CHAPTER 1

Diagnostic Tests in Chronic Kidney Disease

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OVERVIEW

- Urinary protein excretion of < 150 mg/day is normal (~30 mg of this is albumin and about 70–100 mg is Tamm-Horsfall (muco)protein, derived from the proximal renal tubule). Protein excretion can rise transiently with fever, acute illness, UTI and orthostatically. In pregnancy, the upper limit of normal protein excretion is around 300 mg/day. Persistent elevation of albumin excretion (microalbuminuria) and other proteins can indicate renal or systemic illness.
- Repeat positive dipstick tests for blood and protein in the urine two or three times to ensure the findings are persistent.
- Microalbuminuria is an early sign of renal and cardiovascular dysfunction with adverse prognostic significance.
- Microscopic haematuria is present in around 4% of the adult population – of whom at least 50% have glomerular disease.
- If initial GFR is normal, and proteinuria is absent, progressive loss of GFR amongst those people with microscopic haematuria of renal origin is rare, although long-term (and usually community-based) follow-up is still recommended.
- Adults 50 years old or more should undergo cystoscopy if they have microscopic haematuria (MH).
- Any patient with MH who has abnormal renal function, proteinuria, hypertension and a normal cystoscopy, should be referred to a nephrologist.
- Blood pressure control, reduction of proteinuria and cholesterol reduction are all useful therapeutic manoeuvres in those with renal causes of MH.
- All MH patients should have long-term follow-up of their renal function and blood pressure (this can, and often should be, community-based).
- Renal function is measured using creatinine, and this is now routinely converted into an estimated glomerular filtration rate (eGFR) value quickly and easily.
- The most common imaging technique now used for the kidney is the renal ultrasound, which can detect size, shape, symmetry of kidneys, and presence of tumour, stone or renal obstruction.

Symptoms of chronic kidney disease (CKD) are often non-specific (Table 1.1). Clinical signs (of CKD, or of systemic diseases or syndromes) may be present and recognised early on in the natural history of kidney disease but more often, both symptoms and signs are only present and recognized very late – sometimes too late to permit effective treatment in time to prepare for dialysis. However the most commonly performed test of renal function – plasma creatinine – is typically performed in every hospital inpatient and as part of investigations or screening during many GP surgery or hospital clinic outpatient episodes.

Table 1.1 Signs and symptoms of chronic kidney disease

Symptoms	Signs
Tiredness	Pallor
Anorexia	Leuconychia
Nausea and vomiting	Peripheral oedema
Itching	Pleural effusion
Nocturia, frequency, oliguria	Pulmonary oedema
Haematuria	Raised blood pressure
Frothy urine	
Loin pain	

Unlike 'angina' or 'chronic obstructive airways disease' where a history can be revealing (e.g. walking distance; cough) there is little that is quantifiable about CKD severity without blood and/or urine testing.

This is why serendipitous discovery of kidney problems (haematuria, proteinuria, structural abnormalities on kidney imaging, or loss of kidney function) is a common 'presentation'. A full understanding of what these abnormalities mean and a clear guide to 'what to do next' are particularly needed in kidney medicine, and filling this gap is one of the aims of this book.

Correct use and interpretation of urine dipsticks and plasma creatinine values (by far the commonest tests used for screening and identification of kidney disease) is the main focus of this chapter. Renal imaging and renal biopsy will also be described briefly.

Urine testing

Urinalysis is a basic test for the presence and severity of kidney disease. Testing urine during the menstrual period in women, and within 2–3 days of heavy strenuous exercise in both genders, should be avoided to avoid contamination or artefacts. Fresh 'mid-stream' urine is best, again to reduce accidental contamination. Refrigeration of urine at temperatures from +2 to +8°C assists preservation. Specimens that have languished in an overstretched hospital laboratory specimen reception area, before eventually undergoing analysis, will rarely reveal all of the potential information that could have been gained.

