Q&A
Chemical pathology

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Q What are the relative merits of using HbA_{1c} as opposed to fasting plasma glucose or the oral glucose tolerance test for diagnosis of diabetes?

HbA_{1c} can be considered an alternative diagnostic tool to fasting plasma glucose (FPG) or the oral glucose tolerance test (OGTT), but it is not generally considered superior to these tests and has advantages and disadvantages comparatively. HbA_{1c} tends to be used as a first-line screen for diagnosis due to convenience as it does not require fasting and is not a dynamic function test. HbA_{1c} also is able to capture chronic hyperglycaemia more effectively than a standard paired assessment of FPG or an OGTT as it is describing a longer period of time. In addition, both microvascular and macrovascular disease are better associated with HbA_{1c} than FPG (DECODE Study Group, 2001; International Expert Committee, 2009). It is not necessary to fast prior to testing HbA_{1c}, and the analyte itself has greater pre-analytical stability than glucose. It has also been argued that using the same analyte for both diagnosis and then subsequent monitoring of diabetes may be beneficial (Bonora and Tuomilehto, 2011).

Two-hour post-glucose concentration as seen during OGTT better reflects the abnormal physiology seen in diabetes as it describes the post-prandial state with insights into beta-cell function, information that HbA_{1c} does not provide. It has also been shown that HbA_{1c} has poorer sensitivity than OGTT (Tuomilehto et al, 2001) and FPG (Zhou at al, 2010) for detecting asymptomatic cases in the early stages of disease. Issues with assay standardisation for HbA_{1c} unfortunately remain, although the situation has markedly improved in recent years. This is an important issue given the implications for an incorrect diagnosis. HbA_{1c} can be unreliable in certain clinical scenarios, as expanded upon below.

Q In what circumstances is HbA_{1c} unreliable?

Any pathophysiological process leading to reduced erythrocyte lifespan has the potential to lead to a falsely low reported HbA_{1c} concentration. This includes chronic and haemolytic anaemia, renal anaemia with use of erythropoietin, acute blood loss and recent transfusion. General recommendations are to not perform HbA_{1c} testing within 3 months of a blood transfusion. In addition to this, chronic liver disease, dialysis and chronic malaria can lead to a falsely low HbA_{1c}. Conversely, iron-deficiency anaemia may cause a falsely high HbA_{1c}, possibly due to altered glycation rates (Weykamp, 2013). However, if haemoglobin and mean cell volume are normal in someone previously treated for iron-deficiency anaemia or vitamin B12 deficiency, HbA_{1c} should be reliable.

In some cases where HbA_{1c} is potentially unreliable, there are solutions to consider. When HbA_{1c} cannot be measured reliably due to the presence of a haemoglobin variant, it may be possible to analyse a sample via an alternative method which may be able to provide a valid result and this should be discussed with the local biochemistry laboratory as appropriate. For cases where this is not possible, it may be necessary to use standard capillary blood glucose techniques alone to assess glycaemic control. Other options are available, such as fructosamine or glycated

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albumin, but there are interpretive issues that limit their usefulness and it is unclear how they relate to the development of long-term complications. Whether alcoholism can cause a falsely high HbA_{1c} is under debate, with conflicting reports on its effect on HbA_{1c} testing. The mechanism at work is not currently known, nor is it clear if it is a genuine effect rather than a confounding factor, such as background nutritional status.

When should HbA_{1c} not be used to diagnose diabetes?

HbA_{1c} should not be used to diagnose gestational diabetes. In this situation OGTT should be used in accordance with NICE guidelines (2015). If there has been a rapid onset of symptoms, then HbA_{1c} may not be able to detect the change until several weeks later and, as such, it should not be used as it may delay diagnosis. Such situations could include suspected type 1 diabetes, suspected steroid-induced diabetes and diabetes potentially arising from pancreatitis or following pancreatic surgery.

HbA_{1c} gives an indication of "average" blood glucose levels over a period of 2–3 months and, therefore, it is generally not appropriate to repeat the test more frequently than this.

