



Assessment of kidney function in type 2 diabetes

Date written: April 2009

Final submission: April 2009

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GUIDELINES

Kidney status in people with type 2 diabetes should be assessed by: (Grade B)*

a. Annual screening for albuminuria by:

Albumin Excretion Rate (AER) – timed urine collection.

Microalbuminuria is indicated by:

AER 30–300 mg/24 h or

AER 20–200 µg/min in timed collection

Macroalbuminuria is indicated by:

AER > 300 mg/24 h or

AER > 200 µg/min in timed collection

OR

Albumin: Creatinine Ratio (ACR) – spot urine sample.

Microalbuminuria is indicated by:

ACR 2.5–25 mg/mmol in males

ACR 3.5–35 mg/mmol in females

Macroalbuminuria is indicated by:

ACR > 25 mg/mmol in males

ACR > 35 mg/mmol in females

If AER or ACR screening is positive for microalbuminuria:

Perform additional ACR or AER measurements one to two times within 3 months. Microalbuminuria is confirmed if at least two of three tests (including the screening test) are positive.

If AER or ACR screening is positive for macroalbuminuria:

Perform a 24 h urine collection for quantitation of protein excretion.

AND

b. Annual estimation of the Glomerular Filtration Rate (eGFR).

eGFR < 60 mL/min per 1.73 m² indicates at least moderate kidney dysfunction (Stage 3–5 chronic kidney disease [CKD]).

eGFR 60–90 mL/min per 1.73 m² may indicate mild kidney dysfunction (Stage 2 CKD if albuminuria also present).

Continue annual screening for albuminuria and eGFR in the event of negative screening tests.

*Refer to Table A1: Definition of NHMRC grades of recommendation. Also refer to NHMRC 'National Evidence Based Guidelines for Diagnosis, Prevention and Management of Chronic Kidney Disease in type 2 diabetes' (see <http://www.cari.org.au>) for Levels of Evidence and Evidence Grading which were undertaken in accordance with the NHMRC Hierarchy of Evidence procedure.

SUGGESTIONS FOR CLINICAL CARE

- Screening for microalbuminuria and glomerular filtration rate (GFR) should be performed on an annual basis from the time of diagnosis of type 2 diabetes.
- ACR should be measured using a morning urine sample, however, random urine samples can be used.
- Measurement of urinary albumin can be influenced by a number of factors including:
 - urinary tract infection,
 - high dietary protein intake,

- congestive heart failure,
- acute febrile illness,
- menstruation or vaginal discharge,
- water loading, and
- drugs (NSAIDs, ACEi).

- Tests such as albumin concentration >20 µg/litre or a dipstick test for albuminuria are semi-quantitative and should be confirmed by ACR or AER measurements.
- GFR is most commonly estimated using the Modification of Diet in Renal Disease (MDRD) equation which is based on serum creatinine, age and sex. The MDRD

formula tends to underestimate GFR at levels greater than 60 mL/min but is more accurate at lower levels.

- GFR can be estimated using the Cockcroft-Gault (CG) formula, which is based on serum creatinine, age, sex and body weight. The CG formula tends to underestimate GFR at levels less than 60 mL/min but is more accurate at higher levels.
- Interpretation of eGFR should refer to the Kidney Health Australia report, 'CKD Management in General Practice' (<http://www.kidney.org.au>), in brief:
 - eGFR < 30 mL/min per 1.73 m² indicates severe CKD (Stage 4–5) and if persistent should prompt referral to a nephrologist,
 - eGFR 30 to 59 mL/min per 1.73 m² indicates moderate kidney dysfunction (Stage 3 CKD). Referral to a nephrologist or endocrinologist interested in kidney disease should be considered, and
 - eGFR 60–89 mL/min per 1.73 m² may indicate mild kidney dysfunction. A detailed clinical assessment of glycaemic control, blood pressure and lipid profile is recommended in such cases.

BACKGROUND

Aim of the guideline

This guideline topic has been taken from the NHMRC 'National Evidence Based Guidelines for Diagnosis, Prevention and Management of CKD in type 2 diabetes' which can be found in full at the CARI website (<http://www.cari.org.au>). The NHMRC guideline covers issues related to the assessment and prevention of CKD in individuals with established type 2 diabetes. The NHMRC guidelines do not address the care of people with diabetes who have end-stage kidney disease (ESKD) or those who have a functional renal transplant. In addition, the present guideline does not provide recommendations regarding the management of individuals with established CKD, with respect to the prevention of other (non-renal) adverse outcomes, including retinopathy, hypoglycaemia, bone disease and cardiovascular disease. It is important to note however, that in an individual with type 2 diabetes, the prevention of these complications may be a more important determinant for their clinical care. Consequently, the recommendations made must be balanced against the overall management needs of each individual patient.

How should kidney function be assessed and how often in people with type 2 diabetes?

Screening for CKD aims to identify abnormal urine albumin excretion and declining GFR, so that interventions can be given to slow progression of kidney disease, to prevent ESKD and to reduce the risk of CVD. Assessment of kidney function in people with type 2 diabetes includes measurement of urinary albumin excretion and estimation of GFR for the purposes of screening, diagnosis and monitoring response to management.

In a significant proportion of people with type 2 diabetes, CKD may progress (i.e. declining GFR) in the absence of increasing albuminuria. Thus both GFR and albuminuria are important in screening, diagnosis and monitoring. Albuminuria may be assessed by measurement of the AER or the ACR with AER being regarded as the gold standard. The GFR is most commonly estimated rather than measured.

