The investigation of proteinuria

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Summary  Proteinuria can be a major clue to underlying renal disease or a transient finding in normal children. This article will deal with the evaluation of a child with proteinuria, what basic investigations are needed and when to refer to a paediatric nephrologist.

Urinalysis sticks for ward testing are quite sensitive for proteinuria. Suspected proteinuria should always be sent for laboratory quantification, and the simplest method is to measure with the protein:creatinine ratio on a spot sample.

Physiological proteinuria is not usually detected on urinalysis or dipstick testing. Causes of non-physiological proteinuria include benign transient proteinuria, orthostatic, renal and non-renal causes. Persistent proteinuria is more significant. It is also likely to be more significant if associated with haematuria or hypertension. Orthostatic proteinuria can only be diagnosed when the urinary protein from a recumbent sample reduces to normal.

When there is persistent non-orthostatic proteinuria, baseline investigations would normally include a renal ultrasound and a plasma chemistry profile. Plasma albumin gives a good indication of the significance of proteinuria. If haematuria is also present, a basic nephritis screen should also be carried out. Any child with persistent non-orthostatic proteinuria should be referred to a paediatric nephrologist for consideration for renal biopsy.

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KEYWORDS
Proteinuria; Orthostatic proteinuria; Nephrotic syndrome; Glomerulonephritis; Chronic renal failure/chronic kidney disease; Urinalysis

Practice points
• All persistent proteinuria should be quantified in the laboratory and a urine protein:creatinine ratio is the simplest method
• Many children with benign or orthostatic proteinuria will require no more invasive investigations beyond urinary protein quantification
• The presence of haematuria and/or hypertension significantly increases the likelihood of underlying renal disease
• Any child with persistent non-orthostatic proteinuria or any other sequelae of renal disease should be referred to a paediatric nephrologist

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Introduction

Proteinuria can be a major clue to underlying renal disease or a transient finding in normal children. It is likely to be much more significant if associated with haematuria. The spectrum of referrals is illustrated in Box 1.

Routine urinalysis is now part of the registration process with general practitioners and hence the first type of referral is not uncommon. Due to the association between renal disease and proteinuria, there is often considerable anxiety about the finding of asymptomatic proteinuria on the part of doctors and families. Nephrotic syndrome is a particular scenario of heavy proteinuria in childhood that carries additional risks. It can cause confusion in terms of the investigations required and management.

This article will deal with the evaluation of a child with proteinuria, what basic investigations are needed and when to refer to a paediatric nephrologist.

Physiology

The glomerular basement membrane provides both a physical and electrical charge barrier to the passage of most plasma proteins. A small number of proteins do pass into the proximal tubule, depending on their size and plasma concentration. Globulins are large molecules and as such are effectively retained in the plasma. Albumin is a smaller molecule. It is not known how much albumin is filtered in healthy individuals, but there appears to be a mechanism for resorption through proximal tubular epithelial cells.

Smaller proteins such as N-acetyl-D-glucosaminidase, retinal-binding protein and β2-microglobulin may be filtered at rates approaching the glomerular filtration rate (GFR). Up to 98% of these are reabsorbed in the proximal tubule. One hallmark of tubular disease is to see a marked increase of these proteins in the urine. There is a further source of protein secretion from the distal loop of Henle (Tamm–Horsfall protein).

Definitions of proteinuria

Urinalysis sticks for ward testing are quite sensitive for proteinuria. They are impregnated with bromocresol green that changes colour in the presence of protein and is used as an indicator dye. They are intended to correlate as follows: + with 0.3 g/l, ++ with 1 g/l, +++ with 3 g/l and ++++ with ⩾ 20 g/l. False-positive results may be obtained with concentrated or alkaline urine, and false-negative results may be obtained with dilute or markedly acidic urine.

Suspected proteinuria should always be sent for laboratory quantification. The gold standard in adults is a 24-h collection so that protein excretion may be expressed per unit body surface area per day (mg/m²/day). There is frequently confusion about how to carry out a timed urine collection (see Box 2). For an afebrile patient at rest, the urinary protein excretion should not exceed 60 mg/m²/day. Body surface area may be estimated from nomograms that are found in most paediatric formularies. It can also be estimated from the following

Box 1

Dear Consultant Paediatrician,
Re: Sally Smith
This 12-year-old girl has recently joined our practice. On her registration appointment, her urine showed ++ protein. Does she need further investigation?