Table 1.2 The main causes of differently coloured urine

Pink–red–brown–black	Yellow–brown	Blue–green
Gross haematuria (e.g. bladder or renal tumour; IgA nephropathy) **Haemoglobinuria** (e.g. drug reaction) **Myoglobinuria** (e.g. rhabdomyolysis) **Acute intermittent porphyria** **Alkaptonuria** **Drugs**: phenytoin, rifampicin (red); metronidazole, methyldopa (darkening on standing) **Foods**: beetroot, blackberries	**Jaundice** **Drugs**: chloroquine, nitrofurantoin	**Drugs**: triamterene **Dyes**: methylene blue

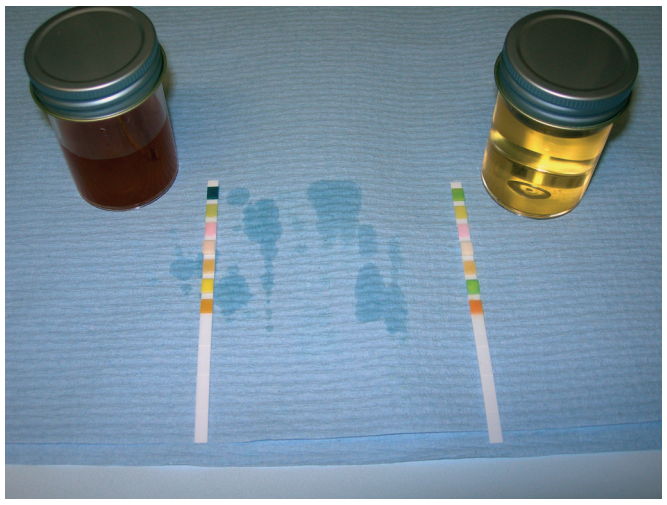


Figure 1.1 Urine dipstick – the urine on the right is normal and the colours of all of the squares on the urine dipstick are normal/negative. The urine on the left is from someone with acute glomerulonephritis, looks pink-brown macroscopically, and has maximal blood and protein on the dipstick.

Table 1.3 The main causes of false negative and positive testing from use of urine dipsticks

Test	False positive	False negative
Haemoglobin	Myoglobin Microbial peroxidases	Ascorbic acid Delayed examination
Proteinuria	Very alkaline urine (pH 9) Chlorhexidine	Tubular proteins Immunoglobulin light chains Globulins
Glucose	Oxidizing detergents	UTI Ascorbic acid

Discounting contamination from menstrual – or other – bleeding, and exercise-induced haematuria and proteinuria

Changes in urine colour are usually noticed by patients. Table 1.2 shows the main causes of different coloured urine. For information concerning changes in urine turbidity, odour and other physical characteristics consult a reference source.

Chemical parameters of the urine that can be detected using dipsticks include urine pH, haemoglobin, glucose, protein, leucocyte esterase, nitrites and ketones. Figure 1.1 shows the dipstick in its 'dry' state, and also an example of a positive test. Table 1.3 shows the main false negative and false positive results that can interfere with correct interpretation.

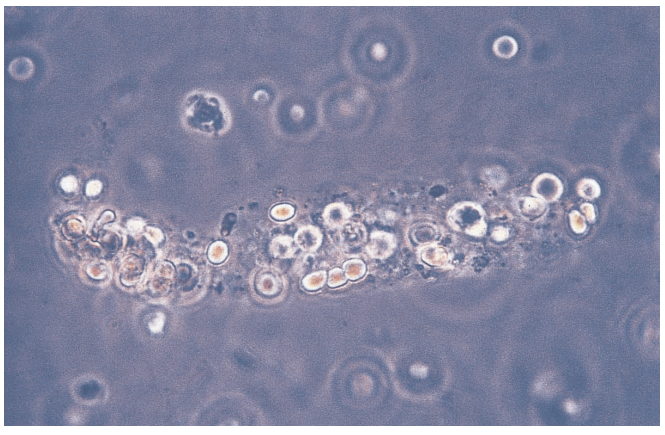


Figure 1.2 Microscopy of centrifuged fresh urine. There is a red cell cast (protein skeleton with incorporated red blood cells). This is characteristic of acute glomerulonephritis.