When is it appropriate to perform an HbA_{1c} test earlier?

There will be times when more frequent requesting of HbA_{1c} may be indicated. For instance, tight glycaemic control is advised for people with diabetes who are considering pregnancy. Recent NICE guidelines (2015) suggest monthly requesting of HbA_{1c} in the preconception period.

In the event of a normal thyroxine (T4) but raised thyroid-stimulating hormone how would you monitor, and how frequently, for potential hypothyroidism?

Thyroid function tests which demonstrate increased thyroid-stimulating hormone (TSH) with an FT4 that falls within the reference range are suggestive of subclinical hypothyroidism. There is no consistent evidence from the literature that an raised TSH, which is <10 mU/L, is associated with symptoms of hypothyroidism, or secondary biochemical effects, such as hyperlipidaemia, or cardiac dysfunction. For patients where the TSH concentration is >10 mU/L with a normal FT4, there is evidence of greatly increased risk of progression to overt hypothyroidism, and also deterioration in hyperlipidaemia (Association for Clinical Biochemistry, 2006). Thus if the TSH concentration is >10 mU/L then treatment with thyroxine replacement is advised in most cases. When the TSH concentration is raised but <10 mU/L, treatment is usually not recommended unless the patient has a goitre or is planning pregnancy.

Patients with subclinical hypothyroidism and a TSH <10 mU/L require monitoring to assess for progression of thyroid disease. It is advised that such individuals should have a repeat TSH and FT4 after 3–6 months along with measurement of thyroid peroxidase (TPO) antibody. Those with a persistent raised TSH, which remains <10 mU/L, who are TPO antibody positive should have repeat thyroid function tests in 1 year. Those who are TPO antibody negative should have repeat thyroid function tests (TFTs) in 3 years. Those with worsening symptoms may require testing sooner as appropriate. Patients whose TSH has increased to >10 mU/L should be considered for thyroxine replacement therapy (Association for Clinical Biochemistry, 2006).

When and how is chronic kidney disease diagnosed and classified?

Chronic kidney disease (CKD) is an all-encompassing term for describing a heterogeneous collection of disorders which result in abnormal renal function or structure. CKD can be detected and assessed using routine laboratory tests. Renal function can be assessed by measuring or estimating glomerular filtration rate (GFR); formal measurement of GFR, such as by using radioactive isotopes is cumbersome and resource intensive, so commonly it is estimated from serum creatinine and other parameters such as age, sex, ethnic origin and body size. Several methods are available for estimating GFR, including the Modification of Diet in Renal Disease (MDRD) study group equation and the CKD Epidemiology Collaboration equation (CKD-EPI). The MDRD equation is reasonably accurate when

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the estimated (e)GFR is <60 mL/min/1.73 m² but is significantly limited by imprecision when the eGFR is higher, and consequently is often not reported when at such levels (Stevens et al, 2007). The CKD-EPI equation is less susceptible to bias at higher levels and can be used to report eGFR results >60 mL/min/1.73 m², and is recommended for use in estimating GFR in the recent NICE guidance (2014a) on CKD.

CKD can be diagnosed on the basis of the presence of renal damage, such as albuminuria, or decreased renal function such as an eGFR <60 mL/min/1.73 m², present for at least 3 months irrespective of the underlying aetiology. The eGFR can then be used to classify CKD into a number of distinct stages:

- **Stage 1:** ≥90 mL/min/1.73 m²
- **Stage 2:** 60–89 mL/min/1.73 m²
- **Stage 3a:**: 45–59 mL/min/1.73 m²
- **Stage 3b:** 30–44 mL/min/1.73 m²
- **Stage 4:** 15–29 mL/min/1.73 m²
- **Stage 5:** <15 mL/min/1.73 m²

It is important to note that for individuals with an eGFR ≥60 mL/min/1.73 m² (i.e. stages 1 and 2), CKD cannot be diagnosed unless there is co-existing evidence of renal damage, such as albuminuria.

eGFR can be used in combination with urine albumin–creatinine ratio (ACR) from an untimed spot urine to further classify patients with CKD by stratifying them into various risk groups. This is demonstrated in *Table 1*, adapted from NICE (2014a) guidance. This enables a consistent approach to diagnosis, staging and risk stratification in CKD.

