La Lettre

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DIABETES MELLITUS:
the laboratory's contribution to diagnosis and follow-up

Editorial

Diabetes mellitus is a worldwide disease with a high prevalence that is constantly increasing: from an estimated 135 million adult diabetics in 1995 to probably more than 300 million in 2025.

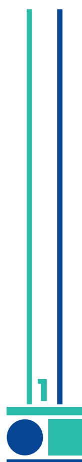
Diabetes mellitus is defined as metabolic disorders of various etiologies characterized by the presence of chronic hyperglycemia. The insulin deficiency that accompanies destruction of the β-cells of the islets of Langerhans in the pancreas, characterizes type 1 diabetes mellitus. Type 2 diabetes is related to disorders of insulin function.

Type 2 diabetes is more frequent: over 80% of diabetics, and its incidence is rising fastest. The rate of increase varies, depending on the country and is related to changing lifestyles and standards of living. In metropolitan France, the prevalence, which was 7.4% of the adult population, in the U.S.A, in 1995, is estimated to be 8.9% in 2025 (2.1% versus 2.6% in France). Prevalence is rising rapidly in children aged 10 to 19 years in some countries, and has increased from 2 - 4% of diabetics to over 15%.

Type 1 diabetes, which accounts for over 80% of diabetes in young subjects, is also increasing, without it being possible to identify any clear causes. The incidence in France, for children aged less than 15 years, is 8/10^5. The incidence will reach 13.7 in 2010. In Europe, prevalence is higher in the North (Finland: 42.9, Sweden: 24.9) than in the South (Spain: 12.3, Italy: 8.4), with Sardinia constituting an exception: 36.6.

Criteria

The diagnosis of diabetes is based on demonstrating chronic hyperglycemia. The cutoff for abnormally high plasma glucose and the manner of determining it in the oral glucose tolerance test (OGTT) remain subject to controversy. Two organizations have defined diabetes: the World Health Organization (WHO) and the American Diabetes Association (ADA).



For WHO, prior to 1999, diabetes was defined by a fasting plasma glucose level (G0) greater than 1.40 g/L (7.8 mmol/L) or plasma glucose at any time of day greater than 2 g/L (11.1 mmol/L), when the patient presented with typical clinical signs (polyuria, polydypsia, weight loss). In the absence of those clinical signs, running a standardized OGTT (75 g of glucose for adults) enabled diagnosis when G0 was greater than 1.40 g/L and plasma glucose at 2 hours (G2h) greater than 2 g/L. Glucose intolerance (IGT) was defined as a G2h between 1.4 and 2 g/L.

In 1997, the ADA formulated new diagnostic and screening criteria and a new classification. The main change consisted in lowering the G0 value defining diabetes from 1.40 to 1.26 g/L (7.0 mmol/L), determined twice in the absence of clinical signs. Patients whose G0 is greater than 1.10 g/L (6.11 mmol/L) and less than 1.26 g/L (hence non-diabetic) and whose G2h is unknown are included in a new category: impaired fasting glucose (IFG).

This classification enabled the ADA to abandon the OGTT, considered difficult to implement and little suitable for population studies and for which the reproducibility at G2h was inferior to that at G0.

In 1999, WHO reviewed its diagnostic criteria and added to those of the ADA by taking into account the OGTT results.

New diagnostic criteria for diabete mellitus (*ADA 1997/WHO 1999*)

Clinical symptoms of diabetes mellitus (polyuria, polydypsia, unexplained weight loss) associated with:

Plasma glucose ≥ 2 g/L at any time of day (without considering the time since the last meal)
or
Fasting plasma glucose ≥ 1.26 g/L (7.00 mmol/L)
(fasting: no calorie intake for at least 8 hours)
or
Plasma glucose ≥ 2 g/L (11.1 mmol/L) hour 2 post-OGTT
(under the WHO conditions: 75 g of glucose dissolved in 250 cc water)

During an OGTT

Time	Normal values	Impaired Fasting glucose	Glucose intolerance	Diabetes mellitus	Units
T0	< 1,10 (< 6,1)	≥ 1,10 < 1,26 (≥ 6,1 < 7)		≥ 1,26 (≥ 7)	g/L mmol/L
T+120	< 1,40 (< 7,8)	?	≥ 1,40 < 2,0 (≥ 7,8 <11,1)	≥ 2,0 (≥ 11,1)	g/L mmol/L

Classification

Until 1980-1985, WHO distinguished between two main types of diabetes: insulin-dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM), which have since been abandoned in order to adopt a classification taking into account not the treatment but the etiology.

