

Proteinuria tests as useful tools in clinical practice

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The kidneys are amongst the most obscure organs in the body. Unlike the lungs, the heart and the intestines, they do not generate any sound. Unlike the liver and the spleen, they are so deeply seated that palpation is not possible unless they are grossly enlarged. It is said (arguably) that kidneys are like the nephrologists looking after them – they work long hours quietly and diligently in the background. The easiest way to examine the kidneys is to check the substance they produce, the urine. Indeed, as a medical student, the author was taught that ‘urine examination is part of a complete physical examination’.

Tests for proteinuria

It is a common belief amongst patients that frothy urine means proteinuria. While it is true that heavy proteinuria causes frothy urine, in many instances the urine froth noted by the patient is only mild and transient. It is usually due to causes other than proteinuria. The most common cause of such frothy urine is the detergent used to clean the toilet pan!

Urine froth caused by proteinuria is thick and persistent. This can be detected by placing urine in a clean test tube and shaking it (Figures 1 and 2). The urine froth will persist for more than ten minutes. This test is neither sensitive nor specific, but it is useful in bringing the patients to seek

medical attention, (‘the screening test of the common folks’).

The commonest urine test performed in the clinics is the common urine dipstick (Figure 3). It detects mainly albumin in the urine. The test is positive when the urine albumin is >150 mg/L. It is simple and specific.

Another time-honoured test is the sulphosalicylic acid test. Protein can be detected by adding one part urine



Figure 2. Urine froth lasts more than 10 minutes (left tube).



Figure 1. Shaking urine placed in a test tube.



Figure 3. Urine test with dipstick.

to three parts 3% sulphosalicylic acid and observing the turbidity (Figures 4, 5 and 6). The test can be made semi-quantitative by comparing the intensity of the turbidity (Figures 7 and 8). This test is not commonly available in most clinics. It tests both albumin and other heavier proteins in the urine. Thus the test will be positive in cases with 'tubular proteinuria' or 'myeloma kidney urine'. Since the proteins in such conditions are heavy proteins, they are not detected by the common urine dipstick.



Figure 4. Sulphosalicylic acid test.



Figure 5. Adding urine to sulphosalicylic acid.



Figure 6. Observing the turbidity.

The traditional use of proteinuria tests

The tests mentioned above are semi-quantitative only. They are useful in the management of glomerulonephropathies and nephrotic syndrome (Figure 9). Relapses can be detected by the proteinuria tests carried out at home by the patients.

These tests only give a measure of the urine albumin (or protein) **concentration**; therefore, they will be affected by the hydration state of the patient. If a patient gives early morning urine, the urine albumin may be 100 mg/dL. After he drinks plenty of water and the same test is repeated on the new urine sample, the concentration may drop to 30 mg/dL.

For this reason, a quantitative estimation of urine protein per day is more reliable and a 24-hour urine collection is useful in this regard.

Advances made in the past two decades provided answers to the following questions:

1. Is the current dipstick urine test sensitive enough?
2. Does the quantitative urine albumin excretion have any bearing on the clinical management of renal patients?
3. Is there a less cumbersome method to measure daily urine protein excretion?
4. Are there any therapies for proteinuria?



Figure 7. Performing multiple tests.

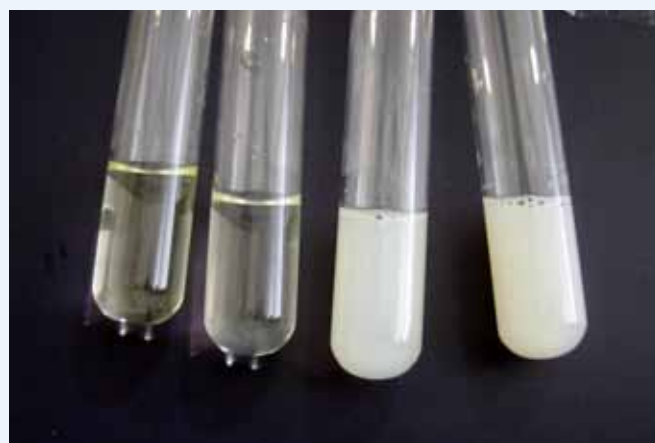


Figure 8. Different concentrations reflected by turbidity.



