Chronic kidney disease - CB-1-C-1

Chronic kidney disease (CKD) is classified into 5 stages. Stages 1 and 2 are defined by the presence of markers of kidney damage (such as microalbuminuria, electrolyte or sediment abnormalities or structural abnormalities). Stages 3-5 are defined by an estimated glomerular filtration rate (eGFR) of >60 ml/min/1.73m^2 with or without markers of kidney damage, on at least two separate occasions separated by a period of at least 90 days (NICE, 2014).

Diseases of the kidney can affect the function of the glomerulus (ultrafiltration) and/or the tubules (selective reabsorption). General assessments of kidney function include urine output and appearance, dipstick analysis, kidney imaging and biochemical tests in the laboratory.

Tables 1 and 2 summarise the biochemical tests used to assess tubular glomerular function. It is relatively uncommon to have impaired tubular function in isolation so glomerular function is usually used to assess overall nephron function.

Tubular function	**Reason for use as a marker**
Urine volume/osmolality	Polyuria is common in early CKD because the kidneys are less able to concentrate the urine. Polyuria is associated with a low urine osmolality. Anuria and oliguria (a urine output of <100mL/day and <400 mL/day respectively) are associated with end-stage renal failure when there is insufficient functioning mass of the kidney to perform any filtration.
Plasma pH	Plasma pH is can be low in renal disease due to impaired excretion of H+. Plasma bicarbonate is can also be low due to the prevailing acidosis.
Urine phosphate	Can be raised due to impaired reabsorption.
Aminoaciduria	
Glycosuria	
Urine β2-microglobulin	

Table 1 Markers of tubular function

Glomerular function	Reasons for use as a marker
Gold standard	Inulin, an inert sugar, is the gold-standard marker of glomerular filtration capacity as it is freely filtered and not reabsorbed, metabolised, synthesised or secreted by the kidney. However, it is not endogenous and requires IV infusion, which limits the assay availability and makes it expensive.
Urea	Urea is the end-product of amino acid metabolism. It is freely filtered from the blood at the glomerulus. However, there is some passive reabsorption at the tubules and urea is raised in GI bleeds and liver disease making creatinine a better marker of glomerular function.
eGFR	The estimated glomerular filtration rate (eGFR) can be calculated using serum creatinine or serum Cystatin C concentrations. Creatinine is a product of muscle metabolism which is freely filtered at the glomerulus. It has a fairly constant production rate proportional to muscle mass. Serum Cystatin C is a small protein filtered by the glomerulus which is then metabolised and reabsorbed by the tubules. Its serum concentrations rise with loss of glomerular function. The assays used to measure creatinine (Jaffe or enzymatic) are relatively insensitive and subject to interferences. Cystatin C has greater sensitivity to changes in GFR and is useful for diagnosing early stages of CKD; however it is a more expensive test than serum creatinine. Creatinine is routinely used to determine eGFR.
Proteinuria	In health, proteins larger than ≈50 kDa are not present in the glomerular filtrate. As the integrity of the glomerular filtration barrier fails, small proteins (in particular albumin) begin to cross the glomerulus, resulting in proteinuria. Proteinuria is assessed by calculating the urine albumin: creatinine ratio (ACR, which is more sensitive than the protein: creatinine ratio). Proteinuria can also be caused by overflow (e.g. Bence-Jones protein), impaired tubular reabsorption (e.g. β2-microglobulinuria) or secretion from the tubules (e.g. Tamm-Horsfall proteinuria, see http://www.uptodate.com/contents/chapter-4d-tamm-horsfall-mucoprotein).

Table 2 Markers of glomerular function and reasons/limitations for their use

Tests for investigating glomerular function

The glomerular filtration rate (GFR) decreases with severity of CKD due to loss of function of the glomerulus. NICE guidelines (July 2014) categorise CKD as follows (table 3).

GFR category	GFR (ml/min/1.73m^2)	Renal function
G1	>90	Normal or high
G2	60-89	Mildly decreased relative to young adult level
G3a	45-59	Mildly to moderately decreased
G3b	30-44	Moderately to severely decreased
G4	15-29	Severely decreased
G5	<15	Kidney failure

Table 3 Kidney disease improving global outcomes GFR categories, NICE 2014

A. Calculating the estimated GFR (eGFR) using serum creatinine

The first equation which was used for calculating the eGFR was the Cockroft-Gault equation:

$$eGFR = \frac{(140 - \text{age in years})\ (\text{wt in Kg})}{\text{serum creatinine umol/L} \times 0.81}$$

However this is no longer used since accurate measurements of patient weight is rarely available.