Albumin excretion typically increases in a continuous manner over several years, rather than showing an abrupt transition from normal to abnormal values. The average increase in AER ranges from 10 to 30% per year until overt nephropathy develops. However, in some people, the rate of increase in AER slows after the stage of microalbuminuria.¹ Regression from microalbuminuria to normoalbuminuria may occur in people with newly diagnosed type 2 diabetes due to interventions or for unknown reasons,^{2,3} while in others regression does not occur.⁴ Regular monitoring of albuminuria in people with type 2 diabetes is warranted on the basis of the rate of progression of albuminuria in type 2 diabetes and ESKD associated with increasing albuminuria and the increased risk of CVD.⁵

There is a high intra-individual variability in 24 h albumin excretion with a coefficient of variation of 40–50%, therefore a diagnosis of persistent microalbuminuria should be based on repeated measurements, especially if long-term treatment of normotensive individuals are being considered. While increasing albuminuria is a risk factor for both CVD and ESKD, cross sectional studies have also shown a high degree of heterogeneity in people with type 2 diabetes compared with type 1 diabetes with respect to CKD. As such a significant proportion of people with type 2 diabetes may have CKD and be normoalbuminuric.^{3,6,7} In the recently reported ARIC study (a population based prospective biracial long-term observational study of 2187 individuals with predominantly type 2 diabetes), 30% of incident CKD (defined as eGFR < 60 mL/min per 1.73 m² or kidney disease at hospitalization) did not have albuminuria (ACR ≥ 30 mg/g).⁸

Cross-sectional studies in people with type 2 diabetes and microalbuminuria have generally shown GFR to be normal, however, increased GFR (hyperfiltration) have been observed. For example in a Danish study 158 microalbuminuric patients had an increased GFR of 139 ± 29 mL/min compared with 39 normoalbuminuric patients (115 ± 19 mL/min) and 20 control subjects without diabetes (111 ± 23 mL/min).⁹ However, the cross-sectional study by Premaratne *et al.*¹⁰ of 662 Australian people with type 2 diabetes showed no significant difference in AER and prevalence of microalbuminuria between hyperfilters and normofilters. Although not recognized as a stage of CKD, hyperfiltration (GFR > 130 mL/min per 1.73 m²) represents an early phase of kidney dysfunction in diabetes. However, its clinical significance remains controversial. By definition, this phase can only be detected by measurement of GFR.

In people who do not have diabetes, the expected rate of decline in GFR with ageing is approximately 1 mL/min per year.¹¹ A proportion of people with type 2 diabetes show a more rapid decline in GFR, in the absence of microalbuminuria or macroalbuminuria.¹² In people with type 2 dia-

betes and established nephropathy, some but not all longitudinal studies have documented a decline in GFR without intervention of about 10 mL/min per year.¹³ In people with type 1 diabetes, and overt kidney disease, the extent of early reduction in AER by ACEi predicts the degree of protection from subsequent decline in GFR.¹⁴ Whether this occurs in people with type 2 diabetes is not yet known.

Lack of uniformity in results on decline in GFR in longitudinal studies is in part due to study design, since most studies have focussed on albuminuria and have been too short to document clinically significant changes in GFR. In a Japanese study over 48 months, no change in GFR was demonstrated in 48 patients who were either untreated or treated with nifedipine, enalapril or both drugs.¹⁵ In another study of 103 normotensive Indians over 5 years, there was no change in GFR during treatment with placebo or enalapril.¹⁶

By contrast, two studies have shown a significant decline in GFR in at least one study arm. In a 5 year study of 94 middle aged normotensive Israelis, GFR remained stable in those treated with enalapril but declined in those treated with placebo.¹⁷ This study used the inverse of the serum creatinine level as an index of GFR. In a 3-year study of 18 hypertensive Italians, the GFR (measured isotopically) decreased in those treated with cilazapril or amlodipine.¹⁸ In 3 long-term studies of microalbuminuric kidney function in people with type 2 diabetes there was no change in serum creatinine over 5 years in 102 hypertensive patients from Hong Kong,¹⁹ 3 years in 10 hypertensive Italian patients²⁰ and 3 years in both normotensive and hypertensive French patients.²¹

Screening will result in identification of individuals who have an increased risk of kidney and cardiovascular morbidity and mortality. In people with type 2 diabetes and microalbuminuria, a reduction in AER has been documented with improved glycaemic control, blood pressure control, lipid profile optimization and specific renoprotective therapy with ACEi, or ARB.¹ Thus screening should not be reserved for known high risk populations (e.g. age >40 years, Australian Aborigines, positive family history of kidney disease) but should be offered to all people with type 2 diabetes.

Laboratory methods for albuminuria

The methods which can be used to assess urinary albumin and protein excretion include:

- Dipstick,
- Measurement of AER on timed urine samples, and
- Measurement of ACR on spot urine.

Timed urine collection, either 24 h or overnight (usually 8 h) is considered the gold standard for the measurement of albuminuria.²² Shorter timed collection periods can be used (e.g. 4 h) but these are time consuming for both patients and staff. AER and ACR on early morning urine are preferred as these tests are not subject to concentration bias.

Considerations in choosing a particular test for assessment of albuminuria include:

- The purpose for which the test is being performed,
- The performance of the assay, and
- The convenience and practicalities of specimen collection.

The evidence for how kidney function should be assessed consists mainly of cross sectional studies assessing various diagnostic tests against a reference method. In various clinical situations, ACR has been proposed as both a screening and diagnostic test for kidney disease.²³ However, many have recommended the use of ACR only in screening,^{24–27} as the test has a high false positive rate and low specificity. Albumin-to-creatinine ratio is also considered to have a useful monitoring role in diabetes with respect to detecting kidney disease progression and the evaluation of treatment effects.²⁸

All of the original assessments of microalbuminuria were based on AER measurements in timed urine collections. AER measurements performed in this way are still regarded as the gold standard for assessment of microalbuminuria. This presumes that the assay technique is sufficiently sensitive, the inter-assay coefficient of variation is less than 15% and at least 2 of 3 urine samples are in the appropriate range before a diagnosis of microalbuminuria is made.²⁹

Albuminuria is commonly measured in the clinical laboratory by one of the following methods: radioimmunoassay (RIA), nephelometry (NEPH), immunoturbidimetry (IT) or radial immunodiffusion (RID). All of these methods are available as commercial kits. RIA is considered as the reference method for albumin measurement as it is the longest established assay. In an evaluation of RID, IT, NEPH against RIA the intra and inter-assay coefficient of variation (CV) of the methods were not found to be significantly different.³⁰ A second study has also found similar degrees of precision and accuracy between the RIA, RID, and IT methods. The IT method was found to be consistently lower than the RIA method (the difference was greatest for albumin concentrations >30 mg/L) although the difference was considered to be not clinically important.³¹ Comparison of albumin concentrations measured by the different methods has however, shown greater variability.^{30,31}