Dear On-call Paediatric SHO,
Re: Fred Brown
This 9-year-old boy has had puffy eyes on waking over the last 2 weeks which has not responded to antihistamines. Today his urinalysis shows protein ++++ and blood ++. Please assess and manage.
Body surface area (m²) = \sqrt{\frac{\text{height (cm) × weight (kg)}}{3600}}

Timed urine collections may be carried out in older children but are not always practical, particularly in young children. The simplest means of quantifying proteinuria is with a spot sample, measuring the protein:creatinine ratio. The urinary concentration of protein or albumin is referred to urinary creatinine, and with the assumption that urinary creatinine concentrations are proportional to body surface area, there is no need to correct these samples for body size. Normal values for protein:creatinine ratios are shown in Table 1.

Some laboratories measure the albumin:creatinine ratio instead and this is more sensitive when there is a need for accurate quantification of albuminuria in the follow-up of glomerulopathies or diabetic nephropathy.

Causes of proteinuria

The protein that is excreted in urine under physiological conditions is not usually detected on urinalysis or dipstick testing. Pathological proteinuria has been classified into four groups: glomerular, tubular, overflow and benign.¹

- Glomerular proteinuria occurs because of increased glomerular permeability to proteins.
- Tubular proteinuria is due to decreased tubular resorption of proteins contained in glomerular filtrate and is seen in tubulo-interstitial diseases.
- Overload proteinuria is secondary to increased production, or release, of low-molecular-weight proteins. The myeloproliferative conditions that cause this form of proteinuria are rare in children.
- Benign proteinuria implies proteinuria that is detected on urinalysis but which has no serious underlying pathology. It includes proteinuria seen in fever or after exercise, idiopathic transient proteinuria, and orthostatic or postural proteinuria.

Causes of proteinuria are shown in Table 2.

Benign proteinuria

Proteinuria may occur in febrile children or in the context of disease states such as cardiac failure. It may also occur after strenuous exercise. Proteinuria may prove to be transient. Before further investigations are carried out, there must be documented proteinuria on at least two occasions. In some, there may be intermittent proteinuria. This is generally believed to be associated with a good prognosis. So long as there are no associated sequelae of renal disease, these children do not need a renal biopsy or long-term follow-up.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Normal ranges for protein:creatinine ratios in different age groups.</th>
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<tbody>
<tr>
<td>Protein:creatinine ratio</td>
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<tr>
<td>0–6 months</td>
<td>Up to 50 mg/mmol*</td>
</tr>
<tr>
<td>6–24 months</td>
<td>Up to 50 mg/mmol</td>
</tr>
<tr>
<td>&gt;2 years</td>
<td>Up to 25 mg/mmol</td>
</tr>
<tr>
<td>Nephrotic range</td>
<td>&gt; 360 mg/mmol</td>
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</table>

*May be even higher.
Orthostatic proteinuria

Urine protein excretion increases with activity and upright posture, and so the protein content of urine passed late in the day will always be greater than that passed first thing in the morning. This is not usually detectable on dipstick testing but the physiological increase is exaggerated occasionally.

The diagnosis is particularly common in adolescents and would need to be excluded in the first case above. This is best done by carrying out a split 24-h urine collection (Box 2). If this is not possible, a simpler method is to measure the protein:creatinine ratio in the first voided urine sample of the morning and compare this with a urine sample voided later in the afternoon or evening.

It should be remembered that proteinuria with a pathological basis will also frequently have an orthostatic component. Therefore, the diagnosis of orthostatic proteinuria can only be made safely when urinary protein during the recumbent period returns to a normal value.

Orthostatic proteinuria is commonly transient but may persist. The data on long-term follow-up of these patients have been confusing. For some studies showing minor glomerular abnormalities on renal biopsy, it is apparent that not all patients had true orthostatic proteinuria. What is clearer is that the long-term follow-up of patients with pure orthostatic proteinuria has shown an excellent prognosis and it is no longer true to suggest that these patients need long-term review. If orthostatic proteinuria is confirmed and there is no significant proteinuria on a recumbent sample, the child and family may be reassured and no follow-up is necessary.

