RANDOX

EDUCATIONAL GUIDE

Diabetes Solutions

Differentiating Type 1 and Type 2 Diabetes Mellitus

Introduction

Diabetes Mellitus (DM) is a collection of chronic diseases which occur when the pancreatic production of insulin is inhibited, or the insulin produced is not effectively processed by the body. In 2019, 1.5 million deaths were directly attributed to DM, and it is estimated that around 422 million individuals are living with DM today[1]. Insulin is responsible for a variety of essential physiological processes related to glycolysis and protein synthesis.

The main forms of DM are Type 1 Diabetes Mellitus (T1DM) and Type 2 Diabetes Mellitus (T2DM) which result from different mechanisms and require different therapeutic strategies. The misdiagnosis of T1DM as T2DM will result in poor glycaemic control, frequent healthcare contact for increased treatment, inappropriate insulin regimes and a risk of life-threatening ketoacidosis.

In this guide, we will cover the pathogenesis and pathophysiology of both T1DM and T2DM, the challenges faced in their diagnosis, and new methods of risk stratification which aim to assist clinicians in providing a fast, accurate diagnosis.

The normal insulin signalling pathway, shown in figure 1, is responsible for the processing and transport of glucose in the body. Briefly, insulin binds to the insulin receptor and activates PI3K and, subsequently, serine-threonine kinase (AKT). AKT is responsible for the phosphorylation of glycogen synthase kinase 3-β (GSK-3β), inhibiting its activity and promoting the synthesis of glycogen leading to a reduction in blood glucose concentration. Failing to inhibit GSK-3β will result in hyperglycaemia and eventually T2DM[2].