Size-exclusion High-Performance Liquid Chromatography (HPLC) has been shown to give consistently higher urinary albumin concentrations particularly in people with diabetes when compared with the routine immunoassay techniques.^{32–35} The difference has been attributed to the presence of immunochemically nonreactive albumin which if measured has been postulated to allow for earlier prediction of microalbuminuria in people with type 1 and type 2 diabetes.³⁴ However, whether HPLC detects a form of albumin not detected by immunoassay (i.e. non-immunoreactive) or other molecules of approximately the same size as albumin, remains unresolved.³⁶ An analysis of the AusDiab cohort, identified both HPLC-detected albumin and albumin detected by immunonephelometry as risk factors for mortality, however, HPLC detected albumin identifies some people at increased risk of mortality that are not detected by immunonephelometry.³⁷ The clinical significance of HPLC *versus* immunoassay detected urinary protein has not been established.²²

The choice of method to be used by a particular laboratory depends on factors such as equipment availability, the number of samples to be processed and the required turn-over time for results.

There are advantages and disadvantages for each of the methods and these are discussed below:

1. Radioimmunoassay (RIA)

Advantages: established reliability.

Disadvantages: assay time of 2 h; rapid deterioration of reagents; handling precautions; needs a gamma counter; expensive; not suitable for a few samples a day; time consuming.

2. Radial immunodiffusion (RID)

Advantages: no sophisticated equipment required; convenient for a small number of samples.

Disadvantages: assay time of 2 h.

3. Nephelometry (NEPH)

Advantages: wide range; assay time of 0.5 h; simple calibration.

Disadvantages: expensive equipment required.

4. Immunoturbidimetry (IT)

Advantages: assay time of 1 h; wide range; least expensive.

Disadvantages: requires multiple samples for standard curves with each assay.

In summary, any of the four methods are suitable for routine use. Variation between methods, however, may influence comparison of results between laboratories or by different methods within the one laboratory.

A number of groups have demonstrated that storage of frozen urine samples (for 2 weeks to 6 months) at -20°C results in lower measurements of microalbuminuria compared with freshly analysed samples.^{38,39} However, one group has reported that adequate mixing (3–4 hand inversions) after thawing of frozen aliquots resulted in the same albumin values as unfrozen aliquots measured by nephelometry.⁴⁰ This same group found however, that a small number of samples (2–9), despite mixing, gave falsely low urinary albumin results by up to 50%. It is postulated that freezing may distort the target albumin antigen in such a way that antibodies may not detect all of the albumin present.

Studies of unfrozen urine samples stored at 4°C for up to 8 weeks have shown no significant effect on urinary albumin.³⁹ It has also been reported that albumin in urine is stable when stored at room temperature for 1 week.⁴¹ In view of these findings, it is considered that urinary albumin measurement should either be analysed as fresh specimens or stored unfrozen at 4°C and assayed within 8 weeks. Timed urine collection (either overnight or 24 h) or a single void early morning urine sample should be obtained.

Confounding factors in assessment of albuminuria

Urinary albumin results can be affected by several confounding factors and the interpretation of albuminuria should take these into consideration. The following factors may affect urinary albumin results.^{26,42}

- Urinary tract infection,
- High dietary protein intake,
- Congestive heart failure,

- Acute febrile illness,
- Menstruation or vaginal discharge,
- Water loading, and
- Drugs (NSAIDs, ACE inhibitors).

In addition it is advisable to avoid assessing AER within 24 h of high-level exercise or fever.

Glomerular Filtration Rate

An accurate measure of GFR can be undertaken using low molecular weight markers of kidney function such as inulin, iothexol or technetium (labelled DTPA), however, the methods are time consuming, expensive and generally not available.⁴³ In addition to direct measurement of GFR by isotopic methods there are several methods for estimating GFR. The measurement of 24 h creatinine clearance tends to underestimate hyperfiltration and overestimate low GFR levels and is subject to errors in urine collection unless great care is taken. The regular measurement of serum creatinine levels is simple to perform and is currently the most common method. However, because creatinine is invariably reabsorbed by the renal tubules, serum creatinine and creatinine clearance measurements tend to underestimate the GFR in the context of hyperfiltration and over estimate the GFR in the context of hypofiltration.⁴⁴

In addition, for optimal approximation of GFR from serum creatinine measurements allowances need to be made for age, gender, height and weight of the individual. If the variables are taken into account, as in the CG and MDRD equations, a satisfactory index of GFR can be achieved. This is particularly important in thin elderly female people whose baseline serum creatinine levels may be as low as $40\text{--}50\ \mu\text{M}$. In these people delay in referral until the serum creatinine rises above $110\ \mu\text{M}$ would imply that more than 50% of kidney function had been lost.⁴⁵

The 6 variable and 4 variable MDRD equations used for the estimation of GFR were developed from general populations (i.e. not specifically people with type 2 diabetes). The 6 variable equation, which is the most commonly used equation for the estimation of GFR, was derived from the MDRD study and includes the variables: creatinine, age, gender, race, serum urea nitrogen and serum albumin as follows:⁴⁶

- $\text{eGFR} = 170 \times \text{serum creatinine (mg/dl)} - 0.999 \times \text{age (years)} - 0.176 \times 0.762 \text{ (if female)} \times 1.18 \text{ (if male)} \times \text{serum urea nitrogen (mg/dl)} - 0.17 \times \text{albumin (g/mL)} + 0.318$

The 6 variable MDRD equation correlated well with directly measured GFR ($R^2 = 90.3\%$).