Figure 9. A patient with nephrotic syndrome.

The discovery of microalbuminuria

One of the most significant recent discoveries in nephrology is microalbuminuria in diabetic patients. It was known that in patients with incipient diabetic nephropathy, there was increased urine albumin excretion at a concentration not yet detectable by the common urine dipstick. It was called 'microalbuminuria'. The term 'micro' referred to the low urine albumin concentration and not the molecular size, as the albumin detected are normal albumin molecules [1].

In quantitative terms, normal urine contains around 20 mg/L of albumin and dipsticks can only detect around 150 mg/L of albumin. A patient is said to have **macroalbuminuria** if urine albumin reaches the concentration which can be detected by the dipstick. Albumin excretion between 20–150 mg/L is thus called **microalbuminuria**. It is an early indicator of diabetic nephropathy as the

risk of diabetic nephropathy increases 10–20 times with microalbuminuria. It is amenable to treatment at this stage.

The detection of microalbuminuria required radioimmunoassay when it was first discovered and was only available in reference laboratories. With the development of immunoturbometric methods, the test is now within the reach of most laboratories. To avoid interference caused by the urine hydration, it is common to test for both the urine (micro) albumin and urine creatinine concentration and the result is expressed as a ratio. Under such circumstances, urine albumin:creatinine ratio >2.5 mg/mmol in men, >3.5 mg/mmol (30 mg/g) for women is regarded as microalbuminuria.

With improvements in medical technology and automation, the test can now be performed in renal clinics as a valuable point-of-care test. In the author's clinic, the early morning urine sample is first tested for albumin using the common urine dipstick. If the test is positive, there is macro-albuminuria and there is no need to do the expensive microalbuminuria test. If the test is negative, a screening test using the Clinitek 50 (Bayer diagnostics) machine is performed (Figure 10). It is a reagent strip test which tests both the albumin and creatinine. The colour changes are read by the machine and the urine albumin:creatinine ratio is calculated (Figure 11). The test takes one minute and is relatively cheap, but the accuracy is not too high.

If the test is positive, it is immediately confirmed with the DCA 2000 test (Figure 12), which takes 7 minutes and is considerably more expensive, but the accuracy is much higher.

Apart from incipient diabetic nephropathy, the presence of microalbuminuria also signals the increased risk of cardiovascular disease. Microalbuminuria is an inflammation marker like the C-reactive protein. It is thus a predictor of a cardiovascular event in a hypertensive patient.



Figure 10. Urine microalbuminuria screening with Clinitek 50.



Figure 11. Reagent strip with Clinitek 50 machine.



Figure 12. Urine microalbuminuria confirmation with DCA2000.

Urine albumin excretion as a clinical tool

For renal patients with macro-albuminuria, the daily urine albumin excretion correlates with the renal outcome. Patients with proteinuria <1 g/day have a much better renal prognosis than a patient with urine protein >3 g/day. Thus urine protein is of paramount importance in the prevention, diagnosis and risk stratification of renal patients.

In his excellent review article [2], Biff Palmer summarized the clinical significance of urine albumin excretion:

1. The magnitude of baseline proteinuria is an accurate predictor of the renal outcome (the renal prognosis).
2. Reduction in urinary protein excretion correlates with long-term preservation of renal function.
3. Apart from renal protection, reduction in urine albumin excretion was associated with reduction in cardiovascular risk.

From the above, it is clear that increased urine albumin excretion is a marker of poor renal prognosis. It is an indication for active interventional measures. Alternatively, reduction of proteinuria is an indication of the response to treatment and signifies improved renal prognosis.

Measurement of daily urine protein excretion

Though urine protein excretion is a useful tool, the collection of 24-hour urine is cumbersome. Recently it was suggested that we send early morning urine for estimation of the urine albumin and creatinine concentration. If both can be expressed in the same unit, (e.g. mg/L), then the urine albumin:creatinine ratio will be a 'pure' number. This number correlates closely with daily protein excretion in g/1.73 m² of body surface area. Thus, a ratio of 3.0 (as with respective urinary albumin and creatinine concentrations of 300 and 100 mg/dL) represents daily protein excretion of approximately 3 g/1.73 m². The accuracy of the total protein-to-creatinine ratio is related to the fortuitous occurrence that daily creatinine excretion is only slightly greater than 1000 mg (8.8 mmol)/day per 1.73 m².

