Diagnosis of Monoclonal Gammopathies

Julio C. Delgado, MD, MS
Assistant Professor of Pathology
Director Immunology Laboratory, ARUP Laboratories
Associate Director H&I Laboratory, University of Utah Health Care

The Accreditation Council for Continuing Medical Education accredits ARUP Laboratories to provide and sponsor continuing medical education for physicians. This activity has been designed in accordance with ACCME Essentials and Standards and is scientific in nature.

This speaker has disclosed there are no relationships with the products or services that will be discussed. The balances of activities are under the auspices of ACCME accredited providers.

Outline

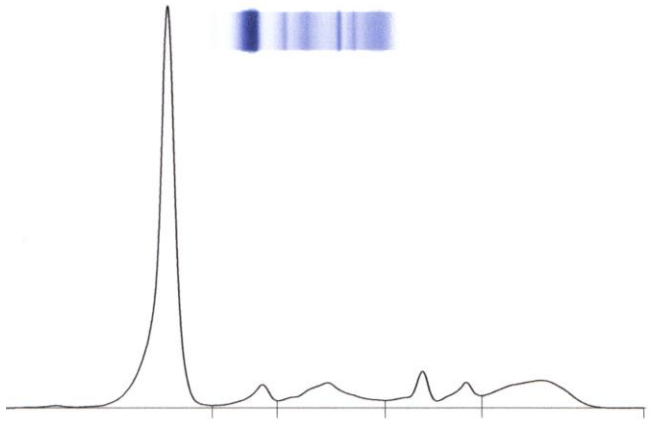
- Laboratory screening
  - Protein Electrophoresis
    - Serum
    - Urine
  - Immunofixation
- Free light chain ratio assay

Monoclonal Gammopathy: Clonal expansion of plasma cells secreting a monoclonal Ig

- Monoclonal Gammopathy of Undetermined Significance (MGUS)
- Multiple Myeloma
  - Light chain multiple myeloma
  - Non-secretory multiple myeloma
- AL Amyloidosis
- Plasmacytoma
- Others:
  - Waldenström Macroglobulinemia
  - POEMS syndrome
  - Heavy chain disease

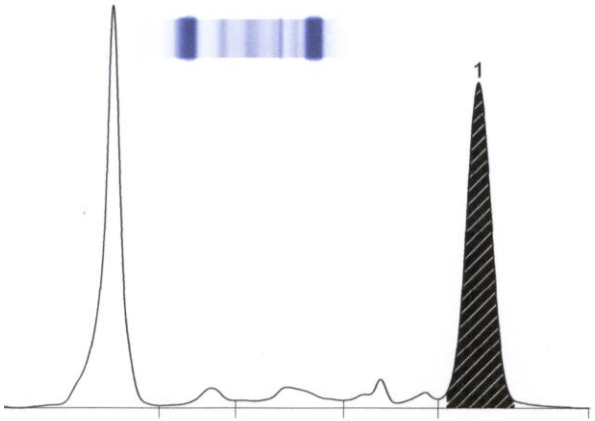
Serum protein electrophoresis (sPEP)

- Intended for the separation of human serum proteins
- Divides proteins in five major fractions:
  albumin, alpha-1, alpha-2, beta and gamma



Total Protein:	7.4	g/dl	T.P. Ref. Range:	6.00 - 8.30 g/dL
Fractions	Rel %	g/dl	Ref. %	Ref. g/dl
Albumin	60.2	4.45	55.1 - 65.7	3.75 - 5.01
Alpha 1	4.5	0.33	3.1 - 5.6	0.19 - 0.46
Alpha 2	10.6	0.78	8.0 - 12.7	0.48 - 1.05
Beta	10.4	0.77	8.5 - 12.8	0.48 - 1.10
Gamma	14.3	1.06	10.3 - 18.2	0.62 - 1.51

sPEP provides quantitation of the M-protein (m, monoclonal spike)



Total Protein: 10.1 g/dL T.P. Ref. Range: 6.0 - 8.3 g/dL

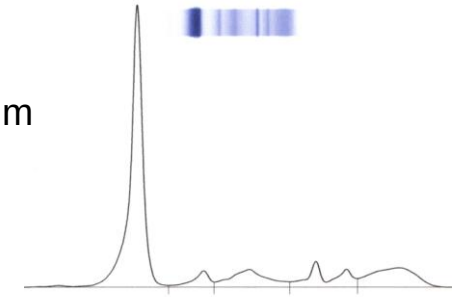
Fractions	Rel %	g/dl		Ref. %	Ref. g/dl
Albumin	40.3	4.07		55.1 - 65.7	3.75 - 5.01
Alpha 1	3.2	0.32		3.1 - 5.6	0.19 - 0.46
Alpha 2	6.0	0.61		8.0 - 12.7	0.48 - 1.05
Beta	5.3	0.54		8.5 - 12.8	0.48 - 1.10
Gamma	45.2	4.57	+	10.3 - 18.2	0.62 - 1.51
1	44.1	4.45			

