

Serum Free Light Chain estimation in plasma cell disorders: What have we learned?

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Disclosures

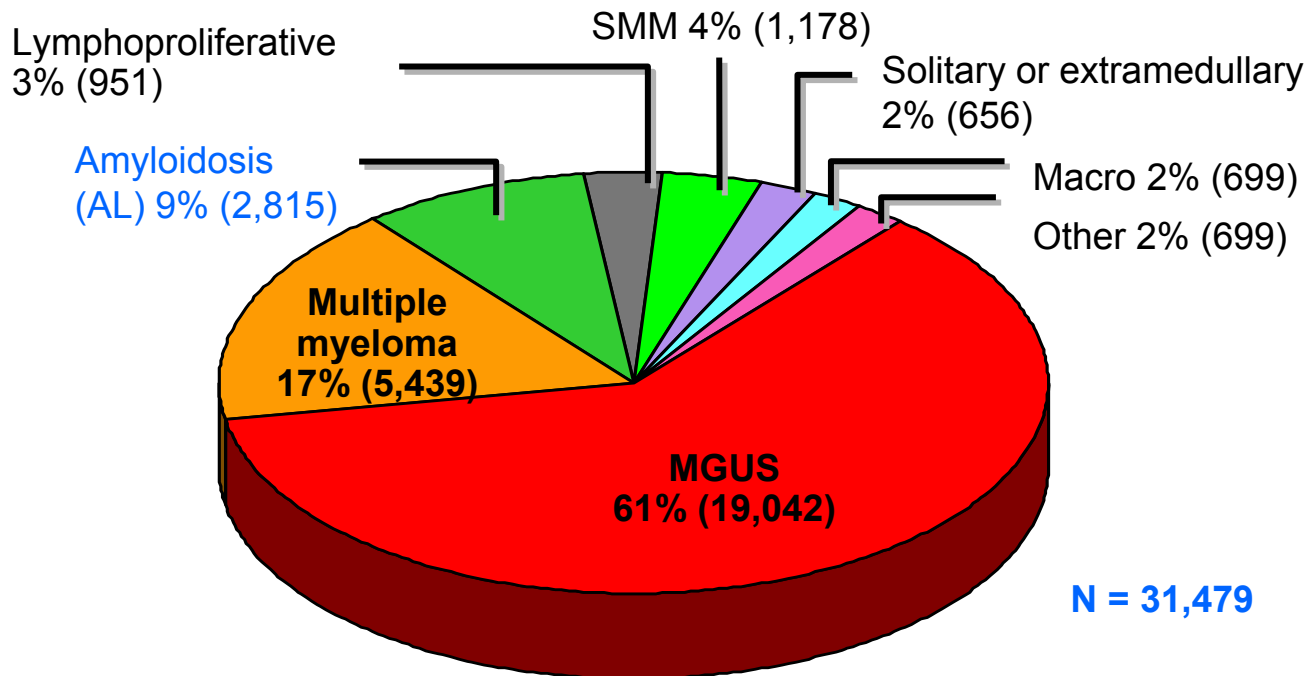
No relevant disclosures

Objectives

- Concept of immunoglobulin free light chains
- Describe the serum free light chain assay
- Discuss the role of serum free light chains in plasma cell disorders for
 - Screening
 - Diagnosis
 - Prognosis
 - Treatment Response