With the new terminology, type 1 diabetes mellitus is defined by destruction of the β-cells of the islets of Langerhans due to an autoimmune or unknown (idiopathic diabetes) mechanism. The diabetes related to destruction of β-cells of known origin is not considered type 1 diabetes mellitus.

Type 2 diabetes mellitus is defined by the presence of insulin resistance and possibly relative insulin deficiency associated in a variable manner. Gestational diabetes are diabetes discovered during pregnancy.

Type 1 diabetes mellitus
- defined by the destruction of the β-cells of the islets of Langerhans (ketoacidosis in the absence of treatment)
- autoimmune type 1 (90%)
- idiopathic type 1
- excluded from type 1: diabetes resulting from β-cell destruction of known etiology (cystic fibrosis, etc.)

Gestational diabetes
- defined as diabetes discovered during pregnancy and which disappears sponteanously after its end..

Type 2 diabetes mellitus
- component of the insulin-resistance syndrome
- associated, to a variable degree, with relative insulin deficiency
- insulin-resistance syndrome (syndrome X or metabolic syndrome):
  . carbohydrate disorder (IGT or diabetes type 2)
  . male-pattern obesity
  . lipid anomalies (↑TG, ↑VLDL, ↓HDL, ↑LDL, etc.)
  . hypertension
  . fibrinolysis disorders (↓fibrinolysis, ↑PAI-1)
  . hyperuricemia, albuminuria
  . 'metabolic' liver disease (steatosis)

Other types of diabetes

- β-cell dysfunction of genetic origin (single gene):
. mutation of the HNF-4α gene (MODY* 1)
. mutation of the glucokinase gene (MODY 2)
. mutation of the HNF-1α gene (MODY 3)
. mutation of the IPF-1 gene (MODY 4)
. mutation of the HNF-1β gene (MODY 5)
. mutation of the NEUROD 1 gene (MODY 6)
. mutation of mitochondrial DNA (MODY 7)
. other

- Defective insulin activity of genetic origin:
. insulin resistance of type A
. leprechaunism
. Rabson-Medenhall syndrome
. lipoatrophic diabetes
. other

- Exocrine pancreatic diseases:
. pancreatitis
. pancreatectomy/injury
. cancer
. cystic fibrosis
. hemochromatosis
. pancreatic fibrosis and calculi
. other

- Endocrine diseases:
. acromegaly
. Cushing's syndrome
. glucagonoma
. pheochromocytoma
. hyperthyroidism
. somatostatinoma
. hyperaldosteronemia
. other

- Drug-induced diabetes:
. nicotinic acid
. pentamidine
. glucocorticoids
. thyroid hormones
. diazoxide
. β-adrenergic agonists
. thiazides
. phenytoin
. interferon
. other

- Diabetes of infectious origin:
. congenital rubella
. cytomegalovirus infection
. other

- Rare autoimmune forms:
. stiff-man syndrome
. anti-insulin receptor antibodies
. other

- Other syndromes of genetic origin sometinmes associated with diabetes:
. trisomy 21
. Klinefelter's syndrome
. Turner's syndrome
. Wolfram's syndrome
. Friedreich's ataxia
. Huntington's chorea
. Lawrence-Moon-Beidel syndrome
. myotonic dystrophie
. porphyria
. Prader-Willi syndrome
. other

Type 1 diabetes mellitus

In the vast majority of cases, type 1 diabetes mellitus has an autoimmune etiology. Insulitis occurs: lymphocyte (CD4, CD8) infiltration, mainly cytotoxic CD8 cells, inducing apoptosis of the β-cells of the islets of Langerhans. This is a long process progressing almost silently over years before onset of the disease.

The autoantibodies detected are markers of the autoimmune process but have no proven pathogenic role.

