

Investigation of B cell malignancies

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The B cell

progression from

- « Pluripotent stem cell
- « Lymphoid committed stem cell
- « B lineage committed
- « Heavy chain rearrangement
- « Light chain rearrangement
- « Encounters antigen in lymphoid tissue
- « Affinity maturation
- « Class switching
- « End differentiated plasma cell

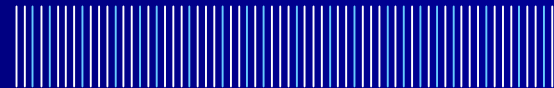
The B cell

- Many opportunities for an “error” to occur
- Some errors result in a malignant transformation of the B cells
- Some errors result in a clonal expansion of B cells.....and B cell malignancies
- Monoclonal proteins are one of the features of B cell malignancies

Beta-gamma region of EP

Normal (polyclonal)

(kappa > lambda approx. 2:1)



Polyclonal raised

(kappa > lambda approx. 2:1)



Oligoclonal banding



Monoclonal protien



History of the lab

- 1847 – Bence Jones protein described
- 1937 – electrophoresis separates plasma proteins
- 1940 – the term paraprotein introduced by Apitz
- 1959 - the immunoglobulin introduced by Heremans
- 1965 - measurement of immunoglobulin concentration by radial immunodiffusion described by Mancini
- 1966 – measurement of proteins by rocket electrophoresis 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 electrophoresis
- « 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 (preferable 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.