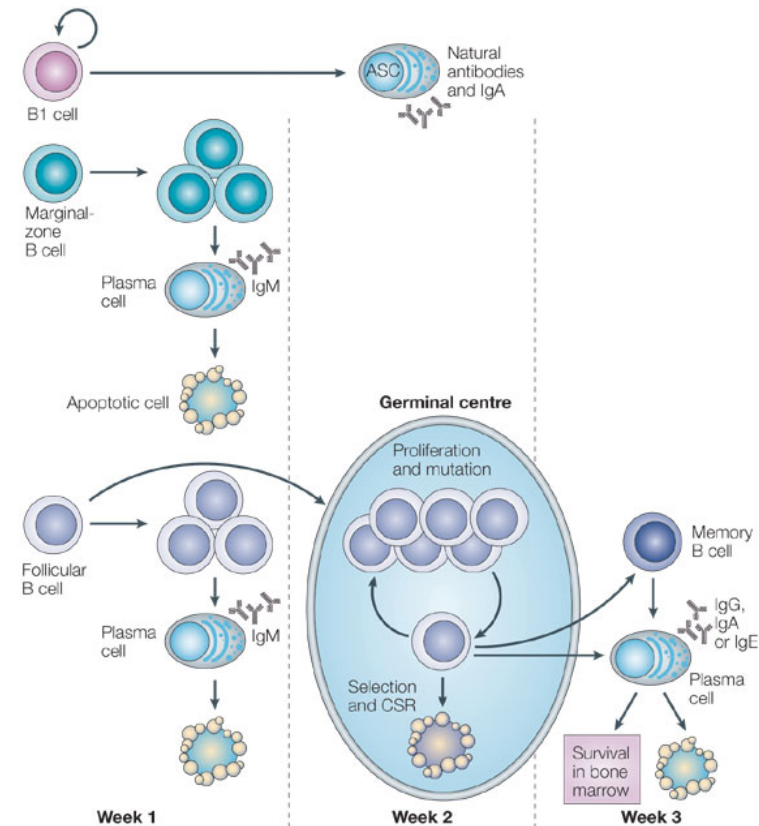
Monoclonal Gammopathies

- Spectrum of plasma cell proliferative disorders



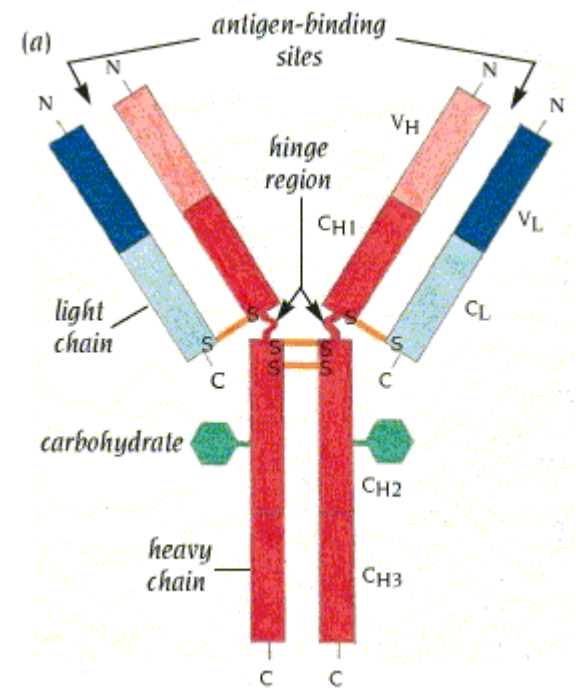
Pathology

- Proliferation of terminally differentiated plasma cells
- Clonal plasma cells found mostly in the marrow
- Late stages characterized by presence of large numbers of circulating plasma cells



Immunoglobulins

- Secreted by plasma cells
- Heavy Chain: G, A, M, E, D
- Light Chain: kappa or lambda
- Circulate as intact immunoglobulin, and as separate kappa or lambda free light chains