Genetic

While less than 15% of new cases of type 1 diabetes mellitus occur in diabetic families, the existence of a familial component has been clearly demonstrated: the risk of developing diabetes for a member of a diabetic's family is 6 to 7%, while it is only 0.4% in the overall population. This relatively low risk is explained by the multiple genes involved and by the role of environmental factors. Thus, the risk for the monozygous twin of an affected subject is only 50-70%, with long-term follow-up.

There are numerous candidate predisposing genes, among which the locus of the major histocompatibility complex is by far the most incriminated. Thus, in the siblings of a diabetic subject, the risk of developing the disease is 1.8% for different HLA, 5% for semi-identical HLA and 16% for identical HLA.

The predisposing haplotypes consist in HLA specificities of class II: these associations are, in fact, much stronger than those initially described with HLA antigens of class I: A1, B8, B18 and B15. Two main predisposing haplotypes have been described:
DRB1*03-DQA1*0501-DQB1*0201
DRB1*04-DQA1*0301-DQB1*0302
They are present in 80 to 95% of cases. The greatest risk is associated with the heterozygous configuration DR3/DR4, which is more synergistic than the homozygous combinations, DR3/DR3 and DR4/DR4.

In contrast to the predisposing genes, certain haplotypes have been shown to confer almost absolute protection, including in subjects, DR3-DQB1*0201 and DR4-DQB1*0302. This is the case for haplotype DRB1*1501-DQA1*0102-DQB1*0602. This haplotype protects, in particular, against progression toward autoimmune destruction of β-cells even by the diabetics' relatives having anti-islet autoantibodies).

Autoantibodies

Even though autoantibodies do not seem to intervene in the pathogenesis of the disease, they constitute a valuable diagnostic marker, including during the asymptomatic period when the cellular autoimmunity of insulitis is not accessible to analysis.

The autoantibodies consist in:
- anti-islet of Langerhans autoantibodies (islet cell antibodies, ICA)
- anti-glutamic acid decarboxylase autoantibodies (anti-GAD)
- anti-insulinoma associated protein 2 autoantibodies (anti-IA2 or ICA 512) and anti-phogrin antibodies
- anti-insulin autoantibodies.

- ICA

ICA may be detected by indirect immunofluorescence (IIF) on a freezing microtome section of primate pancreas. Their sensitivity at the start of the disease is 60 to 70%, but they disappear in the weeks or months following emergence of the disease. When use is legitimate, the human substrate (group O) has higher sensitivity than the monkey substrate.

The reproducibility of the method is poor. Numerous autoantigens are recognized. The Juvenile Diabetes Foundation (JDF) is working on international standardization.

In relatives, 10% positive results are observed. Titer is related to the risk of developing diabetes. Discrimination in result interpretation is enhanced when concomitant investigation is conducted for other autoantibodies.

- Anti-GAD antibodies

Investigation is conducted by a RIA method (liquid-phase immunoprecipitation). The autoantigen is the 65-kD isoform of glutamic acid decarboxylase (GAD 65), present in β-cells and the central nervous system.
The 67-kD isoform, present in the peripheral nervous system, is recognized by different autoantibodies due to epitopes that are shared with GAD 65.

However, their structural homology gives rise to antigen crosses.
Anti-GAD 67 autoantibodies are observed in stiff-man syndrome.

The sensitivity of the antibodies is 90%. They emerge early and are therefore good markers for screening subjects at risk, particularly in combination with ICA. However, their predictive value with respect to the course toward diabetes is poor.

- Anti-IA2 antibodies

Investigation is again conducted by a RIA method (liquid-phase immunoprecipitation). The autoantigen is a trans-membrane protein of the tyrosine phosphatase series (PTP) located in neuroendocrine tissues. Phogrin has 80% homology with IA2 and the same location.
The prevalence of those antibodies is markedly lower than that of anti-GAD antibodies, but their presence is considered a marker of a fast course toward diabetes: the positive predictive value over the 5 years to come in populations at risk is 75 to 100%. Anti-IA2 are reported to be more specific markers of β-cell destruction than anti-GAD antibodies.

