

Paraproteinaemia & Serum Free Light Chains

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Paraproteinaemia

- Increased production of a single monoclonal immunoglobulin/fragment
- Due to a diverse group of disorders: Myeloma, Waldenstrom's, Lymphoma, CLL, Cryoglobulinaemia, AL Amyloidosis & MGUS
- MM: Presence of malignant plasma cells in the bone marrow, usually secrete a monoclonal immunoglobulin/fragment, diagnostic criteria includes detection/typing of paraprotein

Presenting clinical features of MM

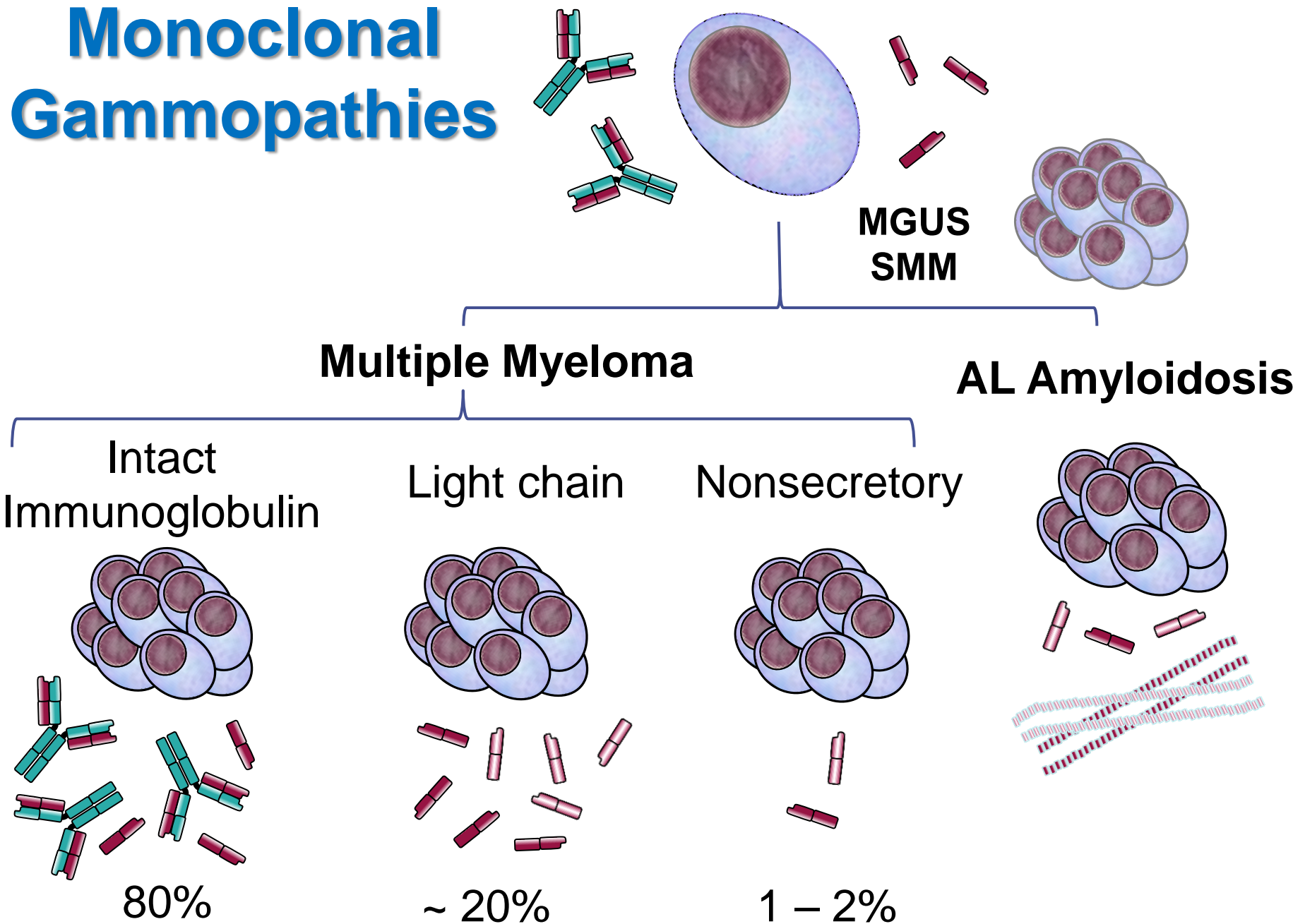
- HyperCalcaemia (>2.75 mmol/L)
- Impaired Renal function (Creat >173 mmol/L)
- Anaemia (Hb <10 g/dL)
- Bone disease (Lytic Lesions/Fractures)