Renal causes

Plasma albumin gives a good indication of the significance of proteinuria. For many hospital biochemical departments, this is not part of a routine chemistry profile and needs to be requested separately. Hypoalbuminaemia in the context of proteinuria suggests proteinuria to the extent that hepatic synthesis of albumin is not able to keep up with urinary losses.

The co-existence of haematuria is also important. The presence of haematuria with proteinuria significantly increases the likelihood that there is underlying renal pathology.

Chronic glomerulonephritis

A number of chronic glomerulopathies may present with asymptomatic proteinuria. For many of these conditions, one would expect to see further evidence of renal disease such as co-existent haematuria, hypertension or impaired renal function. However, conditions such as membranous glomerulonephritis and focal and segmental glomerulonephritis might present without other sequelae of renal disease.

Table 2 Causes of proteinuria.

<table>
<thead>
<tr>
<th>Benign causes</th>
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<tbody>
<tr>
<td>Transient proteinuria (e.g. fever or post-exercise)</td>
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<tr>
<td>Intermittent proteinuria</td>
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<tr>
<td>Orthostatic proteinuria</td>
</tr>
<tr>
<td>Glomerular causes</td>
</tr>
<tr>
<td>Damage to glomerular basement membrane (chronic glomerulonephritides, e.g. MPGN, lupus, IgA nephropathy)</td>
</tr>
<tr>
<td>Diseases of deposition (e.g. amyloidosis, Fabry disease)</td>
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<tr>
<td>Loss of glomerular anionic charge (e.g. idiopathic or congenital nephrotic syndrome)</td>
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<tr>
<td>Increased permeability of residual nephrons (chronic renal failure)</td>
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<tr>
<td>Tubular proteinuria</td>
</tr>
<tr>
<td>Tubulo-interstitial nephritis</td>
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<tr>
<td>Poisoning (vitamin D, heavy metals, non-steroidal analgesics)</td>
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<tr>
<td>Tubulopathies (e.g. Fanconi syndrome, Dent’s disease)</td>
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<tr>
<td>Overflow proteinuria</td>
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<tr>
<td>Myeloproliferative conditions</td>
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<tr>
<td>Rhabdomyolysis</td>
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<tr>
<td>Multiple transfusions or albumin infusions</td>
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MPGN, membranoproliferative (mesangiocapillary) glomerulonephritis.
Many have specific serological markers. Low complement is a feature of membranoproliferative (mesangiocapillary) glomerulonephritis (MPGN) and lupus nephritis, and there are usually raised anti-nuclear antibodies (ANA) or evidence of other auto-antibodies such as double-stranded DNA, extracted nuclear antibodies or lupus anticoagulant in lupus nephritis. IgA may be raised in IgA nephropathy and Henoch–Schönlein purpura nephritis but it is not a very sensitive test. With other vasculitic illnesses such as polyarteritis nodosa or Wegener’s granulomatosis, there may be associated systemic features such as recurrent fevers, rashes or upper respiratory tract symptoms. Vasculitis is usually associated with raised erythrocyte sedimentation rate (ESR) as well as C-reactive protein (CRP), whereas in lupus nephritis, there is typically only raised ESR in the absence of infection. Antinuclear cytoplasmic antibodies (ANCA) may be present in vasculitides. Hepatitis B or C can result in a secondary MPGN or secondary membranous glomerulonephritis.

Streptococcal illnesses may precipitate a number of glomerulonephritides. The hallmark of post-streptococcal glomerulonephritis is an initially depressed complement that normalizes by 2–3 months. Proteinuria usually clears within several months although it is common for haematuria to persist for a year or longer. Asymptomatic proteinuria may present in the context of recovering post-streptococcal glomerulonephritis when the initial illness was not severe enough to come to medical attention.

Clinical evaluation of a child with persistent proteinuria

If evaluation proceeds in a stepwise manner through a careful history taking, examination and urinalysis, many children will not need invasive investigations.

When taking a history, important areas to ask about include episodes of illness, swelling (oedema), urinary tract infections, use of drugs such as non-steroidal anti-inflammatory agents and family history of renal disease. Renal disease may be associated with a wide range of other conditions and a careful systemic enquiry is important. Examination must include blood pressure measured with an appropriate-sized cuff, the presence of oedema and parameters of growth. Clinical assessment of the circulatory status is described below.