- Anti-insulin antibodies

Testing for anti-insulin antibodies is conducted by radioimmunological methods, which are more closely correlated with the clinical picture than immunoenzymatic assays. Unlike anti-GAD and anti-IA2, anti-insulin antibodies cannot be detected by IIF.

The antibodies are directed against the B chain of insulin when they are autoimmune markers of diabetes. In contrast, they recognize epitopes of region A or an epitope present on both chains, A and B, in the event of insulin therapy.

Autoimmune antibodies are observed in 30 to 40% of recently diagnosed subjects prior to insulin therapy initiation. Antibody level varies in a manner inversely proportional to age. When antibody level is high in young children, it may reflect a faster rate of β-cell destruction. Autoimmune antibody prevalence remains lower than that of the other antibodies. Their predictive value is limited, but when combined with a high ICA level, they indicate a further risk of progression toward diabetes.

β-cell destruction may progress almost silently for years. Destruction is not linear. The final acceleration provides the rationale for emergency insulin therapy in order to preserve the remaining insulin secretion capability. Autoantibodies are the reflection of insulitis. However, there is no preventive treatment. The value of the autoantibodies resides in close monitoring and initiation of insulin therapy as soon as it becomes necessary.

100% of anti-GAD positive relatives presenting with fasting plasma glucose greater than 1.10 g/L on several occasions experience the emergence of diabetes mellitus within a year.

Type 2 diabetes mellitus

Type 2 diabetes mellitus is a disease in which loss of plasma glucose control is frequently associated with obesity, with familial predominance.

The hyperglycemia is the consequence of two interdependent anomalies: a disturbance in insulin secretion (functional anomaly of β-cells) and insulin resistance: relative tissue insensitivity to the insulin secreted, resulting in increased glucose production and decreased glucose use in muscle and adipose tissues.

Etiological Factors

Type 2 diabetes mellitus is a genetically transmitted disease as demonstrated by the almost absolute concordance of its emergence in monozygous twins.
The risk of becoming diabetic is 30% if one parent is affected and 50% if both parents are. The disease is polygenic and requires promoting circumstances such as lifestyle and obesity.

Over 90% of cases of type 2 diabetes mellitus are related to lifestyle: sedentary lifestyle and dietary habits. The predisposition is revealed when the subject switches from a traditional diet to a diet rich in fats and rapidly-digestible sugars. Obesity is one of the modifiable risk factors for type 2 diabetes mellitus. In this case also, the genetic component is decisive.

The risk of diabetes and cardiovascular disease mainly increases in the event of a male-pattern fat distribution. The prevalence of obesity perfectly matches that of diabetes. In developed countries, the change in dietary habits and the more sedentary lifestyle of adolescents and young adults account for the increase in the prevalence of obesity and the lower age of type 2 diabetes mellitus discovery.

Gestational diabetes remains a separate disease entity, and is to be distinguished from pregnancy of a diabetic woman. Gestational diabetes is defined as a glucose tolerance disorder, of variable severity, emerging or diagnosed for the first time during pregnancy. Women having presented with that type of diabetes are at risk of developing hypertension, pre-eclampsia and, subsequently, diabetes, most frequently of type 2. The child is exposed to a high risk of macrosomatia (in over 30% of cases) and significant complications at birth (hypoglycemia, hypocalcemia, polycythemia, etc.).
The risk of obesity is also higher than normal: 50% of those infants have a birth weight greater than the 90th percentile and, among them, 50% develop obesity and glucose intolerance before the age of 8 years.

Taking into account the various risk factors, WHO recommends systematic screening for diabetes in the following cases:

- descendents of a subject with type 2 diabetes mellitus, as of age 30 to 35 years
- obesity
- hypertension or dyslipoproteinemia
- history of gestational diabetes or history of an infant of birth weight greater than 4 kg
- history of glucose intolerance or moderate fasting hyperglycemia
- all subjects aged over 45 years (once every 3 years, if all the results are normal).

Laboratory follow-up of diabetes mellitus

In addition to plasma glucose determination, the main investigations of value in the context of diabetes mellitus are:

- insulin assay
- C peptide assay
- challenge tests (OGTT, etc.)
- glycated hemoglobin assay
- fructosamine assay

Insulin and C peptide

- Insulin

Insulin is secreted by the β-cells of the islets of Langerhans in the form of pro-insulin, a polypeptide formed of 2 chains, A and B, connected by connecting C peptide. Enzymatic cleavage releases insulin and C peptide in equimolar quantities.