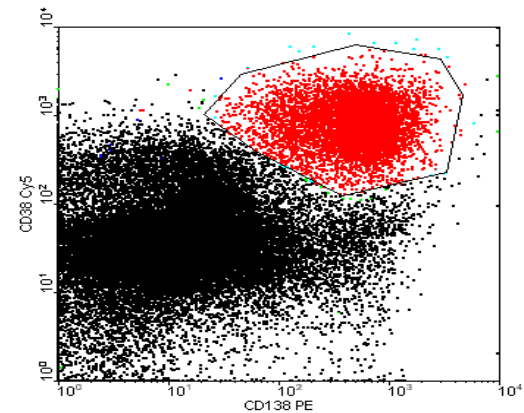
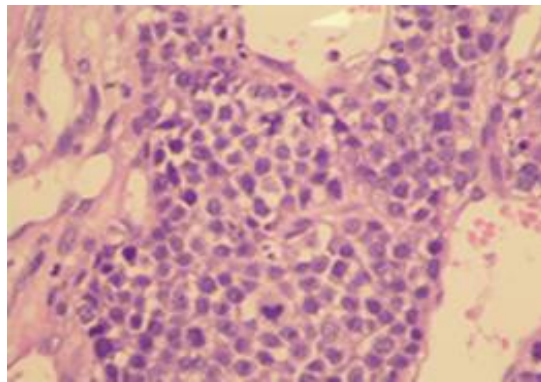
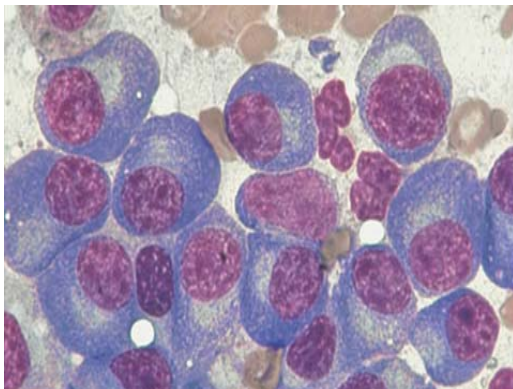
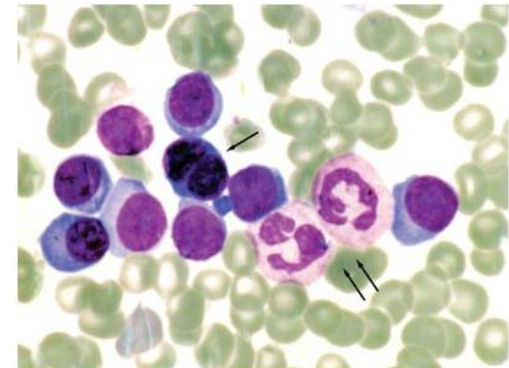
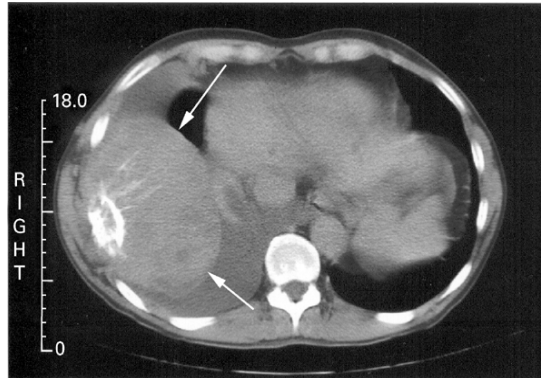
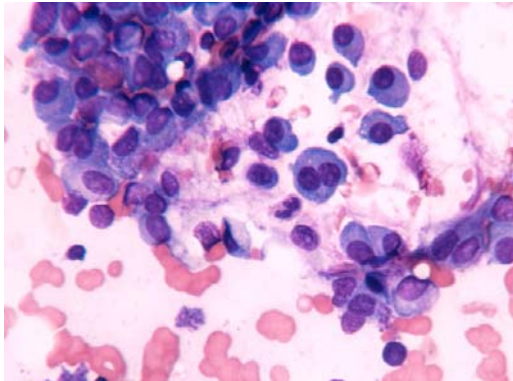
Diagnosis of Monoclonal Gammopathy