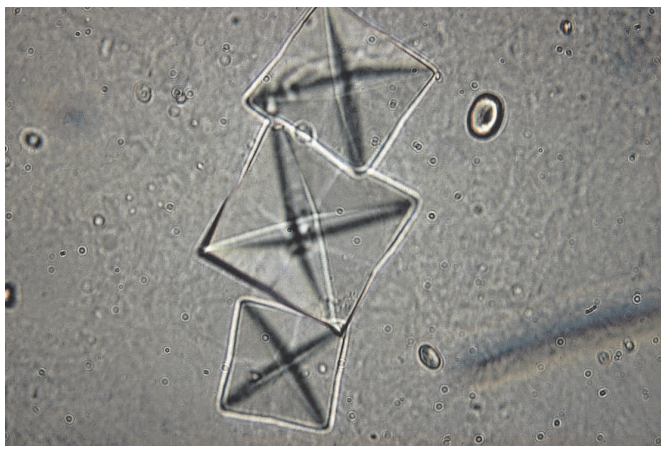


Figure 1.3 Crystalluria.

Urine microscopy can only add useful information to urinalysis when there is a reliable methodology for collection, storage and analysis. This is often lacking, even in hospitals. Early morning urine is best, with rapid sample centrifugation. Under ideal circumstances *cells* (erythrocytes, leucocytes, renal tubular cells and urinary epithelial cells), *casts* (cylinders of proteinaceous matrix), *crystals*, *lipids* and *organisms* can be reliably identified where present in urine. Figure 1.2 shows a red cell cast in urine (indicative of acute renal inflammation). Figure 1.3 shows urinary crystals.

Microscopic haematuria (MH)

Definition and background

In healthy people red blood cells (rbc) are not present in the urine in >95% of cases. Large amounts of rbc make the urine pink or red.

MH is commonly defined as the presence of greater than two rbcs per high power field in a centrifuged urine sediment. It is seen in 3–6% of the normal population, and in 5–10% of those relatives of kidney patients who undergo screening for potential kidney donation.

MH can be an incidental finding of no prognostic importance, or the first sign of intrinsic renal disease, or urological malignancy. It always requires assessment, and most often also requires referral to a kidney specialist or to a urologist.

Clinical features

The finding of MH is usually as a result of routine medical examination for employment, insurance or GP-registration purposes in an otherwise apparently healthy adult. Initially, therefore, MH is an issue for primary healthcare workers. The goal of an assessment is to understand whether:

1. there are any clues available from the patient's history, his/her family history, or from examination, to point to a particular diagnosis, e.g. connective tissue disease, sickle cell disease;
2. the haematuria is transient or persistent;
3. there is any evidence of renal disease, e.g. abnormal renal function, accompanying proteinuria, raised blood pressure (BP);
4. the haematuria represents glomerular (i.e. from the kidney) or extra-glomerular (urological) bleeding.

Investigations

Typically the full evaluation of MH requires hospital-based investigations. Box 1.1 lists these in a logical order.

- *Urine microscopy and culture* should also be undertaken. The presence of dysmorphic red cells in the urine increases the possibility of intrinsic/parenchymal kidney disease as opposed to urological disease. This can only be ascertained in a specialist laboratory.
- *Renal structure* can be assessed with a renal ultrasound scan (this can show stones, cysts and tumours). A plain abdominal film will show radio-opaque renal, ureteric or bladder calculi. Renal function should be assessed by measurement of plasma biochemistry and estimated glomerular filtration rate (eGFR). In addition, proteinuria should be looked for by dipstick analysis of the urine and, if present, a protein/creatinine ratio measured. Proteinuria >0.5 g/24 h (protein:creatinine ratio > 50) suggests glomerular disease and a referral to a kidney specialist is warranted for MH with significant proteinuria, raised BP or abnormal renal function.

Box 1.1 **Investigations required for the work-up of patients with microscopic haematuria**

- Protein:creatinine ratio in fresh urine (if present on urinary dipstick testing)
- Urine microscopy and culture
- Plasma biochemistry and eGFR
- Autoantibody screen e.g. anti-nuclear antibody (ANA) and anti-neutrophil cytoplasmic antibody (ANCA) and complement levels (C3 and C4)
- Renal ultrasound
- Renal CT/MRI (in certain cases)
- Cystoscopy for adults > 50 years of age
- Renal biopsy in certain circumstances

Management

Any patient who presents with persistent microscopic haematuria over the age of 50 should be referred to a urologist. A renal ultrasound and a flexible cystoscopy to exclude urological cancer would normally be undertaken.

Any patient who has abnormal renal function, proteinuria, hypertension and a normal cystoscopy should be referred to a kidney specialist.

