The recognition of the existence of overlapping features and the overarching term diabetes mellitus allowed the introduction of novel diabetes clustering approach to help the clinician to choose the best therapeutic interventions. In this context, Leif Groop and his team proposed in 2018 the subclassification of diabetes in five cluster (ANDIS; the Ahlqvist classification) and based on six clinical biological parameters providing information on the pathogenesis of diabetes[3]. However, although this new clustering has proposed interesting clinical variables (GAD65 autoantibody, age at diabetes diagnosis, BMI, HbA1C at diagnosis, HOMA-B index, and HOMA-IR index) and improved the differentiation between T1D and T2D, this first step in deconstructing the heterogeneity of the disease is based on phenotypic criteria and does not consider genetic forms of diabetes.

Genetic forms of diabetes are less common (up to 5% of all cases) and often confused with T1D[4,5] or DT2[6,7]. Despite the importance of the appropriate etiology - based diagnosis, monogenic diabetes forms remain largely underdiagnosed[8,9]: It is accepted that within a convention of care for patients with diabetes, 2 to 3% of active patients suffer from undetected genetic forms[10]. The diagnosis of a genetic origin of diabetes has multiple consequences, the first being at the therapeutic level: some forms do not require treatment (i.e., GCK-MODY), others respond to oral agents (i.e., HNF1A-MODY) or require specific medical follow-up due to the presence of extra pancreatic manifestations (i.e., HNF1B-MODY). In addition, for certain genetic forms of diabetes, the risk of micro- and macro-vascular complications of diabetes can be negligible, which improves disease outcomes for the patient. Finally, the discovery of a dominant or recessive mutation in an index case may imply the transmission of the disease to the offspring or the presence of the gene mutation in family relatives[11].

Compared to T1D, monogenic diabetes is often associated with better glycemic parameters, absence of anti-islet antibodies and a relatively preserved beta cell function[12]. Several studies have advised to genetically analyze all patients with diabetes without anti-islet antibodies or with high residual C-peptide secretion to optimize the diagnosis of monogenic diabetes and perform targeted therapeutic management[13,14].

To our knowledge, currently, there is no clear criterion or algorithm for diagnosing genetic forms of diabetes. Clinical variables were pooled into a score, the MODY calculator (available on: www.diabetesgenes.org)[15], which provides a probability for a given patient to present monogenic diabetes, but with the disadvantage of not taking into consideration different important parameters as islet autoantibodies, C-peptide secretion, or glycemic variability indices.

In this context, the aim of our GENEPEDIAB study was to create the DIAMODIA score, derived from the MODY calculator, to identify patients with atypical diabetes carrying gene variants, with the aim of optimizing therapeutic management of these patients. Here, we described and evaluated variables of our score which can be implemented in the daily diabetology clinics. Subsequently, these patients with atypical diabetes were characterized based on their clinical and glycemic parameters. This work is the subject of continuous work towards the characterization of genetic forms of diabetes.

MATERIALS AND METHODS

Study context

The GENEPEDIAB study is a multicenter, retro- and prospective, interventional, and diagnostic study to screen monogenic diabetes and to propose a new approach of atypical diabetes detection based on the creation of a new score. For this study, Cliniques universitaires Saint-Luc (CUSL) collaborated with four Belgian hospitals: CHU-UCL Namur, (1) Godinne site (Yvoir) and (2) Saint Elisabeth site (Namur); (3) CHU Liège, ND-des Bruyères site (Liège); (4) Cliniques CHC Mont-Légia (Liège). The GENEPEDIAB study is a sub-part of the DiaType project (multidisciplinary consortium of three Belgian universities [UCLouvain, ULB and VUB]), which aimed to develop and implement personalized diabetes medicine in Belgium. The study protocol is approved by the CUSL central ethics committee and by the different local ethics committees (EC study number: 2018/23JAN/023). The study was performed in accordance with the Declaration of Helsinki followed in conventions of care for patients with diabetes. All children and their parents signed an assent or an informed consent form.

Patients eligible to participate in the GENEPEDIAB study are between 6 months and 18 years of age and are diagnosed with diabetes according to American Diabetes Association (ADA) criteria[1]: fasting blood glucose ≥126 mg/dL or blood glucose ≥200 mg/dL at the 120th minute of OGTT or HbA_{1C} ≥6.5% or symptoms of hyperglycemia/hyperglycemic crisis with random glucose ≥200 mg/dl. Patients participating in the study have been treated for T1D or monogenic diabetes for at least 18 months.

Study design and patient selection

We first created a new diagnostic tool, the DIAMODIA score (for "DIAgnose MOnogenic DIAbetes"), to identify patients who presented an atypical form of diabetes. We based our DIAMODIA score on the incomplete MODY probability calculator (available on www.diabetesgenes.org), which identifies patients with a monogenic diabetes based on clinical history suggestive of non-T1D and non-T2D and based on age, gender, body mass index, HbA_{1C}, insulin treatment vs. oral

antidiabetic drugs, presence of diabetes in a first-degree relative, and duration of insulin treatment[15]. Selected criteria were classified into strong and weak criteria, and patients who presented at least one strong criterion and one weak criterion were considered to have atypical diabetes and formed the ADia cohort (for Atypical Diabetes).

Then, we conducted a retrospective analysis of clinical data from patients treated for T1D or MODY for at least 18 months in the aforementioned centers. For the baseline T1D cohort and the MODY cohort, following data were collected and managed using REDCap (Research Electronic Data Capture) tools[16,17] provided by the Vanderbilt University (Nashville, USA) and hosted at Cliniques universitaires Saint-Luc: (1) Patient information: Sex, date of birth, country of origin; (2) Patient's history: birth height and weight, history of gestational diabetes during pregnancy, history of neonatal hypoglycemia, presence of autoimmune diseases, family history of diabetes; (3) Anthropometric data at diabetes diagnosis: Date of diagnosis, age, weight (kg), height (m), body max index (BMI, kg body weight/m²), Tanner stage; (4) Information on diabetes diagnosis testing: Glycemia (mg/dL), HbA_{1C} (% and mmol/mol), islet autoantibodies – anti-GAD65 (U/mL), IA2 (U/mL), insulin (%) and ZnT8 (U/mL) – HLA genotype, basal C-peptide (pmol/mL), residual insulin secretion (pmol/L), presence of ketoacidosis defined by a venous pH <7.35 or bicarbonate <22 mmol/L[18]; (5) Diabetes management data: treatment start date, type of treatment (insulin, oral antidiabetic); (6) Information about glycemic variability represented by three measures of $IDAA_{1C}$ and $GTAA_{1C}$ (see descriptions below). Z-scores (SDS) for height, weight and BMI were calculated as the number of standard deviations to the mean of the age- and gender subgroups using the Belgian population as reference[19]. BMI score evaluation was based on the international BMI cut-offs (International Obesity Task Force - IOTF) for thinness, overweight and obesity for children and adolescents[20].

Second step, we submitted the baseline T1D cohort to the DIAMODIA score and patients were separated into two cohorts: (1) ADia cohort, including patients with sufficient criteria to the DIAMODIA score to present an atypical diabetes and (2) T1D cohort, corresponding to patients with not sufficient criteria to the DIAMODIA score

to present an atypical diabetes. T1D cohort were described and compared to ADia cohort.

Third step, we applied the DIAMODIA score to a cohort of patients with a positive genetic result for monogenic diabetes, corresponding to our MODY patients, for internal validation of our score and to determine the best clinical variables for the diagnosis of genetic forms of diabetes. For this analysis, clinical data and DIAMODIA variables of MODY cohort were compared to T1D.

Last step, we prospectively genotyped ADia patients using a "classic MODY panel" including the most frequent mutated genes causing monogenic diabetes (described below). ADia patients with a negative result to the "classic MODY panel" were then subjected to a more in-depth genetic analysis with a whole exome sequencing (WES, described below) to identify rare or new variant associated with deregulation of glucose metabolism. Simultaneously, variables of T1D, MODY and ADia cohorts were used to visually classify ADia patients into T1D or MODY groups and identify the variables suggesting a MODY profile rather than a T1D profile.

$IDAA_{1C}$ and $GTAA_{1C}$

The $IDAA_{1C}$ score is used, in conjunction with the $GTAA_{1C}$ score to determine levels of glycemic variability in terms of "daily insulin requirements" and "time in normoglycemic range " for adjustment of standard HbA1C levels as described by the formula of Mortensen[21] and Nielens[22]: $IDAA_{1C} = HbA_{1C}$ (%) + [4 × insulin dose (IU/kg body weight/day)]; $GTAA_{1C} = HbA_{1C}$ (%) – [3 × % of normoglycemic values (70–180 mg/dL)]. For each patient, we selected three consultations dates, at least 18 months after diabetes diagnosis to avoid the influence of partial remission (PR) and we collected information about HbA_{1C}, daily insulin requirement (dose in IU /kg body weight/day) and percent of normoglycemia (defined as a blood glycemic value ranging from 70 mg/dL to 180 mg/dL). We calculated three different values of $IDAA_{1C}$ and $GTAA_{1C}$ to obtain a mean score. A mean score of $GTAA_{1C}$ or $IDAA_{1C}$ inferior to 4.5 or 9 respectively represented a positive criterion of atypical diabetes for the patient.

Classic MODY panel

ADia patients selected with DIAMODIA score (DIAMODIA score ≥2) were screened using a MODY gene-sequencing test called “classic MODY panel”, which includes *GCK*, *HNF1A*, *HNF4A*, *HNF1B*, *KCNJ11*, *ABCC8*, and *INS* gene sequencing, carried out by *Centrum Medische Genetica Antwerpen* (Antwerp University Hospital – Belgium). Mutation analyses were performed using Next Generation Sequencing (NGS) on an Illumina MiSeq sequencer (Illumina Inc., USA) after multiplex-PCR enrichment with the MODY MASTR kit (Multiplicom N.V., Belgium). The genes were checked with a predefined coverage of at least 30X for all encoding exons (including intron/exon transition). For the genes *GCK*, *HNF1A*, *HNF4A*, *HNF1B*, additional deletion/duplication studies were performed using MLPA (Multiplex Ligation-dependent Probe Amplification, SALSA® MLPA® Probemix P241-E1 MODY Mix 1, MRC Holland, Netherlands). For other genes, the analysis did not reveal any presence of large deletions or duplications.

Whole Exome Sequencing

The in-depth genetic analysis for ADia patients with a negative result at the first genetic screening was performed in collaboration with *Institut de Duve - Groupe de Génétique Moléculaire Humaine* (Prof Mikka Vikkula’s Team UCLouvain, Brussels, Belgium). The WES was performed on DNA extracted from the whole blood of our ADia patients using the Wizard genomic DNA purification kit (Promega) and was sequenced by Macrogen (de Duve Institute, Belgium). The exome of our ADia patients was screened by a list of genes involved in monogenic, neonatal, and very rare forms of diabetes and based on the EXETER genes list (https://www.diabetesgenes.org/, accessed 12 May 2022) (Table 1).

Table 1. List of genes involved in monogenic, neonatal, and very rare forms of diabetes and based on the EXETER genes list.

ABCC8	COQ9	FOXP3	IER3IP1	MNX1	PLIN1
AGPAT2	CP	GATA4	IL2RA	MTTL1	POLD1
AIRE	CTLA4	GATA6	INS	NEUROD1	PPARG
AKT2	DCAF17	GCK	INSR	NEUROG3	PPP1R15B
APPL1	DEXI	GLIS3	ITCH	NKX2	PTF1A
BLK	DNAJC3	GLUD1	JAK1	NKX2-2	RFX6
BSCL2	DOCK8	HADH	KCNJ11	PAX4	SIRT1
CDKN1C	DUT	HNF1A	KLF11	PAX6	SLC19A2
CEL	DYRK1B	HNF1B	LMNA	PCBD1	SLC29A3
CISD2	EIF2AK3	HNF4A	LPL	PDX1	SLC2A2
COQ2	EIF2S3	ICOS	LRBA	PIK3R1	STAT1

The WES Bioinformatics analysis was performed using an in-house DNA analysis program, Highlander software and the reads were aligned to the GRCh38 build of the human reference genome.

Variant interpretation

First, read alignments were visually inspected and any weak read was removed. In silico prediction tools as functional scoring system Polyphen2, MutationTaster and CADD were used to score the impact of the variants and the Exome aggregation Consortium (ExAC) to estimate allele frequencies of the variants. To clinically interpret detected variants and their pathogenicity, we relied on the criteria according to the guidelines of the American College of Medical Genetics and Genomics (AMCG). Variants detected with our filter were categorized into five classes[23]: I likely benign, II benign, III Uncertain significance, IV likely pathogenic and V pathogenic. The clinical information of detected variants pathogenicity was assessed with public database ClinVar, LOVD (Leiden Open Variation Database), polymorphism database (dbSNP) and data from literature (pubmed.gov). The ensemble method REVEL score was also used for evaluating variant pathogenicity.

Statistical methods

All statistical analyses were performed in R version 4.2.2[24]. A p-value less than 0.05 was considered statistically significant and all p-values were 2-tailed. Categorial variables are reported as numbers and percentages, and continuous variables were reported as medians with interquartile range (IQR). The Wilcoxon rank sum test was used to compare continuous variables between T1D and ADia groups and T1D and MODY groups. Pearson's Chi-squared test and Fisher's exact test were used appropriately to compare categorical variables between T1D and ADia groups and T1D and MODY groups. An exploratory multivariate analysis (Multiple Correspondence Analysis, MCA) was performed with variables of DIAMODIA score (categorial data) of T1D and MODY cohorts using the FactoMineR package[25]. Univariate logistic regressions were performed to compare T1D and MODY patients based on each DIAMODIA score variable and all resulting p-values were adjusted by False Discovery Rate (FDR) for multiple comparisons. With a similar purpose, a randomForest (R package randomForest[26]) was used to rank the DIAMODIA score variables based on the classification of T1D versus MODY patients. Finally, a multinominal lasso regression analysis was performed with the glmnet package[27] to explain the class of patients (T1D, Adia and MODY) based on the DIAMODIA score variables. Prior to this multinominal lasso regression, some patients were filtered out to avoid too many missing values and the few remaining missing values were imputed by a K-nearest neighbors' algorithm with the VIM package[28].

RESULTS

We included 424 children and adolescents treated for diabetes (Figure 1) and that were divided as such: 34 patients with confirmed monogenic diabetes (MODY cohort) and 390 patients with a diagnosis of T1D (baseline T1D cohort). Clinical characteristics of three cohorts are summarized in Table 2. Most of the entire cohort was Caucasian (87.7 %) and approximately half were male (51.4%).

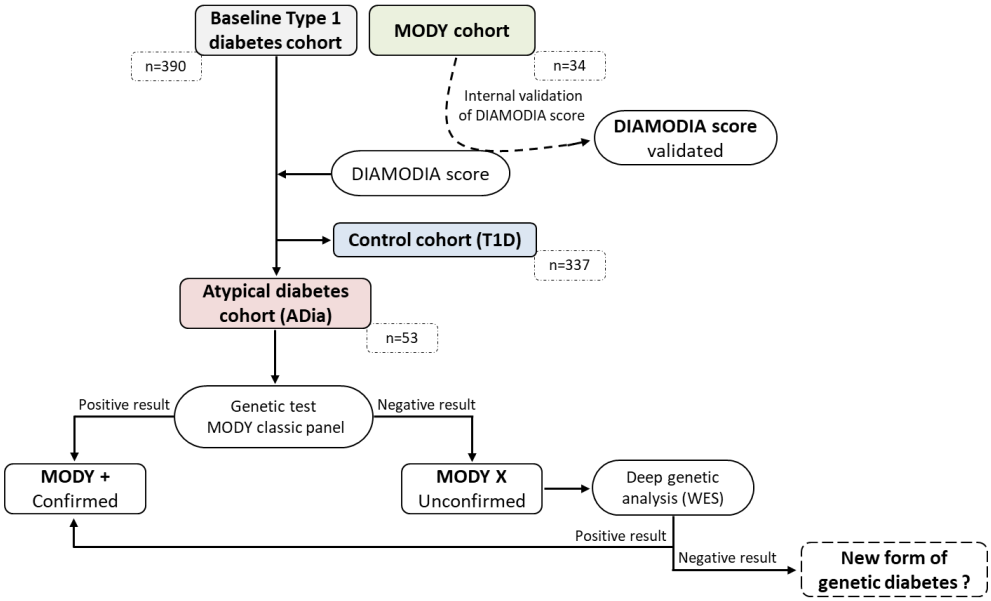

Figure 1: **Flow chart of the study**. Data from pediatric patients treated for a diabetes in five Belgian hospitals were collected. According to the etiology of the diabetes, the patients were separated into two distinct cohorts: 34 patients with confirmed monogenic diabetes (MODY cohort) and 390 patients treated for type 1 diabetes for at least 18 months (the baseline T1D cohort). The baseline T1D cohort was subjected to our DIAMODIA score and 53 patients were identified with an atypical diabetes profile (ADia cohort). The remaining 337 type 1 diabetes patients did not have sufficient criteria to present an atypical diabetes profile and formed the type 1 diabetes cohort (T1D cohort).

Step 1 – Creation of the DIAMODIA score to detect atypical form of diabetes.

To identify patients who presented an atypical form of diabetes, the most representative clinical features of monogenic diabetes were compiled into a new diagnostic tool, the DIAMODIA score. As a result, our DIAMODIA score, illustrated in figure 1, included: (1) the absence of three anti-islet antibodies: GAD65, Ins and IA2, (2) a $IDAA_{1C}$ score ≤ 9, (3) a $GTAA_{1C}$ score ≤ 4.5, (4) age < six month, (5) the presence of first-degree relative with diabetes, (6) the persistence of C-peptide

secretion, (7) the presence of extra-pancreatic manifestations, (8) the absence of ketoacidosis at diagnosis and (9) a history of neonatal hypoglycemia (Figure 2). Criteria (1) to (4) were classified as “strong criteria” and criteria (5) to (9) were classified as weak criteria. Patients who presented at least one strong criterion and one weak criterion were considered atypical diabetes and formed the ADia cohort.

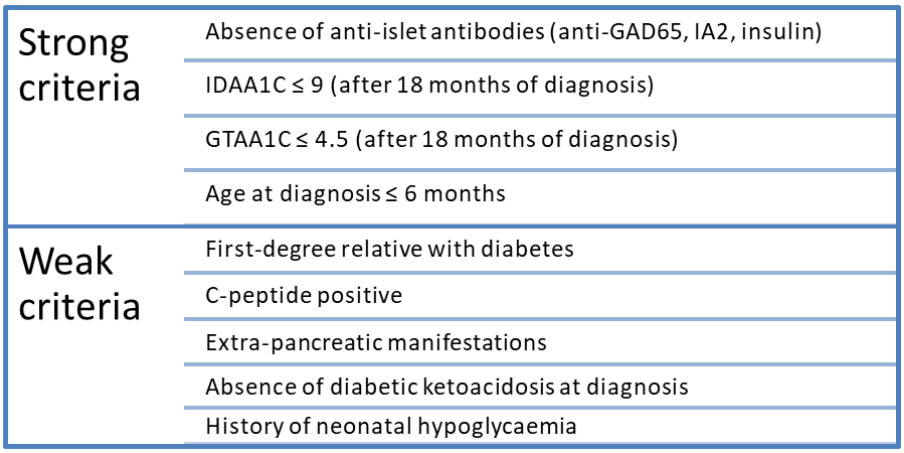

Figure 2. DIAMODIA score (for "DIAgnose MOnogenic DIAbetes"). This score is composed of the most representative clinical features of monogenic diabetes and refers to four strong criteria and five weak criteria. Patients who present at least one strong criterion and one weak criterion are considered “atypical diabetes” and form the ADia cohort.

Step 2 – Screening of atypical diabetes patients in a type 1 diabetes cohort

The baseline TD1 cohort was subjected to our DIAMODIA score, and 53 patients were identified with an atypical diabetes profile (ADia cohort), by presenting at least one weak and one strong criterion to the score. The other 337 T1D patients did not meet sufficient criteria to present an atypical diabetes profile and formed the T1D cohort. The two cohorts were characterized and compared to differentiate DIAMODIA score that discriminate features of atypical diabetes.

Characterization of patients identified as T1D by the DIAMODIA score.

The T1D cohort defined by our DIAMODIA score was composed of 48.4% male (163/337) and all the cohort was under the age of 18 at diabetes diagnosis with a median of 8.3 (5.1; 11.2) years. At diabetes diagnosis, median glycemia (448 [328; 577]) and HbA_{1C} (11.2 [1.01; 12.8]) were above the recommended values based on

American Diabetes Association guidelines[1]. Median blood bicarbonate (20.00 [12.0; 24.0]) and pH (7.33 [7.24; 7.38]) were below cutoff values of 22 mmol/l and 7.35 respectively, suggesting the presence of metabolic ketoacidosis. In clinical follow-up, after the honey-moon period (at least 18 months), median HbA_{1C} (7.4 [6.9; 7.9]) had decreased compared to diabetes diagnosis timepoint but was still above the ADA recommended value. All T1D patients were treated with insulin at a median daily dose per kilogram of 0.92 (0.77; 1.08) and the normoglycemia percentage was 43.0% (37.7; 49.3). Finally, $IDAA_{1C}$ and $GTAA_{1C}$ scores were above the recommended values (9 and 4.5 respectively), confirming the presence of a pathological HbA_{1C}, a high dose of insulin per day and a low percentage of time in normoglycemia (Table 2).

About DIAMODIA variables, all T1D patients were positive for three islet autoantibodies, and none were aged less than six months old. Less than two percent of the T1D cohort had $IDAA_{1C}$ (1.5%) and $GTAA_{1C}$ (1.2%) scores below the recommended values and around 40% had a family member with diabetes (41.0%) and presented a diabetic ketoacidosis at diagnosis (39.5%). At diagnosis, few patients with T1D presented residual C-peptide secretion (15.8%), neonatal hypoglycemia (2.4%) and extra-pancreatic manifestation (8.5%) (Table 3).

Characterization of ADia patients as per the DIAMODIA score

Out of the 53 patients identified with atypical diabetes (ADia cohort) using the DIAMODIA score, 66% were male and all patients were under the age of 18 years at diabetes diagnosis with a median age of 7.8 (5.4; 10.7) years. Out of the ADia cohort, 28 showed no islet autoantibodies and 16 showed only one islet autoantibodies. The nine remaining patients had no AAB test but presented at least one other strong criterion such as $IDAA_{1C}$ (6/9) or $GTAA_{1C}$ (6/9) below the recommended values, accompanied by at least one weak criterion. Three ADia patients were not treated with insulin: two received oral antidiabetic (biguanide, sulfonylureas) and one was not treated for his diabetes.

Clinical data of ADia and T1D patients were compared to characterize the ADia cohort formed by the DIAMODIA score and differentiate it from T1D cohort (Table

2). At diabetes diagnosis, median glycemia (358 [256; 516]) and HbA_{1C} (10.5 [9.3; 12.0]) of ADia patients were also above the ADA recommended values but were significantly lower than those observed in T1D cohort (p=0.004 and p=0.017 respectively). In parallel, the residual C-peptide secretion was significantly higher (0.23 [0.13; 0.35], p=0.04). However, no difference was observed in age, SDS height, weight and BMI, pH and bicarbonate data. In follow-up, after the honeymoon period (at least 18 months); except for three patients, all ADia patients were treated with insulin as T1D patients and their $IDAA_{1C}$ (9.7 [9.1; 10.5]) and $GTAA_{1C}$ (5.1 [4.4; 5.6]) scores were also above the recommended values (9 and 4.5 respectively) but were significantly lower than those observed in the T1D cohort (both p<0.0001). Indeed, ADia patients presented lower value of HbA_{1C} (6.6% [6.1; 7.0], p<0.001), higher percentage of time in normoglycemia (50.0% [40.0; 57.0], p<0.001) and needed lower dose of insulin (0.77 insulin/kg/j [0.66; 1.05], p=0.02).

Then, DIAMODIA variables of ADia and T1D patients were compared to identify variables that classified 53 patients as having an atypical diabetes instead of a T1D (Table 3). In results, six variables were significantly different between ADia and T1D cohort and included: (1) the absence of auto-islet antibodies (p<0.001), (2) $IDAA_{1C}$ score ≤ 9 (p<0.001), (3) $GTAA_{1C}$ score ≤ 4.5 (p<0.001), (5) the presence of diabetes in the family (p=0.024), (6) the persistence of C-peptide secretion (p<0.001) and (8) the absence of ketoacidosis at diagnosis (p<0.037). For all the aforementioned variables, their percentage of presence was significantly higher in the ADia cohort than in the T1D cohort. Briefly, 62% of the ADia cohort showed no islet autoantibodies, approximately 40% presented $IDAA_{1C}$ (44%) and $GTAA_{1C}$ (38%) below the recommended values, around 60% had a member with diabetes (58%) and a C-peptide secretion, and 76% had an absence of ketoacidosis at diagnosis. An independent test was not possible for the criterion ‘diabetes diagnosis before six months old’ because no ADia and T1D patients presented it (Table 3). Moreover, there was no difference for criteria: ‘presence of extra pancreatic disease’ (p=0.13) and ‘neonatal hypoglycemia’ (p>0.9).

Table 2. Clinical data of T1D, MODY and ADia cohorts at diabetes diagnosis and follow-up.