- Recurrent bacterial infection
- Hyperviscosity

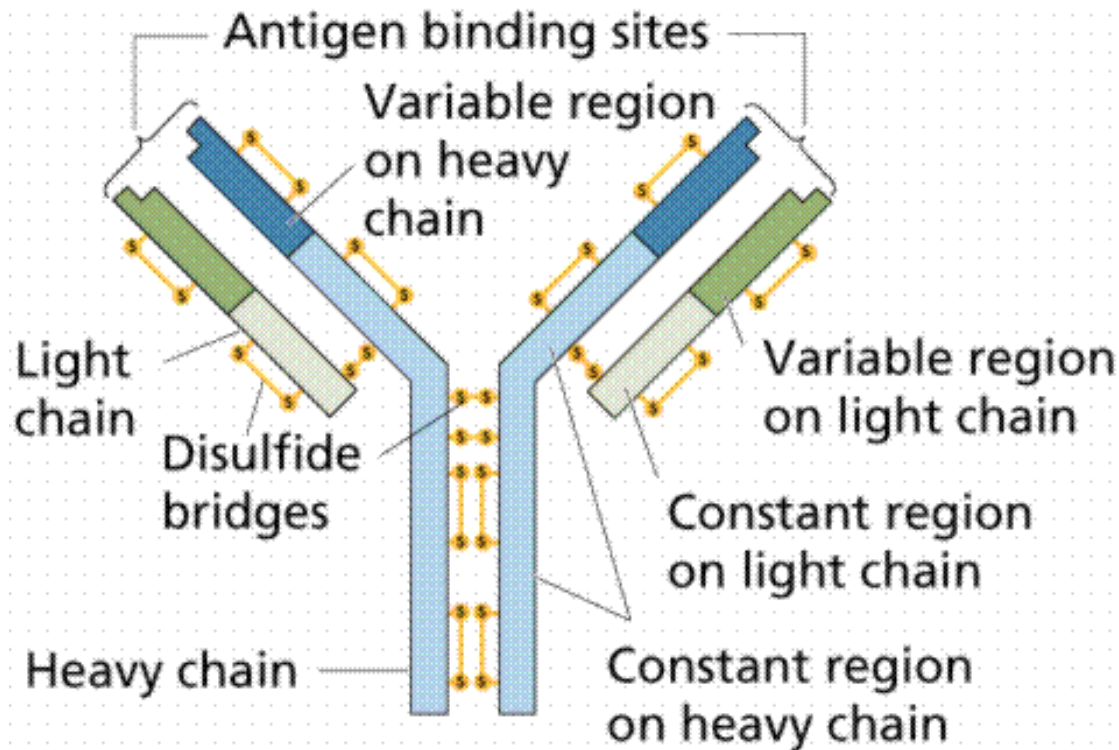
Paraprotein Types & Myeloma

Type	Paraproteins	Myeloma
IgG	53%	53%
IgA	22%	22%
IgM	11%	0.5%
IgD	1%	1.5%
IgE	<0.001%	0.1%
BJP Only	12%	21%
Non-Secretory	-	1%

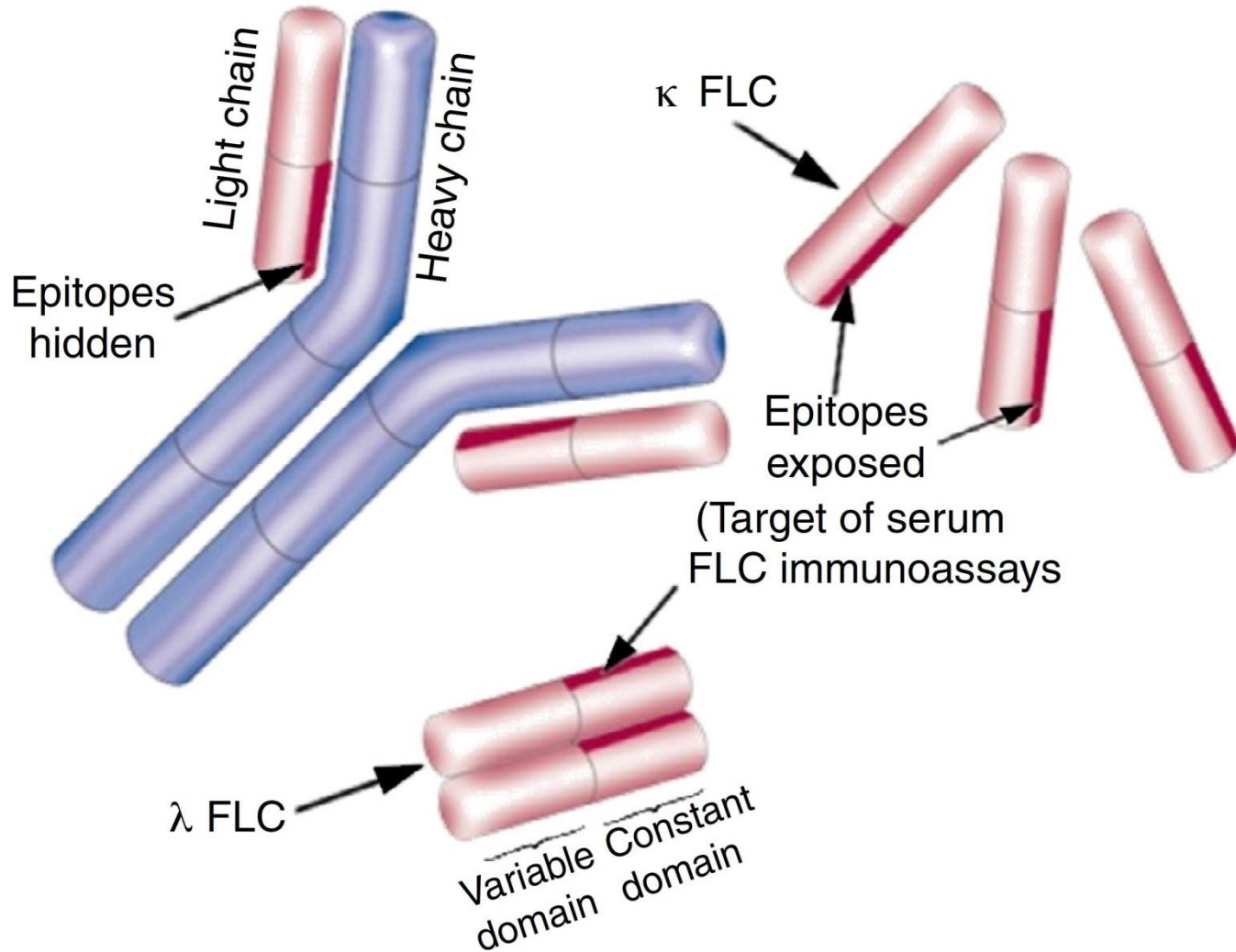
Monoclonal Gammopathies



Immunoglobulin Light Chains



Immunoglobulin Light Chains



Serum Free Light Chain Assay

- Quantitation by turbidimetry on The Binding Site OptiLite analyser



- Rate of \uparrow in light scattering from particles suspended in solution \propto [antigen] in the sample