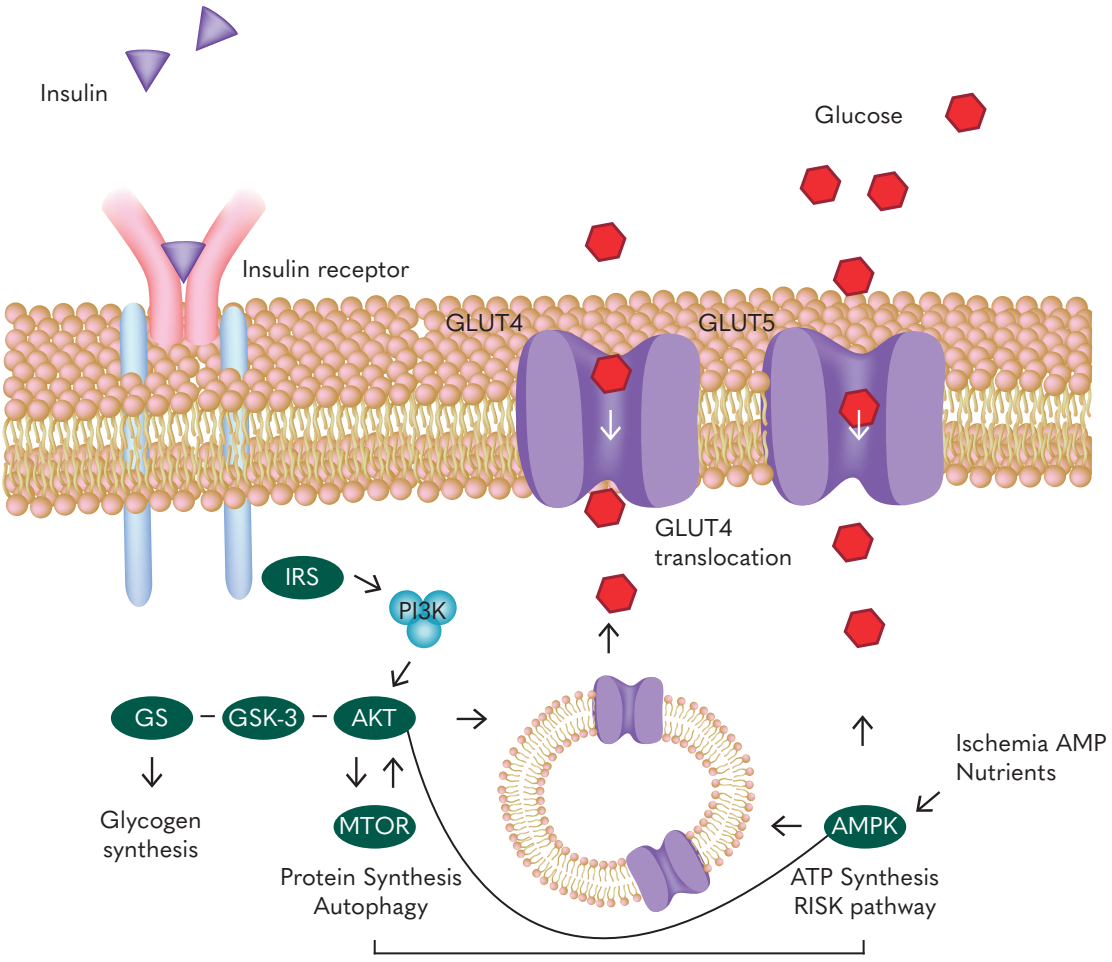

Figure 1. Illustration of the insulin signalling pathway[2]

Type 1 Diabetes Mellitus

T1DM is defined as a lack of insulin secretion by pancreatic β-cells and affects all age groups, however, most people are diagnosed at around 5 years old, in their teens or early adult years. T1DM occurs because of an autoimmune reaction to the proteins of the islet cells of the pancreas. There are 3 types of islet autoantibody related to T1DM described in table 1 below[3]:

Autoantibody	Description
Islet Cell Cytoplasmic Antibodies (ICCA)	ICCA are the primary antibodies seen in 90% of T1DM patients. Their presence is considered a highly accurate, predictive marker for future development of T1DM.
Islet Cell Surface Antibodies (ICSA)	Autoantibodies which act on the islet cell surface antigens are evident in up to 80% of T1DM patients and have been shown to be present in some T2DM patients.
Specific Antigen Targets of Islet Cells	Antibodies to glutamic acid decarboxylase (GAD) are evident in as many as 80% of newly diagnosed T1DM patients. These anti-GAD antibodies decline over time and their presence is a strong predictor of T1DM development in high-risk populations. Anti-insulin antibodies (IAAs) have been shown to be present in T1DM patients and in those at risk of developing T1DM. IAAs are detectable in around 40% of young children with T1DM and are measurable prior to insulin therapy.

Pathogenesis

The lack of insulin characteristic of T1DM is caused by the selective destruction of insulin-producing pancreatic β-cells. The following features of T1DM are used to characterise the disease[3]:

1. Presence of immuno-competent and accessory cells in infiltrated pancreatic islets.
2. Association of susceptibility to disease with the Class II (immune response) genes of the major histocompatibility complex (MHC).
3. Presence of islets cell specific autoantibodies.
4. Alterations of T cells mediated immunoregulation, particularly in CD4+ T cell compartment.
5. The involvement of monokines and TH1 cells producing interleukins in the disease process.
6. Response to immunotherapy.
7. Frequent occurrence of other organ specific auto-immune diseases in affected individuals or their family members.

The activation of $CD4^+$ T cells is an additional prerequisite for T1DM development. This activation is responsible for insulitis (inflammation of pancreatic islets) while $CD8^+$ T cells are thought to contribute to disease severity. Finally, IL-1 and TNFα are responsible for structural alterations of β-cells and the subsequent suppression of insulin secretion. Figure 2 below shows a diagram of the pathogenesis of T1DM.