The modified 4 variable MDRD, again developed from general populations and not specific to people with type 2 diabetes is as follows:⁴⁵

- $\text{eGFR} = 186 \times \text{serum creatinine} - 1.154 \times \text{age} - 0.203 \times 1.212 \text{ (if black)} \times 0.742 \text{ (if female)}$

The 4 variable MDRD equation also correlated well with directly measured GFR ($R^2 = 89.2\%$). By contrast, 24 h creatinine clearance or the CG equation overestimated sub-normal GFR levels by 19% and 16%, respectively.^{45,47}

The position statement of the Australasian Creatinine Consensus Working Group recommend that an eGFR be automatically calculated and reported for every request for

serum creatinine measurement in people of 18 years and over using the abbreviated MDRD equation.⁴³ On the basis of survey and anecdotal information, the group considered that the vast majority of laboratory reports in Australia and New Zealand comply with this recommendation.⁴⁸ Some key aspects of the recommendations from the Australasian Creatinine Consensus Working Group are summarized below:

- Pathology laboratories should automatically report eGFR calculated using the '175' MDRD formula, with every request for serum creatinine.
- eGFR values over 90 mL/min per 1.73 m² should only be reported as >90 mL/min per 1.73 m².
- Pending further studies laboratories also should report eGFR for Australian Aboriginal and Torres Strait Islander peoples and other ethnic groups (previously no recommendation had been made).

Measurement of serum cystatin C can be also used to estimate GFR. This may be more accurate than creatinine based eGFR methods particularly at normal levels (90–120 mL/min) or above normal levels (>120 mL/min) but the assay is more expensive and is not yet generally available. Serial measurements of cystatin C levels have been shown to estimate progressive decline of GFR more accurately than creatinine based methods in both type 1 and type 2 diabetes. As with serum creatinine, the cystatin C is affected by factors other than the GFR and as with creatinine, knowledge of these factors is required in both estimating the GFR and in the interpretation of eGFR in particular populations. Currently the non GFR factors associated with cystatin C are poorly defined which limits the routine application of serum cystatin C in the estimation of GFR both in people with and without type 2 diabetes.^{49–51} The recent review by Stevens *et al.*⁵¹ indicated many factors other than GFR to be associated with serum cystatin-C, including diabetes, measures of body size, higher C-reactive protein, higher white blood cell and lower serum albumin. The impact of these non GFR factors on serum cystatin C appear to be less than the non GFR influences on serum creatinine, however, they remain poorly defined and may introduce significant variability within select sub populations. The recent study by Tidman 2008⁵² concluded that the use of cystatin C only as 'a determinant of eGFR does not yield improved accuracy' over estimation using the MDRD formula alone, however, a formula that combines both serum creatinine and cystatin C may provide greater accuracy, consistent with the conclusions made by.⁵¹

SEARCH STRATEGY

Databases searched: The search strategies were designed to reduce bias and ensure that most of the relevant data available on type 2 diabetes were included in the present review and were similar to those detailed in the Cochrane Collaboration Reviews Handbook (Higgins JPT *et al.*). The electronic databases searched were Medline, EMBASE, Cochrane Library, CINAHL, HTA and DARE. The detailed search strategy, research terms and yields are provided in Appendix 3 of the complete guideline document

that can be found on the CARI website (<http://www.cari.org.au>).

Date of searches: 28 March 2008.

WHAT IS THE EVIDENCE?

Microalbuminuria and CKD

Microalbuminuria is a key predictor for the development of CKD in people with type 2 diabetes, however, CKD may develop in the absence of abnormalities in albumin excretion (Level II – Prognosis).

Two retrospective studies in the early 1980s demonstrated that small increases in urinary AER predicted the development of overt nephropathy in people with type 1 diabetes.^{53,54} This increase in AER was termed microalbuminuria and by consensus, referred to levels of AER of 20–200 µg/min in at least two of three samples. By comparison, in healthy subjects, AER ranges from 3 to 11 µg/min⁵⁴ and routine dipstick tests do not become positive until AER exceeds 200 µg/min (equivalent to total proteinuria of 0.5 g/24h). Subsequent studies showed that microalbuminuria also predicts the development of clinical overt diabetic nephropathy in type 2 diabetes^{55,56} although it is not as strong a predictor as it is in type 1 diabetes. Persistent microalbuminuria confers an approximately 5-fold increase in the risk of overt nephropathy over 10 years in Caucasian persons with type 2 diabetes (approximately 20% cumulative incidence), compared with a 20 fold increase in risk of nephropathy in type 1 diabetes (approximately 80% cumulative incidence). However, in certain ethnic populations with a high prevalence of type 2 diabetes and diabetic nephropathy, including Pima Indians, Mexican Americans, African Americans, Maoris and Australian Aborigines, microalbuminuria is as strong a predictor of nephropathy as in type 1 diabetes.^{56–58}

The prospective cohort type study of 599 normoalbuminuric people with type 2 diabetes,⁵⁹ found the baseline AER as a significant predictor of a subsequent decline in renal function as well as the risk of mortality and CVD (median follow-up of 8 years).

The usefulness of microalbuminuria as a predictor of overt nephropathy in people with type 2 diabetes is shown in the accompanying Table A2 adapted from Parving *et al.*⁶⁰ The selected studies are RCTs of varying size and duration that measured the progression of albuminuria as a primary outcome. Parving *et al.*⁶⁰ concluded that the studies collectively show the value of microalbuminuria as a predictor of overt nephropathy based on the rate of development of overt nephropathy among the placebo groups.

Other prospective studies where the rate of decline in GFR was found to be enhanced in people with microalbuminuria are:

- Murussi *et al.*⁶¹ (n = 65) – normoalbuminuric people with type 2 diabetes showed a similar rate of decline in GFR over a 10 year period (<2 mL/min per 1.73 m² per year) as people without type 2 diabetes. In contrast in people with type 2 diabetes and microalbuminuria a GFR decline of 4.7 mL/min per 1.73 m² per year was recorded.

- Murussi *et al.*⁶² ($n = 193$) – the urinary albumin excretion (UAE) rate (even within the normal limits) was a significant baseline predictor of mortality (rate of 19%) over an 8 year follow up period while eGFR was not significant. Baseline UAE was also a predictor of micro- and macroalbuminuria which had a cumulative incidence of 26%.

While microalbuminuria in people with type 2 diabetes is an important risk factor for CKD and CVD, it is important to recognize that kidney disease in type 2 diabetes is more heterogeneous than in type 1 diabetes and that a significant number of people will develop CKD (i.e. declining GFR) without development of persistent microalbuminuria as shown in the following studies.

