Investigation of monoclonal gammopathies:

what we should know but have probably forgotten!

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History of the lab

- 1847 – Bence Jones protein described
- 1937 – electrophoresis separates plasma protiens
- 1940 – the term paraprotein introduced by Apitz
- 1959 - the immunoglobulin introduced by Heremans
- 1965 - measurement of immunoglobulin concentration by radial immuno diffusion described by Mancini
- 1966 – measurement of proteins by rocket electrophoreiss described by Laurell
- 1976 – automated immunonephelometry for protein measurement introduced

Setting the standards

ALL laboratory methods should be:

- ACCURATE – where possible, be calibrated against an IRP
- PRECISE
  - show CVs of <10% realistic (<5% preferable) within batch
  - show CVs of <20% realistic (<10% preferable) between batch
- CONSISTENT BETWEEN USERS
  - show CVs of <20% realistic (<10% preferable) in EQA
- CLINICALLY SPECIFIC
  - show a low number of false positives
- CLINICALLY SENSITIVE
  - show a low number of false NEGATIVES
- CLINICALLY SENSITIVE
  - show a low number of false POSITIVES
- VALUE FOR MONEY
  - all of the above and cost effective

What is normal?

SERUM

« polyclonal gamma region on electrophresis

« Adult concentrations

- IgG 6-16 g/L, IgA 0.8 – 4.0 g/L, IgM 0.5 – 2.0 g/L

« IgG half life ~ 21 days and dependent on concentration

« IgA and IgM half life ~ 5 days independent of concentration

URINE

«Total protein <0.1 g/L

« a trace of albumin should be detectable in every urine

« normal urine (adequately concentrated) will also show some other protein e.g. transferrin and some polyclonal free light chains

« these free light chains are a normal result of B cell development

Monoclonal proteins

- development of a monoclonal does not happen overnight
  - will start as a small band
  - may develop quickly or very slowly
  - may increase in concentration as clone grows
  - may remain at a low and stable concentration
  - may disappear over time
  - may suppress background B cell population
- the same immunoglobulin concentration may relate to polyclonal, oligoclonal or monoclonal populations
- there is NO antibody that is capable of distinguishing a monoclonal protein from a polyclonal protein

Things to remember

- monoclonal proteins are not (usually) normal proteins (in terms of structure)
- monoclonal proteins do not behave like polyclonal proteins
- presence of a monoclonal does not mean malignancy
- absence of a monoclonal does not exclude malignancy

What are the stages?

- Detection
- Typing
- Quantification
- Monitoring

Detection of monoclonal proteins

- ALWAYS check serum and urine
  - approx. 20% of myeloma only make BJP
  - BJP is small ~22kDa (but can polymerise)
  - BJP can pass easily through the glomerulus
- Serum immunoglobulins should always be done with serum protein electrophoresis
- International Guidelines for BJP analysis recommends 2nd void of the day for detection

Detection of monoclonal proteins

- High quality electrophoresis
  - agarose, cellulose acetate or capillary
  - serum (prefereable to plasma)
  - urine – concentrated or sensitive stain (at least a trace of albumin MUST be seen in all urines)
- monoclonal proteins can appear anywhere from the alpha-1 to the post-gamma areas
- low threshold for immunofixation

IMMUNOFIXATION

- use high quality antiserum
  - anti-total (free and bound) light chain antiserum is better than anti-free light chain antiserum
  - anti-light chain antiserum often shows greater binding to free light chains than to bound light chains
- one antiserum will not detect ALL monoclonals
- immunofixation does increase sensitivity over electrophoresis (by 10-20x)
- good interpretation increases specificity
- immunofixation is not quantitative

Typing of monoclonal proteins

Immunofixation is the only reliable way to type monoclonal proteins – it can

« confirm the clonality of band detected by electrophoresis

« test for α, γ and μ heavy chains and κ and λ light chains

« test for the δ and ε heavy chains where a serum shows monoclonal light chains without a corresponding α, γ or μ heavy chain

« exclude low concentration monoclonal components even where no band is apparent on electrophoresis but with clinical indications e.g. AL amyloidosis

Typing of monoclonal proteins

Immunofixation is the only reliable way to type monoclonal proteins – it can

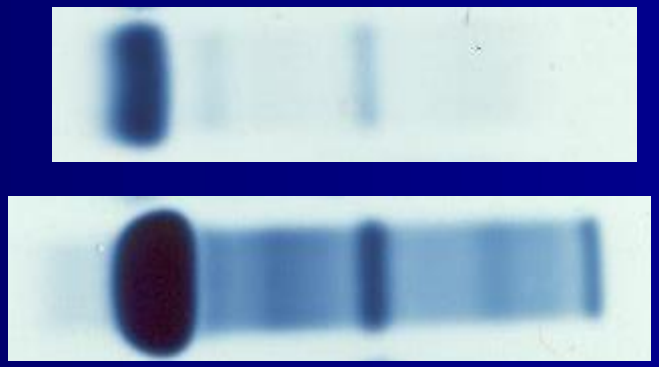
« exclude the presence of monoclonal IgA or IgM if they are showing raised concentrations without increased staining in the beta-gamma region of the electrophoresis

« positively identify other proteins that may be mistaken for monoclonal immunoglobulins e.g. fibrinogen, C-reactive protein, beta-2 microglobulin and complement components

« detect minimal residual disease or complete remission post stem cell transplantation when no monoclonal component is seen on the electrophoretic separation.