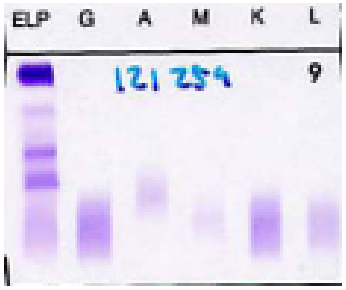
sPEP can be normal in patients with oligo-secretory (~15-20%) on non-secretory myeloma (~1-3%)

Immunofixation Electrophoresis (IFE)

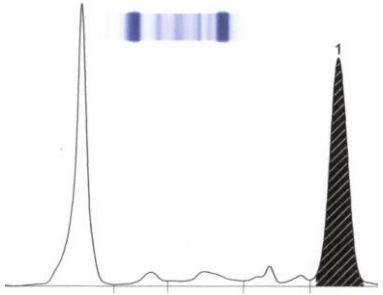
Serum IFE is **more sensitive than sPEP** for detection of M protein
Serum IFE provides characterization of M protein (heavy and light chain subclass)

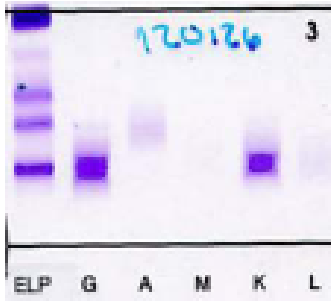
Normal serum





Monoclonal serum





Serum IFE **does not** provide quantification of M-protein
Serum IFE can be normal in patients with non-secretory myeloma (~1-3%)

Urine Protein Electrophoresis (uPEP)

In monoclonal gammopathies, a proteinuria pattern may show a discrete band produced by monoclonal free light chains, or **Bence-Jones Proteinuria (BJP)**