	T1D	MODY	ADia	T1D vs MODY	T1D vs ADia
	n (%) = 337	n (%) = 34	n (%) = 53	p-value	p-value
Gender (M), %	163 (48.4)	20 (58.8)	35 (66.0)	0.2	0.017
At diabetes diagnosis, median (p25; p75)					
Age, year	8.3 (5.1; 11.2)	7.0 (2.3; 12.0)	7.8 (5.4; 10.7)	0.13	>0.9
SDS height	-0.10 (-0.70; +0.70)	-0.35 (-1.18; +0.48)	-0.10 (-0.68; +1.05)	0.14	0.7
SDS weight	-0.50 (-1.30; +0.30)	-0.15 (-0.90; +0.80)	-0.20 (-1.00; +0.63)	0.085	0.2
SDS BMI	-0.90 (-1.90; +0.20)	+0.25 (-0.58; +0.85)	-0.50 (-1.78; +0.88)	<0.001	0.3
Glycemia, mg/dL	448 (328; 577)	126 (107; 164)	358 (256; 516)		0.004
HbA_{1C}, %	11.2 (10.1; 12.8)	6.6 (5.5; 7.4)	10.5 (9.3; 12.0)		0.017
C-peptide, pmol/mL	0.15 (0.09; 0.24)	0.71 (0 .42; 1.05)	0.23 (0.13; 0.35)		0.004
pH	7.33 (7.24; 7.38)	7.35 (7.17; 7.38)	7.36 (7.28; 7.38)	0.8	0.2
$HCO3^-$, mmol/L	20.00 (12.0; 24.0)	23.2 (9.4; 26.2)	21.0 (14.9; 24.0)	0.4	0.4
In clinical follow-up, median (p25; p75)					
HbA_{1C}, %	7.4 (6.9; 7.9)	6.3 (5.5; 6.5)	6.6 (6.1; 7.0)	<0.001	<0.001
Insulin/kg/j	0.92 (0.77; 1.08)	0.00 (0.00; 0.48)	0.77 (0.66; 1.05)		0.002
Normoglycemia, %	43.0 (37.7; 49.3)	82.2 (67.0; 90.0)	50.0 (40.0; 57.0)	<0.001	
$IDAA_{1C}$ score	11.1 (10.4; 12.0)	6.4 (5.9; 8.1)	9.7 (9.1; 10.5)		<0.001
$GTAA_{1C}$ score	6.1 (5.5; 6.7)	3.7 (3.0; 4.4)	5.1 (4.4; 5.6)		
Diabetes treatment, n (%)					
Insulin treatment	337 (100.0)	9 (26.5)	50 (94.3)	<0.001	-
Antidiabetic drugs Biguanide Sulfonylureas Glinides	-	6 (17.6) 3 (8.8) 2 (5.9) 1 (2.9)	2 (3.8) 1 (1.9) 1 (1.9) 0		-

SDS: Age- and gender Standardized Scores, using Belgian references. BMI: Body mass index

Table 3. Variables of DIAMODIA score for T1D, MODY and ADia cohorts.

	T1D, n=337		MODY, n=34		ADia, n=53		T1D vs MODY	T1D vs ADia
	n_{tot}	n (%)	n_{tot}	n (%)	n_{tot}	n (%)	p-value	p-value
Strong criteria, n (%)								
Absence AAB-Gad65	319	73 (23.9)	32	32 (100.0)	44	37 (84.1)	<0.001	<0.001
Absence AAB-IA2	293	54 (18.5)	32	32 (100.0)	44	37 (84.1)		
Absence AAB-ins	161	76 (47.2)	32	32 (100.0)	35	32 (91.4)		
Three AAB negative	154	0 (0.0)	32	32 (100.0)	35	21 (60.0)		
IDAA1c ≤ 9	332	5 (1.5)	34	28 (82.3)	50	22 (44.0)		
GTAA1c ≤ 4,5	333	4 (1.2)	28	23 (82.1)	50	19 (38.0)		
Both ≤ values	332	2 (0.6)	28	21 (75.0)	50	11 (22.0)		
Age < 6 months	336	0 (0.0)	34	3 (8.8)	53	0 (0.0)		NA
Weak criteria, n (%)								
Heredity family	334	137 (41.0)	34	29 (85.3)	52	30 (57.7)	<0.001	0.024
- Mother		9 (2.7)		19 (55.9)		3 (5.7)		0.20
- Paternal GM		29 (8.6)		2 (5.9)		10 (19)	0.80	0.021
C-peptide positive	316	50 (15.8)	32	29 (90.6)	49	31 (63.3)	<0.001	<0.001
Extra pancreatic	331	28 (8.5)	31	12 (38.7)	53	8 (15.1)		0.13
- Kidney		3 (0.9)		4 (12.9)		2 (3.8)	0.002	0.14
- Liver		0 (0.0)		2 (5.9)		1 (1.9)	0.008	0.14
Ketoacidosis negative	327	198 (60.5)	34	30 (88.2)	53	40 (75.5)	0.001	0.037
Neonatal Hypoglycemia	328	8 (2.4)	33	7 (21.2)	53	1 (1.9)	<0.001	>0.99

Step 3 – Internal validation of DIAMODIA score with a MODY cohort.

Data of 34 patients with a diagnosis of monogenic form of diabetes (MODY cohort) were collected for the internal validation of our DIAMODIA score (Figure 2). First, patients from the MODY cohort were identified by gene variants, then their clinical data were compared to T1D cohort to present clinical data predictor of genetic form of diabetes. Finally, the MODY cohort was subjected to the DIAMODIA score and variables were evaluated for internal validation.

Characterization of MODY patients and difference with T1D cohort

Out of 34 MODY patients, 58.8% were male and all the cohort was under the age of 18 at diabetes diagnosis with a median of 7.0 years (2.3; 12.0). The majority of MODY patients presented a mutation in *GCK* (13/34) and *HNF1A* (10/34) genes. Four patients presented a mutation in *HNF4A* gene, two presented a mutated gene in ABCC8, HNF1B and KCNJ11. One patient presented a mutation in *SLC19A2* gene, which was not listed in the "MODY panel" (Figure 3). Out of 15 MODY patients who required a treatment for their diabetes (26.5%), six were treated with antidiabetic drugs (biguanides=3, sulfonylureas=2 and glinides=1) and nine, mostly with the mutated *HNF1A* gene (4/9), were treated with insulin and required a median daily dose of 0.28 (0.0; 0.7) units of insulin per kilogram per day. Clinical data for each mutation were summarized in table 4.

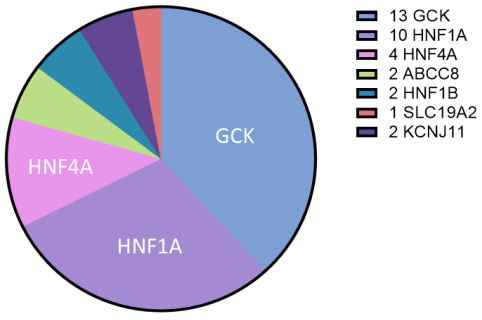

Figure 3. **MODY genes**. The majority of our patients presented a mutation in GCK (38 %) and HNF1A (29.5 %) genes.

Clinical data of MODY and T1D patients were compared. As expected, and showed in table 3 by independent tests, MODY patients presented median values of glycemia (126 [107; 164]) and HbA_{1C} (6.6 [5.5; 7.4]) significantly lower than T1D

patients (both p<0.0001) and higher BMI SDS (+0.25 [-0.58; +0.85], p<0.001) and residual C-peptide secretion (0.71pmol/mL [0.42; 1.05], p<0.001). During follow up and after the honeymoon period, the median of $IDAA_{1C}$ (6.4 [5.9; 8.1]) and $GTAA_{1C}$ (3.7 [3.0; 4.4]) scores for MODY patients were below the recommended values and significantly lower than those observed in the T1D cohort (both p<0.0001). Indeed, MODY patients presented a lower HbA_{1C} (6.3% [5.5; 6.6], p<0.001), a higher percentage of time in normoglycemia (82.2% [69.2; 90.0], p<0.001) and needed lower dose of insulin (0.00 insulin/kg/j [0.00; 0.48], p=0.02) than T1D patients. However, in the case of five patients with mutated genes *GCK* (1), *HNF1A* (2), *KCNJ11* (1) and *HNF4A* (1), $IDAA_{1C}$ score was above 9 and associated to an increase of insulin dose (0.8 insulin/kg/j [0.7; 0.9]) and HbA_{1C} value (7.5% [6.5; 8.2]). In parallel, for four of these patients and another patient with mutated *HNF1A* gene (3 *HNF1A*, 1 *KCNJ11* and 1 *GCK*), $GTAA_{1C}$ score was above 4.5, associated to a low percentage of time in normoglycemia (55.2% [34.6; 58.5]) (Tables 3 and 4).

Submission of MODY patients to the DIAMODIA score

All MODY patients who were subjected to the DIAMODIA score met at least one weak and one strong criterion of atypical diabetes and for the majority of this cohort (28/34) five criteria were met by patients regardless of the mutation. Briefly, MODY patients did not present islet autoantibodies and 82% of the MODY cohort presented values inferior to 9 and to 4.5 for $IDAA_{1C}$ and $GTAA_{1C}$ scores respectively. Three MODY patients were younger than six months at diabetes onset and carry the mutated genes *ABCC8*, *HNF1B* and *KCNJ11*. More than 85% of our cohort had residual C-peptide secretion (29/32) and a family member with diabetes (29/34), mainly a mother with diabetes (19/29). Two of the three patients without residual C-peptide secretion were carrying the mutated *KCNJ11* gene and the third one carried the mutated *HNF1A* gene. Four patients (12.1%) with mutated *KCNJ11* (2), *HNF1A* (1) and *GCK* (1) genes presented a ketoacidosis at diagnosis. Approximatively 40% presented an extra-pancreatic manifestation (12/31) including mainly kidney (4) and liver (2) and 20% (7/33) presented with neonatal hypoglycemia (Tables 3 and 4).

Table 4. Characteristics of mutated genes of MODY cohort.

	GCK n=13	HNF1A n=10	KCNJ11 n=2	ABCC8 n=2	HNF1B n=2	HNF4A n=4	SLC19A2 n=1
Clinical data							
Gender, M	10	3	2	2	0	2	1
Age at diagnosis, average, (min; max)	7 y (13; 16)	10 y (1; 15)	2; 8 mo	0 mo 12 y	2 mo 8 y	5 y (2; 7)	13 y
Glycemia at diagn, mg/dL, median (p25-p75)	118 (108;128)	147 (125; 176)	460; 810	76; 124	108; NA	78 (75;183)	615
HbA1c at diagn, % median (p25-p75)	6.4 (6.2; 6.7)	7 (6.5; 8.5)	9.2; 12.3	4.9; 7.1	5.4; NA	5.2 (4.8; 6.9)	7.9
Antidiabetic treatment, n (%)	2 (15)	6 (60)	2 (100)	1 (50)	1 (50)	2 (50)	1 (100)
- Insulin, n	1	4	1	0	1	1	1
- Oral antidiabetic, n	1	2	1	1	0	1	0
IDAA1c score, median (p25-p75)	6.4 (6.1; 6.6)	7.3 (6.1; 9.4)	10.3; 6.3	4.9; 7	5.4; 8.1	5.3 (4.9; 6.7)	8.2
GTAA1c, score, median (p25-p75)	4.0 (3.6; 4.3)	6.7 (3.0; 4.8)	6.0; 3.9	2.2; NA	5.4; 5.3	2.6 (2.1; 3.4)	5.3
HbA1c HU, %, median (p25-p75)	6.4 (6.1; 6.5)	6.3 (5.4; 7.0)	7.7; 6.3	4.9; 6.5	5.4; 5.3	5.3 (4.9; 5.8)	6.4
Insulin, unit/kg/day	0; 0.5	0.3; 0.7; 0.5; 1.0	0.7; 0	0, 0.1	0; 0.7	0; 0.9	0.44
time in normoglycemia, %, median (p25-p75)	81 (77;85)	79 (60; 89)	56; 82	90; 95	90; 87	90 (79; 95)	38
DIAMODIA score data							
IDAA1c score ≤9	12	7 (NA=1)	1	2	2	3	1
GTAA1c score ≤4.5	8 (NA=4)	7	1	2	2	4	0
Diagnostic<6 mo	0	0	1	1	1	0	0
Familial diabetes	11	9	1	1	2	4	1
Residual C-peptide secretion	11 (NA=2)	9	0	2	2	4	1
Extra pancreatic manifestation	3 (NA=1)	5	0	0	2	1	1

Evaluation of DIAMODIA variables

To evaluate the DIAMODIA score, variables of MODY and TD1 cohorts were first analyzed with exploratory multivariate analysis (Multiple Correspondence Analysis, MCA). Figure 4 showed the formation of two distinct clusters suggesting that, only based on DIAMODIA variables, the two cohorts behaved differently with individualization of MODY and T1D cohorts. Our DIAMODIA variables were therefore sufficient to differentiate MODY patients from T1D whether they met a weak or strong criterion. One patient with the mutated KCNJ11 gene presented characteristics and phenotype similar to T1D patients. He presented $IDAA_{1C}$ and $GTAA_{1C}$ scores above the recommended values, had diabetes in family, an absence of a residual C-peptide secretion and received insulin (0.65u/kg/day). Also, at diagnosis, he was eight months old and presented with ketoacidosis.

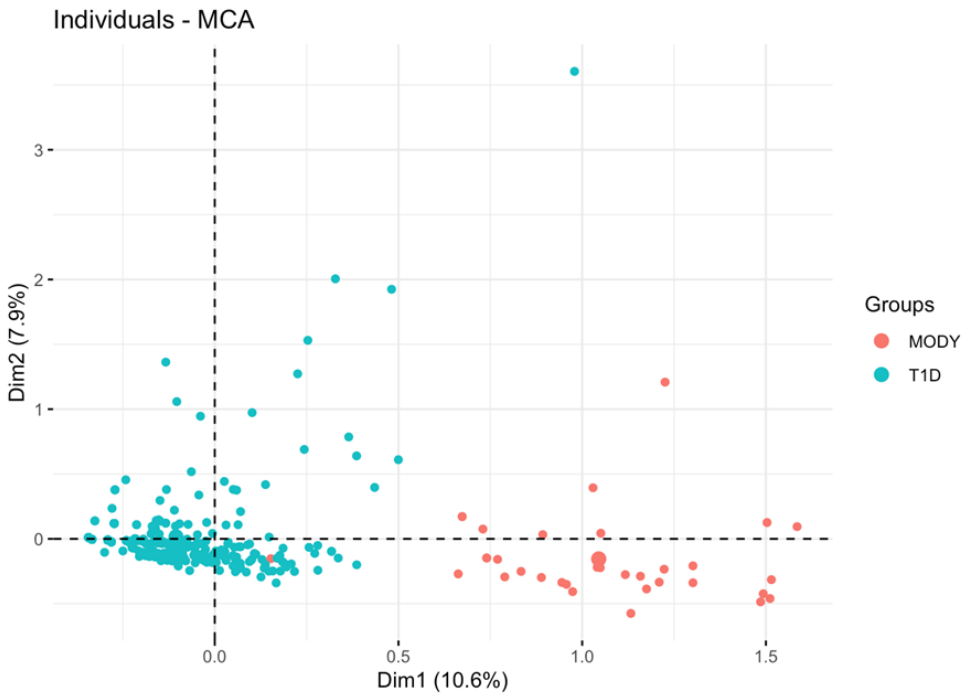

Figure 4. The multiple correspondence analysis shows the formation of two distinct clusters suggesting that only based on DIAMODIA variables, the two cohorts behaved differently with individualization of MODY and T1D cohorts.

Then, the performed independent test (table 3) to compare variables between MODY and T1D cohorts, showed that the MODY cohort presented a significantly higher percentage of presence of all DIAMODIA score variables (all with $p<0.001$) compared to the T1D cohort. To complete these results, univariate logistic regressions adjusted for multiple comparisons were performed to compare T1D and MODY patients based on each DIAMODIA variables. Figure 5 showed that there was a significant difference (all with $p<0.05$) between MODY and T1D cohorts for criteria (2) $IDAA_{1C}$ score ≤ 9, (3) $GTAA_{1C}$ score ≤ 4.5, (5) the presence of a familial diabetes and more specifically if carried by the mother or maternal grand-father, (6) the persistence of C-peptide secretion, (7) the presence of extra-pancreatic manifestations and more precisely the kidney, (8) the absence of ketoacidosis at diabetes diagnostic and (9) a history of neonatal hypoglycemia. These results suggested that the presence of all cited variables, independent of each other, allowed us to differentiate a MODY profile from a T1D profile. Moreover, for the criterion “diabetes diagnostic before 6 months old” logistic regression was not possible due to its absence in T1D cohort, although three MODY patients presented with diabetes before the age of six months. The same observation applied for the absence of the three islet autoantibodies criterion, since all MODY patients were positive to this criterion while none of T1D patients were.

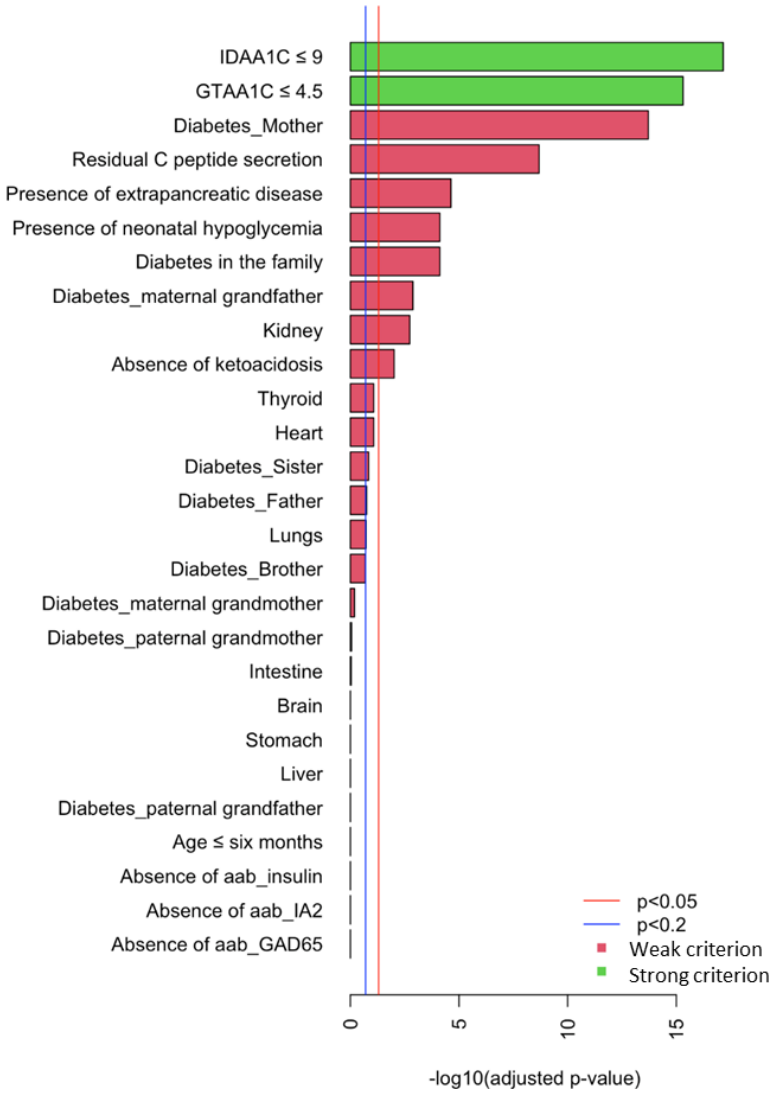

Figure 5. Univariate logistic regression between DIAMODIA variables of MODY and T1D cohorts shows a significant difference for criteria $IDAA_{1C}$ score ≤ 9, $GTAA_{1C}$ score ≤ 4.5, presence of a familial diabetes and more specifically if carried by the mother or maternal grand-father, persistence of C-peptide secretion, presence of extra-pancreatic manifestations and more precisely the kidney organ and absence of ketoacidosis at diabetes diagnostic and a history of neonatal hypoglycemia.

Finally, in parallel, a random Forest analysis ranked the DIAMODIA variables from the most relevant variable to distinguish a MODY profile from a T1D profile to the least relevant. Then, as illustrated in figure 6, the ranking of DIAMODIA variables were in decreasing order: ① $IDAA_{1C}$ score ≤ 9, ② $GTAA_{1C}$ score ≤ 4.5, ③ Absence of the three auto-islet antibodies, ④ Presence of a residual c peptide secretion, ⑤ Having a member family with diabetes, ⑥ Presence of neonatal hypoglycemia, ⑦ Absence of ketoacidosis at diagnosis, ⑧ Presence of extra pancreatic manifestation and ⑨ Age of diabetes diagnosis below six months old. Variables in position 1 to 4 had a higher average decrease accuracy than variables in position 5 to 9, suggesting that variables in position 1 to 4 were the most relevant variables allowing to differentiate effectively a MODY profile from a T1D profile.

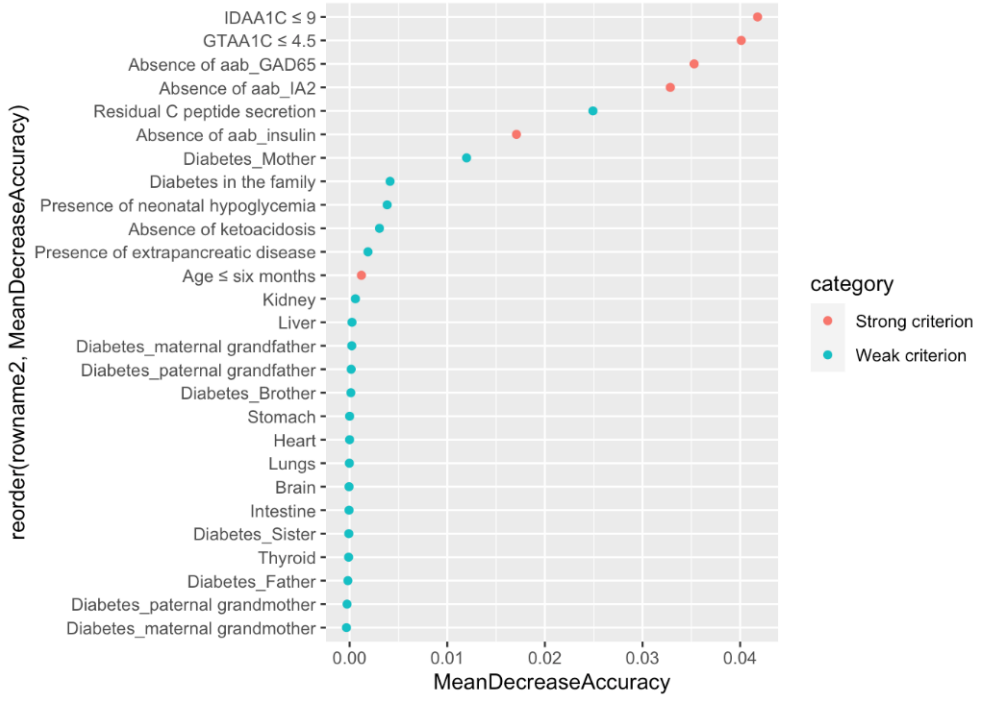

Figure 6. Random forest ranks variables of the DIAMODIA score according to their importance in distinguishing T1D profile from MODY profile. Low glycemic variability ($IDAA_{1C}$ and $GTAA_{1C}$ scores) are the two best criteria to detect monogenic diabetes, followed by the absence of the three-islet autoantibody and the presence of a residual C-peptide secretion.

Step 4 – Screening for genetic forms of diabetes in ADia cohort

For the final stage in screening for atypical forms of diabetes, ADia patients were genotyped using a "classic MODY panel" and no pathogenic variant were discovered. Then, all ADia were subjected to a whole exome sequencing which was filtered with a list of genes involved in monogenic, neonatal, and very rare forms of diabetes and based on the EXETER genes list (table 1). In results of the WES, 57 variants were reported in 32 patients. Among these variants, with the use of ClinVar, we reported 14 benign/likely benign (B) variants, 6 uncertain significance (US), 6 with conflicting interpretation of pathogenicity (CIP), 30 unknown (UNK) and one was reported as a pathogenic variant (PA) (*FOXP3*; c.970T>C; VCV000011416.1). All variants were reported in table 5.

Variants classified as “unknown variant” were not reported in Clinvar, SNP, LOVD or in literature and therefore did not allow to confirm a potential pathogenic variant. They were then sorted into “class I”, “class II” or “class III” variant using the ACMG guidelines. Then, we reported 30 class III variants among which 25 were reported as missense variants (nonsynonymous), three as frameshift with a deletion in CEL (2) and *MAFA* genes, and two as splice site region in *STAT1* and *EIF2B1* genes. Among the 30 variants, 25 were classified as deleterious by MutationTaster (DAMAGING) and CADD (C-score >20), and for 17 of them, allele frequency was less than 0.0006 (ExAC af) confirming that class III variants are very rare. More specifically, a strong pathogenicity prediction by algorithms REVEL (>0.7) and CADD (>24) scores, with no observation in the general population (gnomAD database), was observed for five missense class III variants in *PCBD1* (c.205G>A, p.Val69Met), *WFS1* (c.1124G>A, p.Arg375His), *SLC2A2* (c.1214T>G, p.Phe405Cys), *WFS1* (c.961A>G, p.Thr321Ala) and *ZMPSTE24* (c.505A>C, p.Lys169Gln) genes. For the pathogenic variant in *FOXP3* gene, no assertion criteria were provided in ClinVar. The allele was only included in a submission with an interpretation but without assertion criteria and evidence (DOI: 10.1172/JCI25112). Moreover, *FOXP3* presented a low pathogenicity prediction by Polyphen2 (TOL), REVEL score (0.564) and CADD (17.24).

Table 5. Variants (heterozygous) identified in ADia cohort.