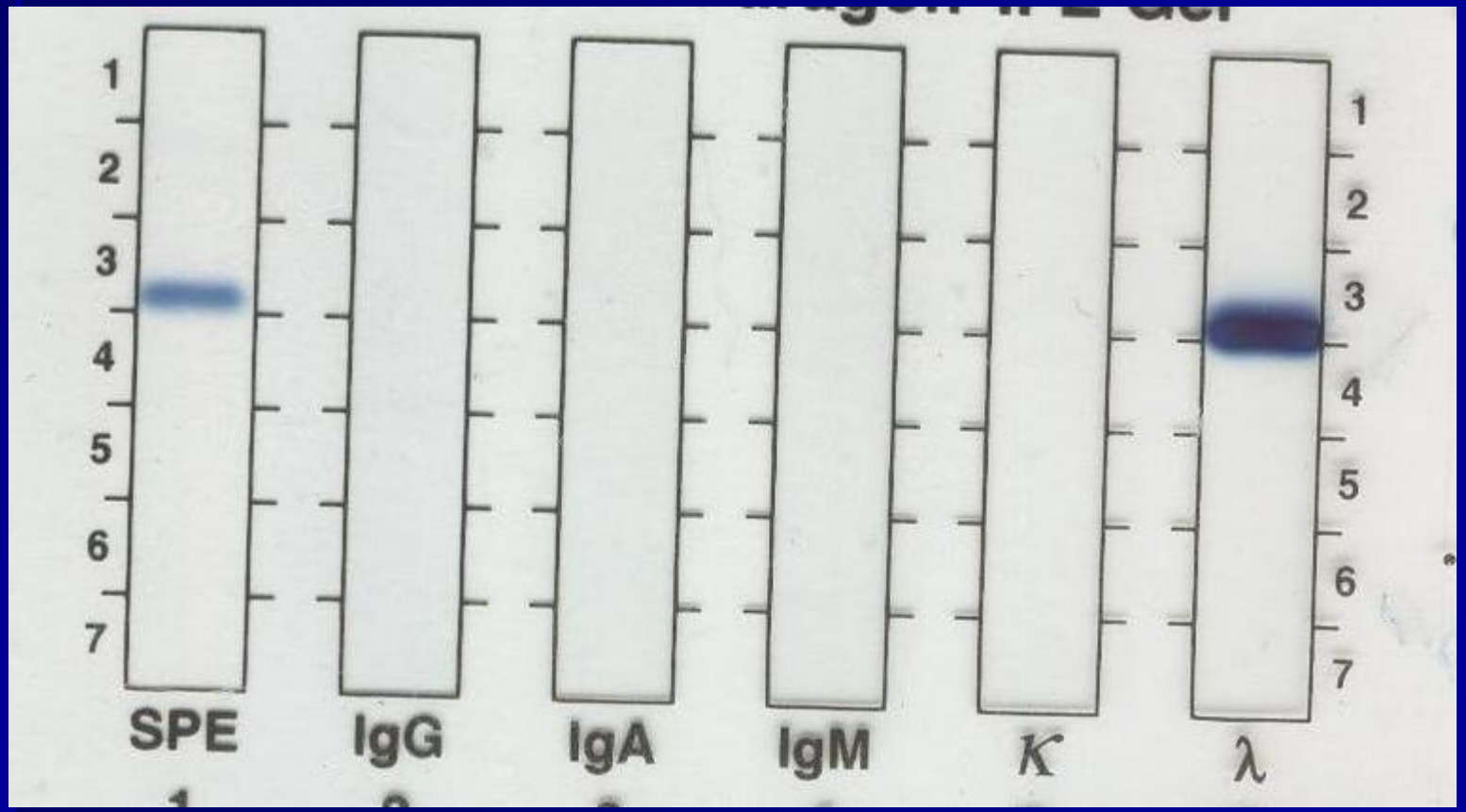
Glomerular proteinuria

- Examples of glomerular proteinuria



Overflow proteinuria

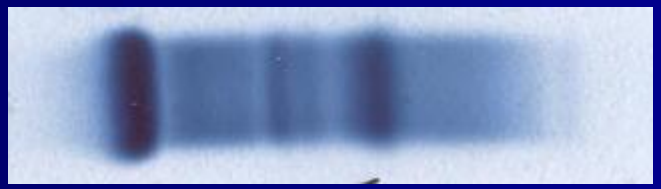
- Examples of overflow proteinuria



Proteinuria

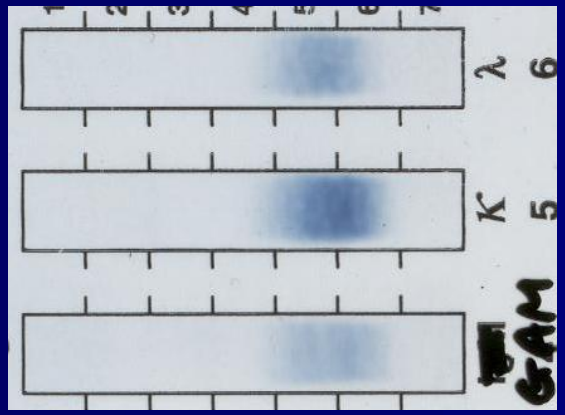
- Mixed proteinuria

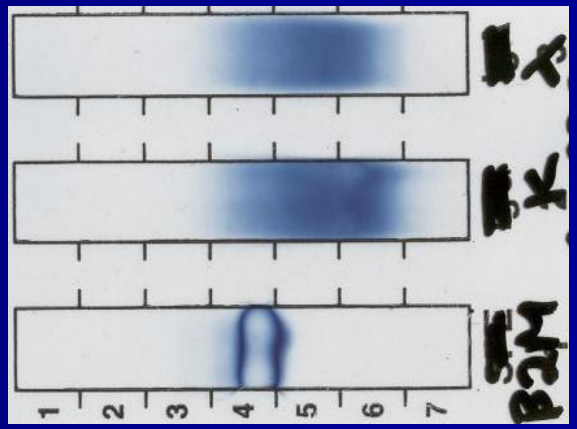
  - glomerular, tubular and overflow
  - can all occur together
  - patterns - hard to classify

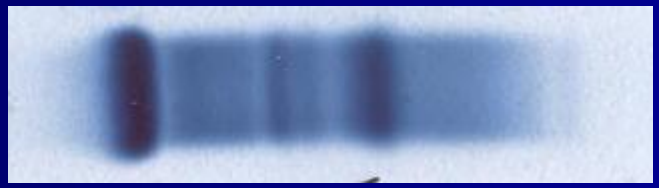


Proteinuria

- Mixed proteinuria







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

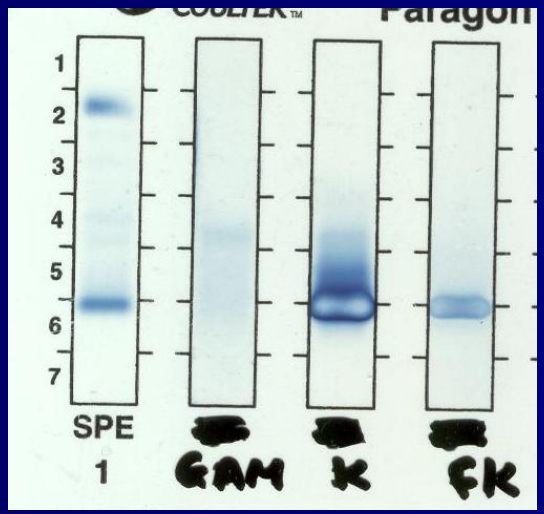
- there is NO antiserum available ANYWHERE that can distinguish monoclonal from polyclonal light chains