Presence of clonal plasma cells

And usually

A monoclonal protein in serum or urine

Detection of Clonal Plasma Cells



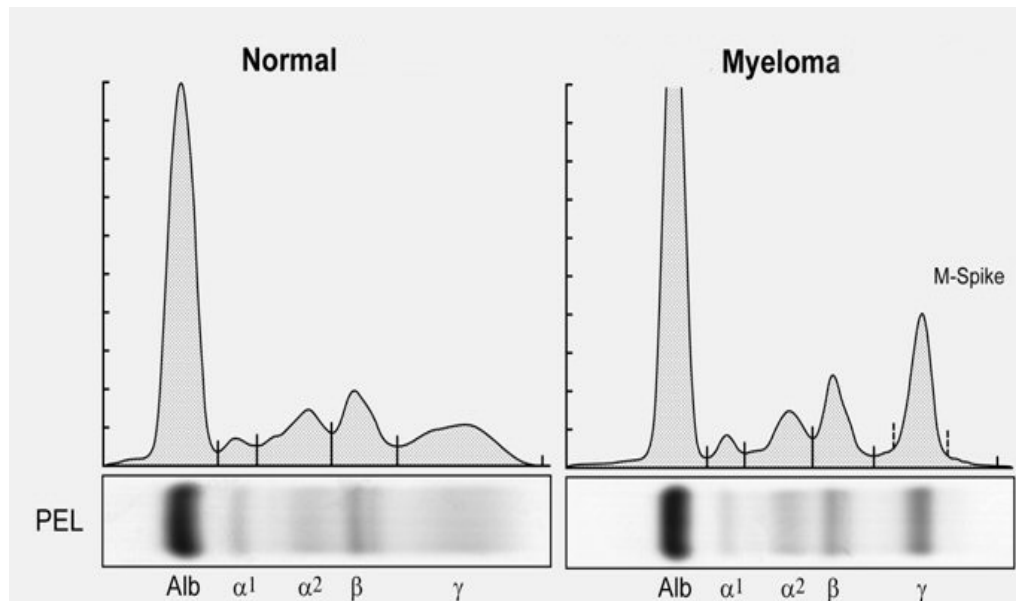
Bone marrow

Plasmacytoma

Peripheral blood

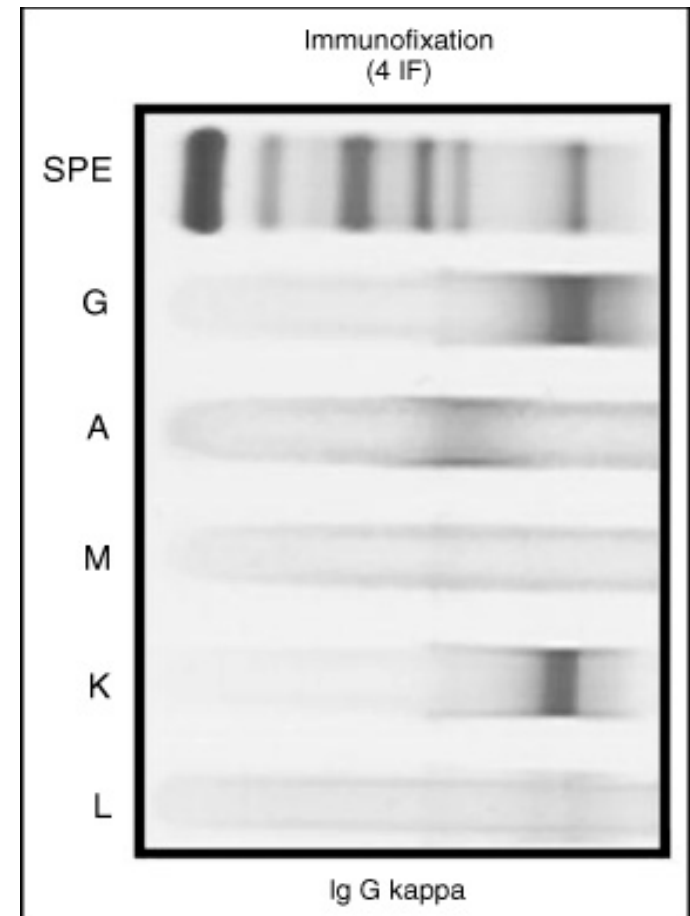
Testing for monoclonal protein

- Immunoelectrophoresis (serum/ 24 hour urine)
 - Gel electrophoresis
 - Quantitative



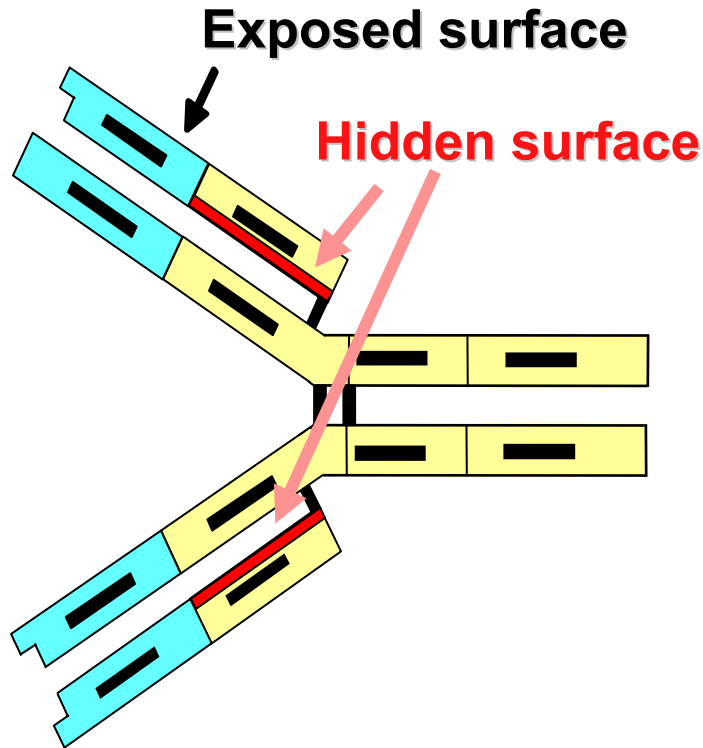
Immunofixation

- Serum/ urine
- Qualitative
- Allows identification of heavy and light chain type