ID	Age	Chr	Pos	Gene	DNA change	AA change	Function	Polyphen 2 HDIV	Mutation taster	REVEL score	CADD	ClinVar	ExAC af(%)	Local af(%)	ACMG class	n AAB
A035	10	3	57269575	APPL1	c.2018C>G	p.Ser673Cys	nonsynonymous_SNV	D	DAMAGING	0.190	26.5	UNK	0.06		3	NA
A037	16	9	133071673	CEL	c.2181delT	p.Val728fs	Frameshift_deletion			-		UNK	-	0.03	3	0
A038	3	4	150310231	LRBA	c.7880C>T	p.Thr2627Ile	nonsynonymous_SNV	D	DAMAGING	0.247	33.0	UNK	0	0.03	3	0
A038		19	40236254	AKT2	c.960+3G>A		Splice site region_SNV			-		UNK	-	0.19	2	
A040	6	11	17393742	ABCC8	c.4629G>T	p.Lys1543Asn	nonsynonymous_SNV		DAMAGING	0.392	21.6	CIP	0.05	0.02	3	0
A051	2	6	116927317	RFX6	c.2176C>G	p.Arg726Gly	nonsynonymous_SNV	D	DAMAGING	0.130	22.8	CIP	0.14	0.1	2	0
A053	9	10	70885163	**PCBD1**	c.205G>A	p.Val69Met	nonsynonymous_SNV	D	DAMAGING	**0.712**	**26.7**	US	0.04	0.03	3	0
A053		4	150868275	LRBA	c.2480G>A	p.Arg827Gln	nonsynonymous_SNV	D	DAMAGING	0.212	27.9	US	-	0.02	3	
A061	13	9	399233	DOCK8	c.3208A>G	p.Asn1070Asp	nonsynonymous_SNV	TOL	DAMAGING	0.104	20.2	B	1.02	0.88	1	0
A061		4	6302130	WFS1	c.2335G>A	p.Val779Met	nonsynonymous_SNV	D	DAMAGING	0.696	23.3	B/LB	0.22		2	
A063		11	17387403	KCNJ11	c.689T>C	p.Val230Ala	nonsynonymous_SNV		DAMAGING	0.571	23.3	UNK	-	0.02	3	
A063	8	7	157009917	MNX1	c.434G>A	p.Gly145Asp	nonsynonymous_SNV	D	DAMAGING	0.347	23.3	UNK	-	0.02	3	0
A063		4	6300919	**WFS1**	c.1124G>A	p.Arg375His	nonsynonymous_SNV	D	DAMAGING	**0.818**	**25.5**	US	0.03	0.1	2	
A063		2	190980615	STAT1	c.1632+5G>A		Splice site region_SNV			-		UNK	-	0.02	3	
A073	4	9	426926	DOCK8	c.4283A>T	p.Asn1428Ile	nonsynonymous_SNV	D	DAMAGING	0.457	26.4	US	0	0.02	3	NA
A078	12	20	44414649	HNF4A	c.635C>T	p.Pro212Leu	nonsynonymous_SNV	TOL	DAMAGING	0.439	18.56	UNK	0.02	0.03	3	1
A078		11	17427923	ABCC8	c.2126C>G	p.Thr709Arg	nonsynonymous_SNV		DAMAGING	0.570	24.8	UNK	-	0.02	3	
A243	13	19	7142813	INSR	c.2542+3A>G		Splice site region_SNV			-		UNK	-	0.92	2	1
A243		16	58558523	CNOT1	c.2282A>G	p.Asn761Ser	nonsynonymous_SNV	D	DAMAGING	0.189	22.8	UNK	0.01	0.02	3	
A305	7	9	136676978	AGPAT2	c.475C>T	p.Arg159Cys	nonsynonymous_SNV	D	DAMAGING	0.583	23.8	B/LB	0.54		1	0
A334	8	5	68273487	PIK3R1	c.427+5C>A		Splice site region_SNV			-		UNK	-	0.08	2	1
A351	11	2	88575349	EIF2AK3	c.2134A>G	p.Ile712Val	nonsynonymous_SNV	TOL	DAMAGING	0.069	20.6	UNK	0	0.02	3	0
C005	8	11	17386938	KCNJ11	c.1154C>G	p.Ser385Cys	nonsynonymous_SNV		DAMAGING	0.369	23.8	CIP	0.48	0.83	2	0
C006	8	3	170998353	**SLC2A2**	c.1214T>G	p.Phe405Cys	nonsynonymous_SNV	D	DAMAGING	**0.830**	**29.3**	UNK	-	0.03	3	0
C006		9	133062009	CEL	c.16C>T	p.Arg6Cys	nonsynonymous_SNV		DAMAGING	0.365	22.9	B/LB	0.11	0.58	1	
C014	15	X	49253200	FOXP3	c.970T>C	p.Phe324Leu	nonsynonymous_SNV	TOL	DAMAGING	0.564	17.24	PA	-	0.02	5	1
C014		4	6302290	WFS1	c.2495G>A	p.Arg832His	nonsynonymous_SNV	D	DAMAGING	0.672	24.3	UNK	0	0.02	3	
C015	15	3	171009957	SLC2A2	c.496+1G>T		Splice site donor_SNV		DAMAGING	-	32.0	UNK	-	0.02	2	0
E009		9	133071304	CEL	c.1819_1982del	p.Val607fs	Frameshift_deletion			-		UNK	-		1	
E009	4	9	133071299	CEL	c.1810delG	p.Ala604fs	Frameshift_deletion			-		UNK	-		1	1
E009		9	4118412	GLIS3	c.1066G>A	p.Gly356Ser	nonsynonymous_SNV	D	DAMAGING	0.272	23.7	UNK	-	0.03	3	
E030	3	4	6291313	WFS1	c.577A>C	p.Lys193Gln	nonsynonymous_SNV	D	DAMAGING	0.695	24.0	B/LB	0.4	0.34	2	0
E037	9	9	304628	DOCK8	c.452G>A	p.Arg151Gln	nonsynonymous_SNV	D	DAMAGING	0.087	23.2	CIP	0.14	0.27	2	1
E063	10	4	151014534	LRBA	c.109C>T	p.Pro37Ser	nonsynonymous_SNV	D	DAMAGING	0.190	23.5	UNK	0	0.02	3	NA
E063		4	6300756	**WFS1**	c.961A>G	p.Thr321Ala	nonsynonymous_SNV	D	DAMAGING	**0.882**	**24.4**	UNK	-	0.02	3	

ID	Age	Chr	Pos	Gene	DNA change	AA change	Function	Polyphen 2 HDIV	Mutation taster	REVEL score	CADD	ClinVar	ExAC af(%)	Local af(%)	ACMG class	n AAB
E096	3	18	22171736	GATA6	c.592C>G	p.Leu198Val	nonsynonymous_SNV	D	DAMAGING	0.716	23.3	LB	0.01	0.02	2	NA
E096		6	116920454	RFX6	c.1327C>T	p.His443Tyr	nonsynonymous_SNV		DAMAGING	0.132	11.78	B	0.46	0.13	1	
E099	7	9	133071303	CEL	c.1818delC	p.Val607fs	Frameshift_deletion			-		UNK	-	0.02	3	NA
E099		1	40270005	**ZMPSTE24**	c.505A>C	p.Lys169Gln	nonsynonymous_SNV	D	DAMAGING	**0.845**	**27.3**	UNK	-	0.02	3	
E126	11	16	57459688	COQ9	c.835G>A	p.Asp279Asn	nonsynonymous_SNV	D	DAMAGING	0.200	25.3	CIP	0.22	0.23	2	1
E138	3	4	99550971	TRMT10A	c.665C>T	p.Ala222Val	nonsynonymous_SNV	D	DAMAGING	0.664	32.0	LB	0.03	0.03	2	0
E138		9	399233	DOCK8	c.3208A>G	p.Asn1070Asp	nonsynonymous_SNV	TOL	DAMAGING	0.104	20.2	B	1.02	0.88	1	
E138		9	133062009	CEL	c.16C>T	p.Arg6Cys	nonsynonymous_SNV		DAMAGING	0.365	22.9	B/LB	0.11	0.58	1	
E138		9	133065052	CEL	c.362T>G	p.Leu121Arg	nonsynonymous_SNV		DAMAGING	0.479	23.1	B	0.5	0.42	1	
E138		13	95763938	DNAJC3	c.1060G>C	p.Glu354Gln	nonsynonymous_SNV	D	DAMAGING	0.125	23.1	B	0.14	0.11	1	
E138		16	57451109	COQ9	c.143A>G	p.Asp48Gly	nonsynonymous_SNV	D	DAMAGING	0.106	25.3	UNK	0.01	0.02	3	
E141	11	20	44414666	HNF4A	c.648+4A>G		Splice site region_SNV			-		UNK	-	0.02	2	1
E141		9	133065052	CEL	c.362T>G	p.Leu121Arg	nonsynonymous_SNV		DAMAGING	0.479	23.1	B	0.5	0.42	1	
E141		9	133062009	CEL	c.16C>T	p.Arg6Cys	nonsynonymous_SNV		DAMAGING	0.365	22.9	B/LB	0.11	0.56	1	
E143	7	19	50402732	POLD1	c.961G>A	p.Gly321Ser	nonsynonymous_SNV		DAMAGING	0.363	25.1	CIP	0.04	0.08	3	1
E143		16	58543705	CNOT1	c.4336C>T	p.Arg1446Cys	nonsynonymous_SNV	D	DAMAGING	0.572	27.2	UNK	0	0.02	3	
E143		10	23193879	PTF1A	c.960C>A	p.Asn320Lys	nonsynonymous_SNV	D	DAMAGING	0.456	23.3	US	0.02	0.06	3	
E153	11	4	150929008	LRBA	c.274A>G	p.Ile92Val	nonsynonymous_SNV	D	DAMAGING	0.122	22.8	UNK	-	0.02	3	1
E242	7	12	123630169	EIF2B1	c.369G>A	p.Ala123Ala	Splice site region_SNV			-		UNK	-	0.02	3	NA
E242		3	12406004	PPARG	c.742G>A	p.Asp248Asn	nonsynonymous_SNV	TOL	DAMAGING	0.317	23.6	UNK	0	0.02	3	
E244	7	4	151014455	LRBA	c.188G>C	p.Arg63Thr	nonsynonymous_SNV	D	DAMAGING	0.226	26.1	US	0	0.02	3	0
E245	12	8	143430124	MAFA	c.282delC	p.Pro96fs	Frameshift_deletion			-		UNK	-		3	0

Finally, out of 53 ADia patients, 21 carried no variants in the genes of interest, even though 12 were negative to islet autoantibodies. Thus, in total of 28 ADia patients negative to islet autoantibodies, none of them showed a known variant based on our genes list involving monogenic, neonatal, and very rare forms of diabetes. Therefore, an exploratory multivariate analysis built with DIAMODIA variables of T1D, MODY and ADia cohorts was performed to visually classify ADia patients in T1D or MODY groups and identify ADia patients similar to MODY patients. Figure 7 showed a gradient of observations in dimensions 1-2 on which ADia patients were situated in between T1D and MODY cohorts. This observation suggested that the three cohorts behaved differently and that ADia patients presented a different profile to T1D and to MODY patients. However, 13% (7/53) of ADia patients showed some specificity, being completely grouped with MODY patients without carrying any variants or mutations from the list of genes used as a filter. These findings suggest that these patients might carry variants or mutations in other genes implicated in diabetes which may imply opportunities for a new discovery.

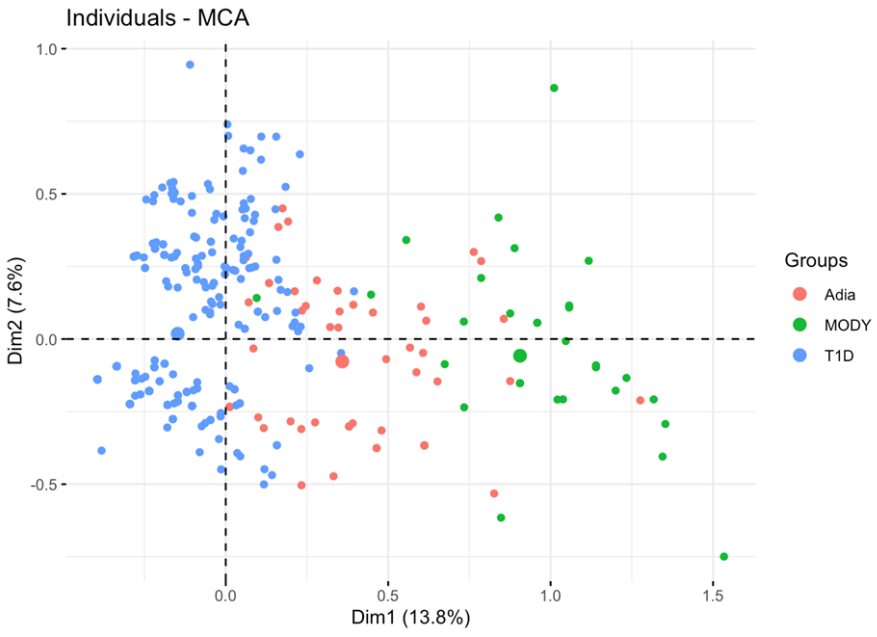

Figure 7. The exploratory multivariate analysis shows a gradient of observations in dimensions 1-2 where ADia patients were in between the T1D and MODY cohorts suggesting that the three cohorts behaved differently and that ADia patients presented a profile different from our T1D but also not so similar than our MODY patients.

The proximity between ADia and MODY patients were analyzed by the multinominal lasso regression analysis based on the DIAMODIA variables of each cohort. Figure 8 showed that the profile of three DIAMODIA variables for ADia patients were similar to MODY patients, but distinctive to T1D patients. These three variables were: (1) the absence of islet autoantibody, (2) the $IDAA_{1C}$ score ≤9 and (6) the presence of a residual C-peptide secretion.

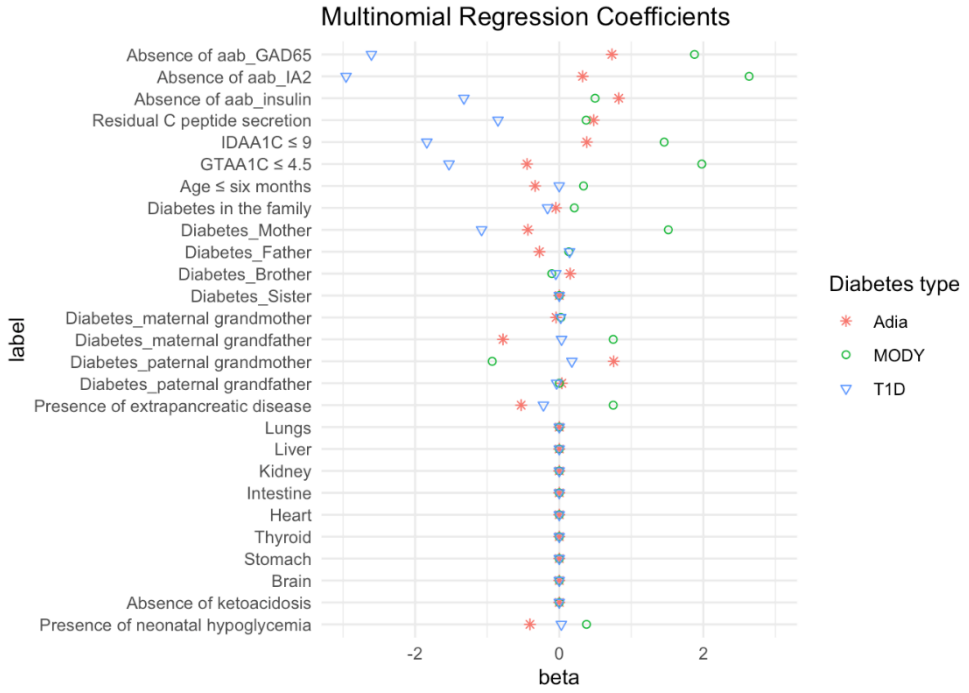

Figure 8. Multinominal regression coefficient shows three DIAMODIA variables (absence of islet autoantibody, $IDAA_{1C}$ score ≤9 and presence of a residual C-peptide secretion) for which ADia patients were close to MODY profile and far from TT1D profile.

DISCUSSION

Diabetes mellitus is a common metabolic disorder characterized by chronic hyperglycemia and includes various forms (i.e., type 2 diabetes, type 1 diabetes, monogenic diabetes, rare forms). In pediatrics, the most common form of diabetes (90%) is type 1 diabetes resulting from insulinopenia due to autoimmune destruction of pancreatic beta cells. More rarely, in 1-4% of patients, a genetic disorder is responsible for diabetes. These disorders include either impaired beta cell function or insulin dysfunction or, less commonly, are of mitochondrial origin[29-31].

In addition to the different etiology, monogenic diabetes is known to be easier to control and theoretically less severe than type 1 diabetes, both at diagnosis and in the chronic course of the disease[1,12]. We observed these differences between the different forms of diabetes in our study. Indeed, clinical presentation, mean glycemia, glycated hemoglobin, and time in normoglycemia are significantly better in our MODY cohort than in our T1D cohort. Moreover, patients with atypical diabetes had intermediate values of the different parameters studied compared to the two other forms of diabetes.

The frequency of genetic diabetes is often underestimated: several studies have shown that monogenic diabetes is often confused with type 1 diabetes and type 2 diabetes due to lack of diagnostic investigation (etiology-based diagnosis)[11]. Under these circumstances, our team developed the DIAMODIA score, based on the incomplete MODY probability calculator and additional clinical parameters, which is able to discriminate atypical diabetes from type 1 diabetes and thus improve the screening of the atypical form of diabetes. The aim of this score is to develop a screening test to identify patients who require a thorough genetic analysis. The genetically confirmed MODY cohort also demonstrated the efficacy of the DIAMODIA score and validated its relevance with 100% accuracy in detecting atypical diabetes.

Subsequently, the use of the DIAMODIA score in our T1D cohort has allowed the identification of atypical forms of diabetes. The criteria that distinguished type 1 diabetes from atypical diabetes were the absence of islet autoantibodies, high residual C-peptide secretion, and $IDAA_{1C} < 9$. The absence of anti-islet antibodies and residual C-peptide secretion are two markers already known to target atypical

diabetes. In addition, some studies have already recommended genetic analysis in all patients considered to have type 1 diabetes who do not have islet autoantibodies at the time of diagnosis[13,14,32].

Unfortunately, no patient could be confirmed as having monogenic diabetes after genetic analysis. Indeed, both the MODY gene panel and the WES were unable to identify known pathological variants in all patients with atypical diabetes. Notably, no genetic variants were found in the seven ADia patients who shared the same DIAMODIA characteristics as the majority of MODY patients. However, these seven patients may carry variants for other currently unknown diabetes-related genes.

However, we were able to show that our patients with atypical diabetes are clinically different from type 1 diabetes but also from monogenic diabetes. Furthermore, these patients with atypical diabetes correspond to a continuum of patients between type 1 diabetes and MODY for all combined DIAMODIA criteria. These intermediate forms of diabetes demonstrate that clinical heterogeneity complicates the precise etiological classification of diabetes and that there is overlap between the different forms of diabetes. Consequently, these forms of diabetes, although etiologically distinct, are difficult to distinguish and classify. Moreover, a recent study has already demonstrated this overlap between the different forms of diabetes such as type 1 diabetes with type 2 diabetes[33].

Our ongoing work has several limitations. First, the retrospective nature of our study was a limitation with the presence of missing values. In addition, our study did not include first-degree relatives of the ADia family for genetic sequencing, limiting the possibility of validating a variant observed in our ADia patients. Also, all our patients were pediatric and under 18 years of age, which prevented us from including clinical features that appear after several decades of diabetes (e.g., as in HNF1B mutations). We did not collect the ZnT8 autoantibodies due to their rare testing.

In conclusion, the GENEPEDIAB study demonstrated the effectiveness of our new DIAMODIA score in the screening for monogenic diabetes with a 100% positive predictive value in our MODY cohort. Unfortunately, no patient with atypical diabetes had a known pathologic gene variant after using the DIAMODIA score, and therefore

no monogenic diabetes was detected. However, the different criteria of the DIAMODIA score revealed a clinical polarization between type 1 diabetes and MODY diabetes, with most patients with atypical diabetes bridging the gap between the two typical forms of the disease.

AUTHOR CONTRIBUTIONS

PA.L. had the idea for the study and reviewed the manuscript. S.W. and P.G designed and performed the study, collected, and analyzed the data. S.W wrote the manuscript, performed the experiences and the whole exome analysis, and decided on the statistical method. M.M. decided on the statistical method, performed, and wrote the statistical analysis. R.H, P.B, and M.V collaborated with Macrogen center, contributed to the analysis of the genome. D.B, T.M, N.S are the doctors of patients, recruited them and reviewed the manuscript.

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CHAPTER 5. CONCLUSIONS

The objective of my research was to better characterize atypical forms of diabetes, and specifically monogenic diabetes and drug-induced diabetes in pediatrics which are currently poorly studied compared to the two common forms and are therefore often misdiagnosed and underdiagnosed. In this dissertation, I conducted three studies to better characterize these two types of diabetes.

Before studying them, I had to establish a brief overview of common subtypes of diabetes such as T1D and T2D to learn their differences and similarities with less common subtypes of interest and, consequently, understand the difficulties encountered in their diagnosis. This overview was followed by three studies, two on drug-induced diabetes, including anticancer and antirejection treatments, and one on genetic forms of diabetes. Our results and discussion, previously described in this manuscript, were correlated and compared with guidelines, data, and hypotheses available in the literature. Thus, the following general conclusion summarizes the findings of my research. In addition, a summary of recommendations will be suggested, and future directions and challenges to improve the characterization of all forms of diabetes will be described.

GENERAL CONCLUSIONS

The overarching term ‘diabetes mellitus’ refers to a heterogeneous group of metabolic disorders characterized by the presence of hyperglycemia. All these metabolic disorders are related to a defect in insulin secretion, insulin action or both. In pediatric, most individuals with diabetes can be classified into two categories: T1D (90 %), characterized by autoimmune destruction of β cells resulting in absolute insulin deficiency, and T2D (5-10%) characterized by an inadequate insulin response resulting from prior insulin resistance. However, rare, and atypical forms of diabetes can also affect children and adolescents (<5%). These atypical forms of diabetes may have similarities to T1D and T2D but differ greatly in etiology and pathogenicity. As a result, these forms can lead to different health issues than the common subtypes and therefore require a completely different therapeutic approach. These atypical cases of diabetes that do not fit into the categories of T1D and T2D (or gestational diabetes in adults) are classified as diabetes due to other specific causes that remain poorly defined. This subtype is defined as a ‘catch-all’ category and includes all atypical forms of diabetes such as, but not limited to, monogenic diabetes and drug-induced diabetes.

Given the current misdiagnosed and underdiagnosed of these two types of diabetes, it was therefore important for our team to improve the diagnosis of atypical forms of diabetes in children and adolescents by using clinical markers to help our clinicians select the best therapeutic interventions for the patient. We know that a correct diagnosis has important implications for predicting disease progression, considering short- and long-term prognosis, deciding on appropriate treatment and possibly for consulting family members about the possible heritability of the disease.

The first objective of my thesis was to characterize drug-induced diabetes in pediatric cohorts of patients requiring anticancer (STUDY 1) and antirejection organ (STUDY 2) treatments. Drug-induced diabetes in pediatric cancer and transplant patients is often understudied and underdiagnosed due to limited information and studies. Indeed, despite all the evidence suggesting that immunosuppressive drugs increase the risk of developing diabetes, its incidence, associated risk factors, and predictive biological markers remain unknown in the pediatric population. Furthermore, the risk of chronic hyperglycemia and overt diabetes in the context of pediatric transplantation and under chemo- and radiation therapy is associated with an unfavorable prognosis (e.g., graft rejection, increased length of hospital day, and an increase in cardiovascular events).

STUDY 1. DIABONCO.

The first research of my thesis was to study the incidence and risk factor of hyperglycemia during treatment of childhood hematologic malignancies. Our DIABONCO study shows that approximately about one fifth of ALL (32/179) and NHL (8/48) patients developed hyperglycemia, and more than half of them developed hyperglycemia within the first month of treatment. In contrast, no hyperglycemia was observed in Hodgkin-lymphoma patients. Our study highlights the importance of considering BMI and pubertal stage as potential markers for the onset of hyperglycemia in children and adolescents receiving diabetogenic cancer treatments. In addition, our study demonstrates the importance of closely monitoring blood glucose levels during treatment intensification when patients have a steroid-resistant disease or relapse, especially when TBI and HSCT are required. Finally, our study identified a reduction in the availability of blood glucose levels in the remission phase and their absence in the Hodgkin lymphoma cohort underscoring the need to monitor blood glucose levels at each follow-up visit to enable the diagnosis of transient, persistent, or late onset diabetes. The next step in our study is to contact cancer survivors who developed diabetes during or after their treatment to observe the presence of insulin resistance or impaired glucose tolerance using an oral glucose tolerance test.

STUDY 2. DIABGRAFT

The second research of my thesis was to describe the incidence and risk factors of hyperglycemia in a pediatric cohort of renal and liver transplant recipients and to prospectively analyze their glycemic profile. Our DIABGRAFT study shows that diabetes is a major side effect after a pediatric renal transplantation (20%) and transient hyperglycemia is frequent after a pediatric liver (25%) and renal (35%) transplantation. Hyperglycemia was characterized by a systematic onset in the post-prandial afternoon period and was associated with the use of glucocorticoids and with acute events such as graft rejection and infection. Hyperglycemia was characterized by impaired glucose tolerance and insulin resistance early after transplantation and could only be detected by oral glucose tolerance tests. In parallel, our study suggests that HbA1C and fasting glucose lack sensitivity for early detection of glucose intolerance and therefore hyperglycemia may be underestimated. We believe that our study may help clinicians to identify liver and renal transplant children at risk for early hyperglycemia, as our study demonstrates the importance of random blood glucose monitoring in the afternoon period when children require glucocorticoids or present critical complications such as graft rejection and infection.

The second objective of my thesis was to better diagnose monogenic diabetes and atypical diabetes in pediatric cohorts of patients treated for a T1D (STUDY 3). Monogenic diabetes results from mutations in a single gene with autosomal dominant inheritance that alter either insulin production or insulin action. Its diagnosis is based on a genetic analysis and is characterized by early neonatal diagnosis (for PND and TND), onset of hyperglycemic symptoms before the age of (for MODY), and a strong familial component. In addition, in contrast to T1D, monogenic diabetes is often described as easier to control diabetes with better glycemic parameters, absence of anti-islet antibodies and relatively preserved C-peptide secretion. Unfortunately, despite these clinical differences, a significant number of patients share many features with T1D or T2D and are therefore misclassified as these two common subtypes, depriving the patient of an appropriate treatment. It is recognized in a care agreement for patients with diabetes that 2 to 3% of active patients suffer from undetected genetic forms. Under these circumstances, the MODY calculator provides a probability score for having a MODY, but with the disadvantage of not taking into consideration different important variables in monogenic diabetes such as low glycemic variability and high residual c-peptide secretion.