Baseline investigations

These should be carried out sequentially in order of increasing complexity and invasiveness as shown below.

- Any concomitant haematuria?
- Confirmation and laboratory quantification of proteinuria (protein:creatinine ratio or timed collection).
- If significant proteinuria confirmed:
  - renal imaging in the form of a renal tract ultrasound scan (any renal scarring?).
  - plasma chemistry profile (to include Na, K, HCO₃, urea, creatinine, Ca, PO₄, alkaline phosphatase and albumin).
- If nephritic presentation (haematuria, hypertension or renal impairment):
  - glomerulonephritis screen (complement levels, ANA, ANCA, streptococcal serology (ASOT/anti-DNAse B), hepatitis B and C serology, ESR and CRP).

If the creatinine appears to be elevated for age, the GFR may be estimated using the Schwartz formula.
Nephrotic syndrome

The classic triad of nephrotic syndrome is heavy proteinuria leading to hypoalbuminaemia leading to oedema. Nephrotic range proteinuria is defined as >3.5 mg/m²/day. This equates to a protein:creatinine ratio of >250 mg/mmol, although it is usually much greater. On dipstick testing, there is protein of +++ or greater. The plasma albumin level is less than 25 g/l. Oedema is rarely present with plasma albumin levels greater than this.

Proteinuria occurs at the ultrastructural level because of so-called ‘fusion’ of podocyte foot processes resulting in disruption of the glomerular basement membrane, leading to a large albumin leak into Bowman’s capsule. The exact mechanisms for this are not completely understood but the main component is believed to be impairment of the charge-specific barrier. The change in glomerular permeability is also not well understood. It is widely believed although not proven that there is an immunological basis for the disease. It is believed to be mediated via a circulating factor such as a lymphokine. The presence of a circulating factor is suggested by the appearance and rapid resolution of nephrotic syndrome in neonates born to mothers with the condition, but the factor has not yet been identified.

Nephrotic syndrome has an incidence of 2–4 per 100 000 population but it is more common in Asian children. It is more common in boys than girls (2:1) and it has a peak incidence between the ages of 2 and 6 years. There is frequently an antecedent upper respiratory infection, following which children typically present with peri-orbital oedema. The diagnosis of allergy is frequently made before the true nature of the condition becomes apparent on urine testing.

The most common lesion causing nephrotic syndrome in childhood is minimal change nephropathy (MCN); this accounts for around 85% of cases. Under the age of 6 months, congenital nephrotic syndromes predominate as a cause of nephrotic syndrome, and from 6 years, other pathology becomes progressively more common.

Investigation of the cause of nephrotic syndrome

The initial investigations of a child with suspected nephrotic syndrome are intended to exclude atypical forms. When there is a nephritic element to the nephrotic syndrome or when the child’s age differs significantly from the typical age range, they need further investigation. Children under the age of 12 months and children over the age of 12 years need further investigation under the supervision of a paediatric nephrologist before commencement of high-dose steroids. Features that suggest a nephritic element include frank haematuria, abnormal renal function and hypertension. Haematuria up to ++ is not uncommon in MCN but even haematuria of higher grades, whilst still not visible to the naked eye, may be associated with a greater incidence of steroid resistance.
Hypertension can only be diagnosed in a euvoaemic state. Both states of hypervolaemia and hypovolaemia are common in nephrotic syndrome, and both are frequently associated with hypertension due to a normal haemodynamic response.

Serum complement, ANA and hepatitis serology need not be carried out in a child with typical nephrotic syndrome prior to commencement of high-dose steroids. However, the presence of antibodies to varicella is worth documenting to avoid unnecessary administration of varicella zoster immunoglobulin for subsequent exposure to chicken pox when the child is immunosuppressed.

Investigation of the consequences of nephrotic syndrome

The nephrotic state causes concern because of the risks imposed by hypoalbuminaemia. These include the risk of hypovolaemia, thrombosis and infection. These have justified the use of high-dose steroids for long periods.