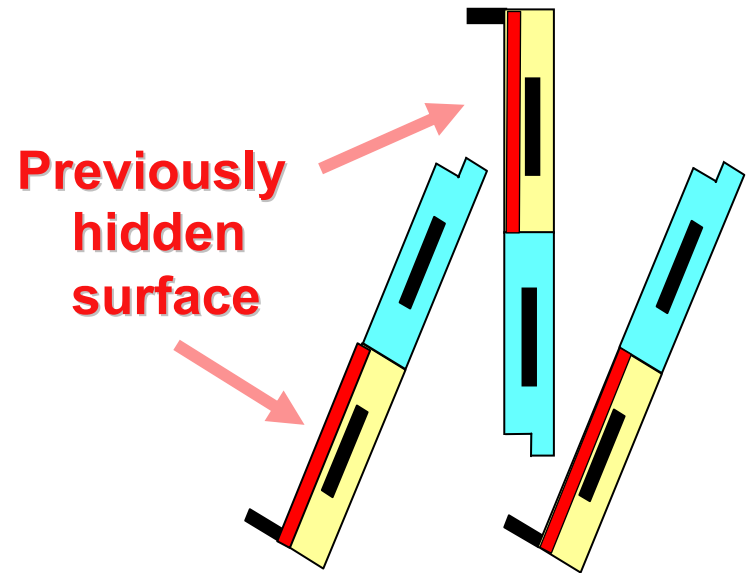
IgG kappa M protein

Intact Immunoglobulin



Free Light Chain

Binding Site®, free light chain assay



FLC reference range:

κ 3.3 – 19.4 mg/L

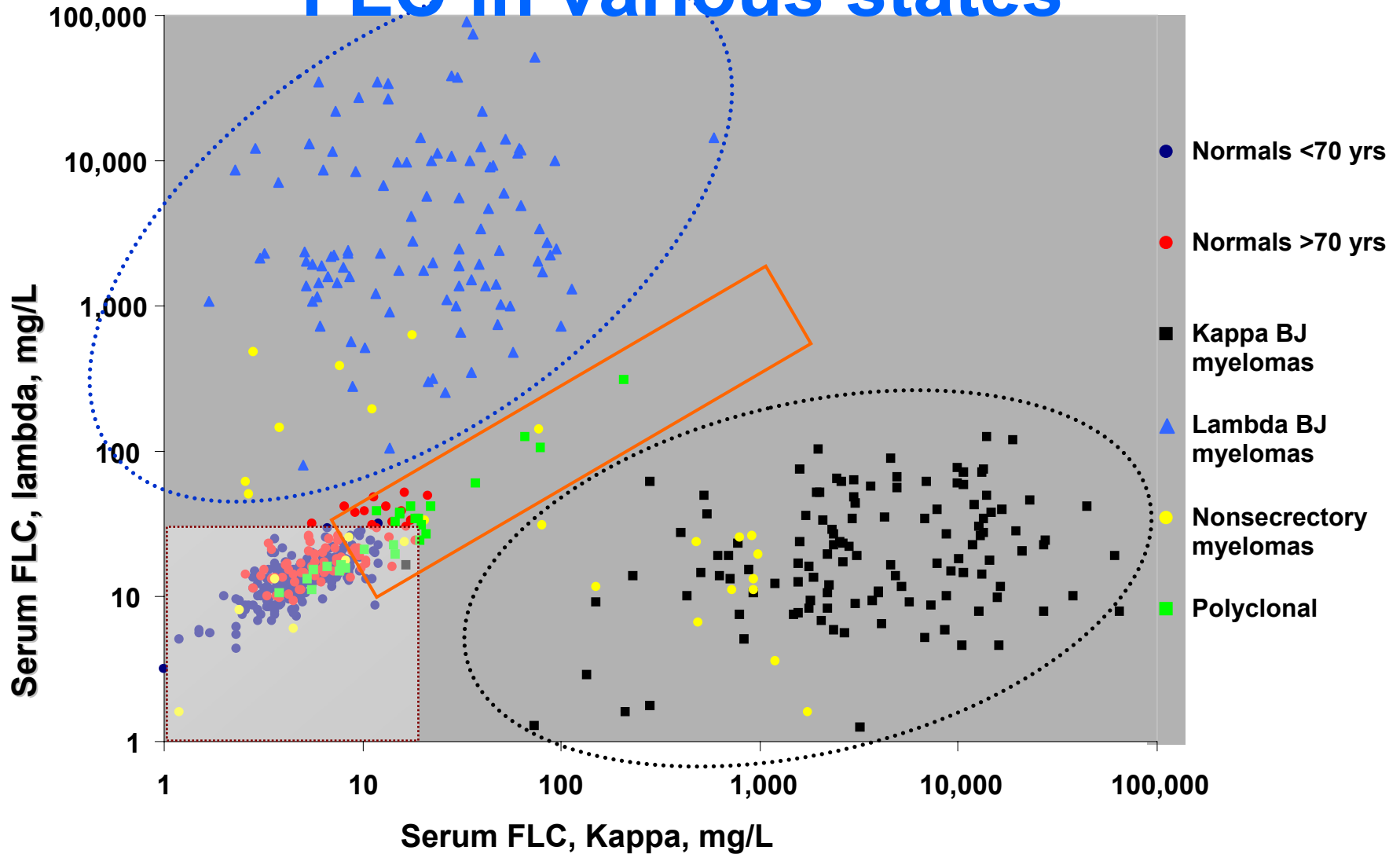
λ 5.7 – 26.3 mg/L

κ/λ ratio 0.26 - 1.65

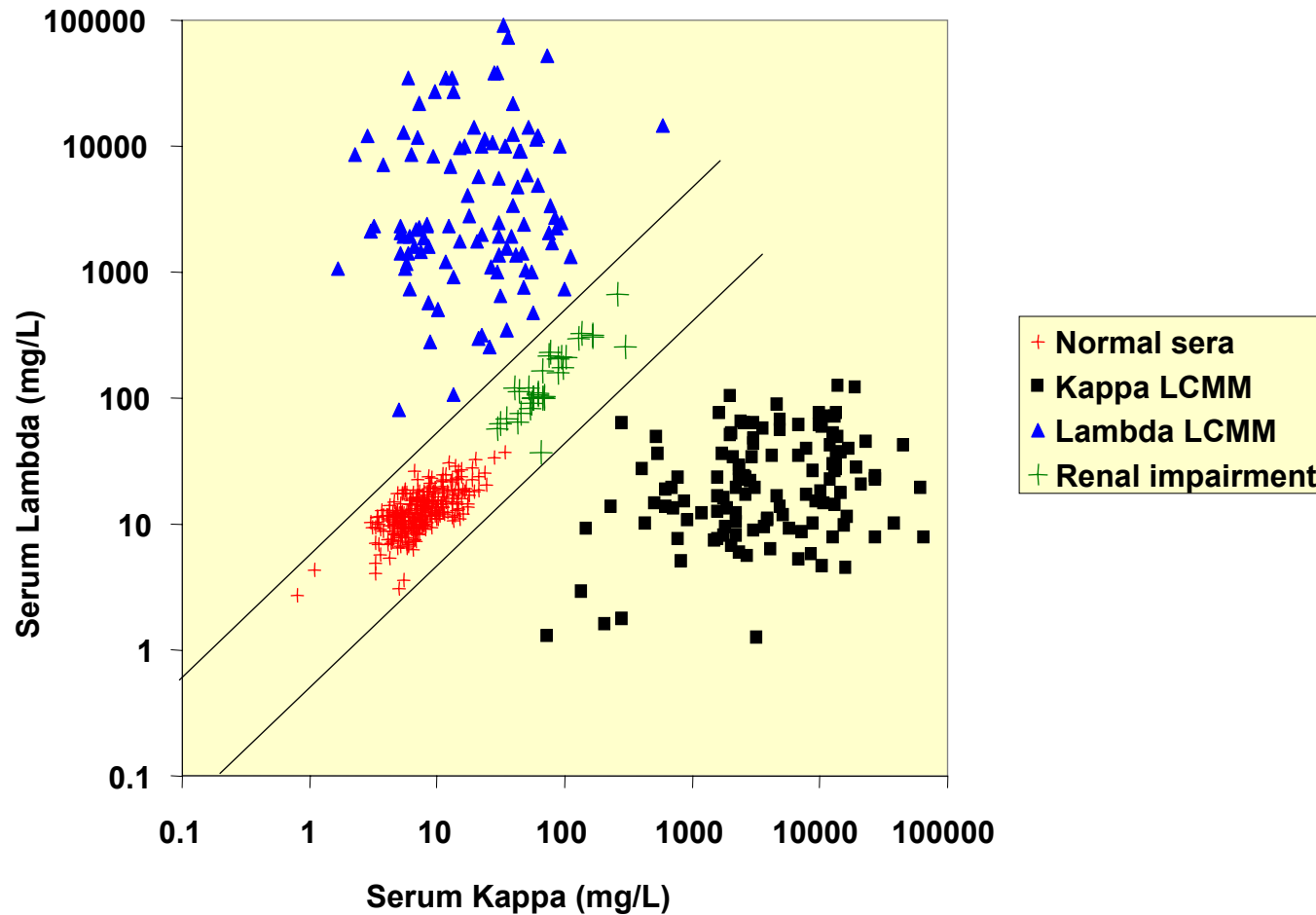
Serum free light chain

- Secreted by plasma cells, cleared through glomeruli and metabolized by proximal tubule
- Normal production 0.5 – 1.0 g/day
- Kidneys can metabolize 10-30 g/day
- Production can be affected in various states
- Reduced renal function will decrease elimination