Normal

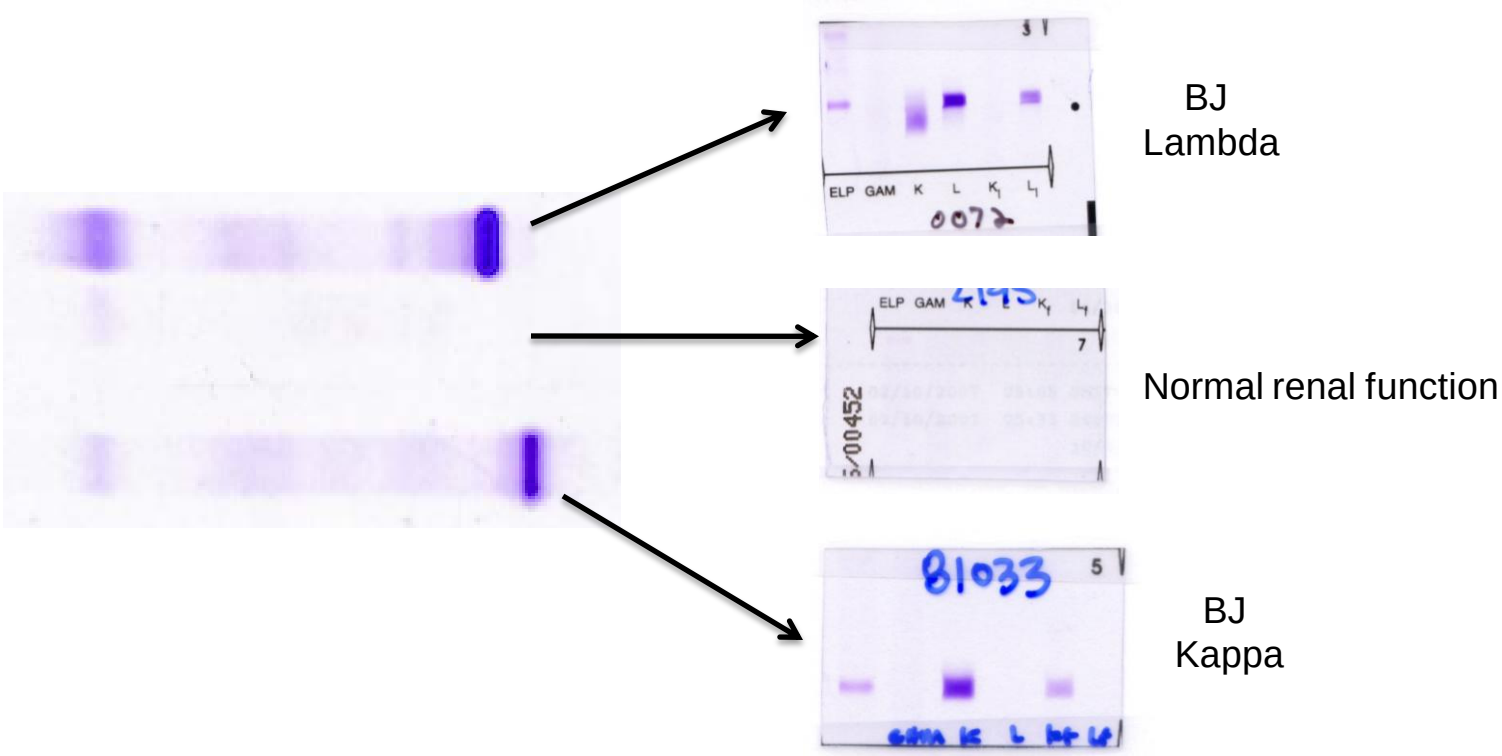
BJP

uPEP can provide quantification of M-protein

Urine IFE (uIFE)

Characterization of BJ protein (kappa or lambda light chain)

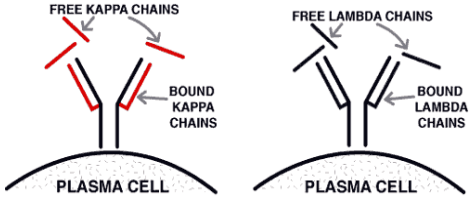
uIFE is **more sensitive than uPEP** for detection of BJ protein



Free light chains

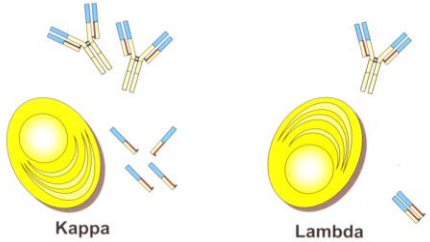
Immunoglobulin molecules consist of 2 identical heavy chains linked to 2 identical light chains (kappa or lambda)

- Majority of light chain in serum exists bound to heavy chain
- Low levels of FLC are found in serum of normal individuals

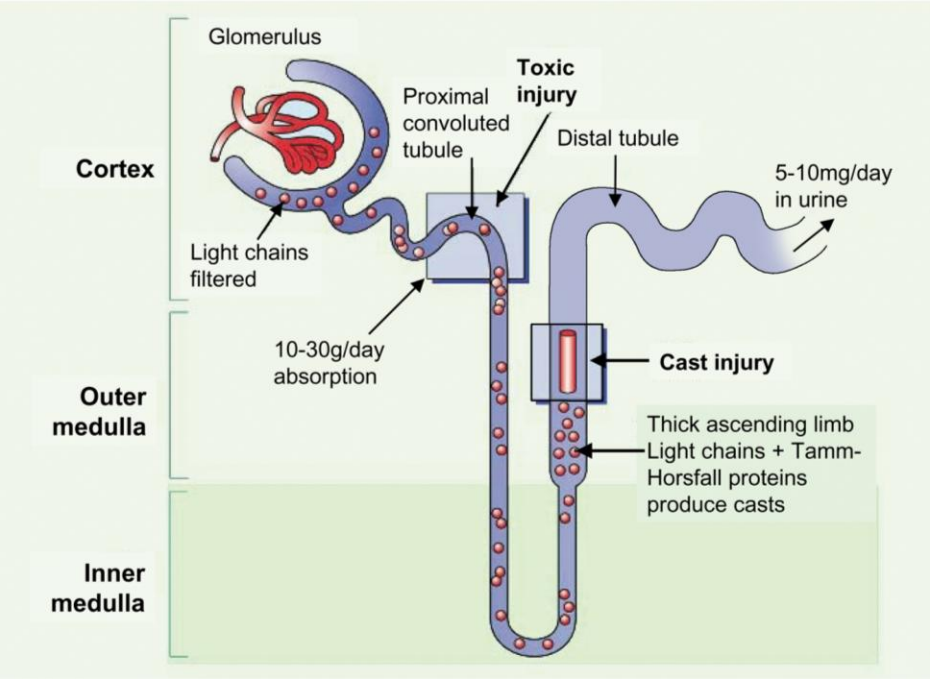


In serum, kappa FLC exists predominantly as monomer and lambda FLC as a dimer

- Resulting in a **differential renal filtration rate**
- Half life of kappa: 2-3 hours; lambda: 4-6 hours
- Serum kappa FLC concentrations are ~50% lower