Hypovolaemia frequently accompanies the initial presentation of nephrotic syndrome. It may present with abdominal pain that is believed to be due to underperfusion of the splanchnic circulation. Examination for hypovolaemia, whilst not easy, is critical in a child with newly diagnosed nephrotic syndrome. Isolated measurement of heart rate and blood pressure are unhelpful. The best assessment is in the temperature of the peripheries, usually taken as the feet. In a warm room, these should be warm to the touch (less than 2°C below central body temperature). In an older child, it is usually possible to position them sitting up at an angle of 45° and assess the height of the jugular venous pressure (JVP); this should be around 2 cm above the sternal angle. Compression of the vein or abdominal pressure may confirm that a JVP is visible but considerably lower than this.

Confirmation of hypovolaemia may be obtained by measurement of urinary sodium ($U_{Na}$). Hypovolaemia results in sodium retention and $U_{Na} < 10$ mmol/l is diagnostic whilst $U_{Na} > 20$ mmol/l makes it unlikely. These values are only valid when no diuretic has been given in the preceding 6–12 h and in the absence of renal impairment.

Investigation for suspected infection should proceed as dictated clinically. Children with nephrotic syndrome should be given prophylactic penicillin until they enter remission. In the nephrotic state, there are urinary losses of antithrombotic proteins such as antithrombin III. There are also increased plasma concentrations of clotting factors and these, together with the presence of hypovolaemia, contribute to the prothrombotic state. However, no investigations are traditionally carried out. Children are not routinely anticoagulated for nephrotic syndrome unless there has been a previous thrombotic event. In the presence of severe hypovolaemia and apparent polycythaemia, it would not be inappropriate to commence a low-molecular-weight heparin until a state of euvoaemia is achieved and maintained.

Hyperlipidaemia is universal in nephrotic syndrome but does not form part of the diagnostic criteria. It is not thought necessary to measure the lipid profile in children with nephrotic syndrome due to the usually transient nature of the nephrosis.

Initial treatment of nephrotic syndrome

The treatment for nephrotic syndrome is outside the scope of this review. However, there is currently no national or international consensus regarding what should be the ideal steroid regimen at disease presentation. The debate is between longer, more-intensive steroid treatment at onset and shorter courses (see Appendix).

Basic investigations in suspected nephrotic syndrome

For a child who fulfills the triad criteria of the nephrotic syndrome, the following basic investigations are suggested.

- Quantification of proteinuria (usually by protein:creatinine ratio).
- Plasma chemistry profile (to include Na, K, HCO$_3^-$, urea, creatinine, Ca, PO$_4^{2-}$, alkaline phosphatase and albumin).
- Varicella serology.

Table 3 Usual indications for renal biopsy in a child with nephrotic syndrome.

<table>
<thead>
<tr>
<th>Atypical features at onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macroscopic haematuria</td>
</tr>
<tr>
<td>Hypertension in a euvoaemic state</td>
</tr>
<tr>
<td>Impaired renal function</td>
</tr>
<tr>
<td>Age less than 12 months or greater than 12 years</td>
</tr>
<tr>
<td>Failure to respond to initial high-dose steroids</td>
</tr>
</tbody>
</table>
Glomerulonephritis screen only if any atypical features.

$U_{Na}$ if hypovolaemia is suspected.

The indications for a biopsy in a child with nephrotic syndrome are shown in Table 3.

Summary

The key to assessment of a child with proteinuria is the appreciation of benign forms of proteinuria and clinical suspicion underlying renal pathology. A suggested schema is shown in Fig. 1. A finding of asymptomatic proteinuria is common. Since proteinuria has become increasingly recognized as a marker of progressive renal injury, there should be close liaison between general paediatricians and paediatric nephrologists in cases of persistent proteinuria.

Appendix

The British Association of Paediatric Nephrology is sponsoring a national multicentre trial of prednisolone treatment in nephrotic syndrome that would be adequately powered to address the issue of the duration of steroid therapy for the initial episode. Recruitment is about to commence for a pilot study to provide data for a power calculation, after which the definitive study will commence. Since most nephrotic syndrome presents to general paediatricians in the first instance, it is hoped that general paediatricians will actively recruit patients for the study. Further information will be included with RCPCH mailings and should be available from all paediatric nephrologists who will act as local facilitators and advisors.

References


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Figure 1 Schema for assessment of a child with proteinuria. Adapted from Postlethwaite RJ, Clinical Paediatric Nephrology, 2nd edn, with permission.

Further reading