STUDY 3. GENEPEDIAB

The last research of my thesis was to demonstrate the efficiency of our internal DIAMODIA score to easily and quickly identify pediatric patients with atypical forms of diabetes associated with genetic polymorphisms and refer them for genetic analysis. Our GENEPEDIAB study first demonstrates the efficiency and relevance of the DIAMODIA score by detecting 100% of our MODY patients. Furthermore, the cross-analysis of DIAMODIA variables between our MODY and T1D cohorts confirms well-documented clinical features such as the absence of islet autoantibodies and the presence of a residual C-peptide secretion, and introduces new clinical characteristics to help clinicians identify the most common forms of monogenic diabetes (*GCK, HNF1A, HNF1B, KCNJ11 and ABCC8*), such as a low glycemic variability ($IDAA_{1C}$ and $GTAA_{1C}$ scores), a family member with diabetes and more specific maternal diabetes, absence of ketoacidosis at diabetes diagnosis and

onset of diabetes before six months of age for neonatal diabetes. Second, our DIAMODIA score identified patients with atypical forms of diabetes, which differ from T1D by the absence of islet autoantibodies, high residual C-peptide secretion and $IDAA_{1C}$ score ≤ 9. However, none of these patients had a known pathological gene variant after the use of both the MODY gene panel and the WES, and therefore no monogenic diabetes was detected. The DIAMODIA score criteria demonstrated a clinical polarization of T1D and MODY diabetes, and that most patients with atypical diabetes bridge the gap between the two typical forms of the disease by being clinically distinct from T1D and from monogenic diabetes. This discovery confirms the heterogeneity of diabetes, which complicates the precise etiologic classification of diabetes, and recognizes the existence of overlap between different forms of diabetes. The next steps of the GENEPEDIAB study are the screening of ADia patients close to MODY patients without the use of filter genes based on the EXETER list, and the validation of the class III variants identified in ADia patients in trio with the exome of the mother and father. Our study is the subject of ongoing work.

RECOMMENDATIONS AND FUTURE DIRECTIONS

In the pathological process of drug-induced diabetes, hyperglycemia occurs rapidly after the initiation of anticancer or antirejection therapy. Therefore, we recommend measuring blood glucose during the initiation phase of anticancer therapy and immediately after the transplantation. For children and adolescents with cancer, as we observed a reduction in blood glucose monitoring during remission phase and its absence in the Hodgkin lymphoma cohort, we suggest that blood glucose be measured at each follow-up visit. For transplant recipients, postprandial blood glucose monitoring is essential to detect the presence of hyperglycemia. In addition, for both conditions, we suggest that blood glucose monitoring should be intensified if glucocorticoids are required or increased. We observed in our studies that $HbA_{1C,}$ and fasting blood glucose lack sensitivity for early detection of glucose intolerance, but their measurements are still indicative of the presence of glucose disturbance and are therefore still recommended. The glucose sensor is a good alternative for simple monitoring of hyperglycemia that can be offered during a period of uncontrolled hyperglycemia to replace repeated finger pricks which can be difficult in young children. Unfortunately, the cost of a glucose sensor is not negligible and is not reimbursed unless the patient is enrolled in a diabetic convention. Therefore, the gold standard for diagnosing early glucose intolerance is the oral glucose tolerance test. OGTT should be routinely offered to patients with the risk factors for developing hyperglycemia previously described in our studies.

For monogenic diabetes, since genetic testing is the only way to diagnose monogenic diabetes, it is important to improve the diagnostic tool that can be guided by this analysis. A positive result can considerably change diabetes treatment towards personalized medicine, anticipate associated complications, and provide information on the hereditary risk for family members. In this context, it is already confirmed that all forms of diabetes diagnosed before the age of 6 months should be screened for neonatal diabetes genes. The diagnosis of MODY is more complex. Several criteria are already well documented to be associated with a monogenic form of diabetes, such as the absence of islet autoantibodies or residual C-peptide

secretion, which is also confirmed by our study. In addition, we validated new criteria related to MODY forms that distinguish T1D. Therefore, we suggest that caregivers be suspicious of atypical forms of diabetes if a child or adolescent presents with an absence of islet autoantibodies, low glycemic variability with the $IDAA_{1C}$ and $GTAA_{1C}$ scores, a strong family history of diabetes and especially maternal diabetes, and diabetes-associated pathologies and especially if kidney or liver are involved. In addition, the absence of classic signs of T1D, such as polydipsia, polyphagia, DKA, and a profile that does not fit the T2D phenotype, including the absence of obesity, insulin resistance, and acanthosis nigricans, may indicate atypical diabetes that should be referred for genetic analysis. The current MODY calculator tool should be further developed to provide more parameters for screening for monogenic forms of diabetes. The introduction of our score reflects the need for accuracy of more variables to reduce the number of patients with unidentified diabetes or with misdiagnosed diabetes. However, further research is needed to validate our clinical prediction tool for monogenic diabetes in various populations to ensure its effectiveness.

In conclusion, the subclassification of diabetes into T1D and T2D is recognized as insufficient to include the heterogeneity of diabetes presentation, disease evolution, response to treatment and risk of complications. However, the growing interest in the study of atypical forms of diabetes is underway and is first step towards a reclassification of diabetes with a better definition of subgroups, incorporating phenotypic and genetic data and including atypical forms of diabetes. In this case, the RADIANT trial was conducted to accurately diagnose individuals with atypical forms of diabetes. RADIANT (for Rare and Atypical Diabetes Network) is a prospective and observational study conducted by the University of South Florida (United States) in collaboration with the National Institute of Diabetes and Digestive and Kidney disease (NIDDK). This study is a network of 14 clinical sites and several laboratories dedicated to the study of atypical diabetes. As explained on the ClinicalTrials.gov website (last visit 03.23.23; identifier: NCT05544266), the objective of this study is to define new forms of diabetes and the unique mechanisms

underlying these forms of atypical diabetes. Like the RADIANT trial, several studies and new algorithms have been developed each year to better identify all forms of diabetes. Through our DIABONCO, DIABGRAFT and GENEPEDIAB studies, we have better characterized monogenic diabetes and drug-induced diabetes and provided new clinical criteria and insights to help clinicians better identify atypical forms of diabetes. However, we know that it takes time to move from theory to practice, and that more studies are needed to better classify all forms of diabetes.

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ANNEXES

Annex 1 – Poster ESPE congress 2021 – DIABONCO STUDY – Final results

Annex 2 – Poster ISPAD congress 2022 – DIABGRAFT STUDY, Liver part

Annex 3 – Poster ESPE congress 2022– GENEPEDIAB STUDY, Preliminary results

Annex 4 – Original article – DIABONCO study, published in DIABETIC MEDICINE journal, 2021. DOI: 10.1111/DME.14720
Characterization and risk factors of hyperglycaemia during treatment of childhood hematologic malignancies.
Sophie Welsch, Kiswendsida Sawadogo, Bénédicte Brichard, Maelle de Ville de Goyet, An Van Damme, Cécile Boulanger, Philippe A. Lysy.

Annex 5 – Original article – DIABGRAFT study, published in FRONTIERS journal in Pediatrics section, 2022. DOI: 10.3389/fped.2023.1080905.
Characterization, evolution and risk factors of diabetes and prediabetes in a pediatric cohort of renal and liver transplant recipients
Sophie Welsch; Virginie Mailleux; Priscilla le Hardy de Beaulieu; Nadejda Ranguelov; Nathalie Godefroid; Annie Robert; Xavier Stephenne; Isabelle Scheers, Raymond Reding; Etienne M. Sokal, Philippe A. Lysy.

Characterization and Risk Factors of Hyperglycaemia During Treatment Of Childhood Hematologic Malignancies

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INTRODUCTION AND AIM

Secondary forms of diabetes mellitus are underdiagnosed in children and adolescents with cancer. Despite the whole body of evidence that asparaginase, steroids and total body irradiation increase the risk of developing diabetes, risk factors are missing and – asides from treatments – understudied (e.g., pre-existing obesity, sex, age, ethnicity, family history of diabetes). The objectives of our study were to study the incidence and associated risk factors of hyperglycaemia in leukaemia and lymphoma patients.

METHODS

We retrospectively collected 15 years of data from paediatric patients aged 0 to 18 years treated in Cliniques universitaires Saint-Luc (CUSL, Brussels) for acute lymphoblastic leukaemia (ALL), Hodgkin's lymphoma (HL), and non-Hodgkin's lymphoma (NHL) immediately at cancer diagnosis.

According to guidelines of the American Diabetes Association, patients developed hyperglycaemia when random glucose levels exceeded 11 mmol/L, for at least two measurements separated by 24 hours.

The variables were compared according to the occurrence or not of hyperglycaemia using Student t test or Mann-Whitney test (as appropriate) for continuous variables and Fisher exact test for discrete variables. A binary logistic regression analysis was performed to predict hyperglycaemia occurrence from all potential predictors available by estimating odds ratios and their 95% confidence intervals. All covariates with a p-value less than 0.10 in univariate analysis were introduced into a multivariate model (Wald Chi-Square).

RESULTS: INCIDENCE, EVOLUTION AND RISK FACTORS OF HYPERGLYCAEMIA

INCIDENCE OF HYPERGLYCAEMIA

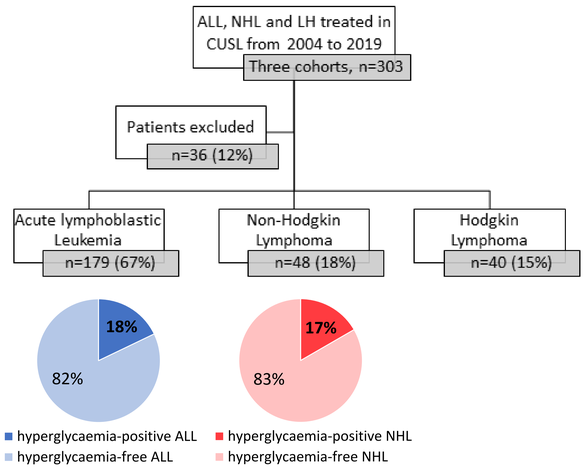

Figure 1. Our study cohort included 267 patients corresponding to 179 patients with ALL, 48 with NHL and 40 with HL. Eighteen percent of ALL patients (32/179) and 17% of NHL patients (8/48) developed hyperglycaemia. No hyperglycaemia was observed in HL patients.

ONSET OF HYPERGLYCAEMIA

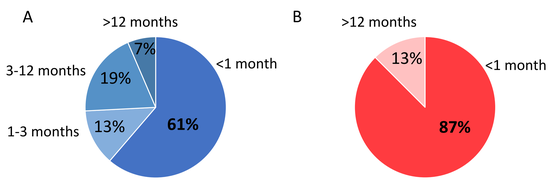

Figure 2. The majority of ALL (A, 61%) and NHL (B, 87%) patients developed hyperglycaemia within the first month of chemotherapy, corresponding to pre- and induction phases that are the most aggressive in terms of steroid doses.

PROBABILITIES OF REMAINING FREE OF HYPERGLYCAEMIA

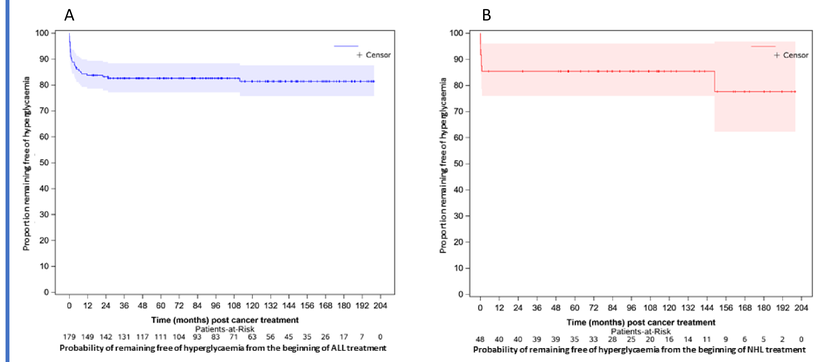

Figure 3. Kaplan-Meier estimates of the probability of remaining free of hyperglycaemia in acute lymphoblastic leukaemia (ALL) and non-Hodgkin lymphoma (NHL) paediatric cohorts.

(A) At 12 months post ALL treatment, the probability of remaining free of hyperglycaemia was 83.8% and remained relatively unchanged thereafter. (B) In the NHL group this probability remained unchanged at 85.4% after one month of cancer treatment.

RISK FACTORS OF HYPERGLYCAEMIA

Table 1. Univariate (Likelihood Ratio) and multivariate (Wald Chi-Square) logistic regression analyzes of factors leading to hyperglycaemia occurrence for ALL and NHL cohorts.

		Univariate Analysis		Multivariate Analysis	
ALL predictors	N	p-value	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)
History of overweight at cancer diagnosis	179	0.008	4.293 (1.464-12.588)	0.046	3.793 (1.026-14.022)
Tanner staging ≥2	179	<0.001	4.880 (2.159-11.032)	0.002	4.269 (1.676-10.875)
Steroid-resistant disease	179	0.014	3.204 (1.265-8.113)	0.032	3.445 (1.114-10.657)
HSCT	179	0.002	5.111 (1.795-14.553)	0.037	4.754 (1.099-20.554)
NHL predictors					
Cancer treatment risk higher vs lower	48	0.038	5.667 (1.104-29.073)		

ALL=Acute Lymphoblastic Leukemia; NHL=Non-Hodgkin Lymphoma; CI=Confidence Interval; BMI=Body Mass Index; SDS=Standard Deviation Score; HSCT=Hematopoietic Stem Cell Transplantation.

Table 1. Multivariate analysis showed that ALL patients with history of obesity/overweight (OR 3.793), a pubertal stage equal to or greater than 2 (OR 4.269) at cancer diagnosis, a presence of steroid-resistant disease (OR 3.445,) and a hematopoietic stem cell transplant (OR 4.754) were associated with a higher risk of developing hyperglycaemia.

CONCLUSIONS

In our cohort, 18% of patients with ALL or NHL developed early-onset hyperglycaemia after chemotherapy/radiotherapy. Our findings may help clinicians to identify patients with acute lymphoblastic leukaemia at risk of early onset of hyperglycaemia, by considering BMI and pubertal stage as potential markers and by monitoring blood glucose levels closely during treatment intensification for steroids-resistant disease or relapse, especially when total body irradiation and stem cell transplantation are required.

ACKNOWLEDGEMENTS

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Characterization, Evolution and Risk Factors Of Hyperglycemia In A Pediatric Cohort Of Liver Transplant Recipients.

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INTRODUCTION AND AIM

Hyperglycemia (HG) and prediabetes (insulin resistance and impaired glucose tolerance) are rarely sought in pediatric liver transplantation (LT), yet their presence indicates a high risk of diabetes and cardiovascular disease. In addition, the use of fasting blood glucose and HbA1C levels might not allow early detection of insulin resistance (IR) as a preamble to prediabetes. The objectives of our study were to assess the incidence and associated risk factors of developing hyperglycemia in liver transplant children and longitudinally analyze the evolution of glycemic profile in these patients during the post-transplant period.

METHODS

DIABGRAFT was constituted of two parts. Its retrospective part (rDIABGRAFT) consisted of collecting data of pediatric patients (<18 years) who benefited from a LT performed at Cliniques universitaires Saint-Luc (CUSL, Brussels) between April 2012 and April 2019. The prospective part (pDIABGRAFT) consisted of a longitudinal glycemic evaluation of four children liver transplant at CUSL between 2020 and 2022 with the use of dynamic endocrine testing. Patients presented HG when fasting plasma glucose or random plasma glucose levels exceeded respectively 126 mg/dL and 200 mg/dL for at least two measurements separated by 24 hours, and not under a condition of stress such as the day of the transplant (American Diabetes Association). Diabetes was defined when a patient presented 2-h plasma glucose levels during OGTT > 200 mg/dL. Logistic regression analysis was performed to predict HG occurrence from all potential predictors.

GLOBAL RESULTS

Our rDIABGRAFT study showed that 25% of LT children developed HG. The occurrence of HG was associated to the use of glucocorticoids, and to acute events such as graft rejection and viral infection. In our pDIABGRAFT cohort, biological markers of diabetes were in the normal range for fasting glucose and C-peptide secretion levels. However, glucose sensors and oral glucose tolerance test showed respectively HG in the post-prandial afternoon period and insulin resistance and diabetes already one month after LT.

INCIDENCE OF HYPERGLYCEMIA

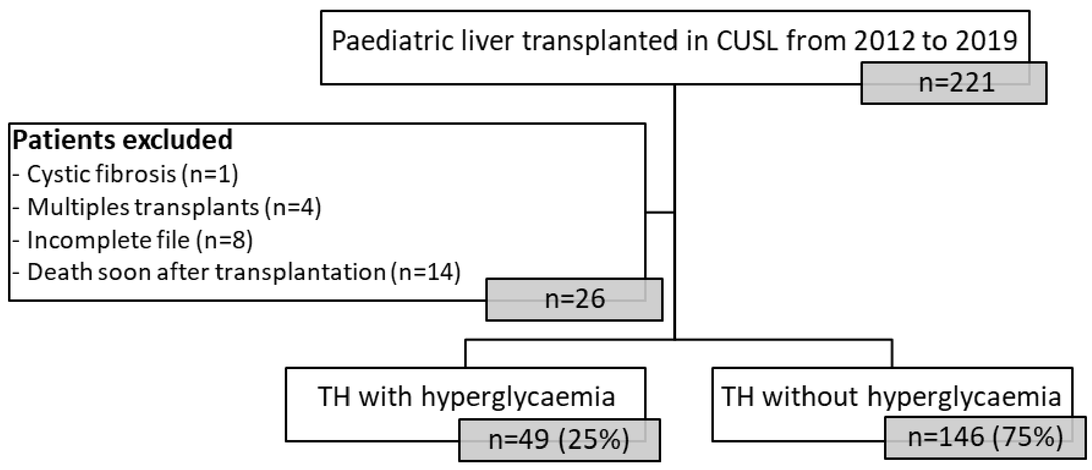


Figure 1: We collected data from 195 pediatric patients treated at CUSL with liver transplantation (LT) and 25% (49/195) of pediatric patients presented HG with no overt diabetes afterward.

EARLY HYPERGLYCEMIA

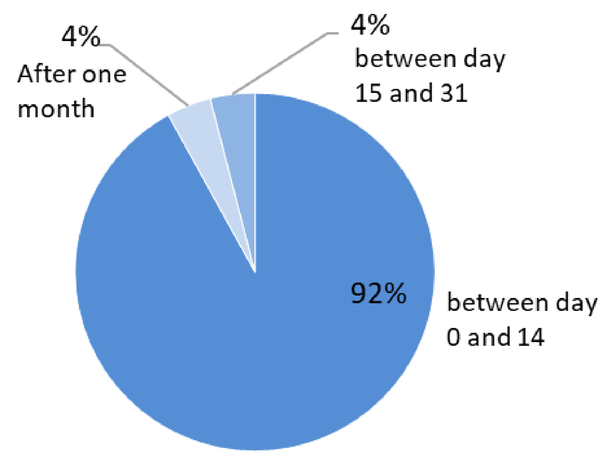


Figure 2: The majority of liver transplant children (92%) developed hyperglycemia within the two weeks after transplant (A).

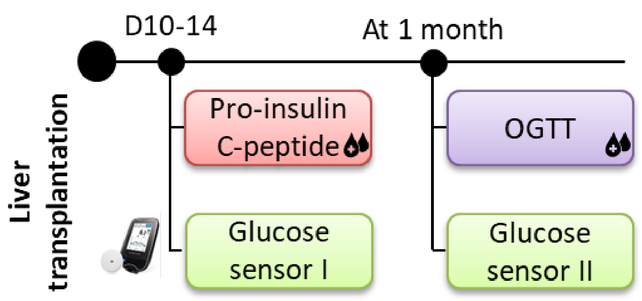
GLUCOCORTICOIDS, GRAFT REJECTION AND VIRAL INFECTION ARE ASSOCIATED TO HYPERGLYCEMIA ONSET

LT predictors	LT HG positive n=49	LT HG negative n=146	Univariate analysis p-value	Univariate analysis OR (95% CI)	Multivariate analysis p-value	Multivariate analysis OR (95% CI)
Glucocorticoids post-LT	39 (79.6)	87 (59.6)	0.01	2.64 (1.23-5.71)	0.01	2.96 (1.32-6.61)
Graft rejection	36 (73.5)	68 (46.6)	0.001	3.18 (1.56-6.48)	-	-
Virus infection	30 (61.2)	56 (38.4)	0.01	2.54 (1.31-4.93)	0.03	2.20 (1.09-4.44)
CMV	22 (44.9)	33 (22.3)	0.003	2.79 (1.41-5.53)	-	-

Figure 3: In univariate analysis, the use of glucocorticoids and the presence of critical condition such as graft rejection and viral infection, in particularly cytomegalovirus (CMV) were significantly associated with the onset of HG. After adjustment with multivariate logistic regression analysis (Wald Chi-Square tests), incidence of transient HG after LT was higher in children who received glucocorticoids and presented a viral infection.

HG IN POSTPRANDIAL AFTERNOON PERIOD, INSULIN RESISTANCE AND DIABETES

A. Study design

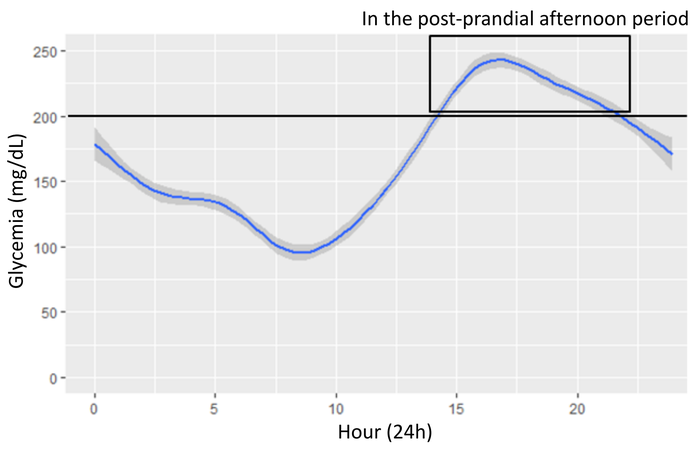


B. C-peptide & HOMA index

	C-peptide (pmol/L)
LT1	665,945
LT2	752,903
LT3	3142,057
LT4	1284,999

	HOMA-IR
LT1	6.3
LT2	2.6
LT3	-
LT4	2.3

C. CGM data regrouped on 24 hours



D. OGTT at one-month post-LT

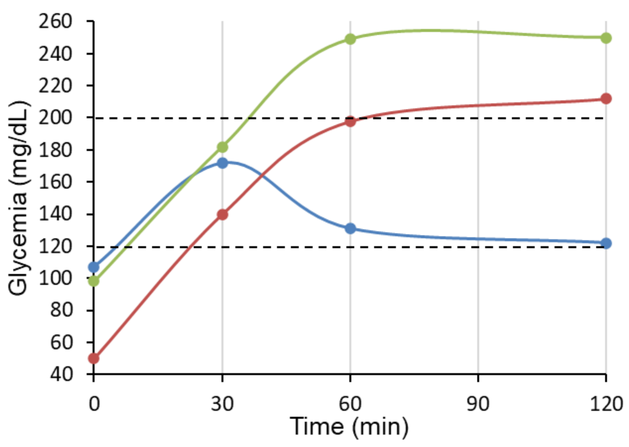


Figure 4. (A) As we observed with rDIABGRAFT that our LT cohort presented HG early after transplantation (i.e., 0-14 days), we performed dynamic testing close to transplantation (day 14 and day 30) in four LT children. (B) All children presented fasting glucose and c-peptide level in normal range whereas (C) CGM placed at day 14 post-LT for one month showed chronic HG (glycemia ≥200 mg/dL) occurring in postprandial afternoon period. (D) During the OGTT performed at one-month post-LT (n=3), all presented IR (HOMA-IR >1.7) during the test while in two of them, glycemia peaked respectively at 212 and 250 mg/dL at 120'. We thus observed in our LT cohort two patients with diabetes at one-month post-LT.

CONCLUSION

In conclusion, transient HG are frequent after a pediatric liver transplant (25%) but underestimated due to fasting measures. The onset of HG systematically occurred in post-prandial afternoon period, was associated to the use of glucocorticoids and was characterized by insulin resistance early after the transplantation only detected by OGTT. Our study suggests that random blood glucose monitoring should be reinforced when children present critical complications such as graft rejection and infections and in afternoon period.

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Etiology-based diagnosis of pediatric patients with atypical diabetes using routine and omic-based phenotyping and genotyping: results from the GENEPEDIAB study.

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INTRODUCTION AND AIM

Among the two main forms of diabetes (type 1 and 2), rare subtypes of the disease called monogenic diabetes (MODY) are hardly diagnosed because of unspecific clinical presentation. This may deprive the patient of an appropriate treatment, which could be simplified (e.g., oral antidiabetics replacing insulin) or etiology-oriented. The objectives of our study were to provide etiology-based diagnostics to pediatric patients with diabetes in Belgium, using routine clinical phenotyping and thorough genotyping.

METHODS

A Belgian GENEPEDIAB study consortium was created to screen, using routine diagnostic tools, for monogenic forms of diabetes in pediatric patients followed in convention centers for type 1 or type 2 diabetes, while presenting atypical biological and clinical features of the disease (e.g., lack of anti-islet antibodies, persistence of C-peptide secretion and low glycemic variability; features not considered by the MODY calculator). We compiled the most representative clinical features of monogenic diabetes into a new diagnostic tool, the DIAMODIA score. Patients fulfilling sufficient criteria were phenotyped (e.g., glycemic variability, glucose tolerance, multiplex serum protein assays) and genotyped (restricted gene panel [University of Antwerp] then whole-exome sequencing using NGS). Gene-phenotype correlations were performed using bioinformatics.