Renal biopsy is required to establish a diagnosis with absolute certainty in most cases of 'renal haematuria'. Those patients who have renal impairment, heavy proteinuria, hypertension, positive autoantibodies, low complement levels or have a family history of renal disease should undergo a renal biopsy.

Prognosis

The prognosis for most patients with asymptomatic MH without urological malignancy and no evidence of intrinsic renal disease is very good. It is beyond the scope of this chapter to discuss the prognosis of all the causes of microscopic haematuria, as listed in Table 1.4. However, some general observations apply for those patients in whom there is no structural cause for microscopic haematuria and bleeding is glomerular, and these are given below.

In the presence of impaired renal function, it is mandatory to try to achieve blood pressure control (< 130/80 mmHg) and reduction of microalbuminuria or proteinuria (if present). Angiotensin converting enzyme (ACE) inhibitors or angiotensin II receptor blockers (ARBs) are useful agents as they achieve both of these desired effects. It is very important to recheck plasma creatinine and potassium about 7–14 days after starting ACE or ARB, and regularly thereafter – an increase of >20% in plasma creatinine from baseline, or similar fall in eGFR, or a rise of plasma potassium to exceed 5.5 mmol/L, should occasion recall to consider abandoning the drugs or reducing the dose, further investigations, and dietary advice for potassium restriction if relevant.

It is important that these patients, whether monitored in the community or at a hospital-based clinic, have their urine tested, BP measured and renal function monitored regularly. If not under renal specialist follow-up, the development of hypertension, proteinuria or deterioration in renal function are all indications for re-referral to a specialist unit (see Chapter 2).

Table 1.4 Causes of microscopic haematuria

Renal causes	Systemic causes	Miscellaneous and urological causes
IgA nephropathy	Systemic lupus erythematosus	Cystic diseases of the kidney
Thin basement membrane disease	Henoch–Schönlein purpura	Papillary necrosis
Alport's syndrome		Urothelial tumours
Focal segmental glomerulosclerosis		Renal and bladder stones
Membranoproliferative glomerulonephritis		Exercise-induced haematuria
Post-infectious glomerulonephritis		

Table 1.5 Equivalent ranges for urinary protein loss

	Urine dipstick	Albumin excretion rate (AER) (µg/min ; mg/24 h)	Urinary albumin:creatinine ratio (mg/mmol)	Protein (mg)/ creatinine (mmol)	Urinary protein (mg/24 h)
Normal	0	6–20 ; 10–30	< 2.5 (m) < 3.5 (w)	< 15	< 150
Microalbuminuria	0	> 20–200 ; 30–300	> 2.5 (m) > 3.5 (w)	< 15	< 150
'Trace' proteinuria	Trace	> 200 ; > 300	15–29	15–29	150–299
Proteinuria	+, ++	N/A N/A	N/A	30–350	300–3500
Nephrotic	+++	N/A N/A	N/A	> 350	> 3.5 g

m: men; w: women.

Microalbuminuria (MAU) and Proteinuria (P)

Protein is normally present in urine in small quantities. Tubular proteins (e.g. Tamm-Horsfall) and low amounts of albumin can be detected in healthy people. Microalbuminuria (MAU) refers to the presence of elevated urinary albumin concentrations (currently between lower and upper limits, see Table 1.5); MAU is a sign of either systemic or renal malfunction.

MAU is measured by quantitative immunoassay – and is an important first and early sign of many renal conditions, particularly diabetic renal disease and other glomerulopathies. It is also strongly associated with adverse cardiovascular outcomes. Around 10% of the population can be shown to have persistent MAU. For confirmation, two out of three consecutive analyses should show MAU in the same three-month period.

UAER (urinary albumin excretion rate) – in a healthy population the normal range for UAER is 1.5–20 µg/min. UAER increases with strenuous exercise, high protein diet, pregnancy and urinary tract infections. Daytime UAER is 25% higher than at night (so for daytime urine, an upper normal limit of 30 µg/min is often used). Overnight timed collections can be performed (and microalbuminuric range is an overnight UAER of 20–200 µg/min), but for unselected population screening the albumin:creatinine ratio (ACR) in early morning urine is preferable. An ACR of > 2 predicts a UAER of > 30 µg/min with a high sensitivity.