In a US population cross sectional study reported by Kramer *et al.*⁶³ 13% of adults with type 2 diabetes had CKD as defined by an eGFR < 60 mL/min per 1.73 m². Of these 30% had neither abnormal albuminuria or retinopathy taking into account the use of ACE inhibitors. Similarly, Tsalamandris *et al.*¹² reports that in 40 adults with worsening kidney disease and both type 1 diabetes ($n = 18$) and type 2 diabetes ($n = 22$), 8 of the 22 people (36%) with type 2 diabetes had normal albumin excretion over the 8–14 year follow-up period, while the creatinine clearance declined at a rate of 4 mL/min per year.

In a small prospective cohort study ($n = 13$) of type 2 diabetes outpatients who were normotensive to borderline hypertensive, in the absence of hypertensive agents, a median rate of GFR decline of 4.5 (0.4–12) mL/min per year with a rise in albuminuria of 494 (301–1868) to 908 (108–2169) mg/24 h ($P = 0.25$) was observed, however, there was no significant correlation between change in albuminuria and decline in eGFR.⁶⁴

In a retrospective cross sectional study of 301 adults with type 2 diabetes attending an outpatients clinic in Melbourne, the majority with reduced measured GFR (< 60 mL/min per 1.73 m²) were found to have microalbuminuria or macroalbuminuria, however, 39% (23% after exclusion of individuals using ACEi or ARB antihypertensives) were found to be normoalbuminuric. The rate of decline in measured GFR in this group was 4.6 mL/min per 1.73 m² per year and was not significantly different to people with microalbuminuria and macroalbuminuria.⁶⁵

A prospective cohort study of 108 people with type 2 diabetes with microalbuminuria or macroalbuminuria found the course of kidney function to be heterogeneous.⁶⁶ Of those who progressed from microalbuminuria to macroalbuminuria a greater number were classified as progressors as defined by an elevated rate of decline of GFR, and of those who regressed from microalbuminuria to normoalbuminuria a greater number were identified as non-progressors as defined by the rate of decline in GFR. However, the level of AER both at baseline and during the 4-year follow-up was a poor predictor of the loss of kidney function among microalbuminuric patients. The authors conclude that the heterogeneity of the course of kidney function meant that abnormalities in AER have a 'different renal prognostic value' among subgroups of people with type 2 diabetes.

These studies demonstrate that a significant decline in GFR may occur in adults with type 2 diabetes in the absence of increased urine albumin excretion. Thus screening of people with type 2 diabetes needs also to include GFR in order to identify individuals at increased risk of ESKD.

Measurement of Albuminuria

AER and ACR are the most common and reliable methods to assess albuminuria based on sensitivity and specificity, however, both methods are subject to high intra-individual variability so that repeat tests are needed to confirm the diagnosis (Level III – Diagnostic Accuracy).

A systematic review of the effectiveness of screening methods for microalbuminuria in the prevention of nephropathy in people with both type 1 diabetes and type 2 diabetes has been undertaken by.⁶⁷ Key findings of the review were:

- No controlled trials of microalbuminuria screening were identified.
- Quantitative tests (AER and ACR) have reported sensitivities of 56% to 100% and specificities of 81% to 98%. Test performance was similar for all types of urine samples.
- Semiquantitative tests (e.g. Micral) have reported sensitivities of 51% to 100% and specificities of 21% to 100%. The sensitivity has been reported to vary with the level of experience of the operators being lowest for general practitioners and highest for laboratory technicians. Thus accuracy may not be reliable in all settings.

Assessment of proteinuria by spot protein: creatinine ratio is appropriate for macroalbuminuria (100% sensitivity, 92% specificity).⁶⁸ However this is not sufficiently sensitive for assessment of microalbuminuria. Previous studies have shown the inherent variability in 24 h AER to be in the range of 40–50%.⁶⁹ This variability is thought to be related to such factors as posture, activity level, diet and glycaemic control. The variability of overnight AER has been shown to be similar to 24 h collections however, the AER in overnight urine samples is 25% lower compared with 24 h urine samples, and has a lower intra-individual variability.⁷⁰

Screening tests are designed to maximize true positive results (i.e. high sensitivity) at the expense of performing a greater number of confirmatory tests. Several studies have examined the relationship between AER and ACR performed on the same timed urine sample,^{23,71–74} however, only 2 of these took gender into account.^{23,71} A number of studies have also compared ACR on a spot urine or early morning sample with a timed AER,^{70,74–77} however, none of these studies were stratified by gender. In these studies timed urine collections were used as the gold standard for comparison. Using the recommended cut-off values, the sensitivities of spot ACR in these studies were $\geq 88\%$. However different definitions for microalbuminuria on the timed collections (15–30 $\mu\text{g}/\text{min}$) as well as varying definitions for a 'positive' ACR level (2.0–4.5 mg/mmol) were used.

Because of high intra-individual variability, transient elevations of AER into the microalbuminuric range occur frequently. The 95% CI for a sample with AER of 20 $\mu\text{g}/\text{min}$

min, assuming a coefficient of variation of 20%, are 12–28 $\mu\text{g}/\text{min}$ (one measurement), 14–26 $\mu\text{g}/\text{min}$ (two measurements) and 15–25 $\mu\text{g}/\text{min}$ (three measurements).⁷⁸ Therefore, clinical assessment should be based on at least two measurements taken over 3–6 months.

Another option for assessment of albuminuria is the ACR which is usually performed on an early morning urine but can also be performed on a random sample. The use of ACR for assessment of microalbuminuria is easier and less time-consuming for the patient than measurement of AER. ACR measurements are particularly useful for screening purposes and for assessing the effects of treatment. For instance, measurements at every visit can be used to evaluate the albuminuric response separately from the blood pressure response during titration of antihypertensive therapy. Comparisons of ACR to the gold standard AER have been made in several studies. All the studies show satisfactory sensitivity (80–100%) and specificity (81–100%) (see Table A3). Table A3 includes a summary of the key components of the cross sectional studies in relation to the assessment of the applicability of ACR.