Table 1. Classification and risk stratification in CKD, adapted from NICE (2014a).

GFR stages (mL/min/1.73 m²), description and range	ACR categories (mg/mmol) description and range		
	A_1 <3 Normal to mildly increased	A_2 3–30 Moderately increased	A_3 >30 Severely increased
G1 ≥90 Normal and high	No CKD in absence of markers of renal damage	Moderate risk CKD	High risk CKD
G2 60–89 Mild reduction related to normal range for a young adult		Moderate risk CKD	High risk CKD
G3a 45–59 Mild–moderate reduction	Moderate risk CKD	High risk CKD	Very high risk CKD
G3b 30–44 Moderate–severe reduction	High risk CKD	Very high risk CKD	Very high risk CKD
G4 15–29 Severe reduction	Very high risk CKD	Very high risk CKD	Very high risk CKD
G5 <15 Kidney failure	Very high risk CKD	Very high risk CKD	Very high risk CKD

ACR=albumin–creatinine ratio; CKD=chronic kidney disease; GFR=glomerular filtration rate.

Q When should you use a cystatin C-based estimate of GFR for diagnosis of CKD (and is it currently available)?

Cystatin C is a proteinase inhibitor and has a low molecular weight. It is freely filtered at the glomerulus, almost completely reabsorbed and undergoes no tubular secretion. It is produced at a constant rate from all nucleated cells in the body. Its serum concentration is largely determined by glomerular filtration and as such it is considered an endogenous marker of GFR. Cystatin C-based GFR estimation is considered to have some advantages over creatinine as its non-GFR determinants seem to be less affected by muscle-wasting and race. There is also some data to suggest that it is more predictive of subsequent cardiovascular mortality and morbidity (Shlipak et al, 2005).

Current NICE (2014a) guidance advises consideration of cystatin C-based eGFR for initial diagnosis of CKD in people with a creatinine-based eGFR of 45–59 mL/min/1.73 m² who also have an ACR of <3 mg/mmol. In this situation, cystatin C-based eGFR can confirm or exclude a diagnosis of CKD. If the eGFR as determined using cystatin C is >60 mL/min/1.73 m² then the diagnosis can be excluded at that time.

There are issues with the use of cystatin C, however. It is considerably more expensive than creatinine and has poor general availability, being offered currently by just three laboratories in the UK. There are also significant issues with assay standardisation, and in addition to this, there is no clear consensus regarding the best equation to use to estimate GFR based on the

serum level. It could also be argued that in those situations where an accurate GFR estimation is required, where one might consider using cystatin C, then the best course of action may be to formally measure GFR using nuclear medicine techniques. For these reasons, cystatin C-based GFR estimation is rarely undertaken in routine clinical practice despite the recommendations in the recent NICE (2014a) guidance.

How frequently should people with CKD undergo monitoring of eGFR and ACR?

It is important to remember that CKD represents a broad spectrum of disease, ranging from rapidly progressive disease leading to end-stage renal failure within months, to those with little to no progression in disease stage over many years of follow-up. It is recommended that all patients with a formal diagnosis of CKD should have regular monitoring to assess for the possibility of disease progression. *Table 2* outlines the monitoring schedule as advised by NICE (2014a).

Table 2. Recommendations for frequency of monitoring per year of GFR and ACR in people with CKD, adapted from NICE (2014a).