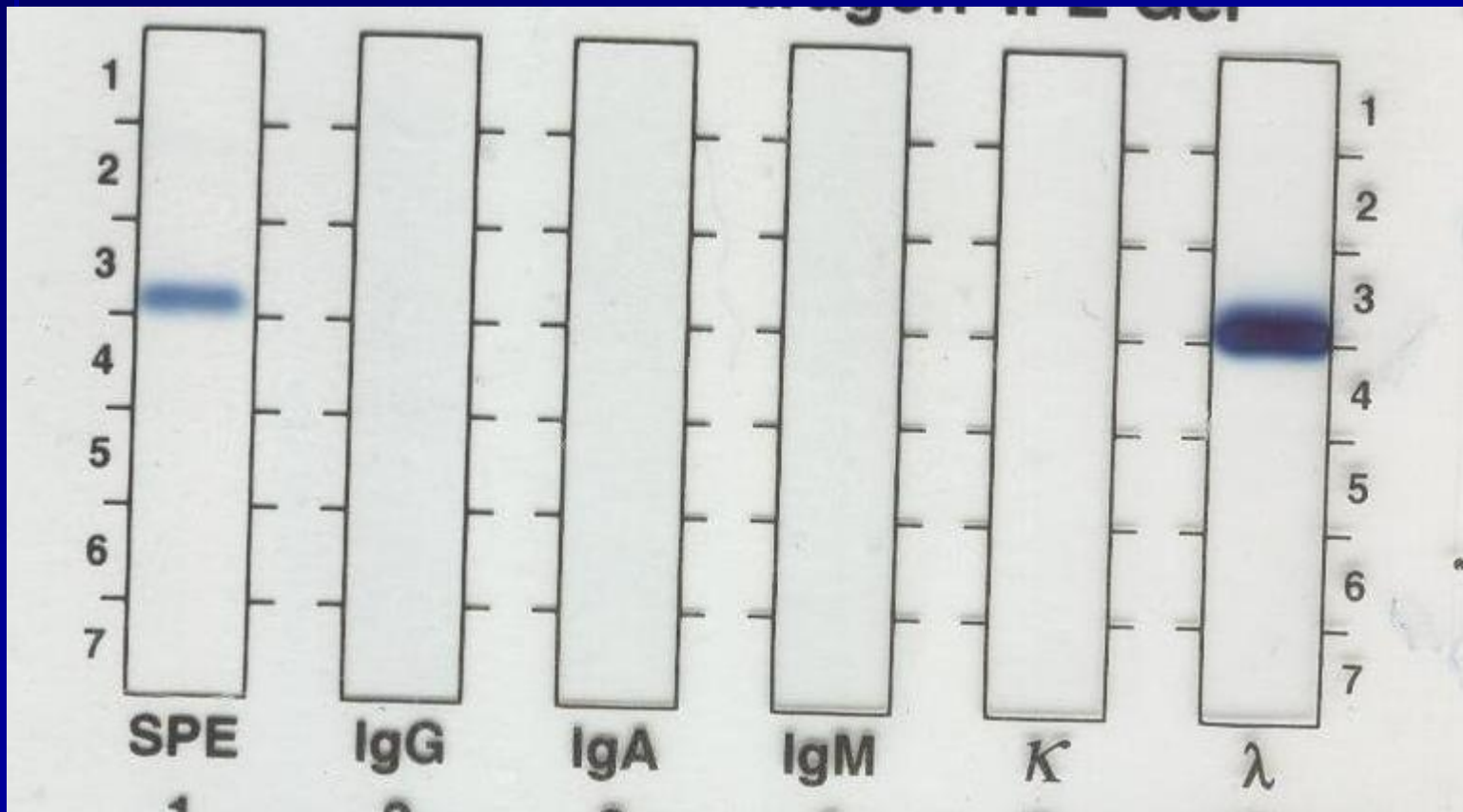
Glomerular proteinuria

- Examples of glomerular proteinuria



Overflow proteinuria

- Examples of overflow proteinuria



Proteinuria

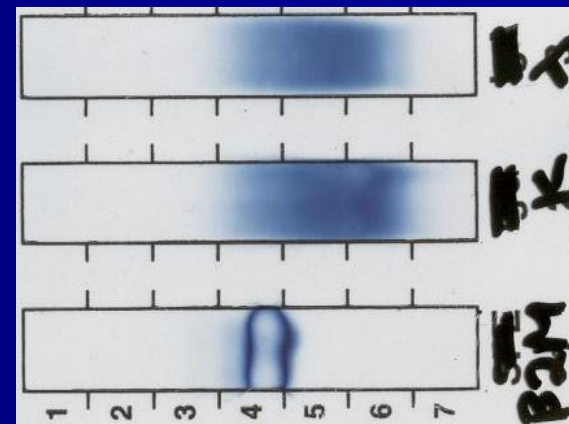
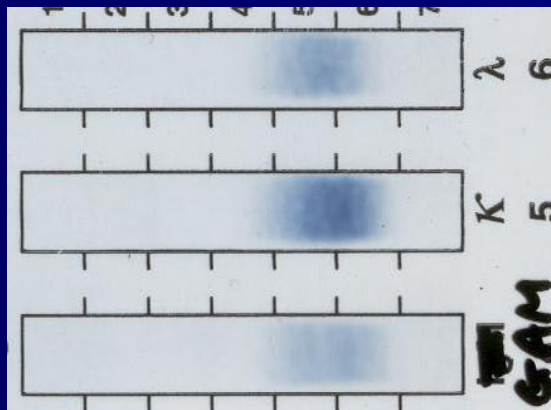
○ Mixed proteinuria

- 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

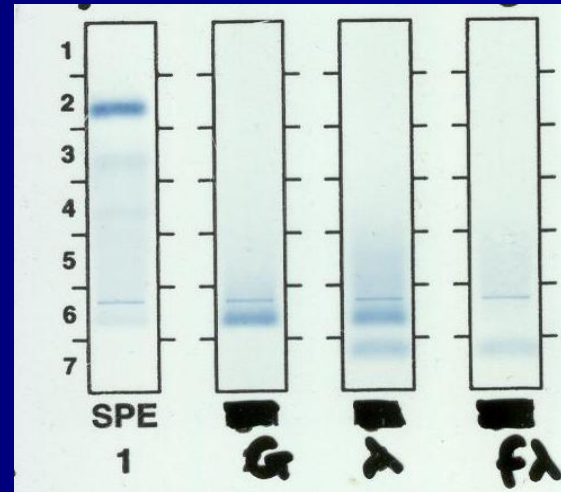
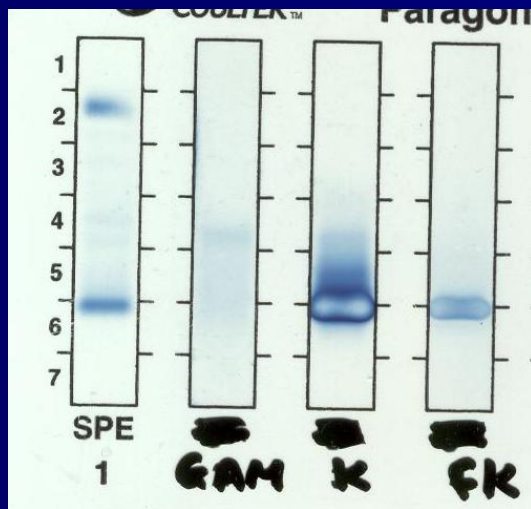
- there is NO antiserum available ANYWHERE that can distinguish monoclonal from polyclonal light chains