1. DIAMODIA SCORE

At least one strong criterion	Absence of anti-islet antibodies
	$IDAA_{1C}$ ≤9 (after 18 months of diagnosis)
	$GTAA_{1C}$ ≤4,5 (after 18 months of diagnosis)
At least one weak criterion	First-degree relative with diabetes
	C-peptide positive
	Extra-pancreatic manifestations
	Absence of diabetic ketoacidosis at diagnosis
	History of neonatal hypoglycemia

Figure 1: The DIAMODIA score. This score refers to weak and strong criteria and can be leveled from “2” to “5” depending on the number of positive criteria encountered by patients. Patients who present at least one strong criterion and one weak criterion are considered “atypical diabetes” and form the ADia cohort (for Atypical Diabetes).

2. FLOWCHART OF THE STUDY

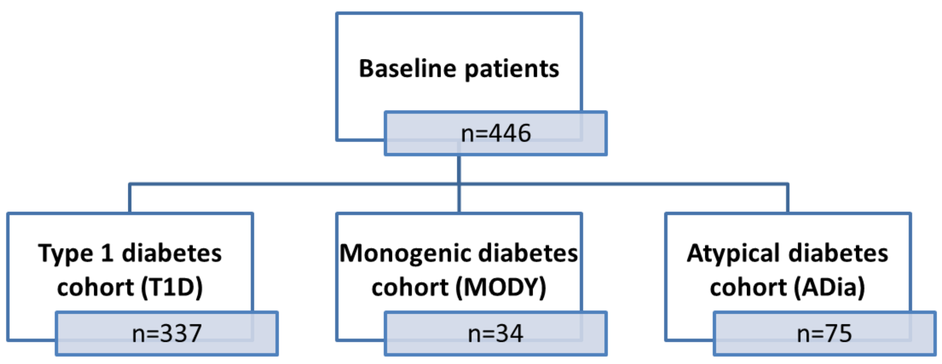
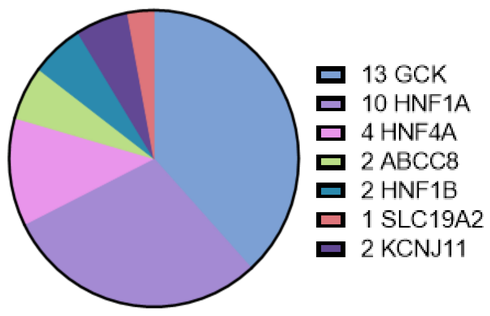


Figure 2. We retrospectively collected data from pediatric patients treated in Cliniques universitaires Saint-Luc (Brussels), CHU Saint-Elisabeth and Mont-Godinne (Namur), CHC Montlegia and CHU Liège (Liège). As results, we categorized 446 patients in 337 with type 1 diabetes (T1D cohort), 34 with monogenic diabetes (Mody cohort) and 75 with a supposed Atypical Diabetes (ADia cohort).

3. MODY GENES

Figure 3. The routine MODY gene panel analysis identified 34 patients with class V variants (pathogenic). Majority of our patients presented a mutation in *GCK* (38 %) and *HNF1A* (29.5 %) genes. One patient presented a mutation in *SLC19A2* gene, which is not listed in the MODY panel gene.



The whole exome of our Adia cohort was sequenced and screened by a list of genes involved in monogenic, neonatal, and very rare forms of diabetes (EXETER list). We observed 37 ADia patients with class III variants (Uncertain significance). Most of the variants designated class III were not reported in Clinvar, in LOVD or in the literature.

4. CHARACTERIZATION OF T1D, MODY AND ADIA COHORTS

	T1D	MODY	Adia total
N	337	34	75
At diabetes diagnosis, median (P25-P75)			
Glycemia (mg/dL)	449 (328; 577)	126 (107; 164)	366 (290; 509)
HbA1c (%)	11,2 (10,1; 12,8)	6,6 (5,5; 7,4)	10,6 (9,7; 12,3)
C-peptide (pmol/mL)	0,15 (0,09; 0,24)	0,71 (0 ,42; 1,05)	0,21 (0,13; 0,35)
Follow-up, median (P25-P75)			
HbA1c (%)	7,4 (6,9; 7,9)	6,3 (5,5; 6,5)	6,5 (6,1; 6,9)
Insulin/kg/j	0,92 (0,77; 1,08)	0,00 (0,00; 0,48)	0,71 (0,59; 0,98)
Normoglycemia (%)	43,0 (37,7; 49,3)	82,2 (69,2; 90,0)	52,8 (44,7; 59,4)

Figure 4. At diabetes diagnosis, our patients with MODY presented lower median glycemic and HbA1c values and higher median C-peptide secretion, compared to patients with T1D.

At clinical follow-up, HbA1c of patients with MODY were significantly lower than in T1D cohort, and their time in normoglycemia was significantly higher, whether they were treated or not (19/34).

5. ABSENCE OF AUTO-ANTIBODIES, RESIDUAL C-PEPTIDE SECRETION, LOWER VALUES OF IDAA1C AND GTAA1C

	T1D		MODY		Adia	
N	n_{tot}	n (%)	n_{tot}	n (%)	n_{tot}	n (%)
Strong criteria						
Three AAB negative	154	0 (0.0)	32	32 (100)	54	21 (38.9)
IDAA1c ≤ 9	332	5 (1.5)	34	28 (82.3)	72	36 (50.0)
GTAA1c ≤ 4,5	333	4 (1.2)	28	23 (82.1)	72	28 (38.9)
Both ≤ values	332	2 (0.6)	28	21 (75)	72	15 (20.8)
Age < 6 months	336	0 (0.0)	34	3 (8.8)	75	0 (0.0)
Weak criteria						
Heredity family	334	137 (41.0)	34	29 (85.3)	74	42 (56.8)
C-peptide positive	306	73 (23.8)	32	29 (90.6)	69	42 (60.9)
Extra-pancreatic	331	28 (8.5)	31	12 (38.7)	74	10 (13.5)
Ketoacidosis negative	327	198 (60.5)	34	30 (88.2)	75	55 (73.3)
Neonatal hypoglycemia	328	8 (2.5)	33	7 (21.2)	75	2 (2.7)

Figure 5. As a result of our DIAMODIA score, the percentage of MODY and Adia patients with absence of auto-antibodies (AAB), residual C-peptide secretion and values inferior to 9 for IDAA1c and 4.5 for GTAA1c were significantly superior from those observed in our T1D cohort.

However, criteria such as diabetes diagnostic before 6 months of life, heredity family, ketoacidosis negative and neonatal hypoglycemia were less able to differentiate ADia to T1D cohorts.

6. CLUSTERING OF MODY WITH DIAGNOSIS AND FOLLOW-UP DATA

A. Diabetes diagnosis data

B. Clinical follow-up data

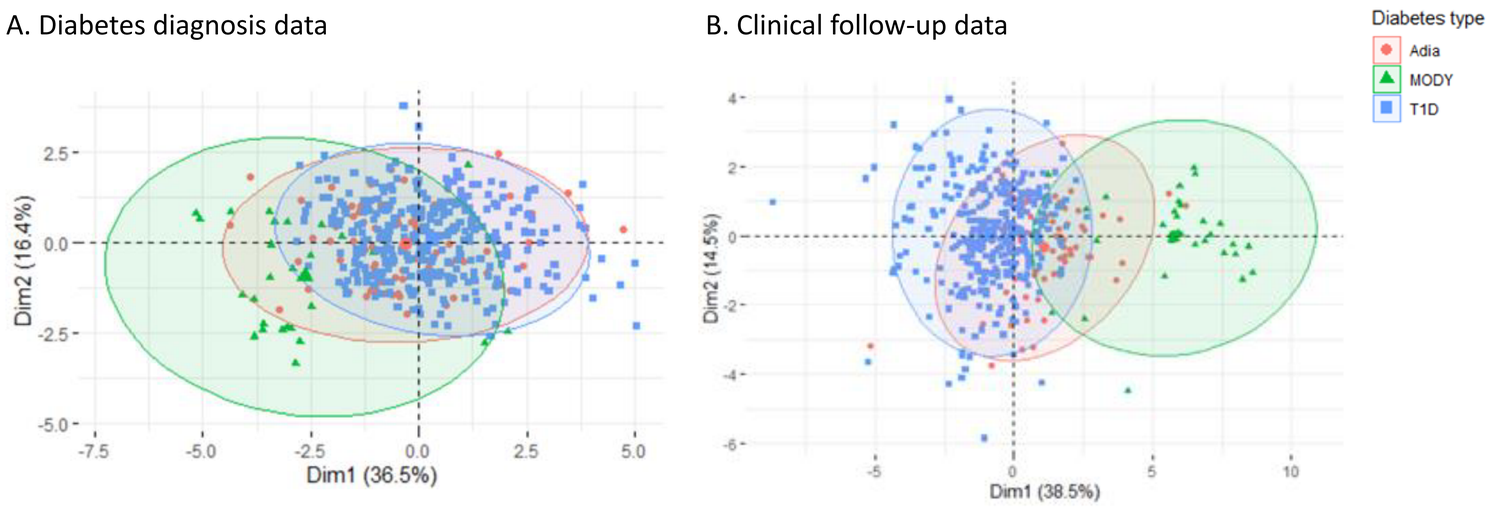


Figure 6. A Principal Component Analysis was performed to observe if data obtained at diabetes diagnosis (A) such as age, glycemia, HbA_{1c}, SDS BMI, C-peptide, number of auto-antibodies (GAD65, IA2 and insulin), pH and bicarbonate were sufficient to differentiate MODY to T1D patients. We observed the formation of two different clusters, in green for MODY and in blue for T1D.

The addition of clinical follow-up data (B) such as medium HbA1c, percentage of normoglycemia, daily dose of insulin, IDAA1c and GTAA1c allowed to strongly differentiate MODY to T1D and more clearly identify Adia close to MODY. We observed a total of eight patients in the MODY group who required further genetic screening.

CONCLUSION AND PERSPECTIVES

In conclusion of our preliminary results, our three cohorts behaved differently with individualization of MODY and T1D patients following diagnostic and clinical follow-up criteria. Adia patients showed some phenotypic similarity with the MODY cohort as 10% were grouped with MODY, without identified variants or mutations known within the EXETER list. These patients require in-depth genetic screening. Our DIAMODIA score is thus effective in clustering patients with MODY and patients carrying novel class III forms of mutations/variants.

ACKNOWLEDGEMENTS

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DIABETIC Medicine

RESEARCH ARTICLE

Characterization and risk factors of hyperglycaemia during treatment of childhood hematologic malignancies

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Abstract

Background: Secondary forms of diabetes are often understudied and underdiagnosed in children and adolescents with cancer. The objectives of our cohort study were to study the incidence and risk factors for hyperglycaemia in leukaemia and lymphoma patients.

Methods: We retrospectively collected 15 years of data from paediatric patients treated for acute lymphoblastic leukaemia (ALL), Hodgkin's lymphoma (HL), and non-Hodgkin's lymphoma (NHL) immediately at cancer diagnosis. We studied risk factors for hyperglycaemia in univariate and multivariate analyses.

Results: Our study cohort included 267 patients corresponding to 179 patients with ALL, 48 with NHL and 40 with HL. Eighteen per cent of ALL patients (32/179) and 17% of NHL patients (8/48) developed hyperglycaemia, with more than 61% developing hyperglycaemia within the first month of treatment. No hyperglycaemia was observed in HL patients. Multivariate analysis showed the following hyperglycaemia risk factors for ALL patients: overweight or obesity (OR 3.793) and pubertal onset (OR 4.269) at cancer diagnosis, steroid-resistant disease (OR 3.445) and hematopoietic stem cell transplant (HSCT) (OR 4.754).

Conclusion: In our cohort, 18% of patients with ALL or NHL developed early-onset hyperglycaemia after chemotherapy/radiotherapy. Patients with ALL with increased hyperglycaemia risk can be readily identified by measuring BMI and puberty stage at cancer diagnosis. Also, glucose monitoring should be reinforced when patients show steroid-resistant disease and/or require HSCT.

KEYWORDS

diabetes mellitus, leukaemia, lymphoma, overweight, radiotherapy, stem cell transplantation, steroids

1 | INTRODUCTION

Children and adolescents diagnosed with acute lymphoblastic leukaemia (ALL), Hodgkin's lymphoma (HL) and non-Hodgkin's lymphoma (NHL) are treated with specific and individual chemotherapy protocols sometimes combined with radiotherapy and/or hematopoietic stem cell transplant (HSCT). Thanks to research initiatives allowing

constant re-evaluation of these protocols, the survival rate of childhood cancer exceed 83%.[1] However, the effectiveness of these treatments is not without consequences: 50% of childhood cancer survivors (CCS) develop endocrine sequelae including metabolic syndrome and glucose metabolism disorders such as diabetes, insulin resistance and impaired glucose tolerance (IGT).[2–4] In the general population, diabetes confers two to three times increased risk of cardiovascular disease and corresponds to 12%–55% of cases of end-stage renal disease worldwide,[5] being as such the 7th expected leading cause of death by 2030.[6]

In CCS, the incidence of hyperglycaemia is still ill-defined and might range between 11% and 35% of cases.[7–13] Moreover, despite the whole body of evidence that asparaginase,[12] steroids[14] and total body irradiation[15] increase the risk of developing hyperglycaemia and diabetes, risk factors are missing and – asides from treatments – understudied (e.g., pre-existing obesity, sex, age, ethnicity, family history of diabetes, etc.).

The purpose of our study was to assess the incidence and associated risk factors of developing hyperglycaemia in children and adolescents diagnosed with ALL, HL and NHL. Deciphering the factors associated with the onset of hyperglycaemia in paediatric patients treated for cancer will provide leverage for lifestyle or therapeutic intervention from a prevention perspective in newly diagnosed patients.

Novelty statement

- It is well known that hyperglycaemia in childhood cancer is caused by the use of steroids, asparaginase and total body irradiation.
- Two new risk factors of hyperglycaemia were identified in paediatric patients with acute lymphoblastic leukaemia: puberty and steroid-resistant disease.
- This work will help clinicians to identify patients with acute lymphoblastic leukaemia at risk of early onset of hyperglycaemia, by considering BMI and pubertal stage as potential markers and by monitoring blood glucose levels closely during treatment intensification for steroid-resistant disease or relapse, especially when total body irradiation and stem cell transplantation are required.

2 | MATERIALS AND METHODS

2.1 | Study design

The DIABONCO retrospective study is being carried out in collaboration with the Paediatric Haematology and Oncology (Institut Roi Albert II) of Cliniques universitaires Saint-Luc in Belgium (Brussels). Our investigations included patients receiving treatment protocols conferring a diabetogenic risk. These included the total body, cranial, and abdominal irradiation (respectively TBI, CI, and AI), steroids and L-asparaginase. Our cohort was, therefore, composed of patients treated for acute lymphoblastic leukaemia (ALL), Hodgkin's lymphoma (HL) and non-Hodgkin's lymphoma (NHL). The local ethical committee (Saint-Luc and UCL Hospital-Faculty Ethics Committee) approved this study protocol (approval number 2018/20MAR/122) and the study was conducted in accordance with the Declaration of Helsinki.

2.2 | Inclusion and exclusion criteria

We included all children and adolescents aged 0 to 18 years treated with the aforementioned diabetogenic treatment protocols and diagnosed at Cliniques universitaires Saint-Luc with ALL, NHL or HL between January 2004 and December 2019. We excluded patients with an incomplete file or a history of the following conditions: previous diabetes (i.e. type 1, type 2, neonatal or monogenic diabetes), pancreatitis, steatosis, Down syndrome, pancreas and liver surgery, kidney disease and previous cancer other than leukaemia and lymphoma.

The patients were stratified according to the presence or absence of hyperglycaemia during the treatment protocol and during clinical follow-up, which ended in August 2020. The groups were called the "hyperglycaemia-positive ALL, NHL or HL" and the “hyperglycaemia-free ALL, NHL or HL".

2.3 | Treatments protocols

In Belgium, ALL, HL and NHL paediatric patients are treated with chemotherapy and radiotherapy according to international guidelines. Several protocols were used for the three pathologies depending on the treatment era, the severity of the disease, the age of patients and the response to treatment. Despite some differences in protocols in the same cohort, the treatment pattern remains unchanged. For ALL patients, the theoretical treatment lasts at least two years and begins with pre-phase with the introduction during seven days of steroids and followed by induction with 21 days of steroids, consolidation, interval, re-induction with also 21 days of steroids and finishes with maintenance phase, which sometimes includes steroids (Table S1 and Figure S1). Treatments for NHL and HL are much shorter

than ALL treatment and last a maximum of six months. If an ALL patient presents steroid-resistance disease at the end of the pre-phase, the protocol will be intensified with an extended consolidation phase with longer doses of steroids and L-asparaginase. When ALL patient presents a relapse during treatment or abnormal cytogenetics, HSCT may be considered, some of them with TBI.

2.4 | Diagnosis of hyperglycaemia

According to guidelines of the international consensus for diabetes of the American Diabetes Association (ADA), we considered that patients developed hyperglycaemia when random capillary blood or plasma glucose levels exceeded 11 mmol/L (200 mg/dl), for at least two measurements separated by 24 h. Hyperglycaemia was identified based on glycaemic measurements during treatment protocols and clinical follow-up. Inpatients are subjected to daily blood analyses, which periodically include the measurement of plasma glucose levels. When hyperglycaemia occurs, the theoretical protocol implemented in clinics requires the confirmation of this hyperglycaemia by plasma glucose measurement and capillary glucose monitoring until resolution of hyperglycaemia.

2.5 | Variables of interests

For all patients, the following data were collected and managed using REDCap (Research Electronic Data Capture) tools[16,17] provided by the Vanderbilt University (Nashville, USA) and hosted at Cliniques universitaires Saint-Luc. We collected personal patient data such as sex, date of birth, country of origin, weight, height and gestation at birth, complications during pregnancy (pre- or post-term, events, foetal macrosomia), dysmaturity, hypoglycaemia and hyperglycaemia in the neonatal period, the presence or absence of previous overweight (BMI >85th centile)/obesity (BMI >95th centile),[18] endocrine disease, autoimmune disease, acanthosis nigricans, sickle cell anaemia, any chronic treatment, date of death if the patient died. Regarding the patient's family history, we registered the presence or absence of previous gestational diabetes, polycystic ovarian syndrome, infertility, dystocia, consanguinity, diabetes, metabolic syndrome, sickle cell anaemia, pancreatic or liver surgery.

We also gathered information about the primary diagnosis and its treatment: type of cancer, diagnosis date, stage and localization of the tumour, anthropometric data on diagnosis, tanner stage, blood pressure (systolic and diastolic), treatments protocols (presence or absence of steroids, asparaginase, radiotherapy and HSCT) and the presence of treatment side effects such as steroid-resistant disease, allergy to asparaginase, pancreatitis and steatosis induced by treatment protocol.

When the patient developed hyperglycaemia more than twice, we reported the date, the anthropometric data of onset, the blood pressure, the treatment for the hyperglycaemia (e.g. insulin therapy, metformin), its doses per day and its duration. To obtain the number of blood glucose levels recorded, we counted all blood glucose measurements from the start of treatment protocol until the end of our study (August 2020). The duration of blood glucose monitoring was evaluated by counting blood glucose measurements performed without an interruption of more than 6 months and deceased patients were excluded. To evaluate the percentage of patients having been tested for blood glucose after the maintenance phase, we included only ALL patients treated before 2015 and HL patients treated before 2017 to have a sufficient delay between the end of the maintenance phase and the end of our study for the metabolic outcome monitoring. Standard deviation score (SDS) for height, weight and BMI were assessed using, respectively, Belgian Flemish reference charts and Cole's Corpulence Curve.[19,20]

2.6 | Statistical analysis

Descriptive statistics were used to summarize the results considering numbers and percentages for discrete variables, means with standard deviations (SD) and medians with interquartile range (IQR) for continuous variables. The clinical characteristics of patients were compared according to the occurrence or not of hyperglycaemia using Student's *t* test or Mann–Whitney test (as appropriate) for continuous variables and Fisher exact test for discrete variables. Kaplan-Meier estimates of the probability of remaining free of hyperglycaemia were plotted. A binary logistic regression analysis was performed to predict hyperglycaemia occurrence from all potential predictors available by estimating odds ratios and their 95% confidence intervals. All covariates with a *p*-value less than 0.10 in univariate analysis were introduced into a multivariate model (Wald Chi-Square). Variance inflation factor analysis was performed to detect a potential multicollinearity problem. A backward elimination strategy was used to estimate the best prediction model. Analyses were performed using SAS V9.4 software (SAS Institute Inc.). All *p*-values were two-sided and values less than 0.05 was considered statistically significant.

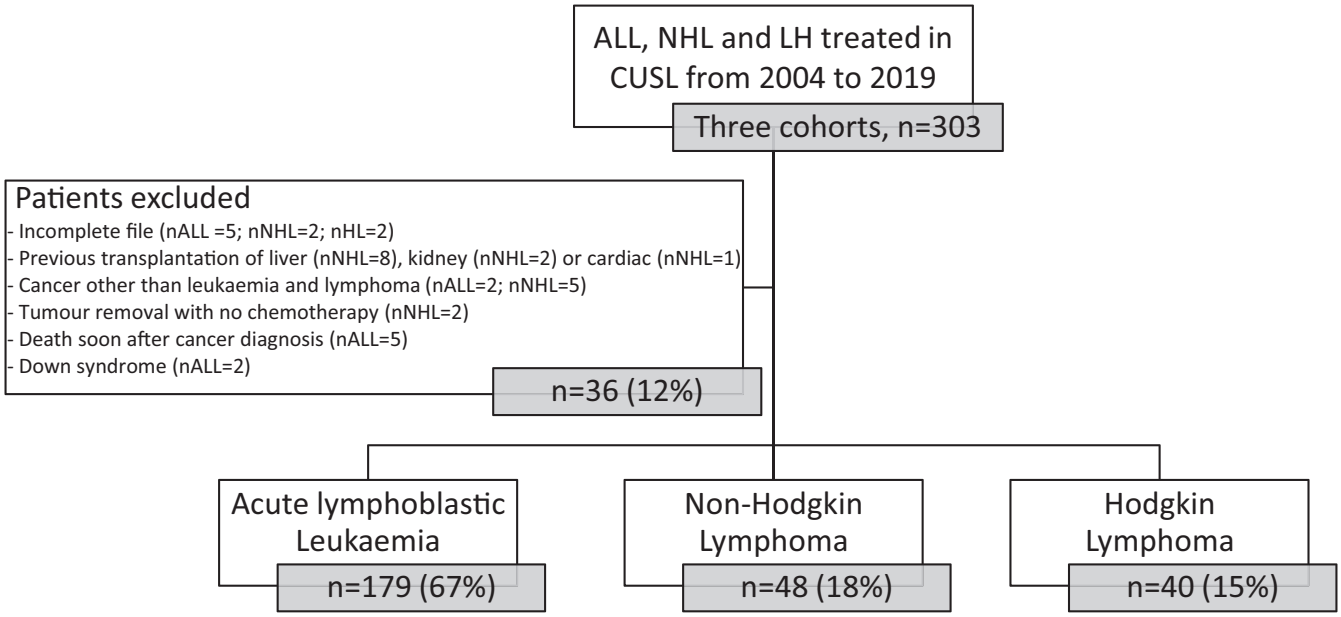

FIGURE 1 Flow chart of the study. Out of 303 patients treated in the Cliniques Universitaires Saint Luc (CUSL) from January 2004 and December 2019, 179 (67%) patients were diagnosed with Acute lymphoblastic leukaemia (ALL), 48 (18%) with non-Hodgkin lymphoma (NHL) and 40 (15%) with Hodgkin lymphoma (HL). *n*, number of patients

3 | RESULTS

3.1 | Patient characteristics

We included 267 children and adolescents out of 303 patients (Figure 1) treated in the Cliniques universitaires Saint-Luc from January 2004 and December 2019, divided as such: 179 (67.0%) patients were diagnosed with ALL, 48 (18.0%) with NHL and 40 (15.0%) with HL. We excluded 36 patients because of an incomplete file (nALL = 5; nNHL = 2; nHL = 2), down syndrome (nALL = 2), death soon after cancer diagnosis (nALL = 5), tumour removal with no chemotherapy (nNHL = 2), previous transplantation of liver (nNHL = 8), kidney (nNHL = 2) or cardiac (nNHL = 1) and cancer other than leukaemia and lymphoma (nALL = 2; nNHL = 5). Clinical characteristics of the three cohorts are summarized in Table 1.

3.2 | Treatments characteristics

Treatment characteristics are presented in Table 2. The median duration of cancer treatment was 32.9 (25.6; 33.7) months for ALL patients, 3.5 (2.6; 13.1) months and 3.8 (2.8; 6.2) months for NHL and HL patients, respectively. All three cohorts received steroids whereas asparaginase was prescribed to ALL (100.0%) and NHL cohorts (33.3%) but not to HL patients. The proportion of patients receiving radiotherapy was 9.5%, 6.3% and 37.5% in the ALL, NHL and HL cohorts. Patients from the ALL cohort required cranial (64.7%) and total body (41.2%) radiotherapy, while HL patients received abdominal (66.7%) and cervical (33.3%) irradiation. Of the three irradiated patients of the NHL cohort, each received radiation at a different site (AI, CI, TBI). The frequency of patients requiring HSCT was 9.5% (17/179), 10.4% (5/48) and 7.5% (3/40) for the ALL, NHL and HL cohorts, respectively.

3.3 | Incidence and evolution of hyperglycaemia during the treatment

Of the 267 children and adolescents, 17.9% (32/179) of the ALL patients and 16.7% (8/48) of NHL patients developed hyperglycaemia (Table 3). No hyperglycaemia was observed in the HL cohort.