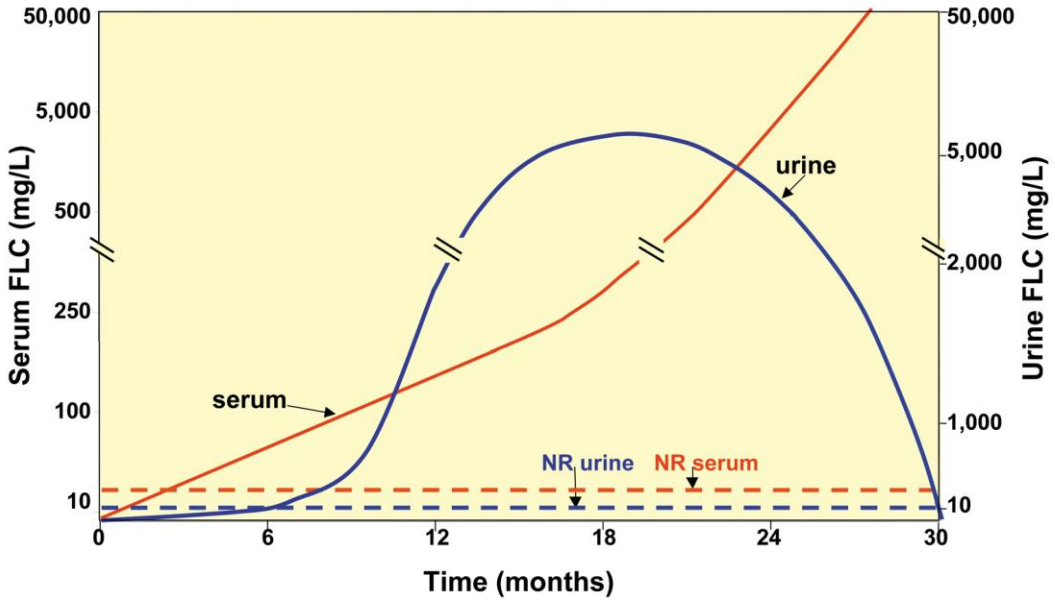


Daily production of FLCs is ~500mg/day
Kidneys can metabolize ~30 times the normal production of FLC



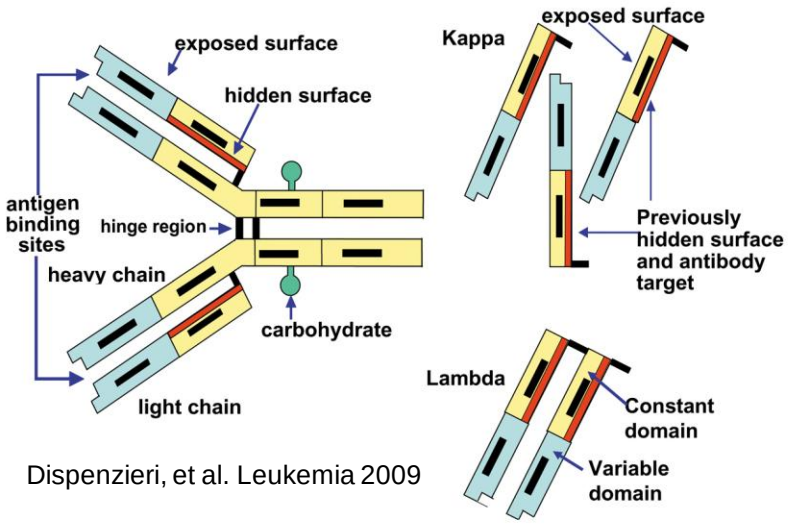
Comprehensive Clinical Nephrolog by Johnson RJ, Feehally J

Serum is preferable to urine for assessing FLC concentrations



Comprehensive Clinical Nephrology by Johnson RJ, et al.