GFR stages (mL/min/1.73 m²), description and range	ACR categories (mg/mmol) description and range: A_1 <3 Normal to mildly increased	A_2 3–30 Moderately increased	A_3 >30 Severely increased
G1 ≥90 Normal and high	≤1	1	≥1
G2 60–89 Mild reduction related to normal range for a young adult	≤1	1	≥1
G3a 45–59 Mild–moderate reduction	1	1	2
G3b 30–44 Moderate–severe reduction	≤2	2	≥2
G4 15–29 Severe reduction	2	2	3
G5 <15 Kidney failure	4	≥4	≥4

ACR=albumin–creatinine ratio; CKD=chronic kidney disease; GFR=glomerular filtration rate.

When should individuals with CKD be referred to a renal physician?

Recommendations from NICE emphasise the need for an open dialogue with the patient, taking into account their wishes and also any comorbidities when considering who to refer for specialist management. People with a diagnosis of CKD should be considered for referral to a renal physician in the following situations:

- eGFR <30 mL/min/1.73 m² with or without diabetes.
- ACR >70 mg/mmol unless known to be associated with diabetes.
- ACR >30 mg/mmol along with haematuria.
- A decrease in eGFR of 25% or more with a change in GFR stage or sustained decrease in GFR of 15 mL/min/1.73 m² or more within 12 months.
- Known or suspected rare or genetic cause of CKD or suspected renal artery stenosis.
- Poorly controlled hypertension despite use of four or more agents at therapeutic doses.

Cholesterol is often raised at the time diabetes is diagnosed or when glycaemic control is very poor – should the cholesterol levels be treated based on these findings or rechecked when glycaemic control improves?

The key issue in this situation is being clear regarding the rationale for treatment. It is not uncommon for individuals with newly diagnosed type 2 diabetes to have co-existent severe hypertriglyceridaemia as a result of their underlying insulin resistance (Subramanian and Chait, 2012). Significant hypertriglyceridaemia will result in a total cholesterol concentration that is often greatly elevated and not representative of the individual's baseline lipid status. As the patient has their diabetes treated and glycaemic control improves, the hypertriglyceridaemia will generally improve and their total cholesterol will reduce. There is no definitive evidence that pharmacological treatment of hypertriglyceridaemia in itself has a beneficial impact on cardiovascular risk (Miller et al, 2011). However, it is advisable that if the triglyceride concentration remains >10 mmol/L after treatment of diabetes has been optimised, treatment with a fibrate should be considered

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Glossary

HDL=high-density lipoprotein

IDL=intermediate-density lipoprotein

LDL=low-density lipoprotein

Lp(a)=lipoprotein-a

VLDL=very low-density lipoprotein

to lower triglycerides due to the risk of acute pancreatitis (Berglund et al, 2012).

Once lipid levels have stabilised, a formal cardiovascular risk assessment using QRISK2 can take place and this will inform the decision to prescribe a statin. Current NICE (2014b) guidance is that all individuals with a calculated cardiovascular risk greater than 10% should be considered for statin treatment. It is worth considering that cholesterol forms just one part of a patient's overall cardiovascular risk package and close attention should be paid to all modifiable risk factors.

Q Is non-HDL-cholesterol a more reliable measure than LDL-cholesterol?

LDL-cholesterol is not generally measured directly in routine laboratory testing; instead it is calculated using the Friedewald equation (Warnick et al, 1990):

LDL-cholesterol=total cholesterol – HDL-cholesterol – (triglycerides/2.2)

The equation has very well-recognised limitations, and is known to be inaccurate in cases of moderate hypertriglyceridaemia, where there are chylomicrons present, and in people with type 3 hyperlipidaemia (Matas et al, 1994). These issues introduce the potential for misclassification of patients on the basis of their cardiovascular risk. This is particularly of concern for people with diabetes as they are more likely to have abnormal triglycerides. Direct LDL-cholesterol assays have been developed which would be theoretically preferable, but analytical issues prevent them being used routinely. However, it is important to consider that LDL-cholesterol remains crucial for the diagnosis of familial hypercholesterolaemia, which is a relatively common monogenic inherited disorder of LDL metabolism.