Such methods avoided the timed collection of urine. It is useful for the follow-up of the same patient.

Therapeutic intervention for proteinuria

Proteinuria is a marker of renal progression. There is also evidence that proteinuria is responsible for the interstitial inflammation and fibrosis, contributing to renal progression [3]. Reduction of proteinuria is therefore of paramount importance in patient management.

From the work in diabetic nephropathy, it was shown that angiotensin converting enzyme inhibitors (ACEI)

like captopril could reduce proteinuria in type 1 diabetes mellitus. Later studies showed the renoprotective effects of angiotensin receptor blockers (ARB) in type 2 diabetes mellitus patients. They were successful in increasing the time for doubling of the serum creatinine and other renal events. Apart from lowering the systemic blood pressure, the drugs dilate the efferent glomerular arterioles, thus reducing the glomerular hypertension caused by the chronic hyperglycaemia. Their anti-proteinuria effect is independent of blood pressure reduction.

ACEI and ARB were subsequently shown to reduce proteinuria in **non-diabetic** renal patients in many studies. Studies also showed that when proteinuria is reduced, the progression to end-stage renal failure (ESRD) is reduced.

ACEI or ARB can **delay** the onset of ESRD but it can be **prevented** only in a minority of cases by either drug alone. In other words, there is a limit to their reno-protective effect. A multi-tier approach is therefore needed.

For many years, there were studies researching the use of a combination of ACEI and ARB, aimed at a complete blockage of the renin-angiotensin system to achieve better renal protection. The beneficial effect was confirmed by a recent meta-analysis. It is recommended that if proteinuria is greater than 500 mg/day, despite monotherapy with an ACEI, an ARB can be added (or vice versa). Under such circumstances, monitoring of the serum creatinine and potassium is mandatory. Another approach is aldosterone blockade with spironolactone or eplerenone. There were a number of small clinical trials which suggested the benefits of add-on aldosterone antagonists, but the danger of severe hyperkalaemia is a serious concern.

Apart from lifestyle changes like stopping smoking and weight reduction, the following measures are useful:

1. Dietary protein restriction – **Moderate** dietary protein restriction would reduce the progression of renal disease. Severe dietary protein restriction (e.g. the Giordano-Giovanetti die') tends to cause protein malnutrition and muscle wasting and should be prescribed with great care.
2. Statin therapy – Some trials have shown the anti-proteinuric effect of statin therapy in early renal disease.

Conclusion

Proteinuria exists as a spectrum from microalbuminuria (>300 mg/day) to severe nephrotic range proteinuria (>3.5 g/day). Early detection of microalbuminuria in diabetic patients is the best way to prevent diabetic nephropathy. For patients with macro-albuminuria, quantitation of the proteinuria is useful in disease stratification and monitoring.

The tests for proteinuria have emerged as cost-effective and reliable ways to study the status of the kidneys and the prognosis. Three decades have passed by, and the teaching of our mentors 'urine test is part of a complete physical examination' still remain a golden principle.

Q&A

Please indicate whether the following questions are true or false

- The easiest way to examine the kidneys is to check the substance they produce, the urine.
- Urine froth can be detected by placing urine in a clean test tube and leaving it to sit for one hour.
- The common urine dipstick detects mainly albumin in the urine, and is positive when the urine albumin is >150 mg/L.
- A quantitative estimation of urine protein per day is more reliable and a 36-hour urine collection is useful in this regard.
- A patient is said to have macro-albuminuria if urine albumin reaches the 150 mg/L. Albumin excretion between 20–150 mg/L is thus called microalbuminuria.
- A urine albumin:creatinine ratio >2.5 mg/mmol in men, and >3.5 mg/mmol (30 mg/g) for women is regarded as microalbuminuria.
- A screening test for microalbuminuria uses the radio-immunoassay.
- Apart from incipient diabetic nephropathy, the presence of microalbuminuria also signals the increased risk of cardiovascular disease.
- A ratio of 3.0 (as with respective urinary albumin and creatinine concentrations of 300 and 100 mg/dL) represents daily protein excretion of approximately 8 g/1.73 m².
- Statin therapy may be a useful intervention tool – some trials have shown the anti-proteinuric effect of statin therapy in early renal disease.