A large study of people with type 2 diabetes from the United States showed that ACR, measured on a random urine sample, in the range 3.0–37.8 mg/mmol was over 88% sensitive and specific for the presence of microalbuminuria.⁷⁷ However it is important to note that the microalbuminuria range for ACR is influenced by both gender and age. There were approximately 30% false positives for ACR in people aged >65 years in a more recent study by Houlihan *et al.*⁷⁹ For these reasons ACR has limitations as a diagnostic test but remains an excellent screening test for microalbuminuria.

ACR performed on overnight urine samples has been reported in a number of studies as the least variable parameter (lowest co-efficient of variation) for measuring microalbuminuria. The coefficient of variation for the day to day variability or urinary creatinine excretion is in the range of 8–13%⁸⁰ and 40–50% for AER.⁶⁹ As discussed by others, the reasons for this variability include changes in blood pressure, activity and fluid intake for albumin excretion, and changes in dietary protein intake for creatinine excretion.^{26,81} Previous studies have shown the intra-individual coefficient of variation for ACR to be 49% in first morning urine samples⁸² compared with 27% in timed overnight urine collections. ACR on overnight urine collections has been found to be the least variable parameter for the measurement of microalbuminuria.^{80,83}

ACR is influenced by gender such that for a similar degree of albuminuria the ACR will be lower in males. Ageing has not been widely recognized as an important predictor of ACR and current guidelines only take gender into account as indicated in the review article by.⁴² In one study examining the inter-individual variability of urinary creatinine excretion and influence on ACR in people with diabetes, only gender and body mass index, but not age, were found to be significant determinants.²³ In that study however, the individuals age range was relatively narrow at 36–68 years. In a more recent study in a clinic population with a wide age range (18–84 years)⁷⁹ and in one recent

large study age was shown to have a significant effect on urinary creatinine excretion and on the relationship between ACR and AER.⁷¹

The gender specific microalbuminuria cut-off values for ACR of ≥ 2.5 mg/mmol and ≥ 3.5 mg/mmol in males and females, respectively, are equivalent to an AER of 20 $\mu\text{g}/\text{min}$. These cut-off values have been supported in a study comparing timed overnight AER and ACR on the same sample in which the values of ACR corresponding to AER of 20 $\mu\text{g}/\text{min}$ were 2.4 (95% CI: 2.2–2.7) in males and 4.0 (95% CI: 3.5–4.7) in females.⁸³ In the study of 314 patients, using regression analysis, a 24 h AER of 20 $\mu\text{g}/\text{min}$ yielded 24 h ACR values of 2.5 (95% CI: 2.3–2.6) mg/mmol for males and 3.6 (95% CI: 3.4–3.7) mg/mmol for females. Spot ACR data, however, produce higher ACR values at 20 $\mu\text{g}/\text{min}$, and had wider confidence limits.⁷⁹

Age influences ACR such that for the same degree of albuminuria, ACR will increase with age. By definition, the ACR is dependent on albumin and creatinine excretion rates. The influence of age and sex on 24 h urinary creatinine is well established. For example, one large population-based Belgian study of over 4000 people (26–60 years) demonstrated significantly lower creatinine excretion in females and significant negative correlation of 24 h urinary creatinine excretion with age.¹¹ Therefore, increases in ACR with age can be explained in part by the age related changes in AER and 24 h urinary creatinine excretion observed in both males and females. Normal ageing is characterized by a progressive decline in skeletal muscle mass and increase in body fat composition. Other age related factors that may influence ACR include the decline in skeletal muscle mass between the 20–80 years of age, which has been estimated to range from 22% to 40%,^{84,85} a decrease in the proportion of muscle in lean body mass⁸⁵ and a lower meat intake in older subjects.⁸¹

Bakker⁷¹ has proposed the use of age-specific cut off values for ACR to help restrict the number of people selected for follow up with timed urine collections. In this large study ($n > 2300$) an increase in the ACR cut-off for each decade, from age group <50 to >70 years, was required to maintain equivalent sensitivities and specificities in each age subgroup. However, the use of both gender and age-specific cut off values for ACR may be confusing and impractical.

The clinical importance of an age-related increase in ACR is an increased false positive rate in older patients (e.g. decreased specificity). Using the recommended cut off values, the age-related increase in false positive rates for spot ACR was approximately 30% for patients of either sex over 65 years limits.⁷⁹

Table A4 presents a summary of studies (including those discussed above) that provide evidence in relation to the use of AER and ACR for the screening and diagnosis of albuminuria. Included in the table is a summary of the key components of the cross sectional studies relevant to assessment of diagnostic accuracy. Where reported the sensitivity and specificity is shown along with the key conclusions made by the authors. It should be noted that only a few of the studies provided PPV and NPV values.

Estimation of GFR

Estimation of GFR (eGFR) based on serum creatinine is a pragmatic, clinically relevant approach to assessing kidney function in people with type 2 diabetes (Level III – Diagnostic Accuracy).

The CG and the MDRD formulas for the estimation of GFR were developed predominantly in individuals without diabetes. Studies involving people with type 2 diabetes, are summarized in Table A5 and are generally consistent with the findings for the large number of studies in non diabetes populations.⁴⁶ Nonetheless, the study by Walser⁸⁶ questioned the acceptability of the CG and MDRD equations for monitoring kidney function in individuals with type 2 diabetes.

SUMMARY OF THE EVIDENCE

- Regular monitoring of kidney function in people with type 2 diabetes is indicated by the high risk of development of CKD and the increased risk of CVD and mortality associated with increasing albuminuria and/or GFR <60 mL/min per 1.73 m².
- The screening, diagnosis and monitoring of treatment is undertaken by measurement of albuminuria and estimation of the GFR (eGFR). AER and ACR are the most common and reliable methods to assess albuminuria, ACR values are affected by gender and thus different values are needed for males and females.
- As a significant proportion of people with type 2 diabetes may have or develop CKD in the absence of albuminuria, estimation of GFR is required in addition to screening for albuminuria.
- There are a range of factors that can influence the values of both ACR and AER in individuals with type 2 diabetes.
- The MDRD equation is the most common method used for the estimation of GFR in Caucasian populations and the most appropriate method for the Caucasian population of Australia.