First attempts to measure FLC in serum were unable to differentiate free vs. bound



Dispenzieri, et al. Leukemia 2009

-FLC assay directly measures the concentration of free kappa or lambda in serum by nephelometry

-To correct for differential kidney filtration rate, the kappa to lambda ratio is calculated from these values. NR= [0.26 – 1.65]

-Patients with monoclonal gammopathies have increased kappa or lambda FLC ratio due to the clonal secretion of a single FLC by malignant plasma cells

Indications of FLC ratio assay

The FLC ratio assay is indicated for the diagnosis of:

- Light chain multiple myeloma
- Non-secretory multiple myeloma
- Light chain (AL) Amyloidosis

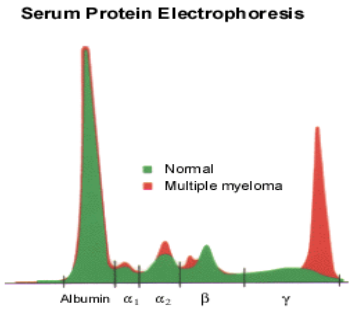
Durie BG, Leukemia 2008

Other indications of FLC ratio assay

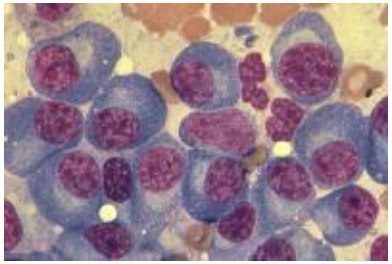
- **Monitoring** of patients with **non-measurable disease**
  - serum M-protein <1g/dl
  - urine M-protein <200mg/24h
  - M-spikes migrating in the beta region (IgA myeloma)
- FLC ratio is useful for **evaluation of residual disease** (complete remission vs relapse)
- Due to short half life of FLC (4-6 h), compared Ig half life (21days), the FLC assay is very useful for **monitoring response to chemotherapy and dialysis**
- Risk factor for progression of MGUS into MM

Diagnostic Criteria of Monoclonal Gammopathies

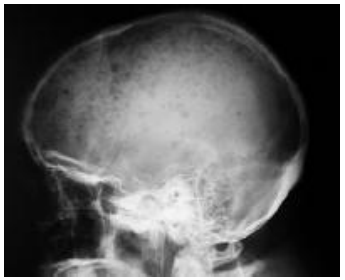
- Serum and/or urine monoclonal (M) protein

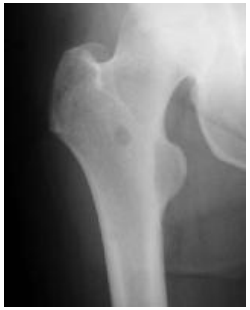


- Bone marrow (clonal) plasma cell proliferation



- Related organ or tissue impairment (**CRAB**)
  - **C**alcium increased
  - **R**enal insufficiency
  - **A**nemia
  - **B**one lesions





Photos courtesy of www.emedicine.com

Monoclonal Gammopathy of Undetermined Significance (MGUS)

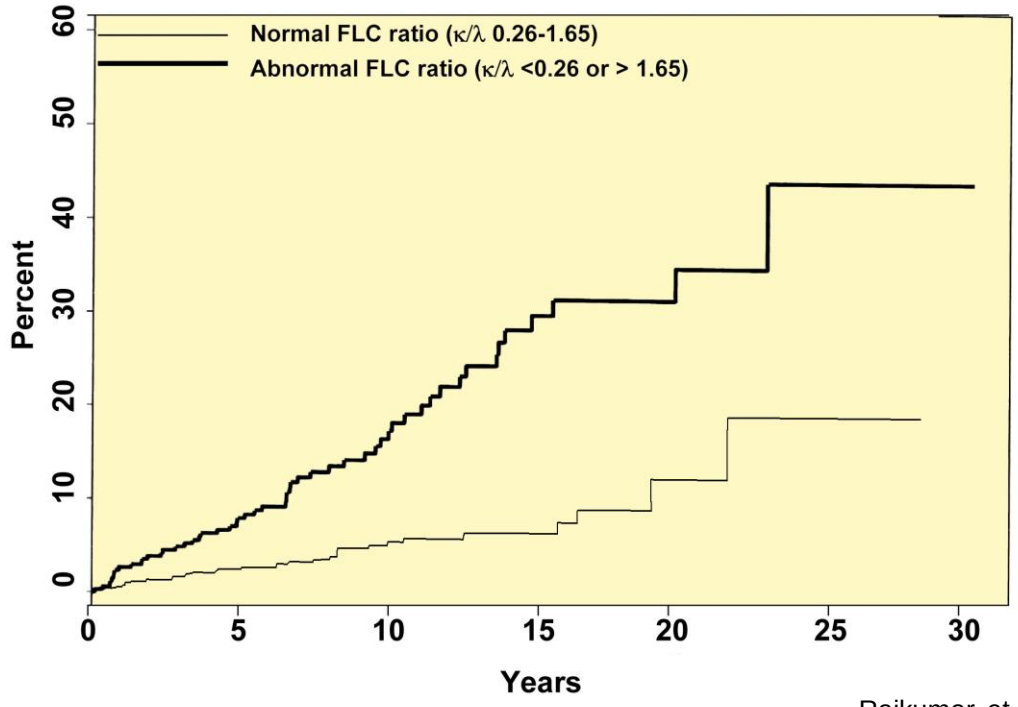
- Dx Criteria
  - Serum M protein **≤ 3g/dL**,
  - bone marrow plasma cells **≤ 10%**
  - **No CRAB**