NICE (2014) recommend that the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation is used to calculate eGFR. This is based on the Modification of Diet in Renal Disease (MDRD) equation, which includes four variables: plasma or serum creatinine, age, gender and race. The CKD-EPI equation uses a different relationship for age, gender and race to determine the eGFR and has been shown to perform better with less bias than the MDRM form, especially in patients with a higher GFR, thus reducing misclassification of CKD (http://nephron.com/epi_equation). The eGFR calculated using either equation is to be interpreted with caution in patients with extremes of muscle mass (e.g. body builders, amputees and those with muscle wasting disorders). Moreover, information about the ethnicity of the patient is rarely available so in practice the scaling factor of 1.210 is rarely included.

MDRM equation

$$eGFR = 175 \times (Scr / 88.4)^{-1.154} \times (Age)^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if black})$$

Scr = serum creatinine (mg/dL)

CKD-EPI equation

$$GFR = 141 \times \min(Scr/\kappa, 1)^{\alpha} \times \max(Scr/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018 \text{ [if female]} \times 1.159 \text{ [if black]}$$

Scr = serum creatinine (mg/dL)

κ = 0.7 (females) or 0.9 (males)

α = –0.329 (females) or –0.411 (males)

Min = the minimum of Scr/κ or 1

Max = the maximum of Scr/κ or 1.

A single serum sample is required to measure the serum creatinine concentration to enable calculation of the eGFR. Creatinine is measured by Jaffe or enzymatic methods, traceable to the gold standard which is isotope dilution mass spectrometry (IDMS). The Jaffe assay is a colorimetric method based on the reaction of creatinine with picric acid under alkaline conditions, to form the yellow-orange product creatinine picrate. The Jaffe assay is cheap, quick and easy to automate and hence remains a popular method for measuring serum creatinine concentration. However, it is highly subject to interference, for example from bilirubin, proteins and glucose, with the interference from glucose being especially acute in diabetic patients at high risk of developing CKD. Haemolysis releases chromogens which react in the Jaffe assay to cause falsely elevated results. Moreover, since it is a spectrophotometric method, lipaemic and icteric samples can interfere with absorbance and cause falsely decreased results.

Colorimetric enzymatic methods are more specific and are recommended in the NICE 2014 guidelines provided they have zero-bias compared with IDMS, which is the reference method (but is too expensive to be used routinely). Nonetheless, enzymatic methods can also be subject to interferences from bilirubin. Moreover, in neonatal samples there is interference from foetal haemoglobin. Unlike adult haemoglobin, foetal haemoglobin is alkali resistant and slowly changes colour over the course of the assay (Peake and Whiting 2006). Another limitation is that both Jaffe and enzymatic assays are relatively insensitive, with the GFR falling to approximately 40 ml/min (CKD stage G3) before creatinine rises to the upper level of normal (the creatinine 'blind spot'), making creatinine an insensitive marker for early stages of CKD.

B. Calculating eGFR using creatinine clearance

The creatinine clearance is approximately equal to the eGFR.

Creatinine clearance = UV/PT ml/min

Where:

U = urine creatinine (mmol/L)

V = urine volume (ml)

P = plasma or serum creatinine (mmol/L)

T = time (minutes = 60 x 24 = 1440 min in 24h)

The specimens required to calculate creatinine clearance are a serum sample (to measure creatinine concentration) and a 24h urine collection (to determine flow rate and urine creatinine concentration). The instructions for the 24h urine collection must be explained clearly to the patient so that the sample is collected correctly. Common problems include forgetting to collect every passage of urine in the 24h period and increasing the volume of the sample (e.g. with water) if the patient feels it is insufficient.

Calculating eGFR using creatinine clearance includes the limitations of the methods to measure serum creatinine levels (i.e. interferences and insensitivity in Jaffe and enzymatic methods). It is also a less convenient method due to the requirement for a 24h urine sample as well as a blood specimen. Therefore creatinine clearance less commonly used as an indicator of kidney function than eGFR calculated using serum creatinine.