WHAT DO THE OTHER GUIDELINES SAY?

KDOQI

Clinical Practice Guidelines and Clinical Practice Recommendations for Diabetes and Chronic Kidney Disease, *AJKD*, Suppl 2. 49(2):S46, February 2007. (Note covers both type 1 and type 2 diabetes)

- Patients with diabetes should be screened annually for CKD. The development of CKD can be attributable to diabetes (diabetic kidney disease, or DKD) or other causes.
- Begin initial screening 5 years after the diagnosis of type 1 diabetes and at the diagnosis of type 2 diabetes.
- Screening should include measurements of microalbumin, urinary ACR (albumin-to-creatinine ratio) in a spot

urine sample and estimation of GFR (eGFR). eGFR alone is not an appropriate screening test for CKD in diabetes.

- An elevated ACR should be confirmed in the absence of urinary tract infection with 2 additional first-void specimens collected over the next 3–6 months.
 - Microalbuminuria is defined as an ACR between 30 and 300 mg/g.
 - Macroalbuminuria is defined as an ACR > 300 mg/g.
 - Two of three samples should fall within the microalbuminuric or macroalbuminuric range to confirm classification.

UK Renal Association: No recommendation.

Canadian Society of Nephrology: No recommendation.

European Best Practice Guidelines: No recommendation.

NICE Guidelines

- Ask all people with or without detected nephropathy to bring in a first-pass morning urine specimen once a year. In the absence of proteinuria/urinary tract infection (UTI), send this for laboratory estimation of ACR. Request a specimen on a subsequent visit if UTI prevents analysis.
- Make the measurement on a spot sample if a first-pass sample is not provided (and repeat on a first-pass specimen if abnormal) or make a formal arrangement for a first-pass specimen to be provided.
- Measure serum creatinine and eGFR (using the method-abbreviated MDRD four-variable equation) annually at the time of ACR estimation.
- Repeat the test if an abnormal ACR is obtained (in the absence of proteinuria/UTI) at each of the next two clinic visits but within a maximum of 3–4 months. Take the result to be confirming microalbuminuria if a further specimen (out of two more) is also abnormal (>2.5 mg/mmol for men, >3.5 mg/mmol for women).

American Diabetes Association

Standards of Medical Care in Diabetes – 2008. Diabetes Care: 31, S1 January 2008. (Note covers both type 1 and type 2 diabetes)

- Perform an annual test to assess urine albumin excretion in type 1 diabetic patients with diabetes duration of 5 years and in all type 2 diabetic patients, starting at diagnosis.
- Measure serum creatinine at least annually in all adults with diabetes regardless of the degree of urine albumin excretion. The serum creatinine should be used to estimate GFR and stage the level of chronic kidney disease (CKD), if present.
- Continued monitoring of urine albumin excretion to assess both response to therapy and progression of disease is recommended.

IMPLEMENTATION AND AUDIT

No recommendation.

SUGGESTIONS FOR FUTURE RESEARCH

No recommendation.

CONFLICT OF INTEREST

None identified.

ACKNOWLEDGEMENT

The Type2 Diabetes Guidelines project was funded by the Department of Health and Ageing under a contract with Diabetes Australia. The development of the 'National Evidence Based Guidelines for Diagnosis, Prevention and Management of Chronic Kidney Disease in Type 2 Diabetes' was undertaken by CARI in collaboration with The Diabetes Unit, Menzies Centre for Health Policy at the University of Sydney.

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APPENDIX

Table A1 Definition of NHMRC grades of recommendation

Grade of recommendation	Description
A	Body of evidence can be trusted to guide practice.
B	Body of evidence can be trusted to guide practice in most situations.
C	Body of evidence provides some support for recommendations(s) but care should be taken in its application.
D	Body of evidence is weak and recommendation must be applied with caution

Table A2 Progression of microalbuminuria to overt nephropathy in people with type 2 diabetes

Study ID	n	Observation period (years)	Individuals developing overt nephropathy (%/year)
Mogensen 1984 ¹	3	9	2.4
Nelson <i>et al</i> . 1996 ²	50	4	9.3
Ravid <i>et al</i> . 1996 ³	49	5	8.4
Gaede <i>et al</i> . 1999 ⁴	80	4	5.8
Ahmad 1997 ⁵	51	5	4.8
Estacio <i>et al</i> . 2000 ⁶	150	5	4.0
The HOPE Study Group 2000 ⁷	1140	4.5	4.5
Parving <i>et al</i> . 2001 ⁸	201	2	7.5
Parving 2001 ⁹	86	5	7.0
Bruno <i>et al</i> . 2003 ¹⁰	1253 (765 normoalbuminuria, 488 microalbuminuria)	7	3.7

Table A3 ACR – sensitivity and specificity for microalbuminuria screening

Study ID	Reference method for AER	Reference level for AER	ACR urine sample	ACR result	Sensitivity (%)	Specificity (%)
Bakker 1999 ¹¹	Immunoturbidimetry (overnight sample)	20 µg/min	Overnight	2.5 mg/mmol (female) 1.8 mg/mmol (male)	94 (female) 94 (male)	92 (female) 93 (male)
Gatling <i>et al.</i> 1985 ¹²	Micro-ELISA (overnight sample)	AER 30 µg/min	Early morning	>3.5 mg/mmol	86	97
Hutchison <i>et al.</i> 1988 ¹³	Radioimmunoassay (overnight sample)	AER 30 µg/min	Early morning	>3.0 mg/mmol	97	94
Nathan <i>et al.</i> 1987 ¹⁴	Radioimmunoassay (24 h sample)	44 mg/24 h	24 h	3.4 mg/mmol	100	100
Parsons <i>et al.</i> 1999 ¹⁵	Immunoturbidimetry (24 h sample)	20 mg/L	24 h	2.65 mg/mmol	95	79
Poulsen & Mogensen 1998 ¹⁶	Immunoturbidimetry (overnight sample)	AER 3.5 mg/mmol (female), 2.5 mg/mmol (male)	Not stated	>3.5 mg/mmol (female) >2.5 mg/mmol (male)	91	98