Insulin is the only hypoglycemic hormone. The secretory response is fast, within seconds of glycemic stimulus, and, conversely, rapidly shut down when the stimulus ceases. Insulin penetrates target cells, mainly in muscle and adipose tissue, via specific receptors. In the cell, insulin promotes glucose use by activating glycolysis. Insulin, 50% taken up by the liver, inhibits gluconeogenesis and promotes lipogenesis at the expense of carbohydrates and protein anabolism. Insulin is degraded by the liver. This enterohepatic cycle confers a very short serum half-life of the order of 4 minutes. Plasma insulin concentration therefore only reflects a small proportion of its secretion, hence the value of determining C peptide.

- C peptide

C peptide is devoid of biological activity. It does not undergo hepatic uptake and its half-life is of the order of 15 minutes. C peptide is totally eliminated by the kidneys: determination of serum or urinary C peptide concentration is thus the best reflection of insulin secretion.

In insulin-treated patients, C peptide assay only reflects residual insulin secretion. Since C peptide is not present in therapeutic insulin, it does not interfere with the assays.

Insulin secretion determination

Insulin secretion determination is indicated for:

- demonstration of defective β-cell secretion and orientation toward type 1 diabetes mellitus
- investigation of residual insulin secretion:
- characterization of insulin dependence and decision to initiate insulin therapy for type 2 diabetics
- investigation of the degree of insulin resistance.

A single assay of insulin or C peptide contributes little. The assay is always to be conducted concomitantly with plasma glucose determination or in the context of a challenge test, of which the most widely used are the oral glucose tolerance test and the glucagon test.

Challenge tests

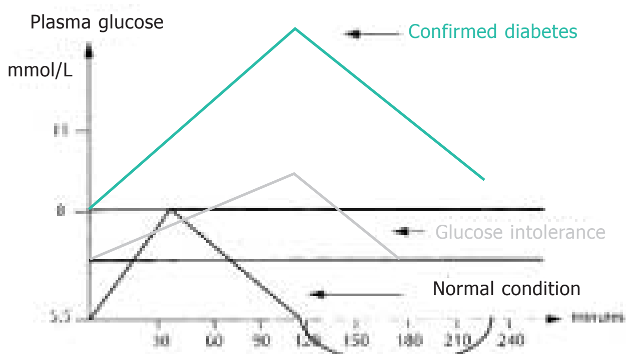
- Oral glucose tolerance test (OGTT)

The conditions have been perfectly standardized by WHO:

- normal carbohydrate intake over the 3 days preceding the test
- discontinuation of interfering medication, if possible
- fasting for 12 hours.

The subject drinks a solution of 75 g of glucose in 250 mL of water in less than 5 min.

Samples are taken at T0, T+30, T+60, T+90 and T+120 min for plasma glucose, C peptide and insulin assay.



The rising portion of the curve shows a gradual increase in plasma glucose due to intestinal absorption and passive diffusion. Insulin limits the amplitude, situated in healthy subjects, at about 8 mmol/L in 30 to 60 min. The falling portion of the curve reflects glucose use by peripheral tissues. The return to baseline occurs in 120 min. In diabetics, the absence or inefficacy of insulin prevents the limitation of the hyperglycemic peak and plasma glucose may rise to 12 mmol/L. The weak peripheral action of insulin results in a return to baseline over 3 or even 4 hours.

The curves enable determination of intermediate conditions, formerly termed 'pre-diabetic' and now known as 'glucose intolerance', in which the curve is right-shifted due to the impaired efficacy of the insulin. The glucose intolerance state can be characterized by 2 points: T0 and T+120 min.

- O'Sullivan's test

Adapted and standardized by O'Sullivan, this specific form of the OGTT is exclusively used for the diagnosis of gestational diabetes. The test is conducted on pregnant women between month 6 and 7 of pregnancy or at any time during pregnancy if there is a risk factor (glucosuria, obesity, etc.).