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
- 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

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

Multiple Myeloma

Summary

a malignant disease of the plasma cells in the bone marrow. Proliferation of one clone of plasma cells (monoclonal proliferation) results in production of a monoclonal immunoglobulin (Ig) molecule which can be detected in the serum or the urine.

frequently associated with bone pain, anaemia, and renal failure.

accounts for about 1% of all cancers; 2,500 new cases of myeloma each year in UK.

incidence increases with age; most patients are over the age of 60 years

the cause is unknown in most patients; radiation exposure is known to increase the risk.

incurable in most patients, average survival 3-4 years.

Monoclonal Immunoglobulins

there are normally many different plasma cell clones, producing many different Ig molecules

In response to infection or inflammation, there is proliferation of a number of different plasma cell clones leading to an increased number of plasma cells in the marrow (a **reactive** increase) and a **polyclonal** increase in Igs, which appear as a broad band in the gamma region on serum electrophoresis.

Monoclonal Immunoglobulins

in myeloma one clone overgrows and produces one particular immunoglobulin molecule - a **monoclonal** immunoglobulin, also termed a monoclonal protein (M-protein) or **paraprotein** - which appears as a dense narrow band on electrophoresis. There is nearly always a reduction in normal polyclonal Igs.

the abnormal plasma cells may also produce free light chains, which are small enough to pass into the urine (**Bence-Jones protein** or BJP). Free light chains can also be measured in the serum using the new Freelite assay. Individual patients may have plasma cells which secrete a whole immunoglobulin alone, BJP alone, or both.

The ESR in Myeloma

a high level of Igs in the blood causes a raised ESR (erythrocyte sedimentation rate). this is measured as mm in 1 hour – the degree of separation of red cells from the plasma that has taken place in 1 hour.

the ESR is therefore raised in most patients with myeloma, but is also raised in conditions where there is a polyclonal increase in Igs (infection and inflammation)

in patients with myeloma who produce only free light chains and no serum paraprotein, the ESR is normal