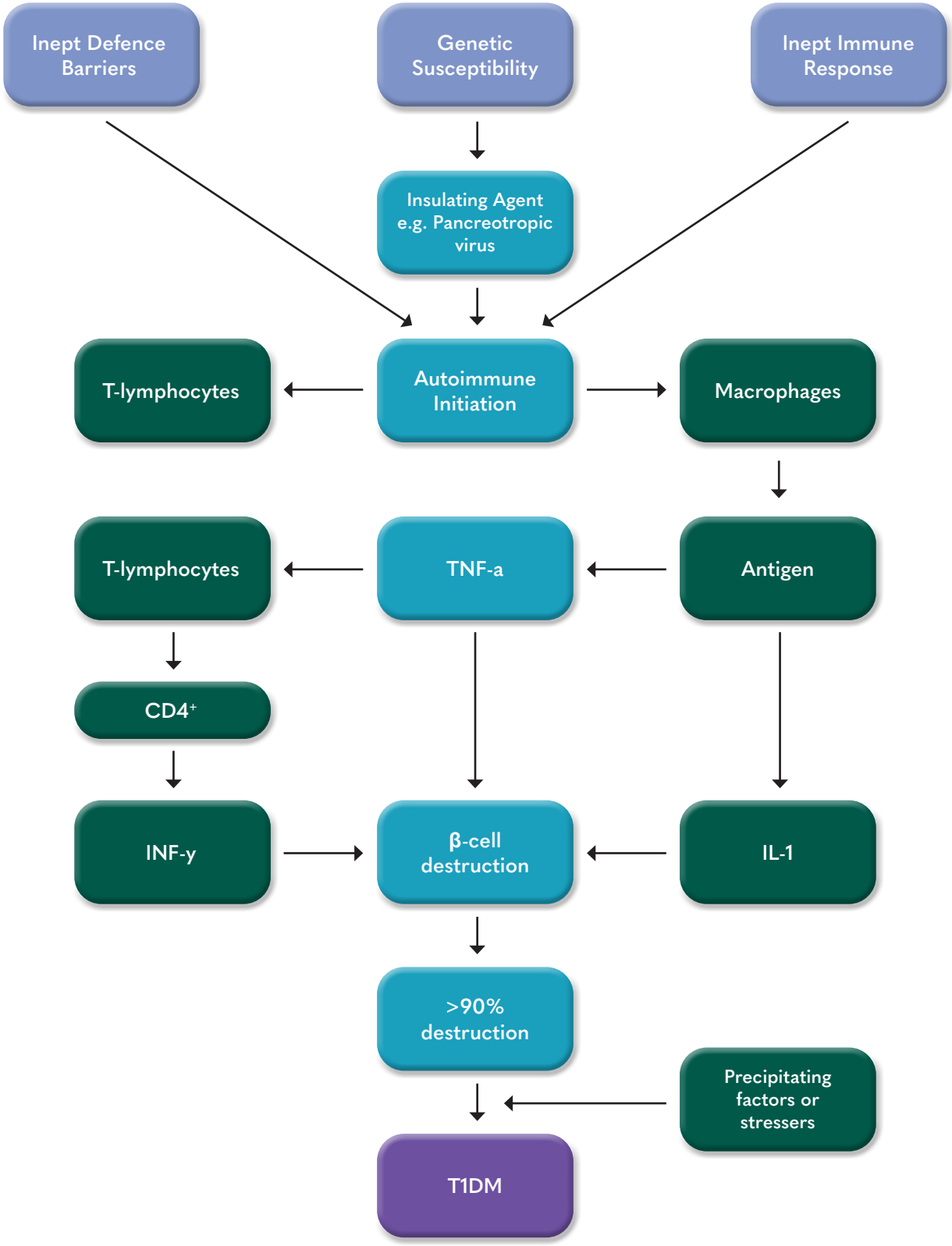

Figure 2. Pathogenesis of T1DM[3]

Pathophysiology

The dysfunction and apoptosis of pancreatic β-cells is facilitated by invading or endogenous macrophages and T cells which release chemokines, cytokines and pro-apoptotic signals in the islet microenvironment which attract the immune cells to the dysfunctional β-cells, resulting in a cessation of insulin production[4]. Insulin deficiency can have effects on the metabolism of glucose and protein synthesis.

T1DM causes an increase in hepatic glucose levels when gluconeogenesis converts glycogen to glucose. A lack of insulin means the subsequent hepatic uptake of glucose does not occur. Additionally, uptake of glucose by other tissues, such as skeletal muscle and adipose tissue is also inhibited as the insulin-mediated movement of glucose transporter proteins to the membranes of these tissues does not take place[3]. Glucosuria occurs when glucose absorption in the kidneys is supressed resulting in increased glucose concentration in the urine. Glucose is an osmotic diuretic, meaning water retention is supressed in concert with glucosuria, resulting in polydipsia. The caloric deficit resultant of glucosuria and tissue catabolism, causes polyphagia[3].

Insulin is also responsible for regulating the synthesis of many proteins. This regulation can be positive or negative but ultimately results in an increase in protein synthesis and a decrease in protein degradation. Therefore, when hypoinsulinemia occurs, decreasing insulin concentration in the blood, protein catabolism is increased leading to increased plasma amino acid concentration. Some of these amino acids serve as precursors for hepatic and renal gluconeogenesis, exacerbating hyperglycaemia[3].