Answer these on page 20 or make an online submission at: www.hkmacme.org

ANSWERS TO AUGUST 2009

Classification of venous disease

- True
- True
- False
- True
- True
- False
- True
- True
- False
- False
- False

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rosiglitazone maleate/metformin HCl

Abridged Prescribing Information

ACTIVE INGREDIENTS

Rosiglitazone maleate and metformin hydrochloride

INDICATIONS

AVANDAMET is indicated as an adjunct to diet and exercise to improve glycaemic control in patients with type 2 diabetes mellitus when treatment with dual rosiglitazone and metformin therapy is appropriate.

DOSAGE AND ADMINISTRATION

AVANDAMET in Drug-Naïve Patients (Initial Therapy): The recommended starting dose of AVANDAMET as initial therapy is 2 mg/500 mg administered once or twice daily. For patients with HbA_{1c} >11% or FPG >270 mg/dL, a starting dose of 2 mg/500 mg twice daily may be considered. The dose of AVANDAMET may be increased in increments of 2 mg/500 mg per day to a maximum of 8 mg/2,000 mg per day given in divided doses if patients are not adequately controlled after 4 weeks.

AVANDAMET in Patients Inadequately Controlled with Rosiglitazone or Metformin Monotherapy (Second-Line Therapy): The selection of the dose of AVANDAMET as second-line therapy should be based on the patient's current doses of rosiglitazone and/or metformin. After an increase in metformin dosage, dose titration is recommended if patients are not adequately controlled after 1 to 2 weeks. After an increase in rosiglitazone dosage, dose titration is recommended if patients are not adequately controlled after 8 to 12 weeks.

For patients inadequately controlled on metformin monotherapy, the usual starting dose of AVANDAMET is 4 mg rosiglitazone (total daily dose) plus the dose of metformin already being taken (see Table).

For patients inadequately controlled on rosiglitazone monotherapy, the usual starting dose of AVANDAMET is 1,000 mg metformin (total daily dose) plus the dose of rosiglitazone already being taken (see Table).

Table: AVANDAMET Starting Dose for Second-Line Therapy

PRIOR THERAPY		Usual AVANDAMET Starting Dose	
Total daily dose	Tablet strength	Number of tablets	
Metformin HCl*			
1,000 mg/day	2 mg/500 mg	1 tablet twice a day	
2,000 mg/day	2 mg/1,000 mg	1 tablet twice a day	
Rosiglitazone			
4 mg/day	2 mg/500 mg	1 tablet twice a day	
8 mg/day	4 mg/500 mg	1 tablet twice a day	

* For patients on doses of metformin HCl between 1,000 and 2,000 mg/day, initiation of AVANDAMET requires individualization of therapy.

When switching from combination therapy of rosiglitazone plus metformin as separate tablets: the usual starting dose of AVANDAMET is the dose of rosiglitazone and metformin already being taken.

If additional glycaemic control is needed, the daily dose of AVANDAMET may be increased by increments of 4 mg rosiglitazone and/or 500 mg metformin, up to the maximum recommended total daily dose of 8 mg/2,000 mg.

No studies have been performed specifically examining the safety and efficacy of AVANDAMET in patients previously treated with other oral hypoglycaemic agents and switched to AVANDAMET. Any change in therapy of type 2 diabetes should be undertaken with care and appropriate monitoring as changes in glycaemic control can occur.

Specific Patient Populations: AVANDAMET is not recommended for use in pregnancy. **Geriatric:** The initial and maintenance dosing of AVANDAMET should be conservative in patients with advanced age, due to the potential for decreased renal function in this population.

Renal Impairment: Any dosage adjustment should be based on a careful assessment of renal function. Generally, elderly, debilitated, and malnourished patients should not be titrated to the maximum dose of AVANDAMET. Monitoring of renal function is necessary to aid in prevention of metformin-associated lactic acidosis, particularly in the elderly (see Warnings and Precautions).