Hyperglycaemia developed rapidly after initiation of chemotherapy protocols: approximatively 61.0% (19/32) of ALL patients and all NHL patients except one (7/8) developed hyperglycaemia within the first month of treatment, corresponding to pre- and induction phases (Figure 2). The median number of blood glucose measurements recorded per patient was 24 (19; 36) for ALL patients, 26 (18; 40) for NHL patients, and 5 (3; 7) for HL patients (Table 3). The median duration of follow-up of blood glucose levels recorded during treatment protocols was 8.6 months (6.2; 12.7) and 3.6 months (2.4; 6.1) for ALL and NHL patients, respectively, and covered the four first phases of cancer

TABLE 1 Patients characteristics

	Acute lymphoblastic leukaemia	Non-Hodgkin lymphoma	Hodgkin lymphoma
N	179	48	40
Age at cancer diagnosis, median (P25–P75)	4.8 (3.1; 10.8)	9.7 (7.1; 13.8)	13.2 (10.4; 15.5)
[0–8] years [*n* (%)]	120 (67.0)	16 (33.3)	6 (15.0)
[9–18] years [*n* (%)]	59 (33.0)	32 (66.7)	34 (85.0)
Gender, male [*n* (%)]	107 (59.8)	34 (70.8)	29 (72.5)
Weight SDS, median (P25–P75)	−0.1 (−0.8; 0.5)	−0.2 (−0.8; 0.8)	−0.1 (−0.7; 0.5)
Height SDS, median (P25–P75)	0.1 (−0.5; 0.6)	0.0 (−0.3; 0.5)	−0.2 (−0.7; 0.5)
Body Mass Index SDS, median (P25–P75)	−0.3 (−1; 0.6)	−0.4 (−1.1; 1.1)	0.0 (−0.8; 0.8)
Tanner staging P < 2 [*n* (%)]	138 (77.1)	28 (58.3)	16 (40.0)
Tanner staging M/G < 2 [*n* (%)]	138 (77.1)	27 (56.2)	17 (42.5)
Death [*n* (%)]	17 (9.5)	5 (10.4)	0 (0.0)

Abbreviations: G, genital; M, mammary; P, pubic; SDS, standard deviation score.

TABLE 2 Treatments characteristics

	Acute lymphoblastic leukaemia	Non-Hodgkin lymphoma	Hodgkin lymphoma
N	179	48	40
Duration of cancer treatment, month, median (P25; P75)	32.9 (25.6; 33.7)	3.5 (2.6; 13.1)	3.8 (2.8; 6.2)
Cancer treatment lower risk [*n* (%)]	151 (84.4)	38 (79.2)	40 (100.0)
Cancer treatment higher risk [*n* (%)]	28 (15.6)	10 (20.8)	—
Treatment with steroids [*n* (%)]	179 (100.0)	48 (100.0)	40 (100.0)
Treatment with asparaginase [*n* (%)]	179 (100.0)	16 (33.3)	—
Treatment with radiotherapy [*n* (%)]	17 (9.5)	3 (6.3)	15 (37.5)
Cranial irradiation [*n* (%)]	11 (6.1)	1 (2.1)	—
Total body irradiation [*n* (%)]	7 (3.9)	1 (2.1)	—
Abdominal irradiation, [*n* (%)]	—	1 (2.1)	10 (25.0)
Cervical irradiation [*n* (%)]	—	—	5 (12.5)
Total irradiation doses, Grays, median (P25; P75)	18 (12; 18)	10 (8; 18)	40 (20; 40)
Treatment with HSCT [*n* (%)]	17 (9.5)	5 (10.4)	3 (7.5)
Allogenic transplantation [*n* (%)]	15 (8.4)	2 (4.2)	—
Autologous transplantation [*n* (%)]	2 (1.1)	3 (6.2)	3 (7.5)
Asparaginase-induced pancreatitis [*n* (%)]	2 (1.1)	1 (2.1)	—

Abbreviation: HSCT, hematopoietic stem cell transplantation.

treatment for ALL patients and all the treatment protocol period for NHL patients (Table 3, Figures S1 and S2). Blood glucose measurements are constantly performed during treatment protocols for ALL and NHL patients, with a peak during the induction phase and a decrease during maintenance and remission phases (Figures S1 and S2). For HL patients, median blood glucose monitoring lasted 4 days (1; 68) and was close to diagnosis (Table 3). The percentage of patients with blood glucose recorded after the maintenance phase for the metabolic outcome monitoring was 77.8% (91/117), 76.7% (33/43) and 88.9% (32/36) for ALL, NHL and HL patients, respectively (Table 3).

At 12 months post ALL treatment, the probability of remaining free of hyperglycaemia was 83.8% and remained relatively unchanged thereafter (end in August 2020). In the NHL group, this probability remained unchanged at 85.4% after one month of cancer treatment (Figure 3). Half (16/32) of the hyperglycaemia-positive ALL cohort and three out of eight hyperglycaemia-positive NHL patients were treated with insulin and all required insulin therapy

TABLE 3 Incidence of hyperglycaemia

	Acute lymphoblastic leukaemia	Non-Hodgkin lymphoma	Hodgkin lymphoma
N	179	48	40
Hyperglycaemia [*n* (%)]	32 (17.9)	8 (16.7)	0
Insulin treatment [*n* (%)]	16 (8.9)	3 (6.3)	0
Number of blood glucose levels, median (P25; P75)	24 (19; 36)	26 (18; 40)	5 (3; 7)
N[a]	162	43	40
Duration of blood glucose monitoring, month, median (P25; P75)	8.6 (6.2; 12.7)	3.6 (2.4; 6.1)	0.13 (0.03; 2.2)
N[b]	117	43	36
Patient with blood glucose recorded after maintenance phase [*n* (%)]	91 (77.8)	33 (76.7)	32 (88.9)

[a]Due to interrupted follow-up, deceased patients ($n = 17$) were excluded.

[b]Dead patients and ALL patients treated after 2015 and HL patients treated after 2017 were excluded.

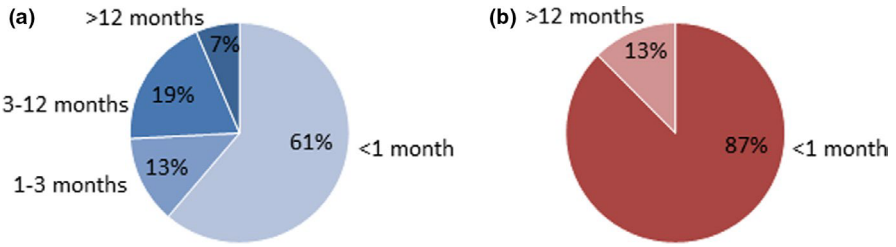

FIGURE 2 Distribution of hyperglycaemia onset over time in acute lymphoblastic leukaemia (ALL) and non-Hodgkin lymphoma (NHL) paediatric cohorts. Most of (a) ALL patients (61%) and (b) NHL patients (87%) developed hyperglycaemia within the first month of treatment

only during a treatment protocol, except one ALL patient who remained insulin-dependent (Table 3). Besides this, only known case from our cohort with persistent diabetes, the median duration of insulin therapy for the 16 patients with ALL and the three patients with NHL was 15 days (3; 30) and 13 days (12;14), respectively.

3.4 | Risk factors for hyperglycaemia

In univariate analysis, age older than 8 years and greater BMI SDS were significantly associated with the onset of hyperglycaemia (OR 1.01; $p = 0.002$ and OR 20.80; $p = 0.008$, respectively) as shown in Table 4 and illustrated in Figure 4. Median age at cancer diagnosis was 10.8 (3.3; 15.1) years for the hyperglycaemia-positive ALL cohort and 4.4 (3.0; 8.7) years for the hyperglycaemia-free ALL cohort and median BMI SDS at cancer diagnosis was 0.2 (−0.8; 1.2) and −0.4 (−1.0; 0.5), respectively (Table 5). Furthermore, the unadjusted odds ratio of hyperglycaemia for a patient over 8 years old was higher (OR 4.62) compared to patients younger than 8 years, and this difference was significant ($p < 0.001$). Other covariates were also significantly associated with the onset of hyperglycaemia such as a Tanner stage at cancer diagnosis equal to or greater than 2 (OR 4.88; $p < 0.001$), a positive history of obesity/overweight (OR 4.29; $p = 0.008$), a steroid-resistant disease (OR 3.20; $p = 0.014$), or HSCT (OR 5.11; $p = 0.002$). Furthermore, high-risk treatment was associated with hyperglycaemia development compared to low-risk treatment (OR 4.01; $p = 0.002$) (Table 4).

After adjustment in the multivariate analysis, the best model to predict hyperglycaemia occurrence included two individual factors and two factors related to treatment. ALL patients with a history of obesity/overweight (OR 3.793, 95% CI 1.026–14.022), a pubertal stage equal to or greater than 2 (OR 4.269, 95% CI 1.676–10.875) at cancer diagnosis, the presence of steroid-resistant disease (OR 3.445, 95% CI 1.114–10.657) and the use of HSCT (OR 4.754, 95% CI 1.099–20.554) were associated with a higher risk of developing hyperglycaemia (Tables 4 and 5).

Due to insufficient statistical power, no association between TBI and hyperglycaemia onset could be demonstrated but five out of the eight patients (seven ALL and one NHL) who received TBI, developed hyperglycaemia (Table 5). The same observation applied for asparaginase-induced

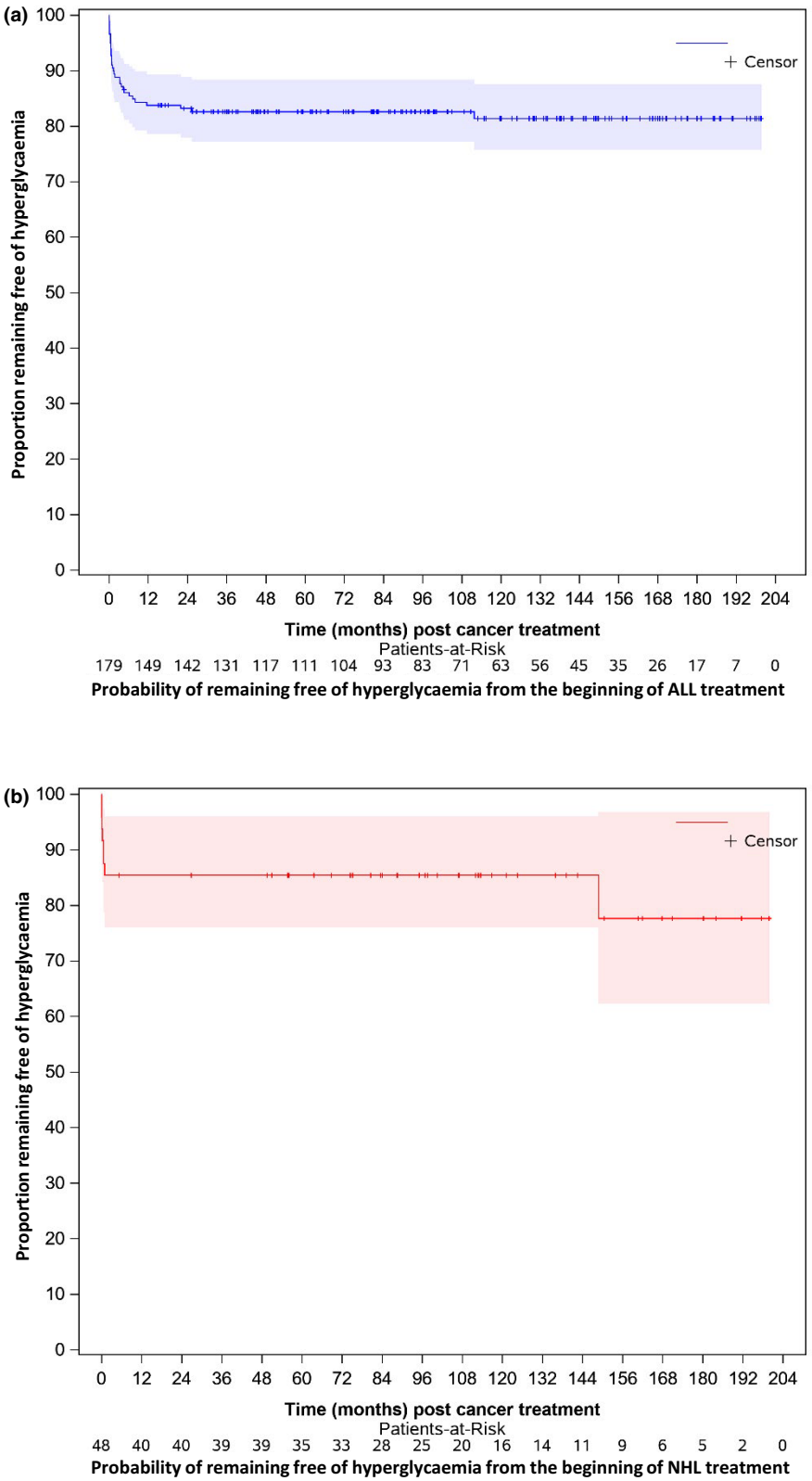

FIGURE 3 Kaplan–Meier estimates of the probability of remaining free of hyperglycaemia in acute lymphoblastic leukaemia (ALL) and non-Hodgkin lymphoma (NHL) paediatric cohorts. (a) At 12 months post ALL treatment, the probability of remaining free of hyperglycaemia was 83.8% and remained relatively unchanged thereafter. (b) In the NHL group, this probability remained unchanged at 85.4% after one month of cancer treatment

pancreatitis as a risk factor, since logistic regression was not possible because no patients in the hyperglycaemia-free ALL cohort developed asparaginase-induced pancreatitis during cancer treatment. However, all three patients (two ALL and one NHL) who developed asparaginase-induced pancreatitis, subsequently developed hyperglycaemia ($p = 0.031$) (Table 5). In contrast, there was no association between cranial irradiation and hyperglycaemia since in a total of eleven ALL patients receiving cranial irradiation, only one developed hyperglycaemia (Table 5).

TABLE 4 Univariate (Likelihood Ratio) and multivariate (Wald Chi-Square) logistic regression analyzes of factors leading to hyperglycaemia occurrence for ALL and NHL cohorts

		Univariate analysis		Multivariate analysis	
ALL predictors	***N***	***p*-value**	**Odds ratio (95% CI)**	***p*-value**	**Odds ratio (95% CI)**
Age at cancer diagnosis	179	0.002	1.010 (1.004–1.017)		
Age: [0–8] versus [9–18]	179	<0.001	4.615 (2.066–10.312)		
BMI SDS	179	0.017	1.486 (1.072–2.059)		
BMI SDS overweight versus Normal weight	179	0.008	20.800 (2.231–193.96)		
Cancer treatment risk higher versus lower	179	0.002	4.006 (1.649–9.730)		
History of overweight at cancer diagnosis	179	0.008	4.293 (1.464–12.588)	0.046	3.793 (1.026–14.022)
Tanner staging ≥2	179	<0.001	4.880 (2.159–11.032)	0.002	4.269 (1.676–10.875)
Steroid-resistant disease	179	0.014	3.204 (1.265–8.113)	0.032	3.445 (1.114–10.657)
HSCT	179	0.002	5.111 (1.795–14.553)	0.037	4.754 (1.099–20.554)
NHL predictors					
Cancer treatment risk higher versus lower	48	0.038	5.667 (1.104–29.073)		

Abbreviations: ALL, acute lymphoblastic leukaemia; BMI, body mass index; CI, confidence interval; HSCT, hematopoietic stem cell transplantation; NHL, non-Hodgkin lymphoma; SDS, standard deviation score.

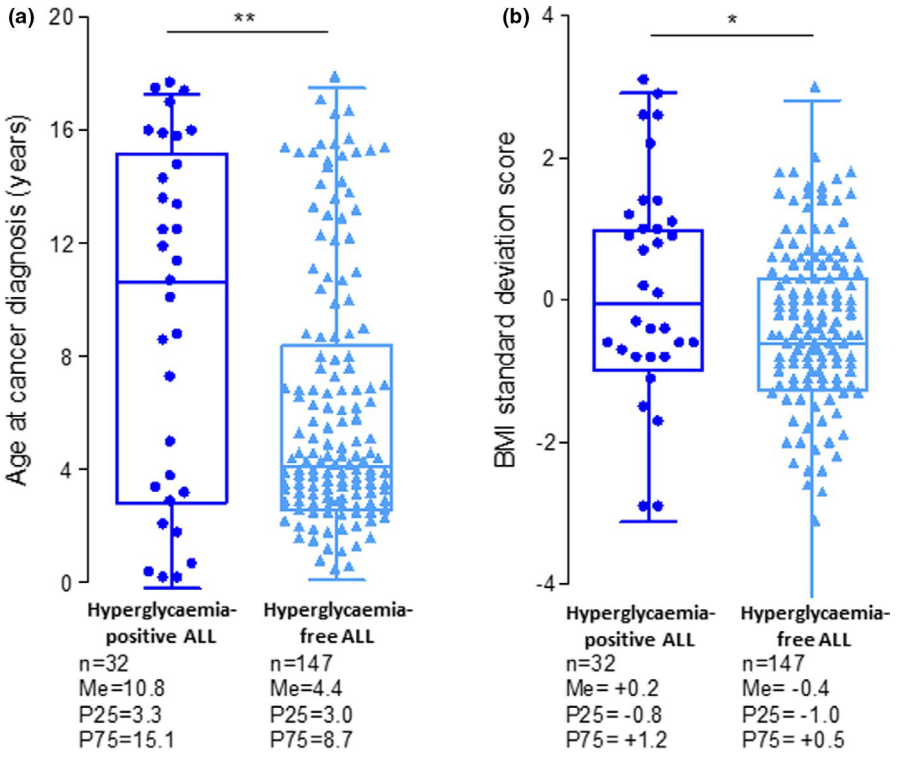

FIGURE 4 (a) Age and (b) BMI as risk factors of hyperglycaemia in acute lymphoblastic leukaemia (ALL) paediatric cohort. ALL patients with older age and higher BMI are more at risk of developing hyperglycaemia. Each point represents a patient. The boxplot represents the median, the minimum and maximum values. Asterisks (*, **) show a significant difference between hyperglycaemia-positive ALL and hyperglycaemia-free ALL cohorts (*$p < 0.05$ and **$p < 0.01$; Mann–Whitney test)

TABLE 5 Comparison of the clinical characteristics of hyperglycaemia-positive ALL and hyperglycaemia-free ALL cohorts

	Hyperglycaemia-positive ALL, $n = 32$	Hyperglycaemia-free ALL, $n = 147$	p-value
Age at cancer diagnosis, median (P25; P75)	10.8 (3.3; 15.1)	4.4 (3,0; 8.7)	0.016
[0–8] [n (%)]	13 (40.5)	108 (73.5)	<0.001
[9–18] [n (%)]	19 (59.5)	39 (26.5)	
BMI SDS, median (P25; P75)	0.2 (−0.8; 1.2)	−0.4 (−1.0; 0.5)	0.017
History of overweight [n (%)]	7 (21.9)	9 (6.1)	0.011
Tanner staging ≥2 [n (%)]	16 (50.0)	25 (17.0)	<0.001
Steroid-resistant disease [n (%)]	9 (28.1)	16 (10.9)	0.021
Cancer treatment lower risk [n (%)]	21 (65.6)	130 (88.4)	0.003
Cancer treatment higher risk [n (%)]	11 (34.4)	17 (11.6)	
Radiotherapy treatment [n (%)]	5 (15.6)	12 (8.2)	0.193
Cranial irradiation [n (%)]	1 (3.1)	10 (6.8)	NA
Total body irradiation [n (%)]	4 (12.5)	3 (2.0)	NA
Treatment with HSCT [n (%)]	8 (25)	9 (6.1)	0.003
Asparaginase-induced pancreatitis [n (%)]	2 (6.3)	0 (0.0)	0.031

Note: Student t test or Mann–Whitney test for continuous variables and Fisher exact test for discrete variables were used to obtain the p-values.

Abbreviations: ALL, acute lymphoblastic leukaemia; BMI, body mass index; HSCT, hematopoietic stem cell transplantation; SDS, standard deviation score.

Due to the low number of NHL patients and hyperglycaemia-positive NHL patients, the univariate analysis only allowed us to identify that high-risk treatment was significantly associated with hyperglycaemia onset compared to low-risk treatment in the NHL cohort (OR 5.67; $p = 0.038$) (Table 4).

There was no difference in the gender, family history of diabetes or metabolic syndrome, type T or B cancer (nature of the disease), type of transplant and between the anthropometric data reported at cancer and hyperglycaemia diagnosis (weight, height, BMI).

4 | DISCUSSION

Our study describes the incidence and risk factors of hyperglycaemia onset, immediately at treatment initiation, in a cohort of paediatric patients treated for ALL, NHL or HL. We showed that 18% of ALL patients and 17% of NHL patients developed hyperglycaemia described as random capillary blood or plasma glucose level exceeding 11 mmol/L (200 mg/dl) for at least two measurements separated by 24 h. The incidence of hyperglycaemia observed in our ALL cohort is similar to a previous study carried out in 2008 by the team of Howard were 16% out of 871 paediatric patients with ALL presented hyperglycaemia during the treatment.[8] More recently, three studies described 16.5% (22/133) and 15.7% (16/102 and 57/363) of ALL paediatric patients with hyperglycaemia (in more than two consecutive measurements).[9,11,21] The impact of NHL treatment protocols on hyperglycaemia onset is less studied; however, the study by Neville et al. showed in a smaller cohort of 20 NHL patients a high incidence of glycaemic dysregulation: 5 patients (25%) developed either hyperinsulinemia, IGT or diabetes.[22]

In our study, the majority of ALL (61%) and NHL (87%) patients developed hyperglycaemia within the first month of chemotherapy, corresponding to pre- and induction phases that are the most aggressive in terms of steroid doses. Only half of hyperglycaemia-positive ALL and NHL patients were given insulin therapy and one hyperglycaemia-positive ALL patient presented persistent non-type 1 diabetes. Also, we observed that the majority of the three cohorts (ALL: 77.8%, NHL: 76.7% HL: 88.9%) benefited from blood glucose control during monitoring of side effects but this monitoring did not include a dynamic test such as the oral glucose tolerance test.

Since all patients treated for leukaemia or lymphoma required steroid treatment but not all developed hyperglycaemia, we sought to identify hyperglycaemia predisposing risk factors in our paediatric cohort. Our multivariate analysis revealed that a history of obesity/overweight at cancer diagnosis is associated with a higher risk of developing hyperglycaemia in ALL patients, as described elsewhere.[7,12,13,23] Also similar to other studies presented by Gregoriou in a recent review,[24] being older than 10 years (Gregoriou) or 8 years (this paper) was identified as a strong risk factor of hyperglycaemia in our univariate analysis ($p < 0.001$) but was not an independent risk factor in our multivariate analyses. Associated with the age factor, we identified a strong correlation between pubertal entry (Tanner stage ≥2) and hyperglycaemia risk. In

normal puberty, rising sex steroid and growth hormone levels are associated with reduced insulin sensitivity, which may predispose to the development of metabolic syndrome in overweight/obese children. Indeed, reduced insulin sensitivity is not uncommon (8%) in extremely obese children.[25]

The stronger treatment-related risk factor for hyperglycaemia that emerged from our study is a steroid-resistant disease for ALL patients. Patients with a steroid-resistant disease receive a more aggressive and risky treatment protocol and "high-risk treatment" was associated with hyperglycaemia onset for ALL patients in our univariate analysis (OR 4.01; $p = 0.002$). Although we do not know if patients received "high risk" treatment due to the more aggressive nature of cancer or because of steroid-resistant disease.

HSCT is also a composite risk factor of hyperglycaemia for ALL patients in our study. Indeed, HSCT is often preceded by TBI and may require the use of steroids in case of graft versus host disease symptoms. Studies carried out on CCS showed that TBI and HSCT together increase the risk of IGT and diabetes.[15,22,26] In our study, we also observed that ALL patients who received TBI followed by HSCT tended to develop hyperglycaemia (5/8), though the number of patients with TBI was insufficient to reach significance. Moreover, in our NHL cohort, HSCT did not emerge as a risk factor of hyperglycaemia, yet two out of five patients with HSCT developed hyperglycaemia.

L-asparaginase induces hyperglycaemia as a result of reduced insulin synthesis due to depletion of the available pool of asparagine concurrent with hyperglucagonemia and probably by a reduction of the number of insulin receptors.[27] In agreement with the results of the study by Irga et al. describing paediatric patients with ALL, NHL and severe aplastic anaemia, we did not find a correlation between L-asparaginase treatment and hyperglycaemia onset.[28] However, as emphasized in a review paper by Hijiya, L-asparaginase-induced pancreatitis is known to affect 2% to 18% of ALL patients and consequently causes the rapid development of diabetes.[29] In our study, it is noticeable that all patients who developed pancreatitis induced by L-asparaginase had subsequently developed hyperglycaemia, although it concerns only three patients.

The absence of hyperglycaemia observed in HL patients can be explained by several hypotheses. Potentially less blood glucose monitoring is done linked to their outpatient status, compared to inpatient treatment for ALL and NHL patients. In addition to less blood glucose testing, there is no exposure to potential diabetogenic treatment protocols such as L-asparaginase and TBI, and there is a discontinuous prescription of steroids with a lower theoretical cumulative monthly dose of steroids for HL patients compared to ALL patients. Also, HL patients required abdominal and cervical irradiation, yet we retrieved only TBI as a risk of hyperglycaemia development in our ALL cohort. Abdominal, cervical and cranial radiation did not appear to induce hyperglycaemia in our study, although this is contrary to findings in other studies which suggested the effect of abdominal radiation.[30–32] *Strengths* of our study include the large sample size of ALL patients, inclusion of risk factors for hyperglycaemia, complete patient records and numerous harmonized blood glucose data for ALL and NHL. Furthermore, we studied the incidence of hyperglycaemia from the initiation of cancer treatment and not only during remission (after 2 years). One *major limitation* of our study was the inclusion of patients who received different ALL, NHL and HL treatment protocols from different treatment eras. The retrospective nature of the study was a limitation although patient records were mostly complete. Moreover, the availability of blood glucose data varied and decreased in maintenance and remission phases for the three cohorts, preventing a potential diagnosis of persistent diabetes or late diabetes (i.e. irradiation treatment) and was limited for HL patients by the ambulatory follow-up.