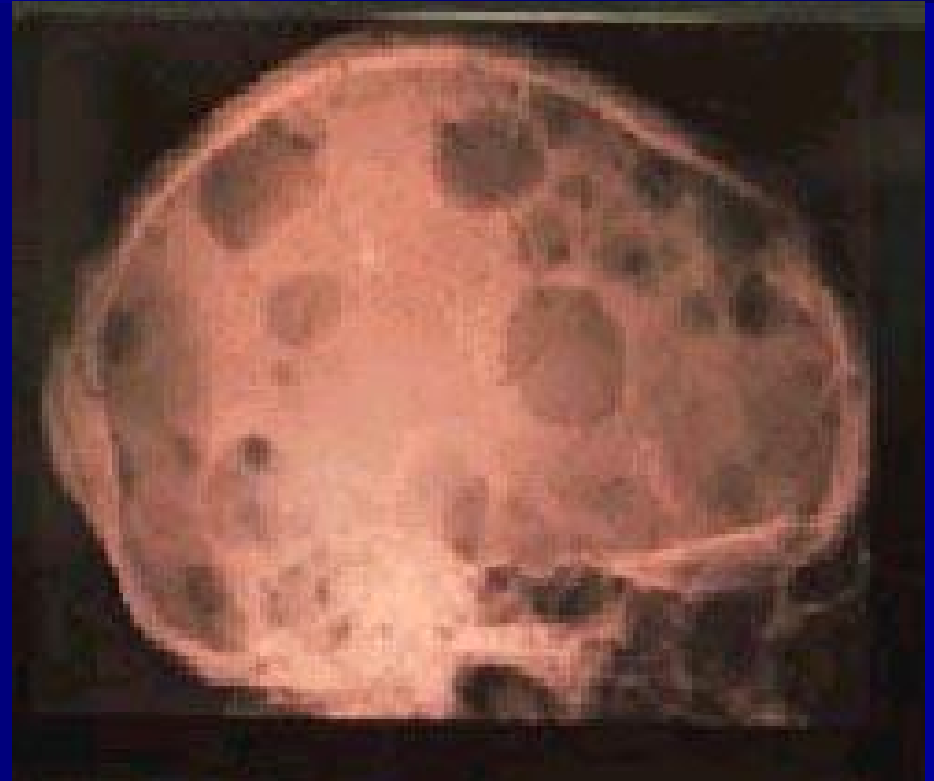
Clinical Features of Myeloma

- **Bone pain.** This affects about 60% of patients. X-rays may show lytic lesions (punched out holes). Generalised osteoporosis (thinning of the bone texture) is also common and there may be compression fractures of the vertebrae, leading to back pain and occasionally to compression of the spinal cord and neurological symptoms. The bone destruction is due to the activation of osteoclasts resulting from interaction between myeloma cells and the bone marrow stromal cells.

Clinical Features of Myeloma



Clinical Features of Myeloma



Clinical Features of Myeloma

- **Hypercalcaemia** - resulting from bone destruction. Causes dehydration, drowsiness, confusion, constipation and renal damage.

Clinical Features of Myeloma

Renal failure. About 30% of patients have some degree of renal impairment and about 5% have severe renal failure. This is most commonly due to BJP which damages the tubules. Other factors which can contribute include hypercalcaemia, infection, dehydration and drugs.

Clinical Features of Myeloma

- **Anaemia.** This is common in myeloma patients.

Clinical Features of Myeloma

- Increased susceptibility to **infection**, both bacterial and viral.

Clinical Features of Myeloma

- **Amyloid.** About 10% of patients with myeloma develop light chain amyloidosis (AL amyloid), in which the light chains are deposited in the tissues in the form of amyloid causes enlargement and stiffness of the tissue. (Amyloid=starch-like) The kidney is usually affected, with glomerular damage leading to loss of albumen, low serum albumen level and consequent oedema (nephrotic syndrome)

Clinical Features of Myeloma

- **Asymptomatic patients** may be picked up by the finding of a raised ESR or abnormal protein electrophoresis.

Diagnosis of Myeloma

Bone pain. This affects about 60% of patients. Confirmation of the diagnosis at least 2 of the following 3 features must be present:

a **paraprotein** in serum or in urine (no specific level)

>10% plasma cells in the bone marrow
lytic lesions on X-ray.

Criteria for MGUS

<30g/l paraprotein

<10% plasma cells in marrow

no bone disease

normal haemoglobin and renal function

Treatment – Specific Aspects

Chemotherapy is the mainstay of treatment in myeloma. Oral or intravenous drugs may be used, often combined with steroids.

Radiotherapy is used for local areas of disease, e.g. for pain or cord compression

Treatment – Specific Aspects

Older / less fit patients are treated with oral chemotherapy. Response is monitored by the paraprotein level, and sometimes by bone marrow tests. Generally a plateau phase is reached after which the level does not decline any further and treatment is stopped. It is unusual for the paraprotein to disappear (complete remission). Sooner or later the paraprotein starts to rise again indicating relapse. Further treatment may induce a second response but this is usually shorter than the first.

Treatment – Specific Aspects

younger patients are treated with initial intravenous chemotherapy to induce a remission followed by high dose chemotherapy and autologous stem cell transplantation. Allogeneic transplantation (from a healthy donor) is an option for a minority of patients who are under 55 years and have a matched donor

Future Treatment

The average survival of patients treated with conventional chemotherapy is around 3 years, although some patients may survive much longer.

A number of factors predict prognosis, of which most important is the serum beta-2 microglobulin level. Others include renal function, serum albumin, calcium and haemoglobin.

High dose therapy appears to prolong survival but is not definitely able to cure the disease.

Monitoring response

response is monitored by the level of paraprotein in the serum or urine (24 hr excretion)

repeat bone marrows or X-rays may be needed occasionally

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Monitoring response

response is graded as follows (simplified criteria)

- no change: <25% decrease in paraprotein
- minimal response: 25-49% decrease in paraprotein
- partial response: 50-99% decrease in paraprotein
- complete response: undetectable paraprotein by EP or by immunofixation and bone marrow showing <5% plasma cells

Monitoring response

in patients with minimal or partial response, a 25% increase in paraprotein is considered progression (relapse)

in patients in complete remission reappearance of the paraprotein is considered relapse.

relapse can also occur without increase in paraprotein, e.g. with new bone lesions.

What is best?

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