- Most common monoclonal gammopathy (50-65% of all gammopathies)
  - MGUS is found in ~3% of individuals aged 50 years

- 1% lifelong risk per year of progression to MM

- Treatment
  - None required
  - Close follow-up to detect appearance of malignancy

MGUS patients with abnormal FLC ratio have higher risk of progression to MM



Rajkumar, et al. Blood 2005

N= 1,148; hazard ratio 2.6

A risk stratification system can be applied to predict the risk of progression of MGUS based on:

- Size of M-protein
- Type of immunoglobulin: IgA or IgM (increased risk)
- Abnormal serum FLC ratio assay

TABLE 2. **Risk Stratification Model to Predict Progression of Monoclonal Gammopathy of Undetermined Significance to Myeloma or Related Disorders***

Risk group	No. of patients	Relative risk	Absolute risk of progression at 20 y (%)	Absolute risk of progression at 20 y accounting for death as a competing risk (%)
Low risk (serum M protein level <1.5 g/dL, IgG subtype, normal free light chain ratio [0.26-1.65])	449	1.0	5	2
Low to intermediate risk (any 1 factor abnormal)	420	5.4	21	10
High to intermediate risk (any 2 factors abnormal)	226	10.1	37	18
High risk (all 3 factors abnormal)	53	20.8	58	27

Rajkumar et al. Blood 2005

Multiple myeloma (MM)

- Dx Criteria
  - Serum M protein **> 3g/dL**,
  - bone marrow plasma cells **> 10%**
  - **CRAB**

- Most common presenting symptoms of MM: bone pain, anemia
  Also: elevated serum creatinine, hypercalcemia, **recurrent infections** (encapsulated bacteria)

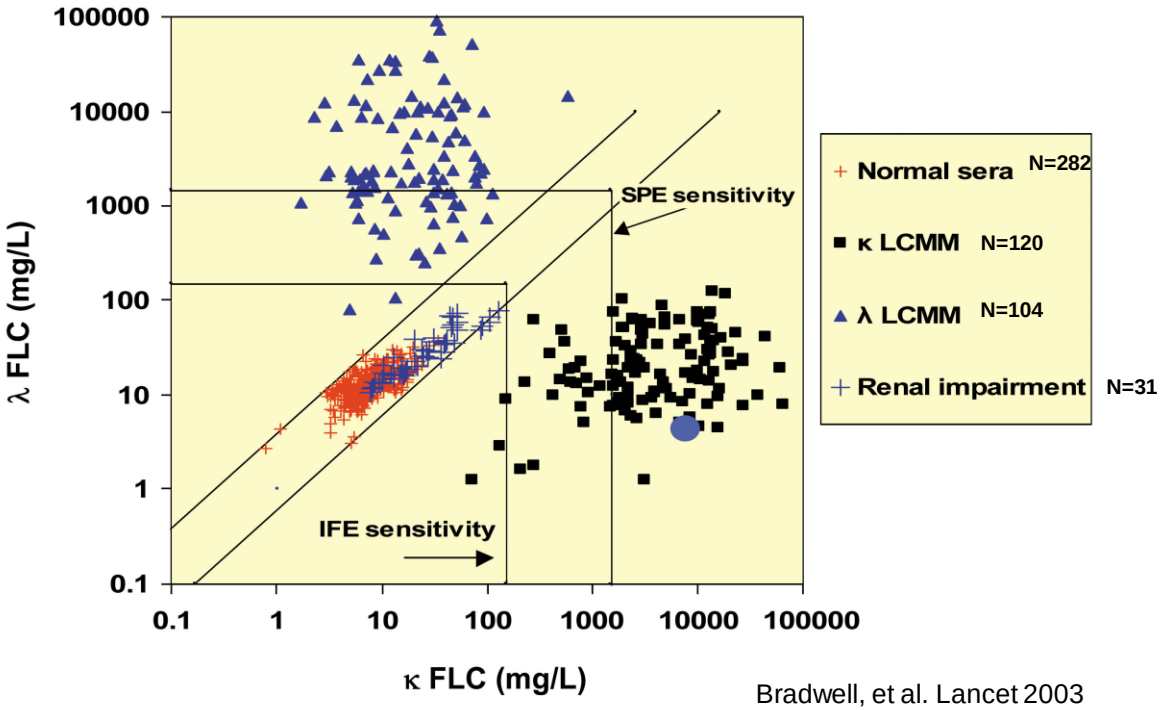
- 60% of MM are IgG, 20% are IgA and < 1 % are IgD or IgE