Result Interpretation

K

λ

κ/λ

10 mg/L

15 mg/L

0.67

3.3 – 19.4 mg/L

5.7 – 26.3 mg/L

0.26 – 1.65

N

N

N

FLC levels and ratio **NORMAL** – No monoclonal FLCs detected

Result Interpretation

κ

30 mg/L

3.3 – 19.4 mg/L



λ

1 mg/L

5.7 – 26.3 mg/L



κ/λ

30

0.26 – 1.65



κ FLC levels high; λ FLC levels low

FLC ratio **HIGH** - Monoclonal **Kappa** FLCs

Myeloma? MGUS? NHL? AL amyloidosis? CLL?

Result Interpretation

K

1.5 mg/L

3.3 – 19.4 mg/L



λ

91.0 mg/L

5.7 – 26.3 mg/L



κ/λ

0.02

0.26 – 1.65






λ FLC levels high; κ FLC levels low

FLC ratio LOW - Monoclonal Lambda FLCs

Myeloma? MGUS? NHL? AL amyloidosis? CLL?

Result Interpretation

K	λ	κ/λ
65.1 mg/L	55.5 mg/L	1.17
3.3 – 19.4 mg/L	5.7 – 26.3 mg/L	0.26 – 1.65
		

FLC Ratio **NORMAL** – No monoclonal FLCs detected

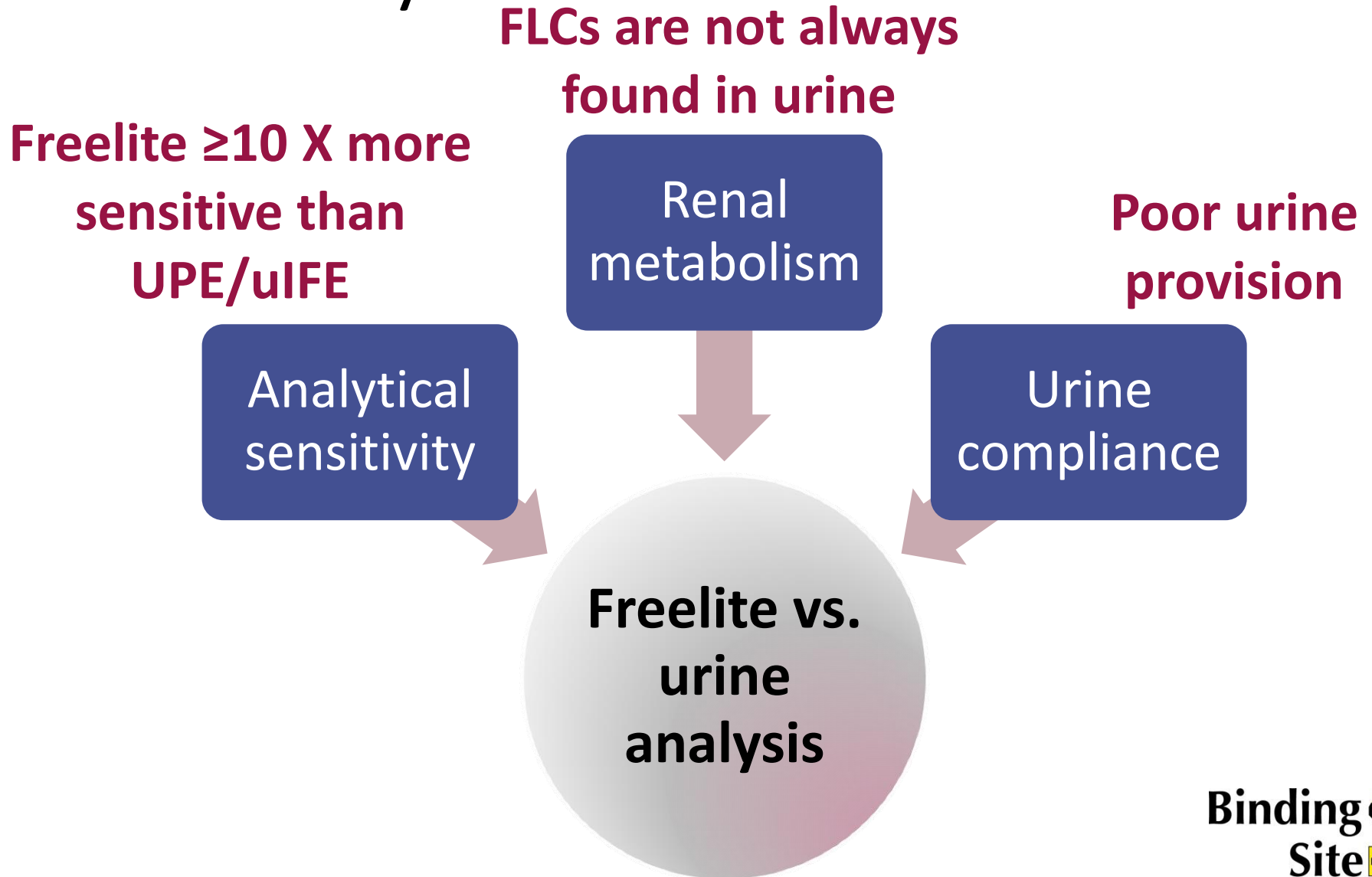
Potential causes of \uparrow sFLC:

Infection, Inflammation, Autoimmune, Renal Impairment

Result Interpretation

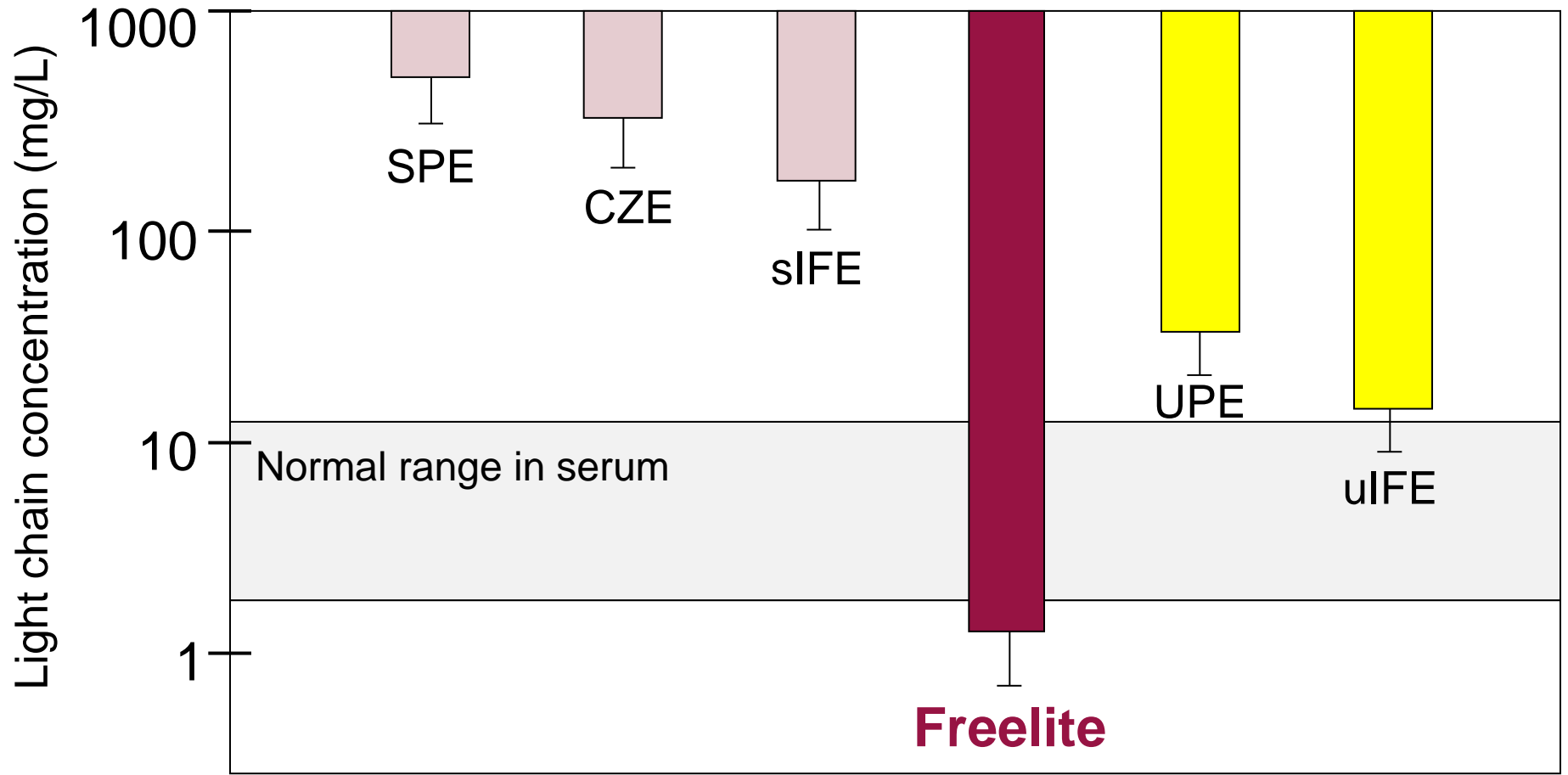
- \uparrow or \downarrow ratios are seen in plasma cells disorders that produce excess monoclonal light chains
- Results should always be interpreted in conjunction with other laboratory and clinical findings
- Slightly abnormal results do not always indicate disease
- Normal results do not always indicate absence of disease
- \uparrow LC and a markedly abnormal sFLC ratio indicate an increased risk of disease progression