The loss of pancreatic β-cells does not occur abruptly, in fact, insulin-containing islets can be detected for more than 1-year post-diagnosis. Data collected from new-onset T1DM patients who died from ketoacidosis show that β-cell concentration was around 50% of normal levels, suggesting that immune-mediated β-cells dysfunction precedes apoptosis[4].

Type 2 Diabetes Mellitus

T2DM is a heterogenous condition induced by a combination of genetic factors related to impaired insulin secretion, insulin resistance and environmental factors like obesity, over-eating, lack of exercise, stress, and aging[3].

Pathogenesis

In normal physiology, plasma glucose levels are regulated and remain stable, regardless of fluctuations in physiological demand. This regulation transpires through interactions between tissue sensitivity to insulin and insulin secretion. In T2DM, these regulatory mechanisms fail and result in impaired insulin secretion through pancreatic β-cell dysfunction and impaired insulin action / insulin resistance.

Mutations in the gene responsible for insulin result in the synthesis and secretion of insulin molecules with abnormal conformation, known as insulinopathies, which are inherited and can cause hyperinsulinemia.

Some reports propose that insulin resistance alone is a secondary event in T2DM as it is also measurable in non-diabetic, obese individuals. This suggests that hyperglycaemia is both a cause and a consequence of impaired islet cell function and insulin resistance[3].

Obesity is considered to be a causal factor in the development of T2DM, not simply a risk factor. The process from obesity to T2DM has been identified as a result of a series of pathological events:

1. Augmentation of the adipose tissue mass, leading to increase lipid oxidation
2. Insulin resistance noted early in obesity, as revealed by euglycemic clamp, as resistance to insulin mediated glucose storage and oxidation, blocking the function of the glycogen cycle
3. Despite maintained insulin secretion, unused glycogen prevents further glucose storage leading to T2DM
4. Complete β-cell exhaustion