In conclusion, in our paediatric study, hyperglycaemia was diagnosed in 18% of ALL patients, 17% of NHL patients but not in HL patients. Puberty and overweight at the time of cancer diagnosis as well as steroid-resistant disease, HSCT preceded by TBI, and asparaginase-induced pancreatitis was identified as risk factors for hyperglycaemia in paediatric patients with ALL. We believe our study may help clinicians to identify ALL patients at risk of early onset of hyperglycaemia since our study highlights the importance of considering BMI and pubertal stage as potential markers for the onset of hyperglycaemia in children and adolescents receiving diabetogenic cancer treatments. In addition, our study shows the importance of closely monitoring blood glucose levels during treatment intensification when patients present steroid-resistant disease or relapse, especially when TBI and HSCT are required. Recognising the reduction of available blood glucose levels in the remission phase and their absence in the Hodgkin lymphoma cohort, we also point out the need to monitor blood glucose levels at each follow-up visit to enable the diagnosis of transient, persistent or late-onset diabetes. The DIABONCO study includes a prospective part with the characterization of survivors inside hyperglycaemia-positive ALL and NHL cohorts and will be able to evaluate the presence of persistent subclinical diabetes or IGT.

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CONFLICT OF INTEREST

The authors declare no potential conflicts of interest.

DISCLOSURE

The authors have nothing to disclose.

AUTHOR CONTRIBUTIONS

All authors have read and approved the final version of the manuscript. PA.L. had the idea for the study, designed the study, wrote and reviewed the manuscript. S.W. designed and performed the study, collected and analysed the data, decided on the statistical method, wrote and reviewed the manuscript. K.S. decided on the statistical method, performed and wrote the statistical analysis. B.B. and M.dV. contributed to the reflection on the results. B.B., M.dV., A.VD. and C.B. reviewed the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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Characterization, evolution and risk factors of diabetes and prediabetes in a pediatric cohort of renal and liver transplant recipients

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Background: Hyperglycemia (HG) and prediabetes are rarely sought in pediatric liver (LT) and renal (RT) transplantation, yet their presence indicates a high risk of diabetes and cardiovascular disease. The objectives of our DIABGRAFT study were to retrospectively (rDIABGRAFT) and longitudinally (pDIABGRAFT) characterize HG and (pre)diabetes in a cohort of children with LT or/and RT.
Methods: We retrospectively analyzed risk factors of HG from 195 children with LT from 2012 to 2019 and twenty children with RT from 2005 to 2019 at Cliniques universitaires Saint-Luc. In addition, we prospectively followed four LT and four RT children to evaluate the evolution of their glucose metabolism.
Results: Our rDIABGRAFT study showed that 25% and 35% of LT and RT children respectively presented transient HG and 20% of RT developed diabetes. The occurrence of HG was associated with the use of glucocorticoids and with acute events as graft rejection and infection. In our pDIABGRAFT cohort, biological markers of diabetes were in the normal range for HbA_{1C}, fasting glucose and insulin levels. However, oral glucose tolerance test and glucose sensors showed insulin resistance, impaired glucose tolerance and HG in the post-prandial afternoon period.
Conclusion: Our study shows that children with LT and RT were more at risk of developing HG when glucocorticoids were required and that HbA_{1C} and fasting glucose lack sensitivity for early detection of glucose intolerance. Also, measurement of glycemia immediately after the transplantation and in postprandial period is key to detect dysglycemia since insulin resistance prevailed in our cohort.
ClinicalTrials.gov ID: NCT05464043.

KEYWORDS

diabetes, hyperglycemia, impaired glucose tolerance, insulin resistance, liver transplantation, renal transplantation, glucocorticoids

Abbreviations
ACR, acute cellular rejection; ADA, American diabetes association; BMI, body max index; CMV, cytomegalovirus; CUSL, cliniques universitaires Saint-Luc; EBV, Epstein-Barr Virus; FPG, fasting plasma glucose; HG, hyperglycemia; HOMA, homeostasis model assessments; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; IR, insulin resistance; LT, liver transplant; OGTT, oral glucose tolerance test; OR, odds ratio; PG, plasma glucose; rDIABGRAFT, retrospective DIABGRAFT; pDIABGRAFT, prospective DIABGRAFT; RT, renal transplant; SDS, standard deviation score; SOT, solid organ transplantation.

Introduction

Solid organ transplantation (SOT) is the therapeutic choice for patient in end-stage renal or liver disease. After transplant, immunosuppression is required to ensure graft survival but is associated with side effects, including glycemic disorders. One of the most frequent complications observed with immunosuppressants is hyperglycemia (HG), which increases the probability to develop prediabetes and overt diabetes. Prediabetes, an intermediate state between normal glucose homeostasis and overt diabetes, represents a major health problem because in 2012 it was estimated that 70% of the prediabetic American citizens (33.5%) will develop diabetes within their lifetime (1–3). Diabetes affects an ill-defined proportion of transplant patients (2%–53%) (4–7) and is common in the context of adult liver and renal transplantation (5, 8, 9). Yet the incidence of transient HG, and the progression to overt diabetes in pediatric liver and renal transplantation remain unknown. However, it is known that both are associated with an unfavorable acute prognosis (i.e., mortality, graft rejection, increased hospital stay) and an increased cardiovascular risk in the long term in adult patients, this risk being correlated to the presence of metabolic syndrome (9–12). In addition, the use of fasting blood glucose and HbA_{1C} levels might not allow early detection of impaired glucose tolerance (IGT) as a preamble to prediabetes. It is therefore essential to gather knowledge on the evolution of glucose in pediatric patients after SOT. The objectives of our DIABGRAFT study were to assess the incidence and associated risk factors of developing hyperglycemia in liver and renal transplant children and longitudinally analyze the evolution of glycemic profile (i.e., HG, IGT and diabetes) in these patients during the post-transplant period.

Materials and methods

Study design

The DIABGRAFT study was conducted in collaboration with the Pediatric Hepatology and Gastroenterology and Specialized Pediatrics (Endocrinology and Nephrology Units) Services of Cliniques universitaires Saint-Luc (CUSL) in Belgium (Brussels). This study was approved by the local ethical committee (CUSL and UCLouvain Hospital-Faculty Ethics Committee; approval number 2019/12MAR/118) and was conducted in accordance with the Declaration of Helsinki. Our study included liver and renal transplant pediatric patients (<18 years of age) at CUSL. Were excluded patients with a history of diabetes (i.e., type 1, type 2, neonatal or monogenic), pancreatitis, Down Syndrome, cystic fibrosis ($n = 1$), a second organ transplantation for our LT cohort ($n = 4$; cardiac, renal), patients deceased shortly after transplantation (<1 year, $n = 14$), and patients with incomplete medical record ($n = 8$).

DIABGRAFT was constituted of two parts. Its **retrospective part** (rDIABGRAFT) consisted of collecting data of pediatric patients who benefited from a liver transplant performed at CUSL between April 2012 and April 2019, or that benefited from a renal transplant in our center between September 2005 and April 2019. The **prospective part** (pDIABGRAFT) of the study consisted of a longitudinal glycemic evaluation of liver and renal transplant children in CUSL between 2020 and 2022 with the use of dynamic endocrine testing (**Figure 1**). Informed consents were collected from parents and from all children over six years of age.

Treatments protocols for pediatric liver and renal transplant patients

At CUSL, liver and renal transplant children receive standard immunosuppression protocol as per international guidelines (13). For LT patients, this protocol includes the association of a monoclonal anti-CD 25 antibody (basiliximab, Simulect®) and a calcineurin inhibitor (tacrolimus, Prograft®) (14, 15). For RT patients, this protocol is based on a combination of Tacrolimus, glucocorticoids, monoclonal anti-CD 25 antibody and a cell proliferator inhibitor as mycophenolate mofetil (Cell-Cept®). Doses of glucocorticoids are introduced or increased when a LT/RT patient presents an acute cellular rejection (ACR). Complete treatment protocol is available in **Supplementary data (Text S1)**.

About glucose monitoring, after a liver or renal transplantation at CUSL, glycemia is measured daily during hospitalization (between two weeks and one month) and for LT patients, glucose monitoring is regularly performed during a month until the patient returns to his home (after three months), after what yearly glycemic control is performed. For RT patients, the measure of fasting glycemia continues once weekly until the 6th month post-transplantation, when the control becomes once a month.

Classification of glucose status

We defined hyperglycemia based on guidelines of the international consensus for diabetes of the American Diabetes Association (ADA): patients presented HG when fasting plasma glucose (FPG) or random plasma glucose (PG) levels exceeded respectively 126 mg/dl (7.0 mmol/L) and 200 mg/dl (11 mmol/L) for at least two measurements separated by 24 h, and not under a condition of stress such as the day of the transplant (16).

For our rDIABGRAFT study, the term "transient hyperglycemia" was used to define patients with HG (as described above) without overt diabetes diagnosed and diabetes was notified when patient required a persistent treatment (i.e., insulin or oral antidiabetics). For our pDIABGRAFT study, as we used dynamic testing, we classified our patient based on ADA guidelines: when a patient presented impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) and/or HbA_{1C} from 5.7% to 6.4% (39–47 mmol/mol), we defined a "prediabetes" state. IFG was defined as FPG between 100 and 125 mg/dl (5.6 and 6.9 mmol/L) and IGT as 2h-PG levels during an oral glucose tolerance test (OGTT) from 140 to 199 mg/dl (7.8 and 11.0 mmol/L) (16). Diabetes was defined when a patient presented FPG $\geq$ 126 mg/dl (7.0 mmol/L) or a random PG or 2-h PG levels during OGTT > 200 mg/dl (11.1 mmol/L) or hemoglobin A1C (HbA_{1C}) > 6.5% (48 mmol/mol) and/or when the patient presents classic symptoms of HG (16).

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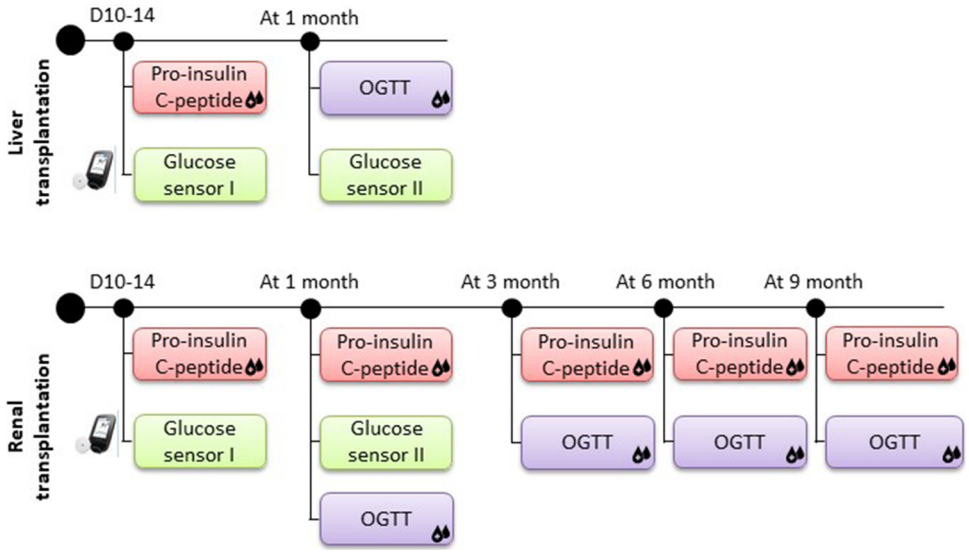

FIGURE 1
Protocol of prospective DIABGRAFT study. For LT and RT cohorts, pro-insulin and C-peptide secretion was measured after two weeks of transplant and measures had continued at one, three, six and nine months for RT patients. A glucose sensor was placed on the patient two weeks post-transplant for one month to detect the presence of early dysglycemia. An OGTT was performed after one month of transplantation and, for RT patients also at three, six and nine months.

Dynamic testing of glucose homeostasis

After obtaining the consent of pediatric patients and their parents, a glucose sensor (The FreeStyle Libre Flash Glucose Monitoring system, Abbott) was placed on the patient two weeks post-transplant for one month to detect the presence of early dysglycemia. Pro-insulin and C-peptide secretion by enzyme-linked immunosorbent assays (ELISA) was measured after two weeks of transplant to analyze the insulin secretion function of beta-cell, and measures had continued at one, three, six and nine months for RT patients. The enzyme-linked immunosorbent assays used for our analyses were performed as per manufacturer's instructions (Proinsulin 10-1118-01 and C-peptide 10-1136-01 kits, Mercodia). To analyze the insulin sensitivity and secretion over time, an OGTT was performed after one month of transplantation and, for RT patients also at three, six and nine months (**Figure 1**). Patients were not treated with insulin during tests. The OGTT was performed after 8 h overnight fast with weight-based glucose load (1.75 g/kg for pediatric patient) (17). Glucose and insulin were measured at fasting and at 30, 60 and 120 min after the ingestion of glucose. Insulin resistance (IR) was evaluated with HOMA-IR (for homeostasis model assessments of fasting insulin resistance; $Ins^{0}_{(\mu U/ml)} \times Gluc(0)_{(mmol/L)}/22.5$) (18). If the HOMA index is less than 1.6, the result is normal. When the HOMA index is between 1.7 and 2.3, the patient presents a moderate form of IR and if the value is greater than 2.4, he suffers from a severe form of IR.

Data collection

Patient history data included sex, date of birth, height, weight and gestation at birth (i.e., term, pre- or post-term), country of origin, date of death if patient deceased, the presence of hypo- and hyperglycemia in the neonatal period, dysmaturity, any chronic and hormonal treatment before the transplant, presence of dialysis for RT, its duration and type (e.g., hemodialysis, hemodiafiltration, peritoneal dialysis), endocrine or autoimmune diseases, acanthosis nigricans and sickle cell anemia. Also, we collected data about familial history as the presence or absence of consanguinity, metabolic syndrome, diabetes (type 1, type 2, gestational and monogenic), polycystic ovarian syndrome, fetal dystocia and sickle cell anemia.

We included information about the liver or renal transplant such as disease etiology, transplant date, the type of immunosuppressants administrated (tacrolimus, cyclosporine A, sirolimus, glucocorticoids), the use and duration of glucocorticoids in pre- and post-transplant period, the presence and date of liver or renal rejection, the type of transplant (living or cadaveric) and the link with the donor. We also collected anthropometric data in pre- and post-transplant period (at one, three, six and nine months after RT): weight, heigh, body max index (BMI) in standard deviation score (SDS) and Tanner stage. To obtain values in SDS score we used Belgian Flemish reference charts and Cole's Corpulence Curve (19, 20).

We collected glycemia and HbA_{1C} data before and after transplantation. When a patient presented HG after the first day of the transplantation, we reported the number of its occurrence, the date of its first and last observation and if a treatment was received (e.g., insulin therapy, antidiabetic oral), its doses per day, and its duration. The number of glycemia recorded was obtained by counting all measurements performed from the first consultation at CUSL (pre-transplantation evaluation) until the end of our data collection (in November 2021 for LT and in April 2022 for RT). The duration between the day of the transplantation and the last glycemia recorded was calculated to obtain the glycemia follow-up. We used REDCap (Research Electronic Data Capture) tool to collect and manage study data (21, 22).

Statistical analysis

Discrete variables are described as numbers and percentages, and continuous variables were presented as medians with interquartile range (IQR). The characteristics of children were compared according to the occurrence or not of HG using Fisher exact test for discrete variables and Student t test or Mann-Whitney test for continuous variables. A binary logistic regression analysis was performed to predict HG occurrence from all potential predictors described in data collection section and results were expressed by estimating odds ratios (OR) with their 95% confidence intervals. Due to the low number of RT patients, only univariate analysis was performed. For our LT patients, covariates with a *p*-value less than 0.10 in univariate analysis were introduced into a multivariate model (Wald Chi-Square). The potential predictors "graft rejection" and "CMV (cytomegalovirus)" were not introduced in multivariate model due to their interaction with "glucocorticoids" and "infection" respectively. All *p*-values were two-sided/2-tailed and values less than 0.05 was considered statistically significant. All statistical analyses were performed with Stata® V17 software (Statacorp, Texas, USA).

Results

Patients and treatment characteristics of our rDIABGRAFT study

Characteristics of our rDIABGRAFT LT and RT cohorts are summarized in **Tables 1**, **2** (**Supplementary Tables S1**: LT countries and **S2**: LT pathologies), respectively. We collected data from 195 pediatric patients treated at CUSL with liver transplantation (LT) (**Figure 2**). The median age of liver recipients was 18 months (10; 36) and the majority (179/195) received a liver from a living donor. All patients were treated lifelong with tacrolimus and 65% (126/195) were temporally treated with glucocorticoids. Regarding acute complications, 44% (86/195) were diagnosed with a viral infection (nCMV = 55/195; nEBV = 42/195) and approximatively half (104/195) of our total LT cohort presented graft rejection, the majority of which was treated with glucocorticoids (91/104) whereas five patients (2.6%) required a second transplantation. About our renal transplantation (RT) cohort, we collected data about 20 pediatric patients (**Figure 3**). The median age of renal recipients was 12 years (9; 15), seven patients (35%) received a renal transplant from a living donor, all patients were treated with tacrolimus and glucocorticoids after the transplantation, and fourteen (70%) were still under both treatment at the end of data collection. For acute complications, fourteen (70%) presented infection: nine (45%) were diagnosed with a viral infection (nCMV = 4/20 and nEBV = 7/20) and seven (35%) presented bacterial infection. Seven (35%) patients presented a confirmed or borderline graft rejection for which they received shots or/and increased doses of glucocorticoids. Two patients (10%) were re-transplanted.

Early transient HG in pediatric LT patients is associated with glucocorticoid use, graft rejection and viral infection

Out of 195 LT pediatric patients, 25.1% (49/195) developed transient HG (**Figure 2** and **Table 3**) and for most of them (92%) HG appeared during the first two weeks after transplantation (**Figure 4** and **Supplementary Figure S1**). No overt diabetes was observed but a third (16/49) of our HG-positive LT cohort was treated with insulin.

In univariate analysis, the use of glucocorticoids (OR 2.64 95% CI, 1.23–5.71) and the presence of critical condition such as graft rejection (OR 3.18 95% CI, 1.56–6.48) and viral infection (OR 2.54 95% CI, 1.31–4.93), in particularly Cytomegalovirus (OR 2.79 95% CI, 1.41–5.53) were significantly associated with the onset of HG as shown in **Table 4**. After adjustment with multivariate logistic regression analysis (Wald Chi-Square tests), incidence of transient HG after LT was higher in children who received glucocorticoids (2.96, 95% CI, 1.32–6.61) and presented a viral infection (OR 2.20, 95% CI, 1.09–4.44) (**Table 4**).

Pediatric LT patients present HG in afternoon, IR and diabetes at one-month

As we observed with rDIABGRAFT that our LT cohort presented transient HG early after transplantation (i.e., 0–14 days), we performed dynamic testing close to transplantation (day 14 and day 30) in four LT children. **Table 5** presents the patients, treatments and characteristics of the pDIABGRAFT LT cohort.

All patients presented fasting glucose and c-peptide level in normal range (**Table 5**) whereas glucose sensor placed at day 14 post-LT for one month showed chronic HG occurring in postprandial afternoon period (**Figure 5**). Parallelly, all children received high doses of glucocorticoids for graft rejection and required insulin. Moreover, during the OGTT performed at one-month post-LT ($n = 3$), all presented IR (HOMA-IR > 1.7) while in two of them, glycemia peaked respectively at 212 and 250 mg/dl at 120′ (**Figure 6**). We thus observed in our LT cohort two patients with diabetes at one-month post-LT.

Chronic HG is associated with graft rejection and infection in pediatric RT patients

Out of our 20 pediatric patients with renal transplantation, 55% (11/20) presented HG (**Table 6**). Out of eleven patients with HG, four of them developed overt diabetes (20% of total cohort, 36% of HG cohort), still treated at the end of data collection with antidiabetic medication (oral antidiabetics in 2/4 and a combination of oral antidiabetics and insulin in 2/4). The remaining seven patients (35% of total cohort, 64% of HG cohort) presented HG without overt diabetes, during a median duration of seven days (6; 12) and four of them (57%) required insulin during a median duration of four days (2; 8).

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TABLE 1 Characteristic and treatment of pediatric liver transplant patients (rDIABGRAFT).

	LT total Cohort, *n* = 195	LT HG positive *n* = 49	LT HG negative *n* = 146
CHARACTERISTIC			
Gender, man, *n* (%)	98 (50,3)	24 (49.0)	74 (50.7)
Alive, *n* (%)	193 (99.0)	48 (98.0)	145 (99.3)
Age of liver transplant, months, median (p25; p75)	18.1 (10.1; 36.2)	11.9 (9.2; 22.4)	20.2 (10.5; 44,3)
Age ≤1 years (%)	72 (36.9)	25 (51.0)	47 (32.2)
Age ≤2 years (%)	122 (62.6)	38 (77.5)	84 (57.5)
Weight SDS, median (p25; p75)	−1.3 (−2.3; −0.4)	−1.5 (−2.7; −0.8)	−1.3 (−2.2; −0.3)
Height SDS, median (p25; p75)	−1.6 (−2.6; −0.6)	−1.8 (−2.6; −0.7)	−1.6 (−2.6; −0.6)
BMI SDS, median (p25; p75)	−0.9 (−1.7; +0.3)	−1.2 (−1.8; +0.1)	−0.8 (−1.7; +0.4)
TRANSPLANTATION AND TREATMENTS			
Treatment before liver transplantation			
Glucocorticoids, *n* (%)	21 (10.7)	6 (12.2)	15 (10.3)
Immunosuppressors, *n* (%)	8 (4.1)	2 (4.1)	6 (4.1)
Living donor for liver transplant, *n* (%)	179 (91.7)	44 (89.8)	135 (92.5)
Father	66 (33.8)	18 (36.7)	48 (32.9)
Mother	79 (40.5)	20 (40.8)	59 (40.4)
Aunt/Uncle	23 (11.8)	6 (12.2)	17 (11.6)
Siblings	2 (1.0)	–	2 (1.4)
Cousin	7 (3.6)	–	7 (4.8)
Grandparents	2 (1.0)	–	2 (1.4)
Immunosuppressive treatments post-transplant			
Tacrolimus, *n* (%)	195 (100.0)	49 (100.0)	146 (100.0)
Glucocorticoids, *n* (%)	126 (64.6)	39 (79.6)	87 (59.6)
Resumption of glucocorticoids	22 (11.3)	9 (18.4)	13 (8.9)
COMPLICATIONS			
Acute graft rejection or suspicion	104 (53.3)	36 (73.5)	68 (46.6)
Glucocorticoids doses elevation or treatment	91 (46.7)	33 (67.3)	58 (39.7)
Viral infection post-transplant, *n* (%)	86 (44.1)	30 (61.2)	56 (38.4)
CMV, *n* (%)	55 (28.2)	22 (44.9)	33 (22.6)
EBV, *n* (%)	42 (21.5)	10 (20.4)	32 (21.9)
Hepatitis C	4 (2.1)	1 (2.0)	3 (2.0)
Second liver transplantation	5 (2.6)	3 (6.1)	2 (1.4)

LT, Liver transplant; SDS, Standard deviation score; BMI, Body max index; PTLD, Post-Transplant Lymphoproliferative Disease; CMV, Cytomegalovirus; EBV, Epstein-Barr Virus.

No precise timing for developing HG was observed with our RT pediatric patients (**Supplementary Figure S2**), but a concomitance with the occurrence of critical events such as graft rejection and infection has been observed. Indeed, univariate analysis (Likelihood Ratio) was performed to evaluate the association between risk factors and HG, and our analysis showed that graft rejection (OR 14.0, 95% CI, 1.25–156.61) and infections post RT (OR 12.5 95% CI, 1.09–143.43) were significantly associated with a higher occurrence of HG (**Table 7**). All our patients with a re-transplantation or bi-organ transplantation (4/20; two second RT and two previous LT) presented chronic HG but logistic regression was not possible because no patient in the HG-free RT cohort required another transplant.

For our LT and RT pediatric patients, there was no difference between occurrence of HG and gender of patient, history of overweight/obesity, BMI (pre- and post-transplant) and the use of glucocorticoids before the transplantation, donor status (cadaveric or living), pathology requiring the transplant, weight gain or family history of diabetes.

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TABLE 2 Characteristic and treatment of pediatric renal transplant patients (rDIABGRAFT).