3 advantages of serum Freelite vs. urine analysis

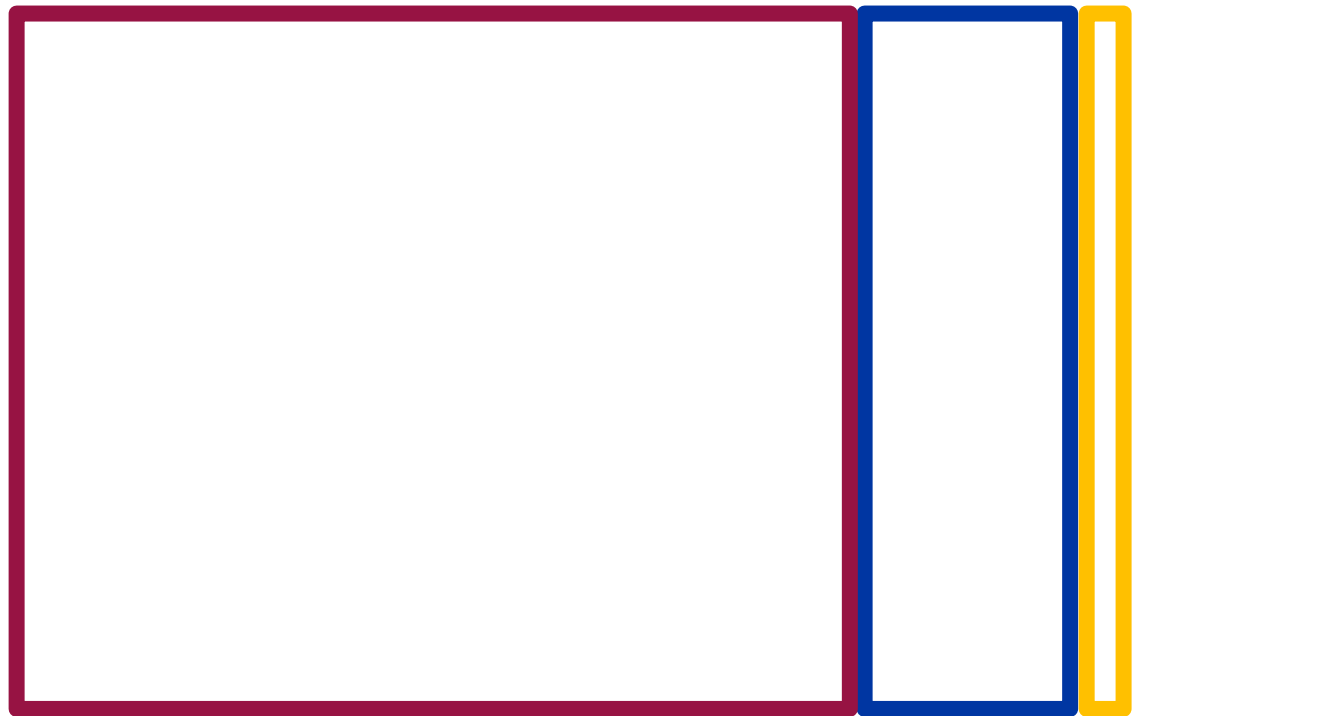


Analytical sensitivity

Freelite is ~10-fold more sensitive than uIFE



Investigation of 'Query MM' Patients



IIMM

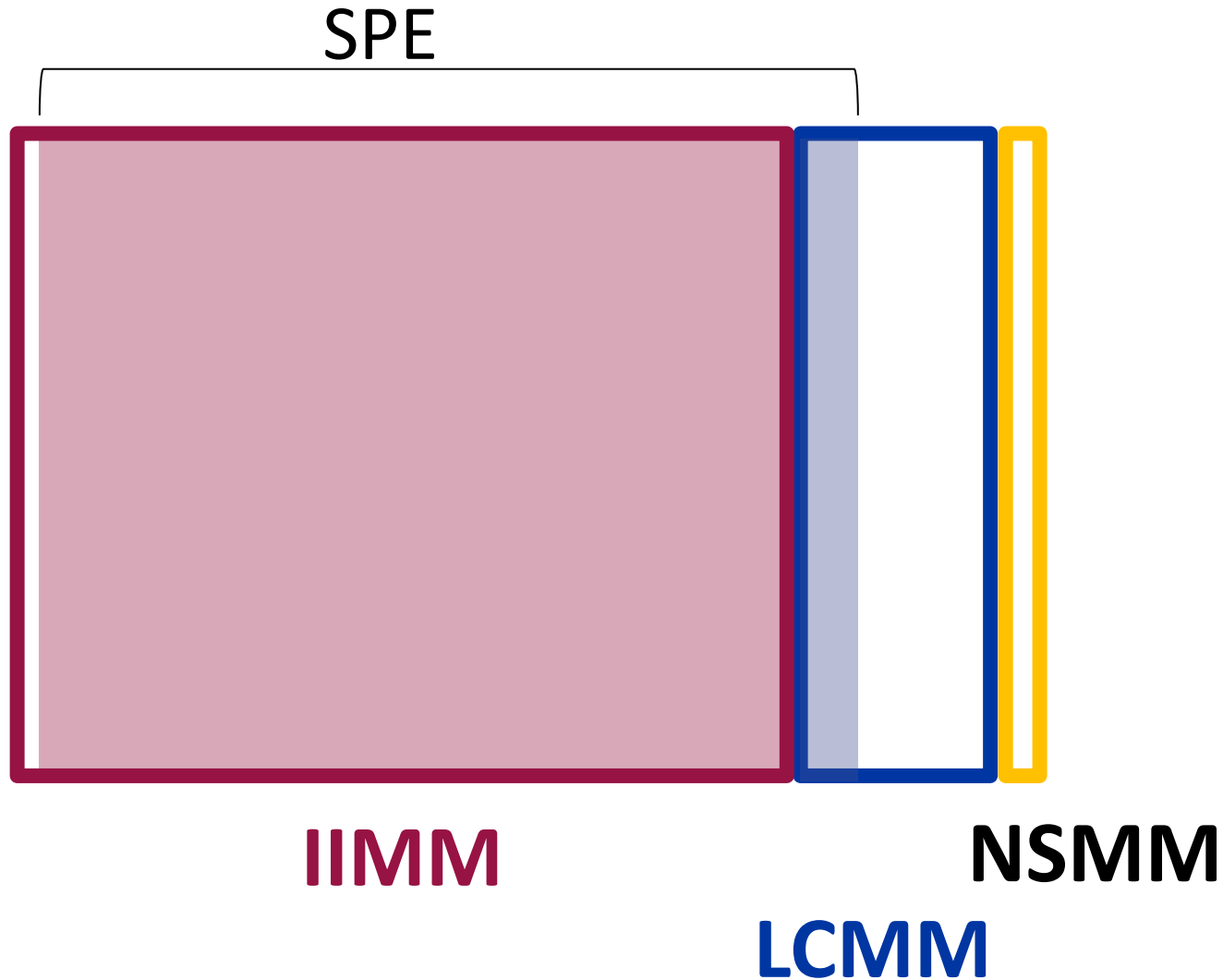