Table A4 Summary of studies relevant to evidence for use of AER and ACR screening

Study ID	Study design and Setting	Test	Reference method(s)	Ref level	Urine sample	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Corr.	Comments
Ahn <i>et al.</i> 1999 ¹⁷	Cross-sectional Korea n = 105	UAC, ACR	AER, Immunonephelometry, 24 h		RUS	77, 77 (mic.) 84, 88 (mac)	82, 92 (mic.) 90, 90 (mac)			0.81, 0.75	Albumin measurements (UAC, UACR) in a RUS were considered as a valid test for screening diabetic nephropathy
Bakker 1988 ¹⁸	Cross-sectional Netherlands n = 159	ACR overnight, ALB albumin conc.	AER Immunoturbidimetry, overnight	20 µg/min	Overnight	(F/M) 94, 94 89, 90	(F/M) 92, 93 90, 89				ACR performs better than ALD in screening for microalbuminuria, however the ACR needs sex- and age-specific discriminator values
Cortes-Sanabria <i>et al.</i> 2006 ¹⁹	Cross-sectional Mexico n = 245	Micraltest II – morning	AER Nephelometry, 24 h		Morning	83	96	95	88	0.81, P < 0.001	Micraltest II is a rapid, valid and reliable method for albuminuria screening
Gatling <i>et al.</i> 1988 ²⁰	Cross-sectional UK n = 842	ACR-random, ACR-overnight	AER MicroELISA overnight	30 µg/mL	RUS	96 (overnight) 80 (random)	99.7 (overnight) 81 (random)				An overnight ACR >2 mg/mmol was the optimal screening test.
Houlihan <i>et al.</i> 2002 ²¹	Cross-sectional Australia n = 314	ACR	AER, immunoturbidimetry 24 h	20 ug/mL	morning	(F/M) 93.35, 95.7					The increase in spot ACR relative to 24 h AER with age supports the use of sex- and age-adjusted cut off values for ACR. The clinical significance of the lack of age-adjusted cut off values for ACR is an increased false positive rate in older subjects (31.8% in men >65 years and 28.2% in women greater than 65 years).
Hurchison <i>et al.</i> 1988 ¹³	Cross-sectional Scotland n = 276	Albumin conc., ACR	AER Radioimmunoassay	30 µg/min	First morning	9.8 96.8	90.7 93.9	58.8 68.2		0.904 0.921	Either method was concluded to be acceptable as an initial screening procedure.
Inceri <i>et al.</i> 2005 ²²	Cross-sectional Brazil n = 278	Micraltest II	Immunoturbidimetry, 24 h		RUS	90	46				Measurement of UAC in a random urine specimen was the best choice for the diagnosis or screening of microalbuminuria.
Jermendy <i>et al.</i> 2001 ²³	Cross-sectional n = 192	UAC, ACR	UAE immunoturbidimetry		First void	79.3 (UAC) 74.6 (ACR)	69.5 (UAC) 68.8 (ACR)				Besides the standard measurement of UAE in timed urine samples, the use of convenient morning urinary spot collection could provide useful results.

Mogensen <i>et al.</i> 1997 ²⁴	Cross-sectional Europe/UK n = 2228	Micraltest II for microalbuminuria	Albumin concentration in urine. Immunoturbidimetry, nephelometer, nephelometry	20 mg/L	Spot, first, second morning sample	96.7	71.0	0.78	0.95	Micral Test II permits an immediate and reliable semi quantitative determination of low albumin conc. In urine samples with an almost user-independent colour interpretation ACR is more suitable for monitoring albumin excretion in longitudinal studies than the AER.
Mosca <i>et al.</i> 2003 ²⁵	Cross-sectional Italy n = 87	ACR	AER Immunoturbidimetry, timed overnight							ACR is a useful method for diagnosis DN, depends on gender
Munder <i>et al.</i> 2001 ²⁶	Cross-sectional n = 214	ACR-first void	AER 24 h							0.93, P < 0.01
Nathan <i>et al.</i> 1987 ¹⁴	Cross-sectional US n = 25	Single void	AER Radioimmunoassay, 24 h	20 µg/mL		94	96			0.82 P < 0.0001
Parikh <i>et al.</i> 2004 ABCD ²⁷	RCT US n = 326	Micratest strips + urine specific gravity determination (dipstick)	AER immunoturbidimetry, timed collections	≥30 mg/d		88	80	69	92	While the use of test strips provides a rapid approach to detecting microalbuminuria, the method has limitations.
Zelmanovitz <i>et al.</i> 1998 ²⁸	Cross-sectional Brazil n = 167, 217 urine samples	Timed 24 h urinary protein (UP), UPC, UPCR	24 h UAER I Immunoturbidimetry, timed collections	20 µg/mmol			95.7, 92.9, 76.2			Protein measurement in spot urine is a reliable and simple method for screening and diagnosis of overt diabetic nephropathy

Table A5 GFR estimation studies with people with type 2 diabetes

Study ID	Study type	Findings
Fontseré <i>et al.</i> 2006 ²⁹	Prospective cohort n = 87	The best prediction equation compared with the isotopic method proved to be MDRD with a slope of GFR of $-1.4/-1.3$ mL/min per year compared with the CG formula -1.0 ± 0.9 mL/min per year. Creatinine clearance presented the greatest variability in estimation $P < 0.001$.
Poggio <i>et al.</i> 2005 ³⁰	Cross-sectional n = 249	MDRD equation performed better than the Cockcroft-Gault equation with respect to bias. (1% vs 22%, $P < 0.05$) and accuracy within 30% (63% vs 53%, $P < 0.05$) and within 50% (87% vs 70%, $P < 0.05$)
Rossing <i>et al.</i> 2006 ³¹	Prospective cohort n = 383	Particularly in microalbuminuric (hyperfiltering) patients, GFR is significantly underestimated with wide limits of agreement by the MDRD equation as well as by the CG formula. The rate of decline in GFR is also significantly underestimated with both equations.

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