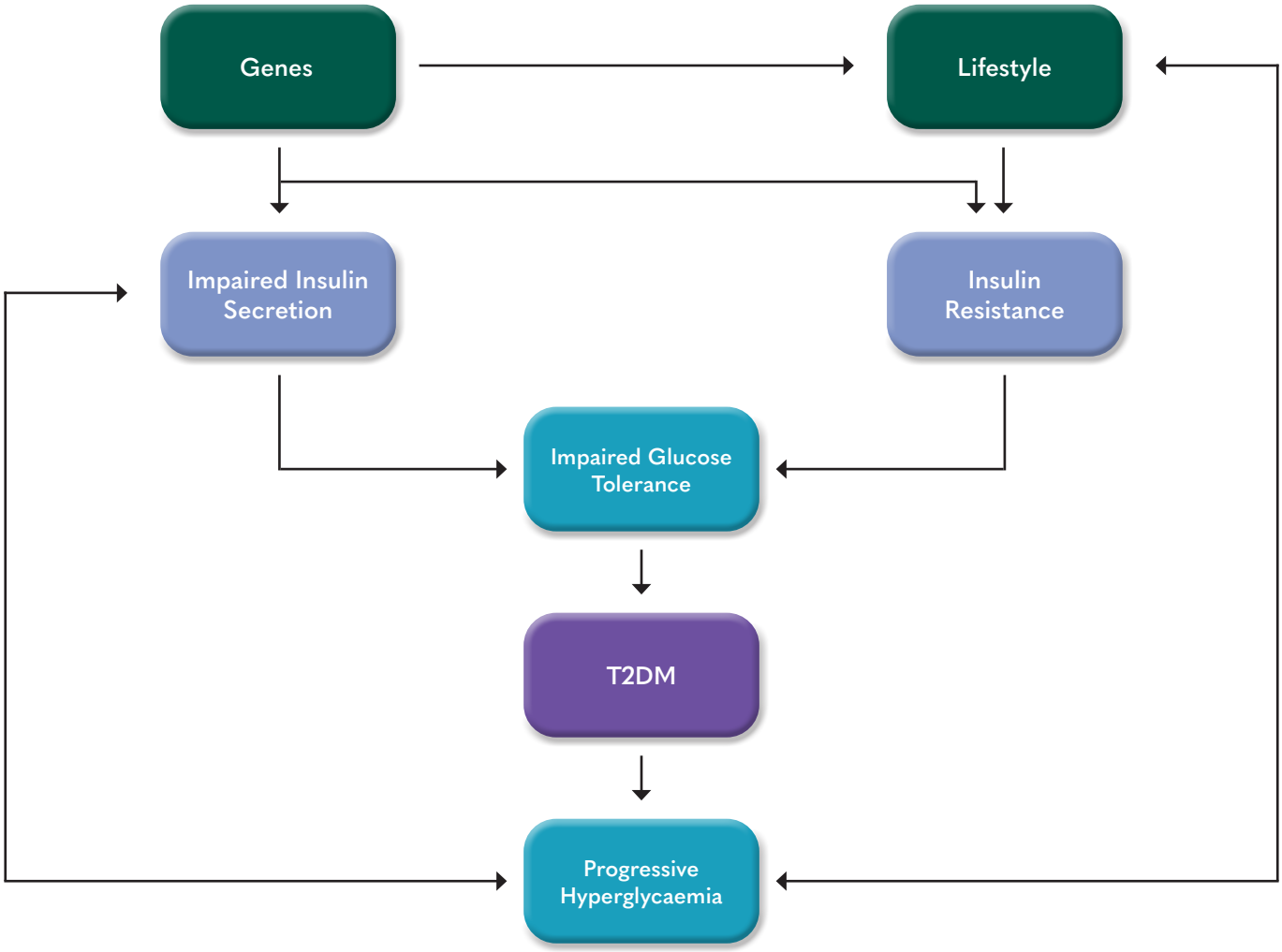

Figure 3. Pathogenesis of T2DM[3]

Pathophysiology

Unlike people who suffer from T1DM, T2DM patients can preserve circulating insulin levels. T2DM patients can be categorised as follows:

1. Those with glucose tolerance
2. Chemical diabetes (called impaired glucose tolerance)
3. Diabetes with minimal fasting hyperglycaemia (fasting plasma glucose less than 104mg/dl)
4. DM in association with overt fasting hyperglycaemia (fasting plasma glucose greater than 140mg/d)

Individuals with impaired glucose tolerance have hyperglycaemia despite preserving high levels of plasma insulin. These levels of insulin decline from impaired glucose tolerance to DM. Insulin resistance is considered the primary cause of T2DM, however, some suggest that it is the insulin deficiency which is the causal factor, stating that insulin resistance alone is not sufficient to cause T2DM[3]. Figure 3 shows the pathophysiological processes of T2DM.

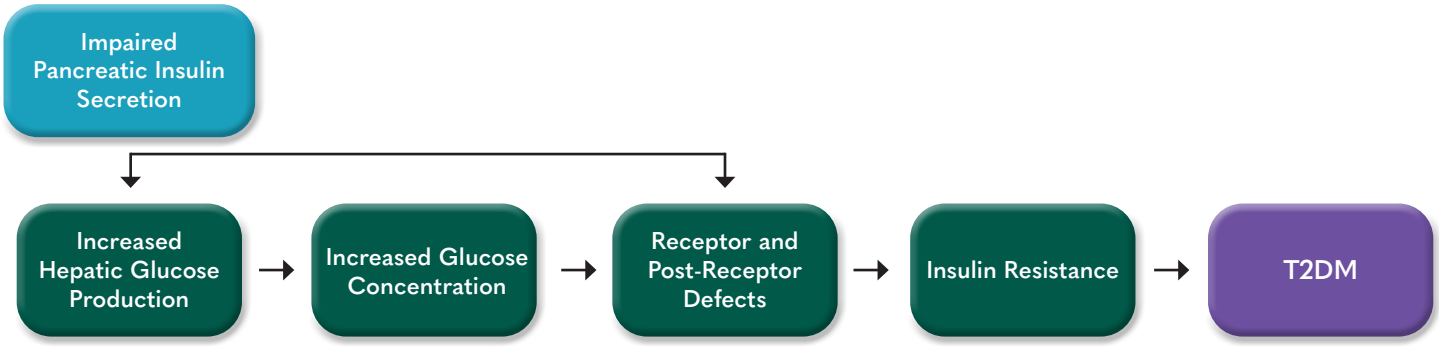

Figure 4. Pathophysiology of T2DM[3]

Misdiagnosis of Diabetes Mellitus

Symptoms of T1DM and T2DM are similar, the main difference is when these symptoms manifest. T1DM patients will experience symptoms much earlier that those who suffer from T2DM. Common symptoms of DM are[5]:

- Frequent urination, particularly throughout the night.
- Polydipsia (excessive thirst)
- Polyphagia (excessive hunger)
- Lethargy
- Sudden weight loss
- Genital itching or thrush
- Blurred vision

The misdiagnosis of these diseases is common, and the rate of misdiagnosis is increasing, largely due to the global increase in obesity, particularly in young adults. Obesity is usually associated with T2DM, but an increased prevalence in T1DM is evident. Furthermore, the average number of people diagnosed with T2DM is decreasing, with many more young people suffering from this condition classically attributed to those over 40 years old[5]. T2DM can be easier to miss due to its slower development, particularly in the early stages of the condition.