Increasingly favoured as a screening tool is the urinary protein-creatinine ratio (**PCR**). This is best done on 'spot' early morning urine samples (as renal protein excretion has a diurnal rhythm - see below). This is now preferable to relying on 24 hour urine collections (which are rarely thus). There is an inherent assumption in using PCR that urinary creatinine concentration is 10 mmol/L (in practice it can range from 5–30) but this is of little practical importance for its use as a screening tool. A PCR of 100 mg/mmoL corresponds roughly with 1 gram per litre of proteinuria.

One question often asked is how to 'convert' an ACR to a PCR. At low levels of proteinuria (< 1 g/day), a rough conversion is that doubling the ACR will give you the PCR. At proteinuria excretion rates of > 1 g/day, the relationship is more accurately represented by 1.3 × ACR = PCR.

Table 1.5 attempts to display all of the different ways to express urinary protein to allow for comparisons between methods.

Please note that the normal range for protein excetion in pregnancy is up to 300 mg/day, with clinical significance (pre-eclampsia or renal disease) being more likely once 500 mg or more is excreted per day. See Chapter 6, page 31.

Tests of kidney function

The kidney has exocrine and endocrine functions. The most important function to assess however is renal excretory capacity which we measure as *glomerular filtration rate* (GFR). Each kidney has about 1 million nephrons and the measured GFR is the composite function of all nephrons in both kidneys and conceptually it can be understood as the (virtual) clearance of a substance from a volume of plasma into the urine per unit of time. The substance can be endogenous (creatinine, cystatin C) or exogenous (inulin, iohexol, iothalamate, ^{51}Cr-EDTA, ^{99m}Tc-DTPA). The ideal substance does not exist – ideal characteristics being free filtration across the glomerulus, neither reabsorption from nor excretion into renal tubules, in a steady state concentration in plasma, and easily and reliably measured. Despite creatinine failing several of these criteria it is universally used, and we shall concentrate on interpreting creatinine concentration in urine and blood as it aids derivation of GFR.

The basic anatomy of the kidney and the anatomy and basic physiology of the 'nephron' (the functional component of the kidney), are shown in Figure 4.1 (page 15).

Table 1.6 shows the different ways in which both plasma urea and plasma creatinine may be 'artefactually' elevated or reduced which

Table 1.6 Problems with sole reliance on plasma concentrations of urea and creatinine to determine renal function

Factors independent of renal function that can affect plasma urea	Factors independent of renal function that can affect plasma creatinine	Other factors that can affect interpretation of plasma creatinine values
Hydration	Diet (meat)	Use of Jaffe reaction in laboratories: interference by glucose, ascorbate, acetoacetate
Burns	Creatine supplements (e.g. body builders)	
Steroids	Age	Use of enzymatic reaction in laboratories: interference by ethamsylate or flucytosine
Diuretics	Body habitus	
Liver disease	Race	
Diet (protein)		

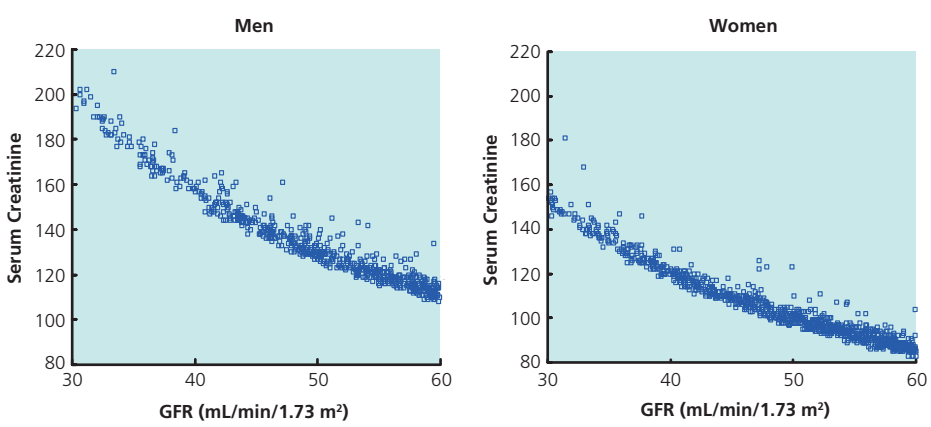


Figure 1.4 Relationship between plasma creatinine and glomerular filtration rate (GFR).

