Urine proteins – how they get there, how we find them and what do they mean?

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Proteinuria

- approx. 25% of cardiac output goes to the kidneys
- complex process of filtration and reabsorption of protein
- normal urine protein of <10 mg/L
Glomerulus

- glomerular membrane
- negatively charged
- glomerular capillary – high pressure filter
- water and low mwt. solutes pass freely
- proteins retained (generally)
- protein passage depends on size, shape and charge
Glomerulus

- Some protein passes through the glomerulus
- approx. 1g of albumin passes into the proximal tubules
- mostly reabsorbed by the tubules
we think of proteins as whole recognisable molecules
fragments and small, low molecular weight peptides are also important
these can represent large amounts of “protein” but are undetectable or unrecognisable by our normal methods
Tamm Horsfall glycoprotein, IgA, lysozyme, urokinase etc
Tubules

- various mechanisms of protein resorption
  - e.g. albumin reabsorbed in the proximal tubules
    - high affinity, low capacity – normal albumin loss
    - low affinity, high capacity – high albumin loss

- reabsorbed proteins are catabolised in the tubular cells
Urine proteins - normally

- Urine total protein should be <10mg/L
- There should be a trace of albumin detectable in EVERY urine
- Very sensitive analytical methods can detect small peptide fragments
Proteinuria

- tiny but abnormal amounts of albumin
- small amounts of low molecular weight proteins and fragments
- high concentrations of low molecular weight proteins
- high concentration of protein
- mixtures of all of the above!
Proteinuria

spectrum of patterns
- tiny but abnormal amounts of albumin
- small amounts of low molecular weight proteins and fragments
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- high concentration of protein
- mixtures of all of the above!
Proteinuria

- normal
- glomerular
- tubular
- overflow
Glomerular proteinuria

- often due to immunologically mediated diseases
- increased glomerular permeability
- urine protein pattern related to serum protein concentration, mwt, shape, charge etc.
- albumin and transferrin predominate in “selective” proteinuria
- all proteins seen in “non-selective” proteinuria
- in extremes, urine protein concentration can be similar to serum protein concentration! ~ 40g/L
- amount of protein in urine will fall as plasma protein concentration falls
Glomerular proteinuria

Causes of glomerular proteinuria
- non-immunoligocal
  - Diabetic nephropathy
  - Amyloidosis
- Immunological (glomerulonephritis)
  - Post strep glomerulonephritis
  - SLE
  - Vasculitides
  - GBM disease
Glomerular proteinuria

Examples of glomerular proteinuria
Tubular proteinuria

- Tubular proteinuria results from disruption of the absorption of the filtered proteins – tubulointerstitial nephritis
- Generally lower protein concentrations <1 g/L
- Presence of low molecular weight proteins
  - β2 microglobulin (mwt 11.8 kDa)
  - α1 microglobulin (mwt 31 kDa)
  - Retinol binding protein (mwt 21 kDa)
- Presence of “cruddy fragments”
- Plus some albumin and other proteins
Tubular proteinuria

- Causes of tubular proteinuria
  - Drugs
  - Heavy metal poisoning
  - Infection and inflammation
  - Tumours
  - Immunological diseases
  - Metabolic diseases
Tubular proteinuria

Examples of tubular proteinuria
Overflow proteinuria

- abnormally high concentrations of protein arriving at the glomerulus
- exceeds any capacity of tubular reabsorption
  - haemoglobin
  - myoglobin
  - paraproteins
  - Bence Jones protein
Overflow proteinuria

Examples of overflow proteinuria
Proteinuria

Mixed proteinuria

- glomerular, tubular and overflow
- can all occur together
- patterns - hard to classify
Proteinuria

Mixed proteinuria
Proteinuria

Detection and quantification:
- Random vs timed collection vs 24 hour collection
- Variable volume so g/L may be misleading
- Dip-stix...detect albumin
- Urine total protein
  - All methods poor!
  - Differences between protein bindings to the dyes
  - Negligible standardisation
  - Poor QA and QC
- Albumin
  - Immunoassay for quantification
  - Reasonable QA and QC
  - Often measured as albumin:creatinine ratio
Proteinuria

What questions are we asking?

Does the patient........
- have a normal or abnormal
  amount of protein in their urine?
  dip-stix and urine total protein
Proteinuria

What questions are we asking?

Does the patient……..

- have a raised albumin excretion
  measure albumin:creatinine ratio
- have raised urine myoglobin concentration
  measure urine myoglobin concentration
- have a tubular proteinuria
  measure alpha-1 microglobulin
- have Bence Jones protein in the urine
  check urine electrophoresis and immunofixation
Light chains

- polyclonal B cells produce a slight excess of light chains as part of their normal processes.
- These free light chains arrive at the kidneys and are filtered by the glomerulus (mwt approx. 25kDa).
- Inflammatory responses can increase the amount of polyclonal free light chains produced.
- Kidneys are important sites of light chain catabolism.
- Light chain catabolism (plus dehydration, acidosis etc) can cause aggregation of excess light chains and tubular damage.
Bence Jones protein

- MONOCLONAL free light chains
- first described in 1846!
- important marker of B cell malignancy
- rarely seen in benign conditions
- can form amyloid or myeloma casts
- kidneys are important sites of light chain catabolism
- light chain catabolism (plus dehydration, acidosis etc) can cause aggregation of excess light chains and tubular damage

there is NO antiserum available ANYWHERE that can distinguish monoclonal from polyclonal light chains
Bence Jones protein

- Free light chains not necessarily BJP
- BJP is *monoclonal* free light chains
- Reliable detection of BJP can only be done by good quality electrophoresis and immunofixation

Finding and typing BJP is probably the hardest thing we do in protein labs.....
Bence Jones protein
Don’t forget…..

- intact monoclonal Ig also appears in the urine (with or without BJP)
- will usually have different mobility BJP
- \(\beta2\) microglobulin can also be a large band on urine EP (especially if patient is on alpha-interferon)
- patients with amyloid may have heavy glomerular or tubular proteinuria and only a small amount of BJP
Urine patterns in the gamma

Normal
(kappa > lambda approx. 2:1)

Light chain banding
(kappa and lambda bands)

Bence Jones protein
Why?

- Patients with infection and inflammatory conditions show increased free light chain excretion – not BJP.
- Patients with B cell malignancies with BJP can have glomerular, tubular, overflow or mixed proteinuria.
- Elderly patients often have some tubular proteinuria.
- Tubular catabolism can make light chains fragments that aggregate.
- Tubular catabolism can make light chains fragments that aggregate and have similar charge.
- Degraded urines show very fuzzy patterns.
- High resolution electrophoresis picks up tiny amounts of protein.
What can we do?

- use an electrophoretic technique that is sensitive...to 10mg/L BJP
- see albumin in every urine
- confirm with immunofixation - increases sensitivity and specificity
- don’t be afraid to ask for a fresh sample if the urine is degraded, smelly or shows an indistinct pattern
- positive identification important – if there is a band, what is it (BJP, Hb, β2M, lysozyme etc.)
Quantification – best of a bad job!

- electrophoresis, scanning densitometry and total protein
- NOT ideal
  - total protein methods are poor
  - EP separation can have a high ‘background’
  - due to protein fragments
  - tubular proteins ‘crud’
  - limitation of urine volume – timed, 24 hour, random
- within a patient, urine patterns are surprisingly stable
What is best?

- high quality electrophoresis
- low threshold for fixation
- skilled interpretation
- quantification by % BJP and TP