Up to 15% of young adults presenting with symptoms associated with DM are incorrectly diagnosed and treated. The misdiagnosis of T2DM as T1DM results in unnecessary initial insulin therapy, higher drug and monitoring costs and often, an increase in number and severity of symptoms. Conversely, the incorrect classification of T1DM as T2DM causes poor glycaemic control, frequent visits to a healthcare service for treatment, inappropriate insulin regimes and a risk of Diabetic Ketoacidosis[6].

Endogenous insulin levels can successfully be quantified through the measurement of serum or urine C-peptide. This method can accurately distinguish between T1DM and T2DM but only after the honeymoon period has passed, eliminating its diagnostic utility. The honeymoon period is defined as the phase immediately after diagnosis in which the patient seems to have an improvement in their condition, however, this is a temporary stage[5].

Diabetic Ketoacidosis (DKA)

DKA is a potentially life-threatening condition caused by an accumulation of ketones in the body due to insulin deficiency, which is common in patients with T1DM, however, an increasing number of cases have been reported in patients with T2DM[7]. DKA is likely to occur when proper diabetic therapy is not followed. Although most of the patients have a history of diabetes, over 25% of those admitted with DKA were considered to have new onset diabetes[8]. Diagnosis of DKA consists of a high anion gap metabolic acidosis, ketone bodies present in serum and/or urine, and high blood glucose concentration (<250mg/dl)[8,9].

Symptoms of DKA

Patients with DKA may present with the following symptoms:

- Polyuria (excessive urination) and polydipsia (thirst)
- Weight loss
- Fatigue
- Dyspnoea (shortness of breath)
- Vomiting
- Fever
- Abdominal pain
- Polyphagia (excess hunger)
- Fruity smelling breath caused by acetone accumulation

Randox Type 1 Diabetes Mellitus Genetic Risk Array

T1DM is largely genetically determined and is associated with over 50 distinct genetic signals. Many of these signals are single nucleotide polymorphisms (SNPs)[4]. Genetic predisposition to T1DM is associated with the human leukocyte antigen (HLA) genes. Genotyping SNPs in the HLA region is a more accurate method of T1DM risk stratification than classic HLA typing. Unlike many biomarkers, genetic markers do not change throughout one's lifetime, providing a robust method for diagnosis and risk-stratification of various diseases. Commonly, genetic data is used to develop genetic risk scores (GRS) which can index an individual's probability of developing the disease in question.

Using this principle, together with our patented Biochip array technology, Randox have developed a T1DM GRS array. Using a combination of 10 SNPs from the HLA region and the non-HLA region commonly detected in T1DM patients, and a selection of other risk factors and biomarkers, this molecular array can accurately discriminate between T1DM and T2DM.

Validation studies[6] were carried out on this array to determine its discriminatory power for T1DM and T2DM. A T1DM risk score, based on the 10 SNPs was found to be highly discriminatory and displayed an area under curve (AUC) of 0.88. A T2DM GRS was found to be less discriminatory with an AUC of 0.64. A combination of both GRS only resulted in a slight increase in discriminatory power (AUC = 0.89). From this data, the investigators determined that a T1DM GRS of >0.280 was indicative of T1DM and a GRS of <0.234 was indicative of T2DM[6]. This study also showed that the same GRS was highly discriminatory for determining the progression of severe insulin deficiency with an AUC of 0.87. Finally, the authors display the impressive discriminatory power of the GRS when used in addition to other common risk factors (autoantibody islets, BMI and age at diagnosis). This enhanced GRS displayed an AUC of 0.96 (95% CI 0.94-0.99), showing an average improvement of 8.9%[6].

A more recent study compared this GRS approach with the gold standard in genotyping, Sanger Sequencing. The Randox T1DM GRS array displayed an impressive 99.7% concordance with Sanger Sequencing[10]. Out of 2980 samples, the Randox array correctly identified 2979. It was later determined that this single inaccuracy was the result of allelic dropout, a common phenomenon in PCR-based target sequencing[10].