- Incidence
  - 10% of all hematological malignancies; 2nd only after non-Hodgkin lymphoma
  - Two time more frequent in blacks. Slight male preponderance.
  - The median age at onset is 66 years; very rare before age 40

- Treatment
  - Oral chemotherapy and transplantation has dramatically increased survival

Light chain multiple myeloma (20% of MM)

FLC ratio is more sensitive than sPEP and IFE combined

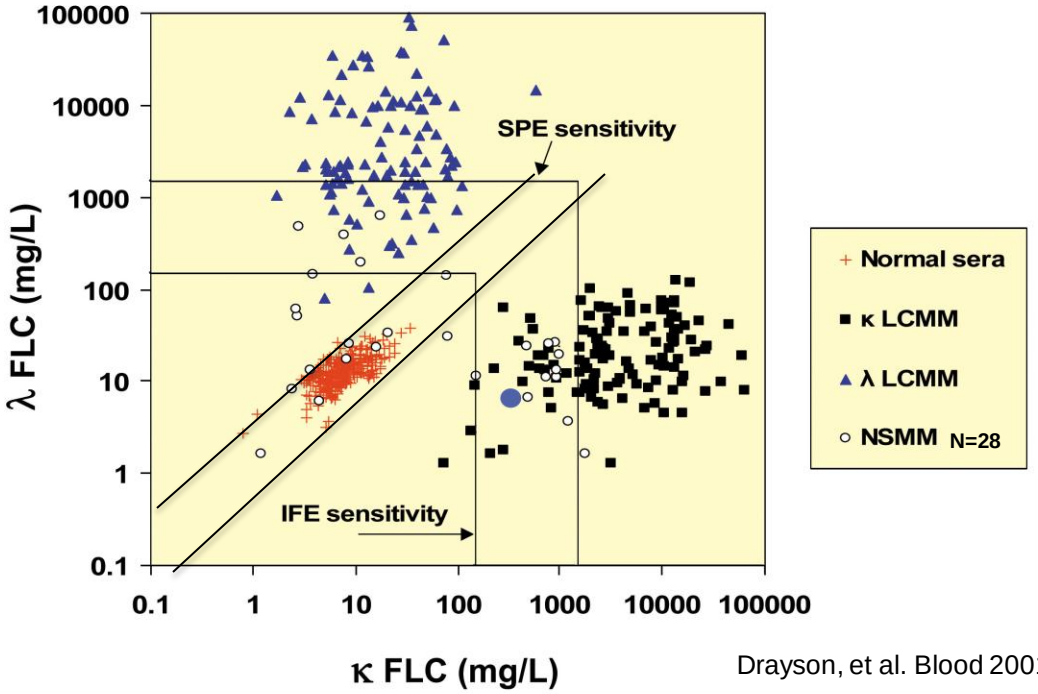


Bradwell, et al. Lancet 2003

Non-secretory multiple myeloma (NSMM)

- Dx Criteria
  - No serum and urine M protein
  - bone marrow plasma cells **> 10%**
  - **CRAB**
- Accounts for ~1-3% of all monoclonal gammopathies
- 10-15% of NSMM are true “non-producers”
  - Most NSMM are M-protein below sPEP sensitivity threshold
- More sensitive diagnosis of NSMM is done using FLC ratio assay

Diagnosis of NSMM by FLC ratio assay

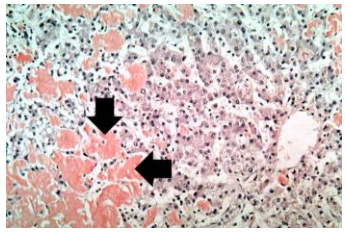


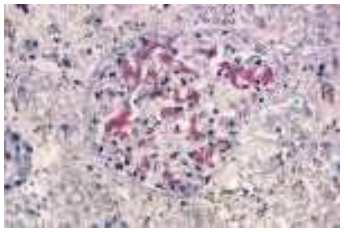
Drayson, et al. Blood 2001

19 out 28 NSMM were diagnosed by FLC ratio assay

Light chain (AL) Amyloidosis

- Malignant disorder of plasma cells (10% of monoclonal gammopathies)
  - Deposit of a fibrillar proteinaceous material (detected by Congo red staining) in various tissues (i.e., liver, kidney, heart, peripheral nerves)