GFR (mL/min/1.73 m²) = 186 × [serum creatinine (μmol/L) × 0.011312]–1.154 × [age]–0.203 × [1.212 if black] × [0.742 if female]

Figure 1.5 Four-variable MDRD equation for eGFR.

can lead to misunderstanding and miscalculation of renal function. Creatinine is measured by two quite different techniques in the laboratory – one, the Jaffe reaction, relies on creatinine reacting with an alkaline picrate solution but is not specific for creatinine (e.g. cephalosporins, acetoacetate and ascorbate), while the other, the enzymatic method, is more accurate. Eventually isotope-dilution mass spectroscopy (IDMS) may render both of these variously flawed techniques redundant, either by direct substitution of method or by allowing IDMS-traceable creatinine values to be reported.

Creatinine is produced at an almost constant rate from muscle-derived creatine and phosphocreatine. However, as can be seen from Fig. 1.4 it is an insensitive marker of early loss of renal function (fall in GFR), and as renal function declines there is correspondingly more tubular creatinine secretion. It varies with diet, gender, disease state and muscle mass.

eGFR

The manipulation of plasma creatinine to derive a rapid estimation of creatinine clearance is very useful clinically, and is now formally recommended (as of April 2006 – see Chapters 2 and 3) to aid appropriate identification and referral of patients with CKD. There are several formulaic ways of doing this, and the formula that has been adopted in the UK, USA and many countries is the four-variable Modified Diet in Renal Disease (MDRD) formula (Fig. 1.5 and Chapter 2), but it must be appreciated that this formula may not be (as) accurate in ethnic minority patients, in the elderly, in pregnant women, the malnourished, amputees, or in children under 16 years of age.

Useful though deriving a value for GFR is, the value derived using the MDRD formula is only an *estimate* whose accuracy diminishes as GFR exceeds 60 mL/min, and values should therefore be viewed as having significant error margins rather than being precise. Values can only properly be used when renal function is in 'steady state', i.e. not in acute renal failure. It is unwise to rely exclusively on the formula between eGFR 60 and 89 mL/min (CKD stage 2) because of its shortcomings, while values > 90 mL/min should be reported thus (i.e. not as a precise figure). There is an urgent unmet need for better markers, and better formulae.

Formal nuclear medicine or research laboratory-derived measures of GFR are expensive, time-consuming and largely (and increasingly) confined to research studies.

Renal imaging

There is a wide range of imaging techniques available to localize and interrogate the kidneys. Table 1.7 gives the preferred methods for a range of conditions. Intravascular contrast studies are still used, though ultrasound has replaced most IVU/IVP examinations. Low osmolar non-ionic agents are less nephrotoxic and better tolerated. Reactions to contrast agents can be severe, though rarely life-threatening. In addition, renal impairment (usually mild and reversible, sometimes severe and irreversible) can be seen after the use of intravenous contrast. In patients with a plasma creatinine > 130 μmol/L (eGFR < 60 mL/min), thought must be given to the wisdom of the investigation. Pre-existing renal impairment, advanced age, diabetes and diuretic use or dehydration significantly increase the risk of contrast-induced nephropathy. The mainstay of prevention is understanding the risk, avoiding dehydration (by judiciously hydrating patients and promoting urine flow) using saline or 0.45% sodium bicarbonate. The dopamine agonist fenoldopam and the anti-oxidant *N*-acetylcysteine have both been proposed as protective agents; oral *N*-acetylcysteine has been widely assessed with conflicting results and its role remains uncertain. However, it is an inexpensive agent

Table 1.7 Renal imaging techniques and their main indications/applications

Condition	Technique
Renal failure	Ultrasound
Proteinuria/nephrotic syndrome	Ultrasound
Renal artery stenosis	MRA
Renal stones	Plain abdominal film
	Non-contrast CT
Renal infection	Ultrasound or CT abdomen
Retroperitoneal fibrosis	CT abdomen

MRA; magnetic resonance angiogram.