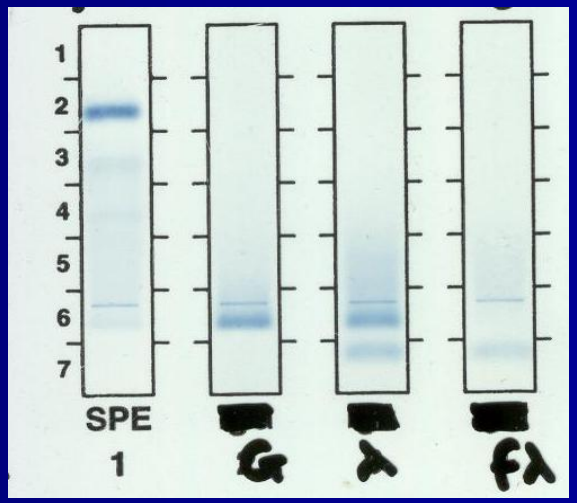
Bence Jones protein

- 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



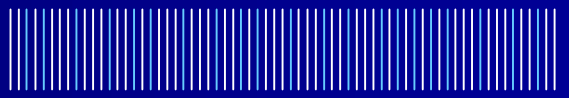


Don’t forget…..

- intact monoclonal Ig also appears in the urine (with or without BJP)
- will usually have different mobility BJP
- β2 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

Beta-gamma region of EP

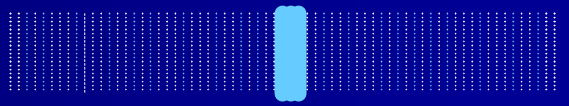
Normal (polyclonal)
(kappa >lambda approx. 2:1)



Polyclonal raised
(kappa > lambda approx. 2:1)

Oligoclonal banding

Monoclonal protien



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