C. Calculating eGFR using Cystatin C

NICE (2014) recommends that cystatin C is used to calculate eGFR to confirm or rule out CKD in patients with stage G3aA1, sustained for at least 90 days, with no proteinuria or other marker of kidney disease. Cystatin C is a small protein (13.3 kDa) that is filtered by the glomerulus, metabolised and then reabsorbed by the tubules. Its serum levels rise as glomerular function worsens due to decreased filtration from the blood. It is measured using immunoassay. The reference range is approximately 0.49 - 1.13 mg/L for adults (Mayo Medical Laboratories).

Unlike creatinine, cystatin C levels are not affected by age, gender, race or muscle mass. Moreover it has greater sensitivity to GFR changes in the early stages of GFR than serum creatinine. Therefore cystatin C is useful for diagnosing CKD in its early stages where intervention is more likely to improve outcomes.

However, measuring cystatin C is a more expensive test than serum creatinine so it is not routinely used. Also, thyroid disease alters its levels (cystatin C is elevated in hypothyroidism and decreased in hyperthyroidism) and some drugs can affect its serum levels (increased by corticosteroids, decreased by cyclosporin).Therefore the eGFR based on cystatin C levels should be interpreted with caution in patients with thyroid disease or on these drugs.

D. Albumin creatinine ratio

The albumin creatinine ratio (ACR) is calculated to assess the degree of microalbuminuria. It is used in preference to the protein creatinine ratio (PCR) because the ACR has greater sensitivity for detection of early CKD. NICE (2014) published ACR categories (table 4) alongside the eGFR categories shown in table 3, to be used in monitoring CKD. Urine albumin can be measured from a random urine sample using immunotubidimetric methods, in which a urine specimen is mixed with buffer and anti-human albumin anti-serum. Insoluble aggregates form which cause scattering of light; the decrease in absorbance is proportional to the concentration of albumin in the specimen. Urine creatinine is measured using Jaffe or enzymatic methods as previously described. Creatinine corrects for the concentration of the urine specimen.

ACR category	**ACR (mg/mmol)**	**Renal function**
A1	<3	Normal to mildly increased
A2	3-30	Moderately increased relative to young adult level

A3	>30	Severely increased

Table 4: ACR categories associated with CKD

Decreased GFR and increased ACR multiply the risk of adverse outcomes (Table 5)