Hepatic Impairment: Therapy with AVANDAMET should not be initiated if the patient exhibits clinical evidence of active liver disease or increased serum transaminase levels (ALT >2.5X upper limit of normal at start of therapy). Liver enzyme monitoring is

recommended in all patients prior to initiation of therapy with AVANDAMET and periodically thereafter.

Paediatric: Data are insufficient to recommend paediatric use of rosiglitazone.

CONTRAINDICATIONS

History of hypersensitivity to rosiglitazone, metformin or any other ingredient of the preparation, diabetic ketoacidosis; renal failure; initiation of rosiglitazone combination regimens (like other thiazolidinedione combination regimens) in patients with NYHA Class III and IV heart failure.

WARNINGS AND PRECAUTIONS

Rosiglitazone-metformin is effective only in the presence of insulin and therefore, should not be used in the treatment of type 1 diabetes mellitus. Rosiglitazone-metformin treatment in premenopausal anovulatory patients with insulin resistance may result in resumption of ovulation. These patients may be at risk of pregnancy. Lactic acidosis is a rare, but serious metabolic complication that can occur due to metformin accumulation. Associated risk factors of lactic acidosis should be assessed prior to initiation of metformin, and therefore rosiglitazone-metformin, therapy. If lactic acidosis is suspected, rosiglitazone-metformin should be discontinued and the patient should be hospitalised immediately. Serum creatinine levels should be determined before initiating treatment with rosiglitazone-metformin and regularly thereafter. Special caution should be exercised in patients likely to have renal impairment, or in situations where renal function may become impaired. Rosiglitazone-metformin is not recommended in patients with functional hepatic impairment. Rosiglitazone, like other thiazolidinediones, can cause or exacerbate congestive heart failure in some patients. After initiation of rosiglitazone-metformin, and after dose increases, patients should be monitored for signs and symptoms of heart failure (including excessive, rapid weight gain, dyspnoea, and/or oedema). If these signs and symptoms develop, the heart failure should be managed according to current standards of care. Furthermore, discontinuation or dose reduction of rosiglitazone-metformin must be considered. Rosiglitazone-metformin is not recommended in patients with symptomatic heart failure. Initiation of rosiglitazone-metformin in patients with established NYHA Class III or IV heart failure is contraindicated. Patients experiencing acute coronary syndromes (ACS) have not been studied in rosiglitazone controlled clinical trials. Since patients experiencing ACS are at an increased risk of developing heart failure, and in view of the potential for rosiglitazone to cause or exacerbate heart failure, initiation of rosiglitazone-metformin in patients experiencing an acute cardiovascular event is not recommended. Furthermore, discontinuation of rosiglitazone-metformin during the acute phase should be considered. There is inconsistent evidence regarding the risk of cardiac ischaemia in patients treated with rosiglitazone. A retrospective analysis of mostly short term integrated clinical trials (ICT) showed rosiglitazone to be associated with an increased risk of myocardial ischaemic events in placebo-controlled but not active-controlled trials. This risk was not confirmed in individual large, longer duration studies comparing rosiglitazone to metformin and sulphonylureas. A causal relationship between cardiac ischaemia and rosiglitazone has not been established. Additionally, there is no conclusive evidence on the comparative effects of oral anti-diabetic drugs, including thiazolidinediones, on macrovascular risks and benefits in patients with type 2 diabetes mellitus. A small number of events typically associated with cardiac ischaemia have been observed with the addition of rosiglitazone to patients already receiving insulin therapy and these events occurred at a higher frequency with the insulin plus rosiglitazone combination (2.77%) compared with insulin alone (1.36%). Therefore, rosiglitazone-metformin is not recommended as add-on therapy to patients already receiving insulin. In a separate study, where insulin was added to patients on established rosiglitazone-metformin therapy, there were no heart failure adverse events and one myocardial ischaemic event (angina) in the rosiglitazone-metformin plus insulin arm. In light of these data, for patients establishing on rosiglitazone-metformin receiving add-on insulin therapy, insulin must be titrated cautiously following appropriate clinical evaluation to assess the patient's risk of developing adverse reactions relating to fluid retention and other cardiovascular events. Type 2 diabetes is a major risk factor for coronary heart disease and adverse outcomes following a myocardial ischaemic event. Thus, independent of the choice of anti-diabetic agent, cardiovascular risk factors should be identified and corrective measures taken where possible. Postmarketing reports of new-onset or worsening diabetic macular oedema with decreased visual acuity have been reported with rosiglitazone. Many of these patients reported concurrent peripheral oedema. In some cases the visual events resolved or improved following discontinuation of the drug. Prescribers should be alerted to the possibility of macular oedema if patients report disturbances in visual acuity. Patients taking