The test is 2-phases:

- screening test: intake of 50 g of glucose in 250 mL of water in less than 5 min and sample 60 min post-intake. A plasma glucose value greater than 1.40 g/L requires a confirmatory test

- confirmatory test: intake of 100 g of glucose in 250 mL of water in less than 5 min and samples at T0, T+60, T+120 and T+180 min. Gestational diabetes is confirmed when at least 2 values exceed the cutoff (cf. table).

INTERPRETATION

Sampling time	Screening with a 50-g oral glucose challenge Cutoffs (g/L)	(mmol/L)
T 0	-	-
T + 60 min	1,40	7,8
T + 120 min	-	-
T + 180 min	-	-

Sampling time	Screening with a 100-g oral glucose challenge Cutoffs (g/L)	(mmol/L)
T 0	1,05	5,8
T + 60 min	1,9	10,5
T + 120 min	1,65	9,2
T + 180 min	1,45	8,1

- Glucagon test

This test is designed to assess the (mainly residual) insulin secretion capability of diabetic subjects. 1 mg of glucagon is injected by the IM or slow IV route and peptide C is determined on samples taken at T0 and T+6 min. If insulin secretion is normal, the C peptide level is inseased by 50% versus the baseline value, 6 min after the injection of glucagon.

Glycated proteins

- Glycated hemoglobin (HbA1c)

Glucose binds to the amine functions of all proteins by ketoamine bonding as the result of a non-enzymatic process known as glycation. Glycation is thus not to be confused with glycosylation (enzymatic binding of sugars to proteins), hence the discontinued use of the term glycosylated hemoglobin and use of the term glycated hemoglobin. Glycation intensity depends on plasma glucose level, the lifetime of the protein, its structure and its accessibility. The concentration of proteins having undergone glycation thus reflects the plasma glucose balance and its variations, and thus constitutes a sort of 'glycemic memory'.

Hemoglobin is the most widely used protein because of its limited intra-individual variations and long lifetime: 120 days. Glucose binds to the N-terminal of the β chains, modifying their physicochemical characteristics, of which the isoelectric pH, which enables separation and assay of HbA1c. The assay methods must specifically recognize glycated HbA1 to the exclusion of other normal or abnormal hemoglobins (A2, F, S, C, etc.).
Numerous methods are available: electrophoresis, chromatography including HPLC, which constitutes the reference method, and immunological methods. There is no real standardization but the recommendations formulated by the learned societies (National Glycohemoglobin Standardization Program (NGSP), Diabetes Control and Complication Trial (DCCT)) and recently adopted by the French Agency for the Safety of Health Products (Afssaps) encourage the use of certain methods.

Afssaps suggested the following items:
- the method and clinical value of the assay (HbA1c for diabetes follow-up)
- accuracy
- repeatability (< 3%)
- reproductibility (< 5%)
- specific interferences with HbA1c (hemoglobin variants)
- normal ranges (required to be compliant with the standardization and recommendations).

The following reagents comply with most of the Afssaps items

Reagent name	MANUFACTURER/DISTRIBUTOR
ABX Diagnostics HbA1cWB	ABX DIAGNOSTIC
DCA 2000Kit reagent HbA1c	BAYER
RA et ope RA HbA1C	BAYER
Glycomat DS5	BAYER
Glycomat 745/G15	BAYER
Dia STAT	BIO RAD
Variant HbA1c test	BIO RAD
Variant II HbA1c	BIO RAD
Dimension HA1C	DADE BEHRING
Set of reagents, HA 8121	MENARINI
Set of reagents, HA 8140	MENARINI
Set of reagents, HA 8160 diabetes mode	MENARINI
Set of reagents, HA 8160 diabetes mode and thalassemia	MENARINI
TSK dgel glyco Hsi variant	TOSOH BIOSCIENCES
TSK gel G7 Hsi variant	TOSOH BIOSCIENCES
HYDRAGEL 7 et 15 HbA1c	SEBIA

HbA1c plasma glucose control is to be conducted every 3 to 4 months. HbA1c is the only parameter affording a reliable reflection of plasma glucose balance and it constitutes a validated index for predicting the emergence or progression of complications. The percentage glycated Hb is directly proportional to the mean plasma glucose value.