FLC in various states



Serum free light chain and renal function



Reading the FLC results

- Test results
 - Kappa / Lambda FLC ratio (Normal: 0.26 - 1.65)
 - Absolute values of kappa and lambda FLC
 - Kappa: 3.3 – 19.4 mg/L
 - Lambda: 5.7 – 26.3 mg/L
- Derived values
 - FLC difference (difference between absolute values)
 - Involved to uninvolved FLC ratio

Rates of Abnormal rFLC in Different Plasma Cell Disorders

Condition	n	Abn rFLC, %
Multiple myeloma (MM)		
Symptomatic MM	1706	96
“Non secretory” MM	33	73
Light chain MM	252	100
MGUS	1262	34
AL amyloidosis	467	95
LCDD	28	93
Smoldering MM	345	90
Plasmacytoma	116	47

How is serum FLC assay useful?

- It has value in
 - Disease screening and diagnosis
 - Disease prognostication
 - Measuring effect of treatment and guiding treatment decisions

Screening for plasma cell disorders

- Serum and 24 hour urine electrophoresis (PEL) and immunofixation (IFE)
- 24 hour urine collection is cumbersome
- Question: Can we eliminate urine studies?
- 428 patients with various monoclonal gammopathies, with no prior treatment
- Had a positive urine IFE, and available concurrent serum assays for PEL, IFE, & FLC

Serum PEL & IFE Results: 428 Patients with Monoclonal Urinary Protein

<u>Lab Test</u>	<u># Abnormal (%)</u>	
• Urine IFE	428	(100%)
• Serum PEL	346	(80.8%)
• Serum IFE	400	(93.5%)

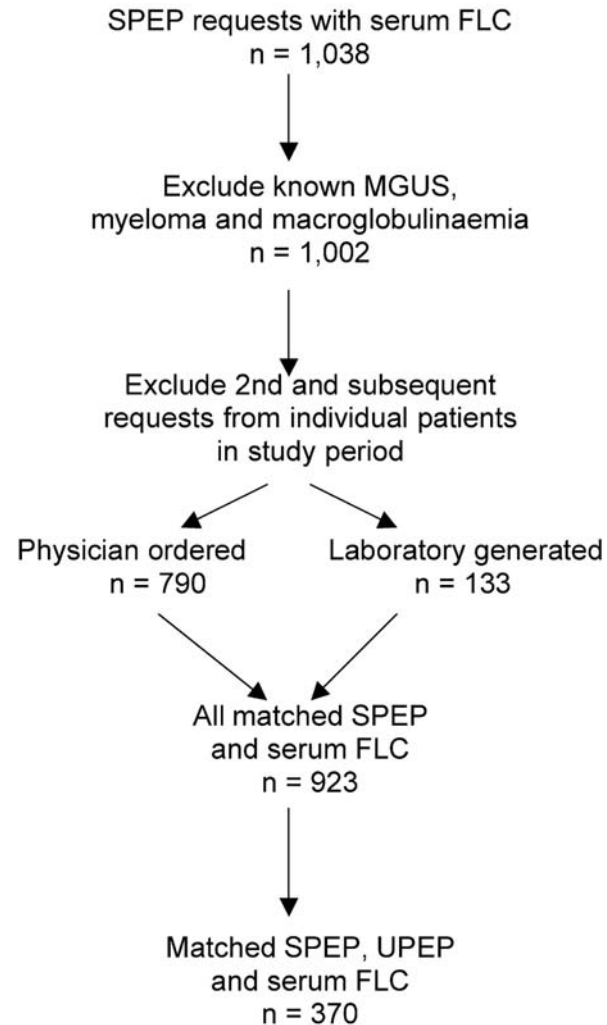
Serum PEL/IFE missed 28 cases (6.5%):

- Primary Amyloid, n = 19
- Plasmacytoma, n = 3
- MGUS, n = 3
- Multiple Myeloma, n = 2
- Smoldering MM, n = 1

Laboratory Results: 428 Patients with Monoclonal Urinary Protein

<u>Lab Test</u>	<u># Abnormal</u>	<u>(%)</u>
• Urine IFE	428	(100 %)
• Serum IFE	400	(93.5 %)
• Serum PEL	346	(80.8%)
• Serum FLC K/L ratio	367	(85.7 %)
• Serum PEL/IFE <u>or</u> FLC ratio	426	(99.5%)
• 1 Idiopathic λ Bence Jones proteinuria, urine M-spike = 83 mg/24 hrs		
• 1 MGUS (?sample contamination: urine IgA kappa, serum neg, subsequent urine neg)		