rosiglitazone-metformin in triple therapy with a sulphonylurea or insulin may be at risk of dose-related hypoglycaemia. A reduction in the dose of the concomitant agent may be necessary. Rosiglitazone-metformin should be discontinued prior to, or at the time of the test and not reinstated until renal function has been confirmed as normal. In a 4 to 6 year study of glycaemic control with monotherapy in recently diagnosed patients with Type 2 diabetes mellitus, an increased incidence of bone fracture was noted in female patients taking rosiglitazone (9.3%, 2.7 patients per 100 patient years) vs metformin (5.1%, 1.5 patients per 100 patient years) or glyburide/glibenclamide (3.5%, 1.3 patients per 100 patient years). The majority of the fractures in the females who received rosiglitazone were reported in the upper arm, hand and foot. The risk of fracture should be considered in the care of patients, especially female patients, treated with rosiglitazone, and attention should be given to assessing and maintaining bone health according to current standards of care. Close monitoring of glycaemic control and dose adjustment of the rosiglitazone or metformin components may be needed when rosiglitazone-metformin is co-administered with CYP2C8 inhibitors or inducers or cationic drugs that are eliminated by renal tubular secretion.

INTERACTIONS

Rosiglitazone: *In vitro* studies demonstrate that rosiglitazone is predominantly metabolised by CYP2C8, with CYP2C9 as only a minor pathway. Co-administration of rosiglitazone with CYP2C8 inhibitors (e.g. gemfibrozil) resulted in increased rosiglitazone plasma concentrations. Since there is a potential for an increase in the risk of dose-related adverse reactions, a decrease in rosiglitazone dose may be needed when CYP2C8 inhibitors are co-administered. Co-administration of rosiglitazone with a CYP2C8 inducer (e.g. rifampicin) resulted in decreased rosiglitazone plasma concentrations. Therefore, close monitoring of glycaemic control and changes in diabetic treatment should be considered when CYP2C8 inducers are co-administered. **Metformin:** Increased risk of lactic acidosis in acute alcohol intoxication. Cationic drugs that are eliminated by renal tubular secretion (e.g. cimetidine) may interact with metformin by competing for common renal tubular transport systems. Therefore, close monitoring of glycaemic control and changes in diabetic treatment should be considered when cationic drugs that are eliminated by renal tubular secretion are co-administered.

PREGNANCY AND LACTATION

Rosiglitazone-metformin treatment in premenopausal anovulatory patients with insulin resistance may result in resumption of ovulation. These patients may be at risk of pregnancy. Rosiglitazone has been reported to cross the human placenta and to be detectable in foetal tissues. There are no adequate data to support the use of rosiglitazone-metformin during pregnancy in humans. Rosiglitazone-metformin should be used during pregnancy only if the potential benefit justifies the potential risk to the foetus. There are no adequate data to support the use of rosiglitazone-metformin during lactation in humans. Rosiglitazone-metformin should be used during lactation only if the potential benefit justifies the potential risk to the infant.

ADVERSE REACTIONS

Rosiglitazone-metformin: In clinical studies, the safety profile of rosiglitazone-metformin was similar to that of the individual components. **Rosiglitazone:** Oedema, anaemia, hypercholesterolaemia, weight gain, hypoglycaemia, increased appetite, congestive heart failure/pulmonary oedema, events typically associated with cardiac ischaemia, constipation, bone fractures, anaphylactic reaction, hepatic dysfunction, primarily elevated by elevated hepatic enzymes, arthralgia, urticaria, rash, pruritus, macular oedema.

Metformin: Gastrointestinal symptoms, nausea, vomiting, diarrhoea, abdominal pain and loss of appetite, lactic acidosis, vitamin B12 deficiency, metallic taste, mild erythema.

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