GLYCATED HEMOGLOBIN + PLASMA GLUCOSE BALANCE

Mean plasma glucose (g/L)	HbA1c (%)
1.2	6
1.5	7
1.8	8
For each 0.3 increment	an increment of 1

The objectives for the diabetic subject can be translated into glycated hemoglobin objectives:
- < 6.5%: optimal objective
- < 8% for 2 successive determinations:
  . acceptable control
  . treatment review possible with evaluation of the advantage/disadvantage ratio
- > 8% on 2 successive determinations:
  . poor plasma glucose control
  . treatment review recommended.

In the particular case of the elderly, the objectives are less strict in order to take into account the risk of treatment-related hypoglycemia and the possible presence of kidney failure.
A degree of caution with respect to interpretation is necessary in diseases associated with reduced erythrocyte life (chronic hemolysis, valve diseases, thalassemia, etc.).

In addition, certain methods do not enable reliable glycated Hb assay in the presence of abnormal hemoglobin. The separative methods avoid this pitfall in case of heterozygotes S or C hemoglobin. For subjects who do not synthesize HbA1 (homozygotes S, C, E, etc.), fructosamine assay is necessary.

- Fructosamines

The term fructosamine covers all the glycated proteins of the body except hemoglobin. The proteins constitute a highly heterogeneous set of dissimilar components with variable half-lives. Fructosamines provide information on the plasma glucose balance over the last 2 to 3 weeks and are thus of value in the rapid assessment of the results of a change in treatment, metabolic decompensation or unstable diabetes.

Fructosamine determination is recommended for the follow-up of pregnant women.

The colorimetric assay is based on the reducing properties of glycated proteins in an alkaline medium. The method is fast and can be automated, but is not very specific. Strict compliance with the analytical conditions recommended by the SFBC (French Society of Clinical Biology) enables assay transferability. The results are always to be interpreted as a function of the total protein level since true or relative hypoproteinemia, as is the case during pregnancy, may induce an excessively low result.

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Bibliography available on request

Comparison of the indications for HbA1c and fructosamine assay

	HbA1c	Fructosamine
Screening or diagnosis	No	No
Regular monitoring	Yes (cumulative image of the last 3 months)	Of interest, if more frequent follow-up is required (cumulative image of the last 20 days)
Treatment change	No (too great inertia)	Yes
Pregnancy, gestational diabetes	No (too great inertia)	Yes
Protein metabolism disorder, thyroid disorder	Yes	No
Mutant hemoglobins, thalassemia, pathological hemolysis	Yes, to be interpreted with caution	Yes
Kidney failure	Both are to be combined and interpreted with caution	

Serum and urines assays in the diabetic patient follow up

Analyse	Frequency	Preanalytical conditions		Interferencies		Relevance
	T : trimester A : annual	BLOOD		Analytical	Physiological	
		Fasting	Tube			
Glycemia		X	with antiglycolytic			Glucometer control
HbA1c	T		EDTA	Certified method NGSP/IFCC (CV< 2%)	- Anemia - Presence of Hb variant	Glycemic balance follow up
Triglycerides*	A	X	dry			Cardiovascular risk factors follow up
Cholesterol*	A	X	dry			Cardiovascular risk factors follow up
HDL cholesterol* (LDL with concentration)	A	X	dry	No LDL calculation if triglycerides > 4 mmol/l		Cardiovascular risk factors follow up
Creatinine	A		dry or heparin	possible (icterus, hemolysis) according to the technique		Screening of complications
Clearance of creatinine	A			Cockroft formula : $\frac{[140-age(years)]xweight(kg)}{0.814xCreatinine(\mu mol/l)}$	(women x 0,85)	Screening of complications
		URINES				
		Sample	24 h or night			
Hematuria	A	X	X	Blood presence		If positive, proteinuria uninterpretable
Proteinuria	A	X	X	Blood presence		Following
Microalbuminuria*,**	A	X	X		high blood pressure, urinary tract infection, diabetes decompensation, major dyslipidemia	Following

** if dyslipemia or/and microalbuminuria : 2 or 3 times per year*
*** microalbuminuria on one sample : one time per year. If positive, dosage on 24 h diuresis*

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