Non-HDL-cholesterol is the difference between total cholesterol concentration and HDL-cholesterol concentration, and thus represents an estimate of the cholesterol present in atherogenic particles such as IDL, LDL, VLDL and Lp(a). With regards to its efficacy as a cardiovascular risk biomarker, there is clear evidence of the superiority of non-HDL cholesterol over LDL-cholesterol (van Deventer et al, 2011). Some clinical trials go as far as to say that non-HDL-cholesterol has equivalent vascular event prediction capabilities as measurement of apolipoprotein B or LDL particle number, both of which are significantly more expensive tests and not routinely available (Blaha et al, 2008). However, much of the established evidence base refers to lipid status in terms of total cholesterol and LDL-cholesterol and this should be considered when interpreting lipid results in an individual. ■

Association for Clinical Biochemistry (2006) *UK guidelines for the use of thyroid function tests*. Association for Clinical Biochemistry, London

Berglund L, Brunzell JD, Goldberg AC et al (2012) Evaluation and treatment of hypertriglyceridaemia: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* **97**: 2969–89

Blaha MJ, Blumenthal RS, Brinton EA et al (2008) National Lipid Association Taskforce on non-HDL cholesterol: the importance of non-HDL cholesterol reporting in lipid management. *J Clin Lipidol* **2**: 267–73

Bonora E and Tuomilehto J (2011) The pros and cons of diagnosing diabetes with A1C. *Diabetes Care* **34**: S184–90

DECODE Study Group (2001) Glucose tolerance and cardiovascular mortality: comparison of fasting and 2-h diagnostic criteria. *Arch Intern Med* **161**: 397–404

International Expert Committee (2009) International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. *Diabetes Care* **32**: 1327–34

Matas C, Cabre M, La Ville A et al (1994) Limitations the the Friedewald formula for estimating low-density lipoprotein cholesterol in alcoholics with liver disease. *Clin Chem* **40**: 404–6

Miller M, Stone MJ, Ballantyne C et al (2011) Triglycerides and cardiovascular disease: a scientific statement from the American Heart Association. *Circulation* **123**: 2292–333

NICE (2014a) *Chronic kidney disease in adults: assessment and management* (CG182). NICE, London

NICE (2014b) *Lipid modification: cardiovascular risk assessment and the modification of blood lipids for the primary and secondary prevention of cardiovascular disease* (CG181). NICE, London

NICE (2015) *Diabetes in pregnancy: management from preconception to the postnatal period* (NG3). NICE, London

Shlipak MG, Sarnak MJ, Katz R et al (2005) Cystatin C and the risk of death and cardiovascular events among elderly persons. *N Eng J Med* **352**: 2049–60

Stevens LA, Coresh J, Feldman HI et al (2007) Evaluation of the modified diet in renal disease study equation in a large diverse population. *J Am Soc Nephrol* **18**: 2749–57

Subramanian S, Chait A (2012) Hypertriglyceridaemia secondary to obesity and diabetes. *Biochim Biophys Acta* **1821**: 819–25

Tuomilehto J, Lindstrom J, Eriksson JG et al (2001) Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Eng J Med* **344**: 1343–50

van Deventer HE, Miller WG, Myers GL et al (2011) Non-HDL cholesterol shows improved accuracy for cardiovascular risk score classification compared to direct or calculated LDL cholesterol in a dyslipidemic population. *Clin Chem* **57**: 490–501

Warnick GR, Knopp RH, Fitzpatrick V et al (1990) Estimating low-density lipoprotein cholesterol by the Friedewald equation is adequate for classifying patients on the basis of nationally recommended cutpoints. *Clin Chem* **36**: 15–9

Weykamp C (2013) HbA_{1c}: a review of analytical and clinical aspects. *Ann Lab Med* **33**: 393–400

Zhou X, Pang Z, Gao W et al (2010) Performance of an A1C and fasting capillary blood glucose test for screening newly diagnosed diabetes and pre-diabetes defined by an oral glucose tolerance test in Qingdao, China. *Diabetes Care* **33**: 545–50