80%

LCMM 1-2%

15-20%

NSMM

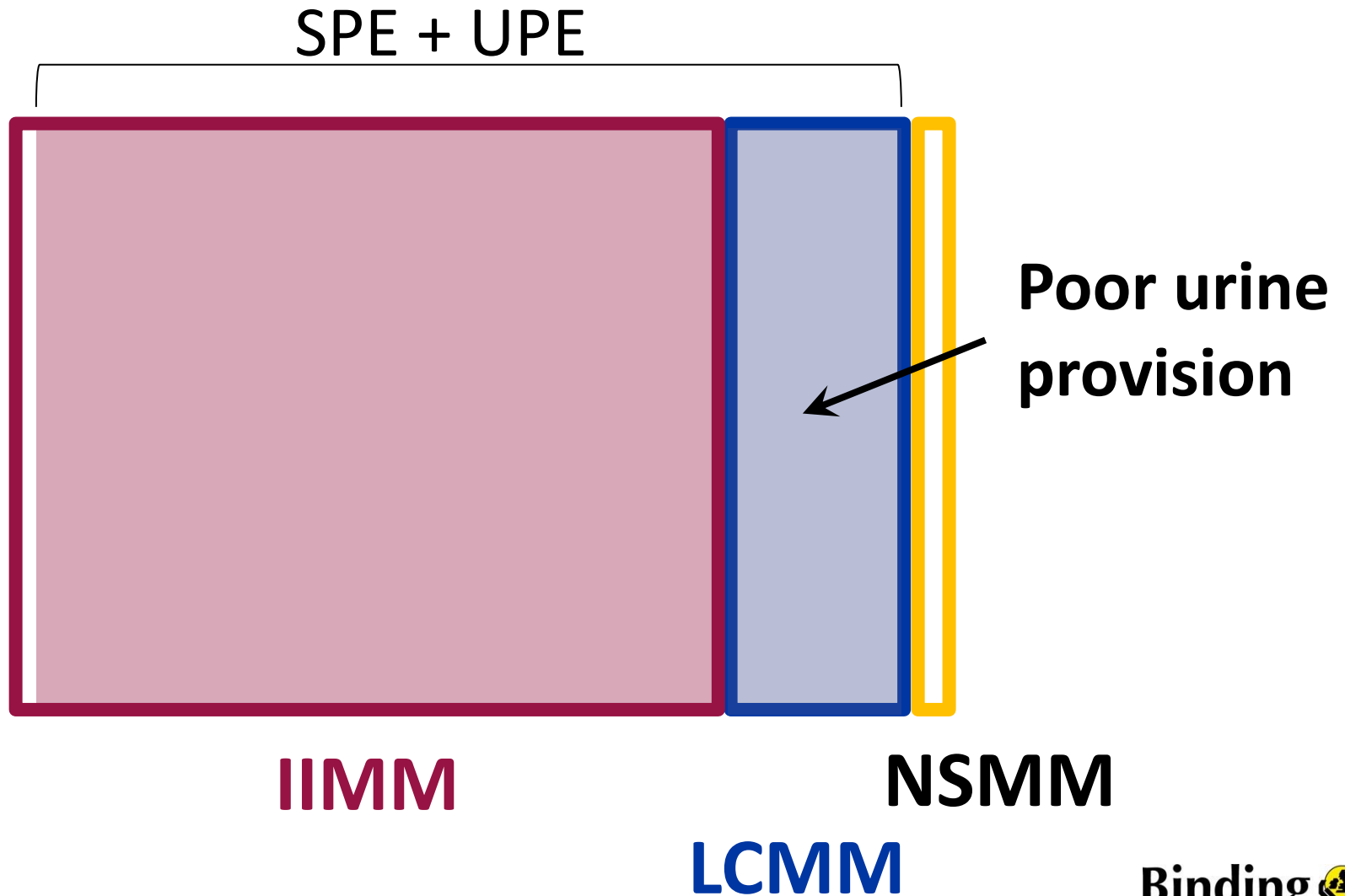
Investigation of 'Query MM' Patients



Abraham Clin Chem 2002;48:655-7

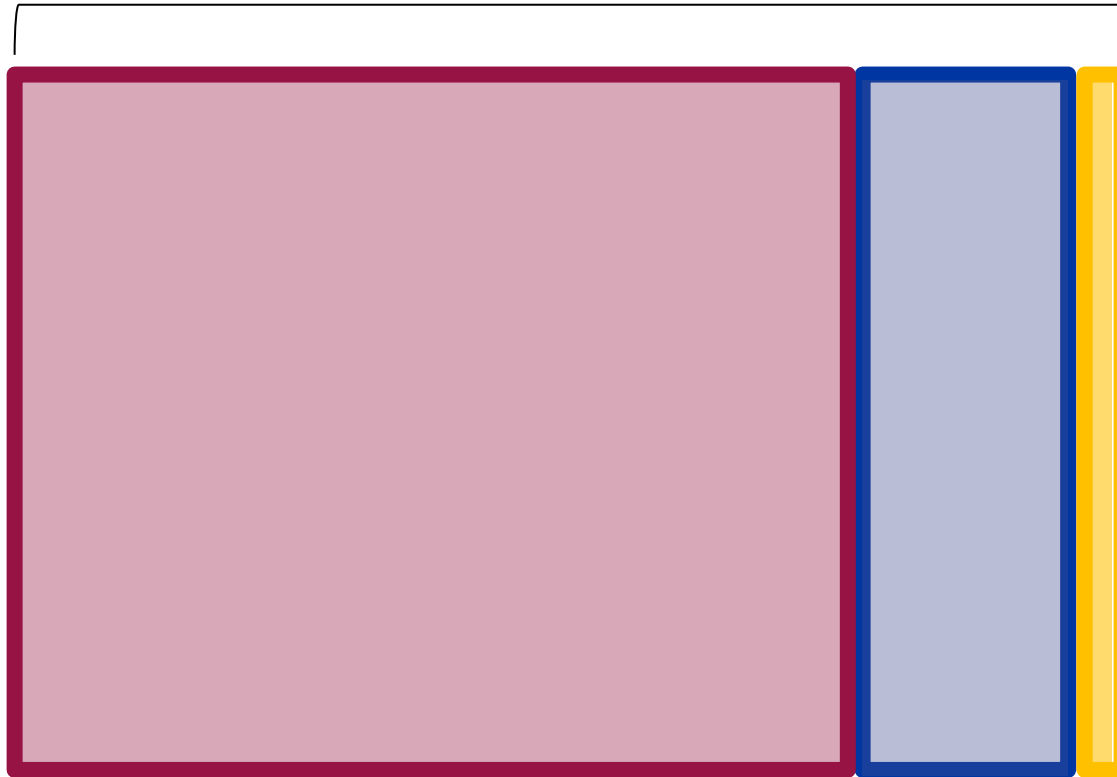
Katzmann Am J Clin Pathol 1998;110:503-9

