

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

Dr. Joanna Sheldon
Protein Reference Unit
St. George's Hospital

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

The bits and pieces

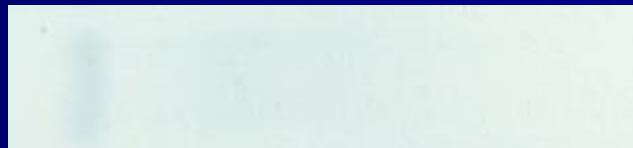
- we think of proteins as whole recognisable molecules
- fragments and small, low molecular weight peptides are also important
- these can represent large amounts of “protein” but are undetectable or unrecognisable by our normal methods
- Tamm Horsfall glycoprotein, IgA, lysozyme, urokinase etc

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

- spectrum of patterns
 - 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!

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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-immunological
 - 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 <1g/L
- presence of low molecular weight proteins
 - β 2 microglobulin (mwt 11.8 kDa)
 - α 1 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

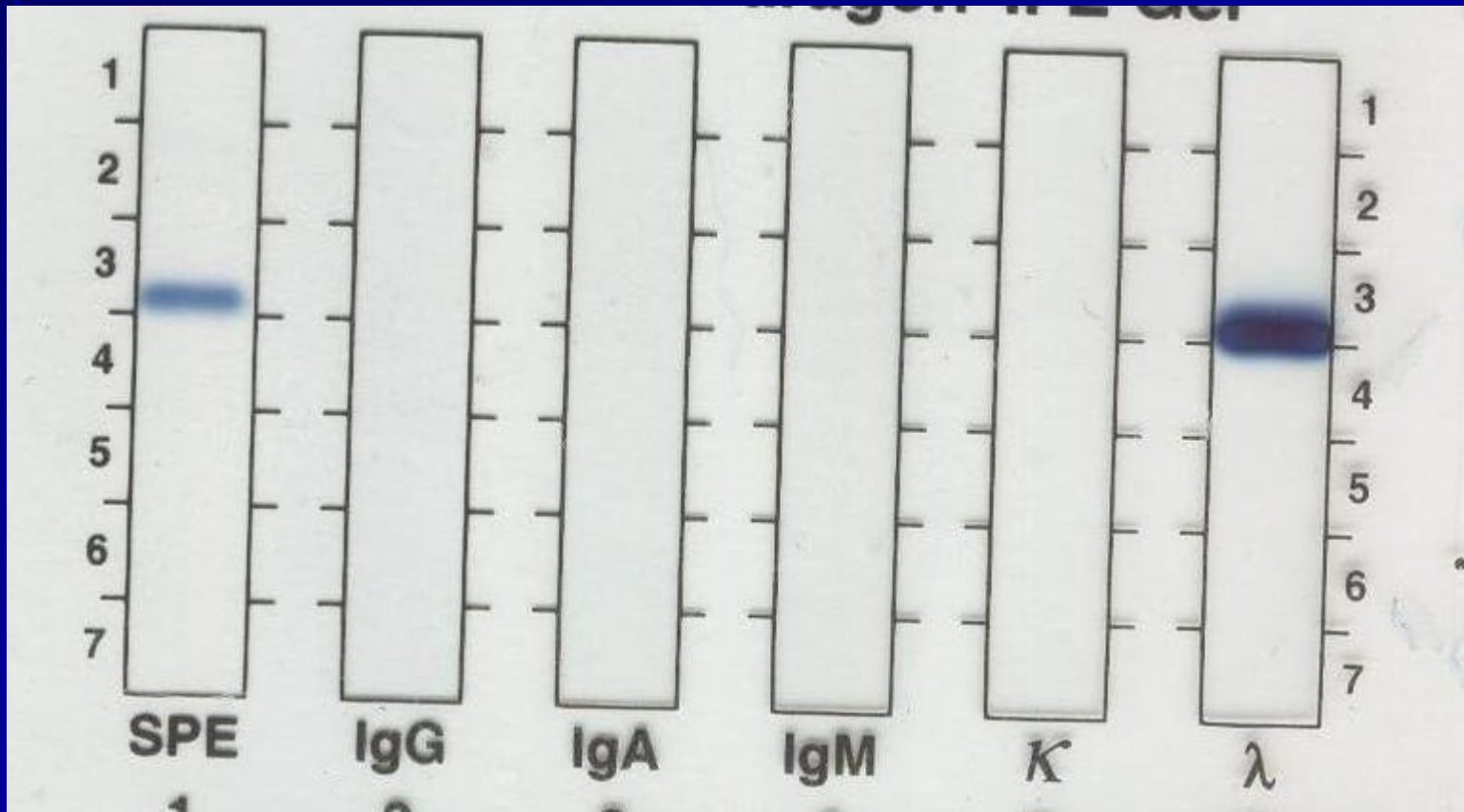


Overflow proteinuria

- causes of 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

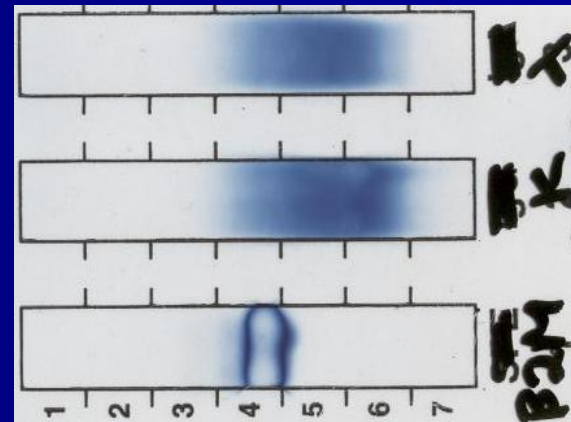
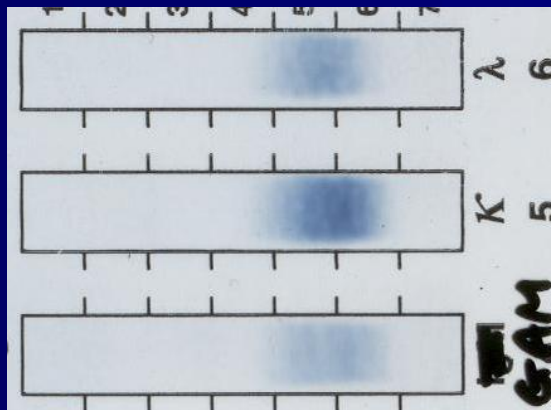
○ Mixed proteinuria

- 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

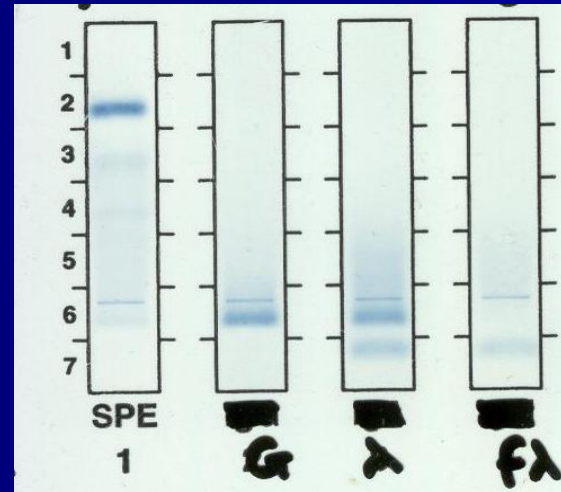
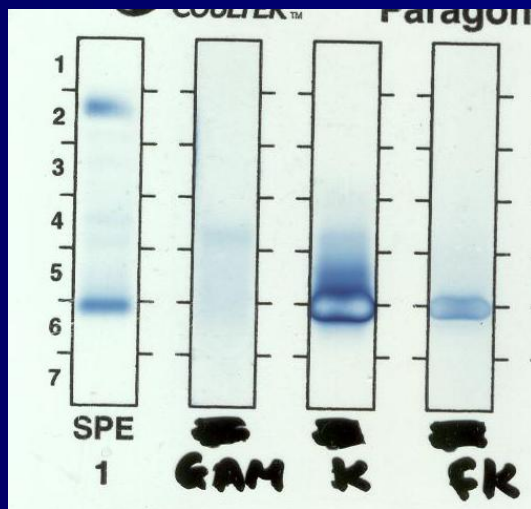
- there is NO antiserum available ANYWHERE that can distinguish monoclonal from polyclonal light chains

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
- β 2 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

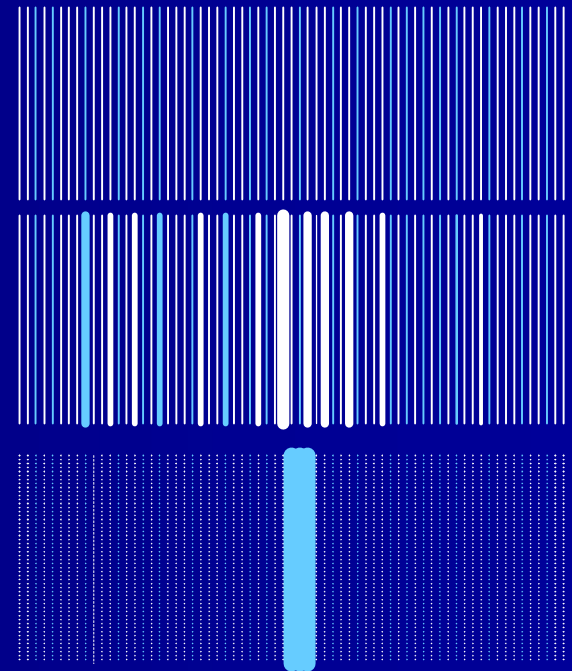
Normal

(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, β 2M, 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