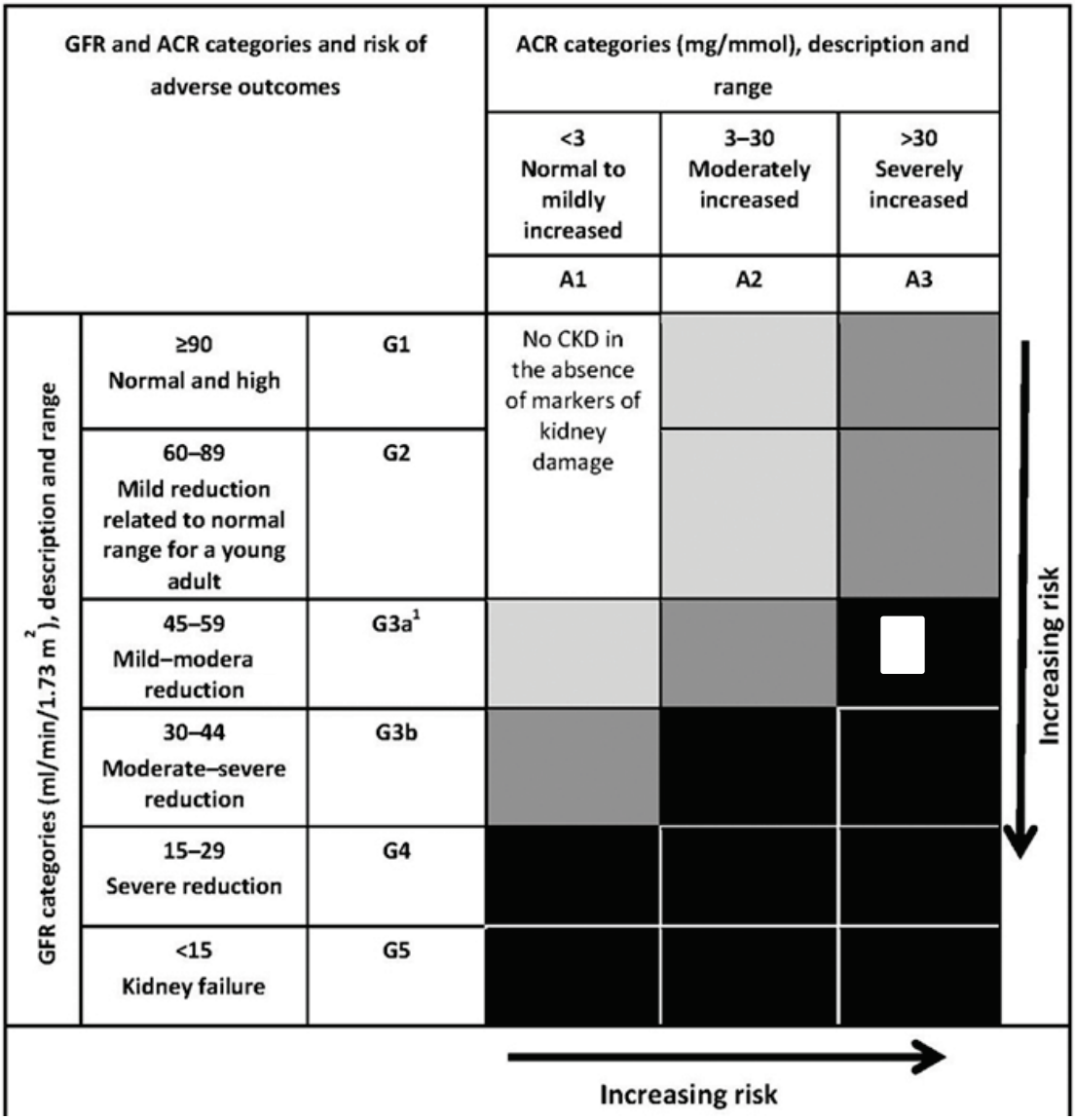

Table 5 Risk associated with GFR and ACR values: from NICE 2014.

Diabetes

Diabetes is characterised by hyperglycaemia and patients typically present with polydipsia (increased thirst) and polyuria (frequent urination). Type 1 diabetes is caused by a failure to of the beta pancreatic cells to produce insulin and is treated by regular insulin injections. Type 2 diabetes is a result of insulin resistance in tissues and can be controlled by diet and exercise and hypoglycaemic drugs, (such as metformin, sulfonylureas, α-glucosidase inhibitors and thiazolidinediones), with insulin treatment being introduced as severity worsens. Studies have shown that maintaining blood glucose concentrations as close to normal as possible slows the onset of complications such as diabetic nephropathy, retinopathy, neuropathy and cardiovascular problems. Diabetes is also managed by addressing and minimising risk factors (such as obesity, smoking, hypercholesterolaemia, hypertension and lack of exercise).

A. Diagnosis

In a symptomatic patient, diabetes can be diagnosed by a random plasma glucose concentration of >11.0 mmol/mol or a fasting plasma glucose of >7 mmol/mol on two separate days. A fluoride oxalate sample (anti-coagulated whole blood) is required. Glucose can be measured by an enzymatic spectrophotometric assay as follows:

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{hexokinase, magnesium}} \text{glucose-6-phosphate} + \text{ADP}$$

$$\text{Glucose-6-phosphate} + \text{NAD}^+ \xrightarrow{\text{glucose-6-phosphate dehydrogenase}} \text{gluconate-6-phosphate} + \text{NADH} + \text{H}^+$$

The NADH produced is measured by a change in absorbance at 340 nm and is proportional to the concentration of glucose in the sample.

In a patient in whom diabetes is suspected but whose fasting glucose is not in the diabetic range, diagnosis is via the oral glucose tolerance test (OGTT). A blood sample is taken under fasting conditions. The patient then drinks a solution containing 75g of glucose and a blood sample is taken two hours later. Diabetes is diagnosed with a blood glucose level >11 mmol/mol in the 2h post-challenge sample. A blood glucose level of 7.8 – 11 mmol/mol 2h post-challenge is diagnostic of impaired glucose tolerance.

B. Monitoring diabetes: HbA1c testing

The extent of glycosylation of haemoglobin (Hb) in the blood is proportional to the blood glucose concentration and the lifespan of red blood cells (which is on average 120 days). Therefore the concentration of glycosylated haemoglobin, HbA1c, gives an estimate of the control of blood glucose concentration over the last 120 days.