Investigation of 'Query MM' Patients



Investigation of 'Query MM' Patients

SPE + Freelite

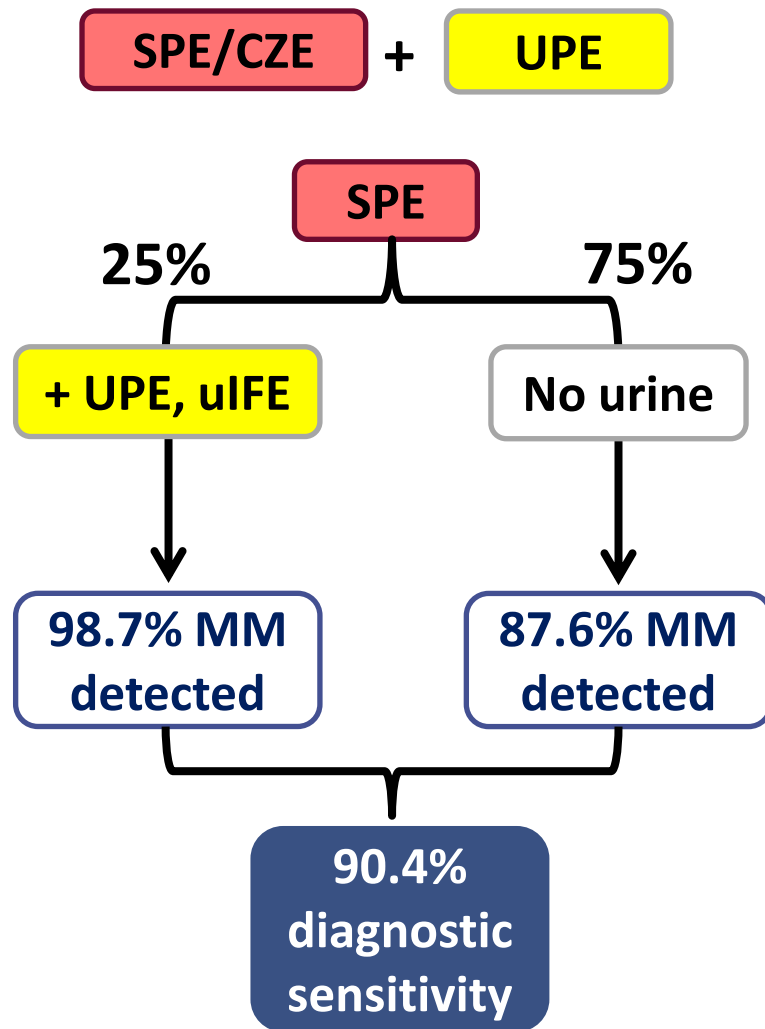


IIMM

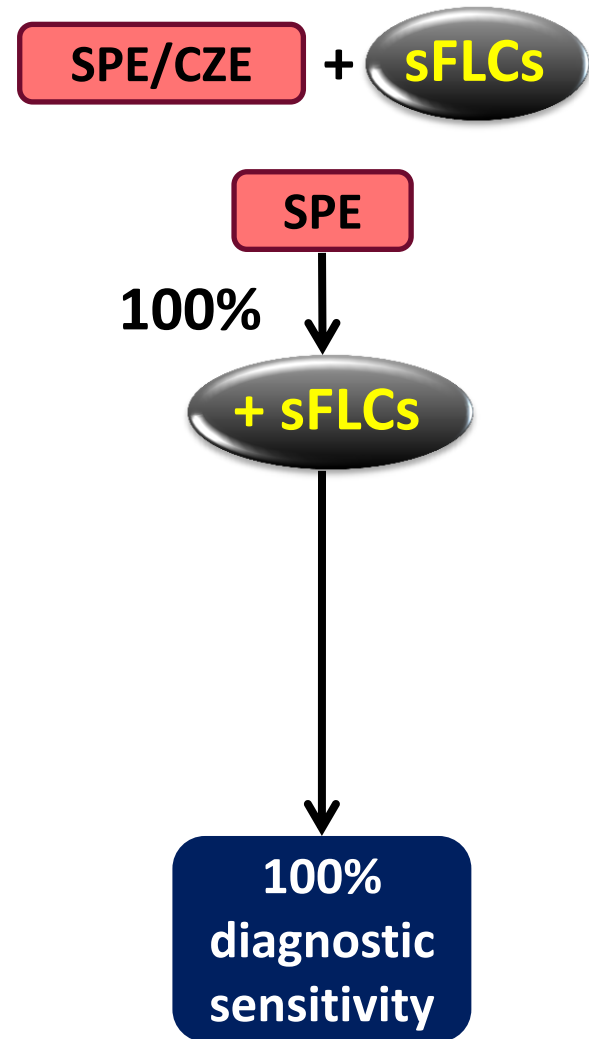
LCMM

NSMM

Sensitivity of 'Query MM' algorithms



vs.



Laboratory Investigation

- Screening Tests: FBC, ESR/Plasma Viscosity, Renal Function, Calcium, Albumin, Serum Immunoglobulins, Serum PEP (CZE or gel), Urine PEP (analysis by gel, 2nd Void/24hr Hr) &/or Serum Free Light Chains
- Establish Diagnosis: Immunofixation of serum/urine, Bone marrow aspirate + trephine biopsy with plasma cell phenotyping
- Estimation of Tumour Burden/Prognosis: FISH, Quantitation of Monoclonal (M) protein, albumin, β_2 M, Serum Free Light Chains

Sample Analysed for Immunoglobulins & Electrophoresis

M-Protein Detected

Equivocal

Negative

Immunofixation for Hv.
Chain G/A/M, + LCs

Negative

IgG/A/M κ/λ
paraprotein

Hv. Chain not
detected, κ/λ LC
detected

Zone(s)/Multiple
Bands of Varying
Isotype

Typed Paraprotein,
quantitation, referral
to haematologist,
send urine for
BJP/**sFLC**, Request:
FBC, Renal/Bone
profiles, LDH

IF for D/E

IgD/E κ/λ
paraprotein or LC
only detected

Repeat
in 3 – 6
months

Analyse Urine for
BJP or measure
sFLC if clinically
suspected

BCSH/UKMF Guidelines: Diagnosis & Management of MM 2014

- Serum and urine PEP/immunofixation
- sFLC: indicated when high suspicion of MM but routine sPEP/immunofixation is negative
- sFLC: additional tool for assessment of LC production/response to treatment, LC only myeloma & oligosecretory/non-secretory disease
- Renal impairment \uparrow sFLC $\frac{1}{2}$ -life, \Rightarrow renal ref. range
- sFLC: Monitoring asymptomatic myeloma

NICE 2016 Guidelines: Laboratory investigations for people with suspected myeloma

- Detection of PP/Myeloma/MGUS: Use serum protein electrophoresis and sFLC assay