Diabetes Reagents

In addition to our T1DM GRS Array, Randox provides a range of reagent assays for the diagnosis and monitoring of diabetes and the complications associated with this disease.

Diagnosis and Monitoring

- **Microalbumin** is an independent predictor of progressive renal disease and is an early biomarker of diabetic nephropathy, the most common complication of type 2 diabetes mellitus. (T2DM)

- **Glucose** is a major source of energy for most cells in the body and is obtained through carbohydrate-enriched foods. Insulin helps control blood glucose levels to ensure they do not get too high, and as such, high levels of glucose in the blood are an indicator of diabetes.

- **HbA1c** is used to identify the average amount of glucose in the blood over a 2-3-month period. It is a good indicator of diabetes, as well as enabling diabetic patients to understand how well their diabetes is being controlled.

- **Fructosamine** is used in the monitoring of diabetes and is particularly useful in reviewing the effectiveness of medication adjustments. This is because it enables average glucose levels to be obtained over a 2–3-week period. In addition, it is used to monitor the glucose levels of pregnant women suffering from gestational diabetes which allows for the monitoring of both, the mother, and baby's glucose levels. This is crucial in decreasing risks associated with gestational diabetes such as premature birth, immediate infant health problems, miscarriage, or stillbirth.

- **Creatinine** measurements are useful in the diagnosis and monitoring of diabetic nephropathy, the leading cause of kidney disease in patients commencing renal replacement therapy, affecting 40% of diabetics (type 1 and type 2)[6]. The RENAAL risk score for end-stage renal disease (ESRD) emphasizes the importance of the identification of elevated SCr, alongside other renal markers, in the prediction of end-stage renal disease (ESRD) development in patients with type 2 diabetes mellitus (T2DM) and nephropathy.

Complications Monitoring – Speciality Tests

- **Cystatin C** is a more sensitive indicator of renal dysfunction than routine creatinine due to the creatinine blind range. Also, the elevated creatinine levels found in stage 2 and halfway through stage 3 renal dysfunction cannot be detected. Therefore, patients can suffer from 50% of kidney dysfunction before elevated levels are detected. Using the Cystatin C test enables more accurate patient results and allows time for treatment to begin before it is too late.

- **Non-Esterified Fatty Acids (NEFA)** are linked to an increased risk of developing diabetes. The measurement of NEFA is important in cases where insulin deficiency results in the metabolism of fat. An increase in NEFA concentration has also been associated with adiposity (high level of body fat), malignant disease (progressive disease) and other metabolic syndromes such as high blood pressure and abdominal obesity. NEFA can be used to assess diabetic patients' risk of developing adiposity (high level of body fat), malignant disease (progressive disease) and other metabolic syndromes such as high blood pressure and abdominal obesity (NEFA test is important in cases where insulin deficiency results in the metabolism of fat).

- **D-3-Hydroxybutyrate** is used in the identification of diabetic ketoacidosis, a serious complication of diabetes which occurs when blood sugar levels are consistently high and insulin levels are severely low. Immediate diagnosis is vital as the condition can lead to coma or death if not treated immediately. Symptoms include nausea, vomiting and abdominal pain.

Benefits

High stability to ensure cost effectiveness for even small throughput labs

A range of methods, kits and ranges for enhanced suitability of all labs

A range of liquid and lyophilised formats for convenience

High quality for accurate results

Controls and calibrators available

Applications available for a wide range of clinical chemistry analysers

ACUSERA

Acusera Diabetes quality controls provide a true third party solution for key tests used in the diagnosis and monitoring of diabetes and haemoglobin variants. Designed for use on multiple platforms, an independent assessment performance is guaranteed. An extended reconstituted stability of four weeks for many controls will not only keep waste to a minimum but will help reduce costs. As with all Acusera controls, laboratories can expect to experience reduced time and costs without compromising on consistency or quality.

Conclusion

The correct classification of T1DM and T2DM is essential to ensure the correct therapeutic strategies are prescribed, and to reduce the progression of adverse symptoms and conditions, such as DKA. The misdiagnosis of these diseases is becoming increasingly common, driving the need for novel methods of risk stratification and classification.

The Randox T1DM GRS array is a multiplex array shown to accurately differentiate T1DM and T2DM to aid in risk stratification, diagnosis and treatment of these diseases.

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