	RT ($n = 20$)	RT HG positive ($n = 11$)	RT HG negative ($n = 9$)
CHARACTERISTIC			
Gender, man, *n* (%)	13 (65.0)	7 (63.6)	6 (66.7)
Alive, *n* (%)	19 (95.0)	10 (90.9)	9 (100.0)
Age of renal transplant, year, median (p25; p75)	12.3 (9.3; 15.6)	11.5 (10.4; 14.3)	13.1 (5.1; 16.5)
[0–8], *n* (%)	5 (25.0)	2 (18.2)	3 (33.3)
[9–18], *n* (%)	15 (75.0)	9 (81.8)	6 (66.7)
Overweight/Obesity before transplant, *n* (%)	4 (20.0)	3 (27.3)	1 (11.1)
Weight, SDS, median (p25; p75)	−0.9 (−2.0; −0.1)	−0.6 (−2.2; +0.0)	−1.1 (−1.9; −0.3)
Height, SDS, median (p25; p75)	−1.3 (−2.2; −0.6)	−2.1 (−2.5; −1.5)	−0.6 (−1.2; −0.4)
BMI, SDS, median (p25; p75)	−0.1 (−1.4; +0.7)	+0.3 (−1.0; +1.1)	−0.3 (−1.6; +0.2)
PERSONAL HISTORY			
Other transplants (liver), *n* (%)	3 (15.0)	3 (27.3)	–
LT before RT, *n* (%)	2 (10.0)	2 (18.2)	–
LT the same day as RT, *n* (%)	3 (15.0)	3 (27.3)	–
Treatment before renal transplantation			
Glucocorticoids, *n* (%)	5 (25.0)	4 (36.4)	1 (11.1)
Immunosuppressors, *n* (%)	4 (20.0)	3 (27.3)	1 (11.1)
Dialysis treatment, *n* (%)	13 (65.0)	6 (54.5)	7 (77.8)
Hemodialysis, *n* (%)	10 (50.0)	6 (54.5)	4 (44.4)
Peritoneal dialysis, *n* (%)	9 (45.0)	5 (45.5)	4 (44.4)
TRANSPLANTATION AND TREATMENTS			
Living donor for renal transplant, *n* (%)	7 (35.0)	3 (27.3)	4 (44.4)
Father, *n* (%)	2 (10.0)	–	2 (22.2)
Mother, *n* (%)	3 (15.0)	1 (9.1)	2 (22.2)
Friend, *n* (%)	1 (5.0)	1 (9.1)	–
Sister, *n* (%)	1 (5.0)	1 (9.1)	–
Immunosuppressive treatments post-transplant			
Tacrolimus, *n* (%)	20 (100.0)	11 (100.0)	9 (100.0)
Glucocorticoids, *n* (%)	20 (100.0)	11 (100.0)	9 (100.0)
Patient treated until the end of the study, *n* (%)	14 (70.0)	7 (63.6)	7 (77.8)
COMPLICATIONS			
Graft rejection or suspicion	7 (35.0)	6 (54.5)	1 (11.1)
Graft rejection	3 (15.0)	3 (27.3)	–
Suspicion with immunosuppressors treatment	4 (20.0)	3 (27.3)	1 (11.1)
Glucocorticoids doses elevation or treatment	7 (35.0)	6 (54.5)	1 (11.1)
Tacrolimus doses elevated	1 (5.0)	1 (9.1)	–
Infection post-transplant, *n* (%)	14 (70.0)	10 (90.9)	4 (44.4)
Bacterial infection, *n* (%)	7 (35.0)	5 (45.5)	2 (22.2)
Virus infections, *n* (%)	9 (45.0)	6 (54.5)	3 (33.3)
CMV, *n* (%)	4 (20.0)	3 (27.2)	1 (11.1)
EBV, *n* (%)	7 (35.0)	5 (45.5)	2 (22.2)

(*continued*)

TABLE 2 Continued

	RT ($n = 20$)	RT HG positive ($n = 11$)	RT HG negative ($n = 9$)
Weigh gain post-transplant, *n* (%)	9 (45.0)	5 (45.5)	4 (44.4)
Second renal transplant	2 (10.0)	2 (18.2)	–

RT, Renal transplant; SDS, Standard deviation score; BMI, Body max index; LT, Liver transplant; PTLD, Post-Transplant Lymphoproliferative Disease; EBV, Epstein-Barr Virus; CMV, Cytomegalovirus.

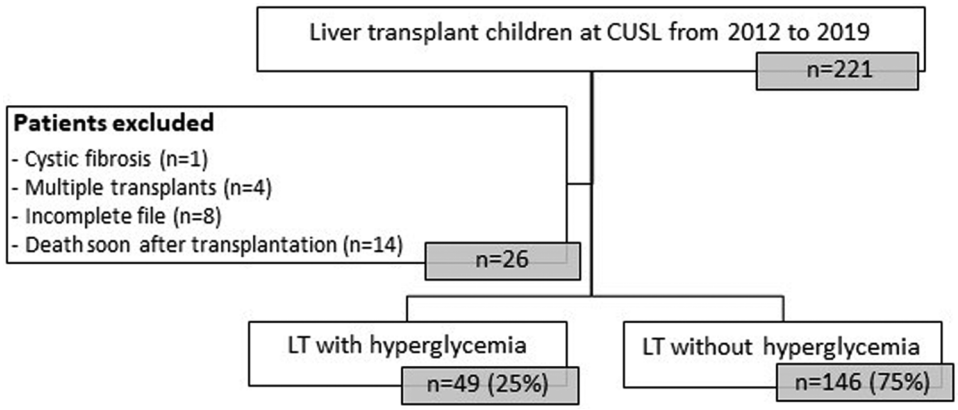

FIGURE 2
Flowchart of rDIABGRAFT pediatric LT cohort. Out of 195 pediatric patients who benefited from a liver transplant in the Cliniques universitaires Saint Luc (CUSL) between April 2012 and April 2019, 25% (49/195) patients presented hyperglycemia. n: number of patients, LT: liver transplant.

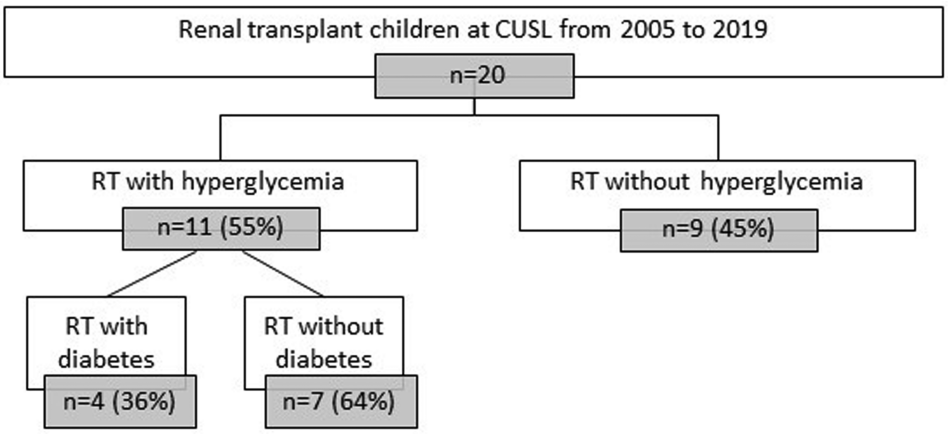

FIGURE 3
Flowchart of rDIABGRAFT pediatric RT cohort. Out of 20 pediatric patients who benefited from a renal transplant in the Cliniques universitaires Saint Luc (CUSL) between April 2004 and December 2019, eleven (55%) presented hyperglycemia. Out of them, four (20%) developed overt diabetes and the remaining seven (35%) patients presented HG without overt diabetes. n: number of patients, RT: renal transplant.

Insulin resistance and diabetes occur early after pediatric renal transplantation

For our RT cohort, as we did not observe a specific moment of HG occurrence but more a concomitance with the presence of critical events, we analyzed the evolution of glucose over time (at one-, three-, six- and nine-months post RT). Then, we followed four RT pediatric patients (**Table 8**). One RT patient (TR1) disagreed to use glucose sensor at one month post RT.

Our analyses showed that all our RT pediatric patients presented normal fasting glycemia and HbA_{1C} levels from all the post-transplant follow-up period (up to 9 months) (**Table 8**). Moreover, glucose sensor (placed after 2 weeks post-RT) data showed HG in the afternoon as illustrated in **Figure 7**, with data regrouped on 24 h. OGTT performed at one-month post RT showed that two patients presented IGT with glycemia above 140 mg/dl at the end of the test, suggesting prediabetes, and one presented glycemia above 200 mg/dl (TR1: 221 mg/dl) at 120′,

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TABLE 3 Incidence of hyperglycemia in LT cohort (rDIABGRAFT).

	Pediatric liver transplant patients, $n = 195$
Hyperglycemia the day of the transplantation, *n* (%)	115 (59.0)
Hyperglycemia, more than two days with glycemia >200 mg/dl, *n* (%)	49 (25.1)
Days in hyperglycemia, median (P25; P75)	4 (3; 8)
Transient insulin treatment, *n* (%)	16 (8.2)
Transient insulin treatment duration, days, median (P25; P75)	8 (2; 16)
Number of blood glucose levels recorded, median (P25; P75)	48 (37; 66)
Duration of blood glucose monitoring, days, median (P25; P75)	675 (325; 1355)

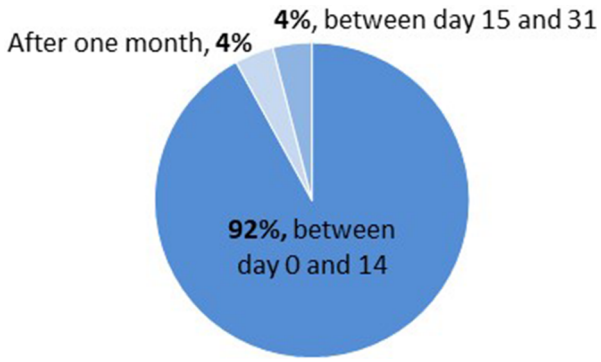

FIGURE 4
Onset of hyperglycemia in pediatric LT cohort (rDIABGRAFT). For most of our pediatric liver transplant patients (92%), hyperglycemia occurred during the first two weeks after transplantation.

corresponding to overt diabetes (**Figure 8**). Dosage of pro-insulin and C-peptide showed that no patient presented a β-cell dysfunction whereas HOMA-IR showed severe IR (HOMA-IR >2.4) for all our RT patients (**Table 8**). At three-, six- and nine-months post-RT, all patients had normalized their glycemia at the end of the test (<140 mg/dl) (**Figure 8**), but they had continued to present moderate and severe IR except one at nine-month (**Table 8**).

Discussion

Our study describes the incidence and risk factors of hyperglycemia and analyzes glycemic profile in a cohort of liver and/or renal pediatric transplant patients. To our knowledge, our study is the only one that combines retro- and prospective parts which include glucose screening test rarely performed in pediatric patients who benefited from a liver or kidney transplant.

In our LT cohort, 25% (49/195) of pediatric patients presented early HG with no overt diabetes afterwards. For our pediatric RT cohort, 55% (11/20) of pediatric patients presented HG. For 35% of them (7/20), HG were transient and the remaining 20% (4/20) developed overt diabetes, currently treated with antidiabetic treatment (insulin and/or oral antidiabetics). Studies performed before 2014 were based on variable definitions of diabetes, but the introduction of recommendation in 2014 by the American Journal of Transplantation and guidelines in 2017 by ADA for "post-transplantation diabetes" induced the observation of rates of diabetes closer to our results (23, 24). Indeed, the recent study by Calani et al. reported 13% (17/127) of diabetes in RT pediatric patients (25).

In a prevention perspective, we sought to identify relevant risk factors of HG onset after a pediatric LT and RT. The first finding of our DIABGRAFT study was, as expected, the association between HG and the use of glucocorticoids for LT cohort. The negative effect of glucocorticoids on glucose metabolism is well documented in transplant children (26–28). Associated to the use of glucocorticoids, graft rejection was also correlated to the risk of HG in our univariate analysis for our both cohorts. According to the immunosuppressive treatment protocol, high doses of glucocorticoids are introduced for LT and increased for RT when a patient presents ACR (14). The other risk factor of HG observed for our both cohorts was the presence of infections and can be explained by two hypotheses. Various studies described that following a metabolic stress such as infection in this case, various hormones such as cortisol, glucagon, catecholamines and pro-inflammatory cytokines are secreted and may provoke HG onset (29–32). In parallel, HG concomitant to an infection also may be related to an intensive prior immunosuppressive treatment (33). Our study suggests that these three risk factors of HG indicated a specific moment when a LT and RT patient has a higher risk of developing HG, when glucocorticoids were required and when a graft rejection and an infection occur.

We did not observe risk factors as older age at the time of the transplant and history of overweight/obesity usually seen in adults

TABLE 4 Uni and multivariate analysis for LT cohort (rDIABGRAFT).

	LT HG positive	LT HG negative	Univariate analysis		Multivariate analysis	
	$n = 49$	$n = 146$	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)
Glucocorticoids post-transplantation	39 (79.6)	87 (59.6)	0.01	2.64 (1.23–5.71)	0.01	2.96 (1.32–6.61)
Graft rejection	36 (73.5)	68 (46.6)	0.001	3.18 (1.56–6.48)	–	–
Virus infection	30 (61.2)	56 (38.4)	0.01	2.54 (1.31–4.93)	0.03	2.20 (1.09–4.44)
CMV	22 (44.9)	33 (22.6)	0.003	2.79 (1.41–5.53)	–	–

LT HG, Liver transplant hyperglycemia; OR, Odds ration; CI, Confidence interval; CMV, Cytomegalovirus.

TABLE 5 Pathology and glycemic profile data of LT cohort (pDIABGRAFT).

		TH1	TH2	TH3	TH4
Medical record	Genrer	Woman	Woman	Woman	Woman
	Country origin	Algeria	Algeria	Romania	Russia
	Pathology	Alagille Syndrome	Biliary cirrhosis, Progressive familial intrahepatic cholestasis	Budd-Chiari syndrome	Alagille Syndrome
	Donor	Living	Living	Living	Living
	Age of transplant, years	11.5	8.0	5.3	5.2
	Graft rejection	Yes	Yes	Yes	Yes
	Glucocorticoids	Yes	Yes	Yes	Yes
Secretion	[C-peptide], pmol/L	665.9	752.9	3142.0	1285
	[Pro-insulin], pmol/L	10.1	5.2	18.8	19.4
OGTT	Fasting glycemia, 0′	107	50	70	98
	Glycemia at 120′	122	212	–	250
	HOMA-IR	6.3	2.6	–	2.3

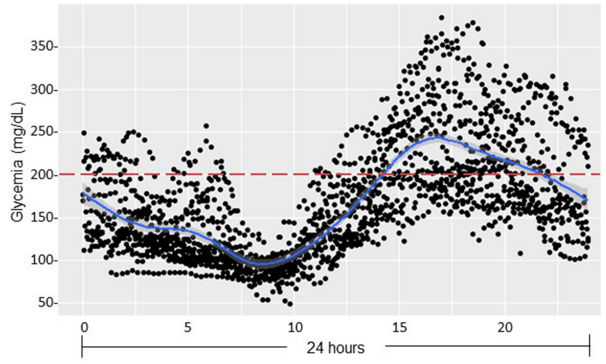

FIGURE 5
Continuous glucose monitoring after pediatric liver transplantation (pDIABGRAFT). Data of the continuous glucose monitor placed at day 14 post-LT for one month were regrouped on 24 h and showed chronic hyperglycemia occurring in postprandial afternoon period.

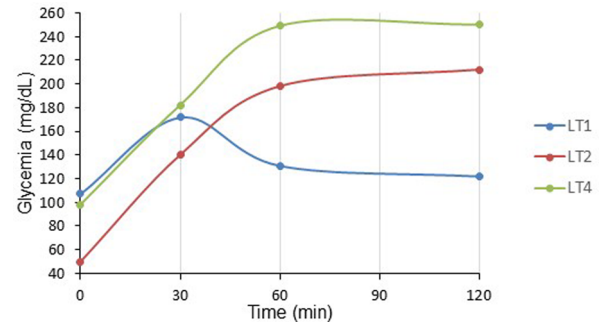

FIGURE 6
OGTT at one-month post liver transplant children (pDIABGRAFT). The oral glucose tolerance test (OGTT) performed at one-month post LT showed that fasting glucose were in the normal range whereas for two of them glycemia peaked respectively at 212 (LT2) and 250 (LT4) mg/dl at the end of the test (120'), corresponding to overt diabetes.

TABLE 6 Incidence of hyperglycemia and overt diabetes in RT cohort (rDIABGRAFT).

	Pediatric renal transplant patients, $n = 20$
Glycemia >200 mg/dl the day of the transplantation, *n* (%)	10 (50.0)
Hyperglycemia: more than two days with glycemia >200 mg/dl	11 (50.0)
Transient hyperglycemia, *n* (%)	7 (35.0)
Days in hyperglycemia, median (P25; P75)	7 (6; 12)
Overt diabetes, *n* (%)	4 (20.0)
Insulin and antidiabetic treatments, *n* (%)	7 (35.0)
Insulin treatment for transient HG, *n* (%)	4 (20.0)
Duration of transient insulin treatment, day, median (P25; P75)	4 (2; 8)
Current antidiabetics treatment, *n* (%)	4 (20.0)
Current insulin treatment, *n* (%)	2 (10.0)
Total dose of insulin, Unit/kg/j, median (P25; P75)	0.30 (0.30; 0.32)
Number of blood glucose levels recorded, median (P25; P75)	136.5 (110; 229)
Years of blood glucose monitoring, median (P25; P75)	8.8 (6.3; 10.9)

(5, 7, 12, 34), potentially because our cohorts were principally composed by liver transplant patients under the age of two years and underweighted. Also, overweight/obese patients waiting for a kidney transplant were on a specific diet to lose weight before transplantation.

In addition, the high proportion of transient HG and overt diabetes observed in our RT cohort compared to our LT cohort can be explained by several hypotheses. RT patients were directly

TABLE 7 Univariate analysis for RT cohort (rDIABGRAFT).

	RT HG positive	RT HG negative	Univariate analysis	
	n = 11	*n* = 9	*p*-value	OR (95% CI)
Graft rejection	7 (63.6)	1 (11.1)	0.03	14.0 (1.25–156.61)
Infection post-transplantation	10 (90.9)	4 (44.4)	0.02	12.5 (1.09–143.43)

RT HG, Renal transplant hyperglycemia; OR, Odds ration; CI, Confidence interval.

administrated glucocorticoids for at least six months after transplantation, although LT patients received this treatment only in some specific cases, as graft rejection (15, 35, 36). In addition, our RT patients were pubertal (12 years, Tanner stage ≥2), whereas the majority of LT cohort was under the age of two (Tanner stage = 1) and in agreement with our previous study, with pediatric patients treated with glucocorticoids for a leukemia, Tanner stage ≥2 is associated with a higher risk of developing HG (37).

The other main finding of our DIABGRAFT study was that pediatric LT and RT patients developed early IGT and IR after the transplant. In our study, the normal C-peptide levels secretion showed that there was no effect of glucocorticoids or tacrolimus on β cell function, but the globally abnormal values of OGTTs showed that all our transplant patients developed IGT by the installation of IR already at one-month post-transplant, until 9-month for our RT cohort. In addition, our glucose sensor and OGTT data confirmed

TABLE 8 Glycemic profile data of RT cohort (pDIABGRAFT).

			TR1	TR2	TR3	TR4
Medical record	Genrer		Man	Man	Man	Woman
	Country origin		Belgium	Romania	Romania	Belgium
	Donor		Cadaveric	Cadaveric	Cadaveric	Cadaveric
	Age of transplant, year		5.1	6.8	16.8	14.9
	Patients	14 days	1-month	3-month	6-month	9-month
C-peptide (pmol/L)	TR1	899	608	317	392	368
	TR2	748	798	344	1887	444
	TR3	1891	1918	1075	1101	848
	TR4	1122	3832	725	541	571
Pro-insulin (pmol/L)	TR1	11.7	4.5	3.9	2.5	2.1
	TR2	9.4	13.0	4.6	27.8	5.2
	TR3	19.0	19.5	9.5	12.2	7.2
	TR4	11.8	48.0	4.3	4.0	4.2
HbA1c	TR1		5.5	4.9	6.0	5.3
	TR2		-	5.8	5.6	5.4
	TR3		5.2	5.1	5.7	5.9
	TR4		5.1	5.0	4.9	5.4
Fasting glucose (OGTT 0′)	TR1		84	91	–	82
	TR2		88	87	84	93
	TR3		98	100	96	84
	TR4		76	80	88	85
OGTT 120′	TR1		221	120	–	81
	TR2		141	126	105	122
	TR3		120	130	113	109
	TR4		172	97	103	125
HOMA INDEX	TR1		1.7	2.1	–	1.2
	TR2		2.8	3.0	2.7	2.8
	TR3		3.7	3.6	5.1	1.7
	TR4		3.0	3.5	3.6	4.3

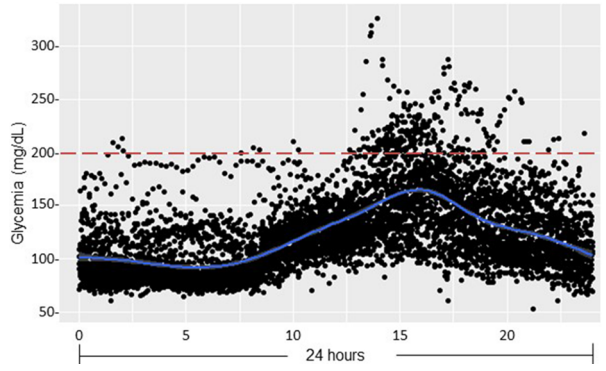

FIGURE 7
Glucose sensor data after pediatric renal transplantation (pDIABGRAFT). Data of the glucose sensor placed at day 14 post-RT for one month were regrouped on 24 h and showed hyperglycemia in postprandial afternoon period.

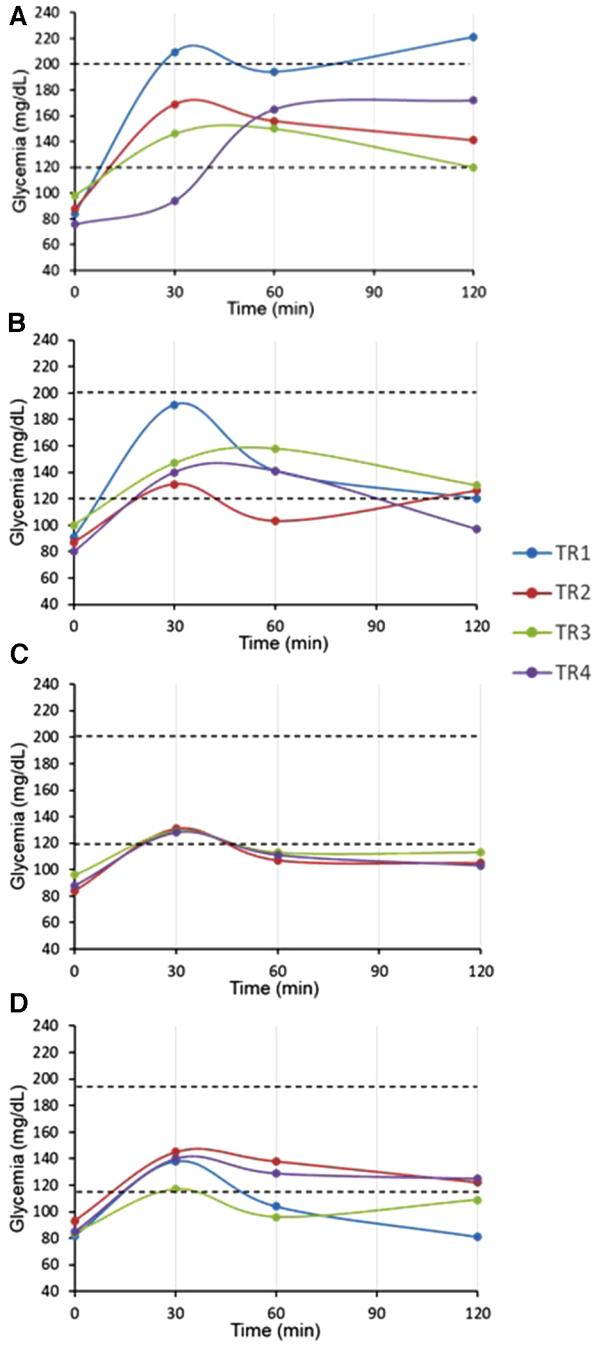

FIGURE 8
OGTT at one, three, six and nine-month post-renal transplant children (pDIABGRAFT). The oral glucose tolerance test (OGTT) performed at (A) one-month post RT showed that fasting glucose were in the normal range whereas two patients (TR2, TR4) presented impaired glucose tolerance (>140 mg/dl) at the end of the test, suggesting prediabetes and one presented glycemia above 200 mg/dl (TR1) at 120', corresponding to overt diabetes. At (B) three-, (C) six- and (D) nine-months post-RT, all patients had normalized their glycemia at the end of the test.

that non-fasting glucose monitoring (i.e., random) should be widely recommended for early detection of glucose abnormalities and that fasting plasma glucose and HbA1C measurements lack power/sensibility to identify post-prandial hyperglycemia. Indeed, in our both cohorts, all pediatric transplant patients had fasting blood glucose and HbA_{1C} in the normal range whereas glucose sensor confirmed the presence of HG in post-prandial afternoon period and values of OGTT indicated the presence of prediabetes and the onset of diabetes. Our findings are similar to a recent study carried out on Egyptian pediatric kidney transplant recipients where OGTT was able to detect a high proportion of abnormalities in glucose metabolism (23.3%) (38). The increase of glycemia in post-prandial afternoon period is widely described and related to the use of glucocorticoids. Studies characterizing the circadian glycemic pattern by Burt et al. showed that the glucose peak after 8 h of the prednisolone administration corresponds to the action peak of prednisolone (39, 40).

Our study presented some limitations. First, the retrospective nature was a limitation although we excluded patients with an incomplete medical record. In addition, like for any surgical intervention, clinical parameters, including glycemia, are frequently recorded close to the surgery and less afterwards. Moreover, it may be expected that patients with a critical condition such as graft rejection and infection had benefited from a closer control of glycemia included in the global clinical parameters compared to patient without complication. Also, we highlighted with our prospective study that HG appeared in the post-prandial afternoon period whereas in our retrospective study, glycemia collected in patient medical record was often carried out in the fasting stage due to the tacrolimus dosing protocol. Thus, we obtained a potential underestimation of the occurrence of HG. Finally, since CUSL is an international center for pediatric liver transplantation, our patients and their parents were mostly foreigners and recruitment could be less effective even with the intervention of a translator. In parallel, since patients were returning home after surgery, the monitoring of glycemia by our center was performed every six months then annually.

In conclusion, diabetes is a major side effects in RT children (20%) and transient HG are frequent after a pediatric liver (25%) and renal (35%) transplant yet underestimated due to fasting glycemic measures and HbA_{1C}. The onset of HG systematically occurred in the post-prandial afternoon period and was associated to the use of glucocorticoids and with acute events as graft rejection and infection. HG was characterized by

IGT and IR early after transplantation, and only detected by OGTT. Our study suggests that random blood glucose monitoring should be reinforced in the afternoon period when children present critical complications such as graft rejection and infections.

Data availability statement

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving human participants were reviewed and approved by Cliniques Universitaires Saint-Luc and UCLouvain Hospital-Faculty Ethics Committee. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

PAL had the idea for the study, designed it and reviewed the manuscript. SW designed and performed the study and assays, collected and analyzed data, followed patients, decided and performed statistical methods and wrote and reviewed the manuscript. VM collected data and with AR performed statistical methods. PlHdB performed dynamic tests and blood sample. NR and NG recruited patients who had undergone renal transplantation. NR, NG, XS, IS, RR and ES were doctors of pediatric patients and reviewed the manuscript. All the authors have read and approved the final version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2023.1080905/full#supplementary-material.

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