- Serum immunofixation to confirm abnormal findings
- Do not use serum protein electrophoresis, immunofixation, sFLC or urine electrophoresis (BJP) alone to exclude a diagnosis of myeloma
- Assess prognosis: sFLC and use sFLC ratio

North Thames ACB Audit Group Guidelines 2009: Laboratory Standards

- Suspected myeloma/plasma cell dyscrasias: investigated by serum & urine PEP (CZE or high resolution agarose gel)
- Quantitation of PPs: densitometry/AUC
- Newly diagnosed patients: β_2 M and sFLC for prognostic use
- sFLC is useful: diagnosis/management of oligo secretory myeloma, plasmacytoma, BJP only myeloma
- Suggest referral to a Consultant Haematologist if a monoclonal band is detected or BJP positive

Summary & Conclusions

- For full investigation of ?Myeloma: request **serum electrophoresis** with **urine BJP** *and/or* **sFLC**
- Patients with high suspicion of MM but negative/ equivocal results sPEP: suggest sFLC
- Positive urine BJP and/or inappropriately \uparrow sFLC ratio: suggest referral to a Haematologist
- sFLC uses: diagnosis/management of MM, oligo secretory myeloma, prognostic marker, assessing response treatment, monitoring of asymptomatic myeloma

References

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- International Myeloma Working Group guidelines for serum-free light chain analysis in multiple myeloma and related disorders. *Leukaemia* 2008; 23: 2
- UK myeloma Forum and Nordic Myeloma Study Group: Guidelines for the investigation of newly detected M-proteins and the management of monoclonal gammopathy of undetermined significance (MGUS). *British Journal of Haematology* 2009; 147, 22-42
- ACB North Thames Audit Guidelines: 2009
- Capillary electrophoresis and its application in the clinical laboratory. 2003, *Clinica Chimica Acta*, 330: 1-2, 1-30