Box 1.2 **Reasons for enlarged or shrunken kidneys on renal imaging**

Large kidneys – symmetrical
Diabetes
Acromegaly
Amyloidosis
Lymphoma

Large kidney – asymmetrical
Compensatory hypertrophy (eg. secondary to nephrecotmy)
Renal vein thrombosis

Large kidneys –irregular outline
Polycystic kidney disease
Other multicystic disease

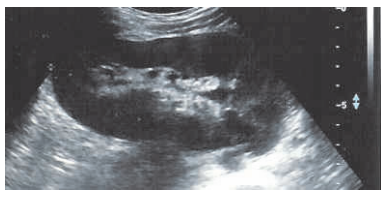
Small kidneys – symmetrical
Chronic kidney disease
Bilateral renal artery stenosis
Bilateral hypoplasia

Small kidney – unilateral
Renal artery stenosis
Unilateral hypoplasia
Scarring from reflux nephropathy

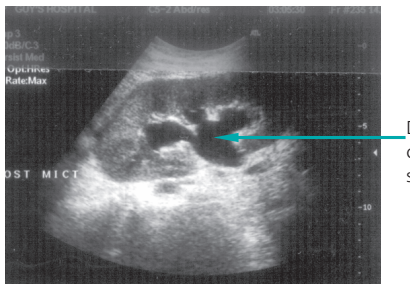
without significant side-effects and its use in clinical practice may not therefore be inappropriate.

A comprehensive review of all imaging techniques is beyond the scope of this chapter. We shall concentrate on *ultrasound* imaging as this is by far the most often used for screening and investigation. Reference to radionuclide imaging, and IVU/IVP is made in Chapter 8. Renal size is usually in proportion to body height, and normally lies between 9 and 12 cm. Box 1.2 shows reasons for enlarged or shrunken kidneys. The *echo-consistency* of the renal cortex is reduced compared to medulla and the collecting system. In adults the loss of this 'cortico-medullary differentiation' is a sensitive but non-specific marker of CKD. Apart from renal size and cortico-medullary differentiation, the other significant abnormalities reported by ultrasound include the presence of cysts (simple, complex), solid lesions, and urinary obstruction. Figure 1.6 shows a normal kidney (a) and an obstructed kidney (b). Examination of the bladder and prostate is usually undertaken alongside scanning of native (or transplanted) kidneys.

Renal angiography and other techniques relevant to renal blood vessels are covered in Chapter 5. Radionuclide imaging is used for



(a)



(b)

Figure 1.6 (a) Ultrasound appearance of a normal kidney - dark areas represent renal cortex, and the central white area is the renal pelvis and collecting system. (b) An obstructed kidney, which shows in its centre a severely dilated renal pelvis and calyces (containing urine which is 'dark' on ultrasound).

renal scars and urinary reflux, which is also mentioned in part in Chapter 8.

Renal biopsy

A renal biopsy is undertaken to investigate and diagnose renal disease in native and transplanted kidneys. Table 1.8 shows the main indications, contra-indications, and complications of this test. It is a highly specialized investigation, which should only be performed after careful consideration of the risk to benefit ratio, and with the close support of experienced imaging and renal histopathological teams.

Further reading

Van de Wal RM, Voors AA, Gansevoort RT. (2006) Urinary albumin excretion and the renin-angiotensin system in cardiovascular risk management. *Expert Opin Pharmacother*; **7**(18):2505–20.
NHS Information National Library for Kidney Disease, www.library.nhs.uk/kidney
www.renal.org/eGFR/haematuria.html
www.renal.org/eGFR/proteinuria.html
www.renal.org/eGFR/refer.html

Table 1.8 Indications for renal biopsy

Indications	Contra-indications	Complications
Nephrotic syndrome	Multiple renal cysts	Pain
Systemic disease with proteinuria or kidney failure	Solitary kidney (relative)	Bleeding – haematoma, haematuria (significant in <5%)
Acute renal failure	Acute pyelonephritis/abscess	Other organ biopsied (e.g. colon, spleen, liver)
Proteinuria (PCR >50–100)	Renal neoplasm	Arterio-venous fistula (0.1%)
Proteinuria and micro/macro-haematuria	Uncontrolled blood pressure	Nephrectomy (<0.1%)
Unexplained chronic renal failure	Abnormal blood clotting	Death (<0.01%)
Transplanted kidney	Morbid obesity (relative)	
	Inability to consent, or to comply with instructions	