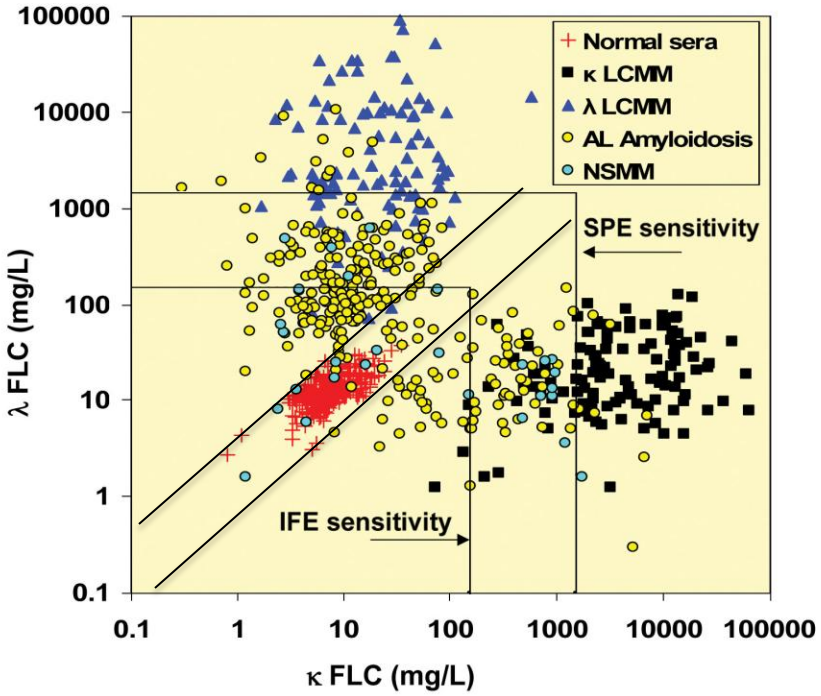


Photos courtesy of IPLAB.net

  - Evidence of a monoclonal plasma cell proliferative disorder by serum or urine M protein, or clonal plasma cells in the bone marrow

- Presentation
  - The clinical presentation of AL amyloidosis varies
    - Depends on the dominant organ involved
    - Nephrotic syndrome, restrictive cardiomyopathy and peripheral neuropathy are common presenting syndromes
    - Oral chemotherapy and transplantation – worst prognosis compared to MM

Diagnosis of AL Amyloidosis by FLC ratio assay



Diagnostic performance in AL (n=110)	
Assay	% Positive
sPEP + IFE	69
uPEP + IFE	83
FLC ratio	91

Katzmann, et al. Clin Chem 2005

Solitary Plasmacytoma

- Dx Criteria
  - Low or no serum and urine M protein
  - bone marrow not consistent with MM
  - No CRAB other than single bone lesion due to clonal plasma cells
- Accounts for ~3-5% of all monoclonal gammopathies
  - Twice more common in women
- Patients with SP are at risk of progression to MM
  - ~50% progress to MM over 3-4 years
- Most common in medullay sites
  - Can also occur in extramedullary sites (upper respiratory tract)
- Treatment
  - Irradiation of involved site

Other diseases associated with clonal gammopathies

Waldenström Macroglobulinemia ("IgM multiple myeloma")

- Serum IgM M-protein of any concentration
- Bone marrow lymphoplasmatic infiltration
- Most common symptoms: anemia, hyperviscosity, lymphadenopathy, hepatosplenomegaly
- Poor response to therapy

POEMS Syndrome

Paraneoplastic syndrome including: **P**olyneuropathy, **O**rganomegaly, **E**ndocrinopathy, **Monoclonal gammopathy** and **S**kin changes

Heavy-chain disease: poor prognosis

Sensitivity of monoclonal gammopathy screening panels

	MGUS (n=524)	MM (n=467)	AL (n=581)
sPEP, sIFE, **uPEP**	100	98.7	94.2
sPEP, sIFE, **FLC ratio**	97.1	100	97.1
FLC ratio	42.4	96.8	88.3

Katzmann, et al. Clin Chem 2009

In the future, FLC ratio assay most likely will replace urine testing during screening of monoclonal gammopathies

Current screening recommendations

sPEP

sIFE

uPEP

Serum FLC ratio assay as indicated (LCMM, NSMM, AL)

Monitoring recommendations

sPEP or uPEP

FLC ratio assay (non-measurable disease)