Glycosylation of Hb occurs in two steps: reversible formation of a Schiff base (labile A1c) followed by the irreversible conversion to a ketoamine (stable HbA1c). Stable HbA1c is measured by high performance liquid chromatography (HPLC) and an EDTA anti-coagulated whole blood specimen is required. The sample is diluted and haemolysed to release the Hb. An ionic gradient of increasing strength is established across the HPLC column through the mixing of three buffers. Variants of Hb in the specimen are separated according to their relative adsorption to the column under the ionic conditions. The retention time for the major Hb variants, including HbA1c is known, and the concentration of HbA1c is calculated as a percentage of the total haemoglobin content.

HbA1c levels used to be expressed as a percentage of HbA (concentration of HbA1c/concentration of total Hb). The new international standard for HbA1c, introduced by the International Federation of Clinical Chemistry and Laboratory Medicine (FICC), is to express HbA1c in mmol/mol. The conversion between the two units is as follows:

HbA1c (mmol/mol) = (HbA1c (%) - 2.15) x 10.929

The reference ranges of HbA1c for diabetics are shown in table 6.

HbA1c range (mmol/mol)	**Level of control**
42 - 52	Excellent
53 - 63	Good
64 - 74	Poor
> 75	Very poor

Table 6 Reference ranges for HbA1c. The target HbA1c level for diabetic patients is below 53 mmol/mol.

Clinical action is taken (e.g. altering the therapeutic dose of insulin or applying a more stringent diet and exercise regimen) when HbA1c levels exceed 63 mmol/mol.

There are limitations to using HbA1c as a biochemical marker for monitoring diabetes. The reference ranges are based on the assumption that the red blood cell lifespan is 120 days. This figure is an average lifespan and therefore there is natural variation in HbA1c levels between individuals with similar levels of blood glucose. For diabetic patients with comorbidities causing abnormal red blood cell survival, HbA1c is a poor indicator of glucose control. For example, haemolytic anaemia (i.e. decreased red cell lifespan) leads to a decreased HbA1c level, whereas polycythemia (i.e. increased production of red cells by the bone marrow) leads to an increased HbA1c level. In neither situation is the abnormal HbA1c level due to glucose concentration, so HbA1c values must be interpreted with caution in these patients.

The use of HbA1c as a biochemical marker is also limited in patients with particular Hb variants. Variant Hbs may themselves become glycated and elute at a similar time to HbA1c, thus giving a falsely elevated HbA1c level. Patients with the sickle cell trait (heterozygous for HbS) or sickle cell anaemia (homozygous for HbS) have additional HbS peaks on the chromatogram. For carriers, this increases the apparent ‘total Hb’ and therefore gives a lower HbA1c value. For affected individuals there is no HbA, therefore no HbA1c peak, meaning HbA1c cannot be used as a marker.

Modern analysers can have built-in software which recognise the more common Hb variants such as HbS and HbE, and can exclude them from the calculation to provide an accurate estimate of the HbA1c level. Nonetheless, HbA1c results from patients with abnormal Hb variants must be interpreted with caution.

C. Monitoring diabetes: kidney function

The detection of microalbumin in the urine in diabetic patients is indicative of early stages of kidney damage. Early detection and appropriate action can slow the progression to diabetic nephropathy and end stage renal failure. The eGFR of diabetic patients is also regularly assessed to monitor kidney function. Tests for microalbuminuria and eGFR have been discussed above, under Chronic Kidney Disease. More stringent glycaemic control and treatment with ACE inhibitors (which control blood pressure and impart reno-protective benefits) have been shown to reduce microalbuminaemia and slow progression to diabetic nephropathy.

Kidney function should be monitored regularly in diabetic patients, in part because proteinuria may not be pathological (non-pathological, non-CKD proteinuria). Types of non-pathological proteinuria include transient and orthostatic proteinuria. Transient proteinuria is not indicative of significant underlying renal disease; rather it is precipitated by high fever or heavy exercise and disappears upon repeat testing. Orthostatic proteinuria is diagnosed if the patient has no proteinuria in the early morning but has a low level at the end of the day. This usually occurs in tall, thin adolescents or adults under 30 years old and may be associated with severe lordosis (curving of the lower back) (http://emedicine.medscape.com/article/238158-overview)

References

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