Serum FLC as screening test



Serum FLC in Urine IFE + patients

- Of the 370 urine samples, urine IFE revealed monoclonal bands in 15
- Four of these patients had a normal FLC
- In 1 patient, serum IFE was positive
- In 1 patient, the BJP resolved when polyclonal hypergammaglobulinemia resolved
- In another, a follow-up urine was negative
- Only 1 of the 3 patients had a persisting low concentration of BJP in follow-up urine samples

Screening algorithm

- When screening for plasma cell disorders, 24 hour urine can be replaced with serum free light chain assay
- When combined with serum PEL + IFE, nearly all patients can be picked up
- Caveat: When screening for AL amyloidosis, 24 hour urine should still be done
- If screening positive, urine should be tested

Diagnosis: Light chain myeloma

- Archived sera from patients in MRC trials
- From a total of 2323 patients, 64 (3.6%) had NSM
- 28 patients with adequate serum samples were selected for study
- In 19 (68%), FLC ratio was abnormal

Diagnosis: Light chain amyloidosis

Test N=110	Abnormal	Normal
Serum IF	76 (69%)	34 (31%)
Urine IF	86 (83%)	17 (17%)
Serum or Urine IF	104 (95%)	6 (5%)
Abnormal FLC ratio	100 (91%)	10 (9%)
S or U or FLC	109 (99%)	1 (1%)

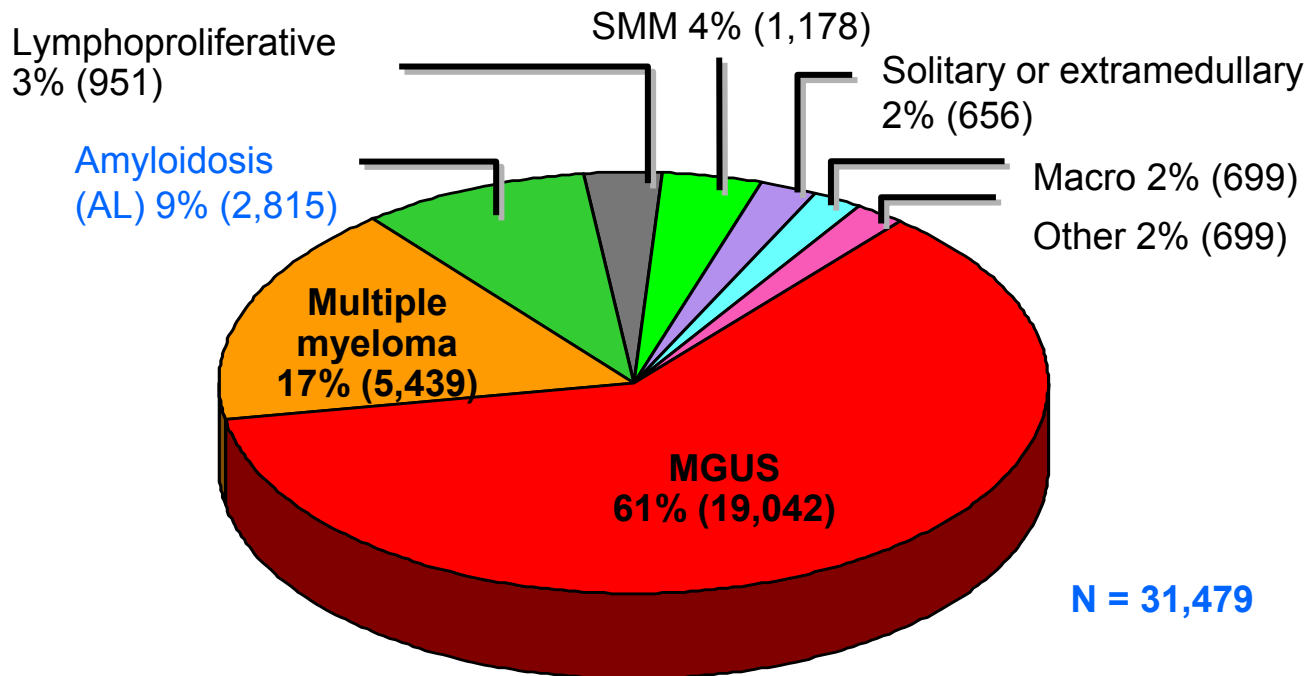
- In patients with AL amyloidosis urine immunofixation should be performed for optimal diagnostic sensitivity.
- Not performing urine immunofixation led to missing 6% of amyloidogenic clones in one study.

Prognostic value of FLC

- Monoclonal gammopathy of undetermined significance (MGUS)
- Smoldering myeloma (SMM)
- Newly diagnosed multiple myeloma
- Solitary plasmacytomas
- Light chain amyloidosis

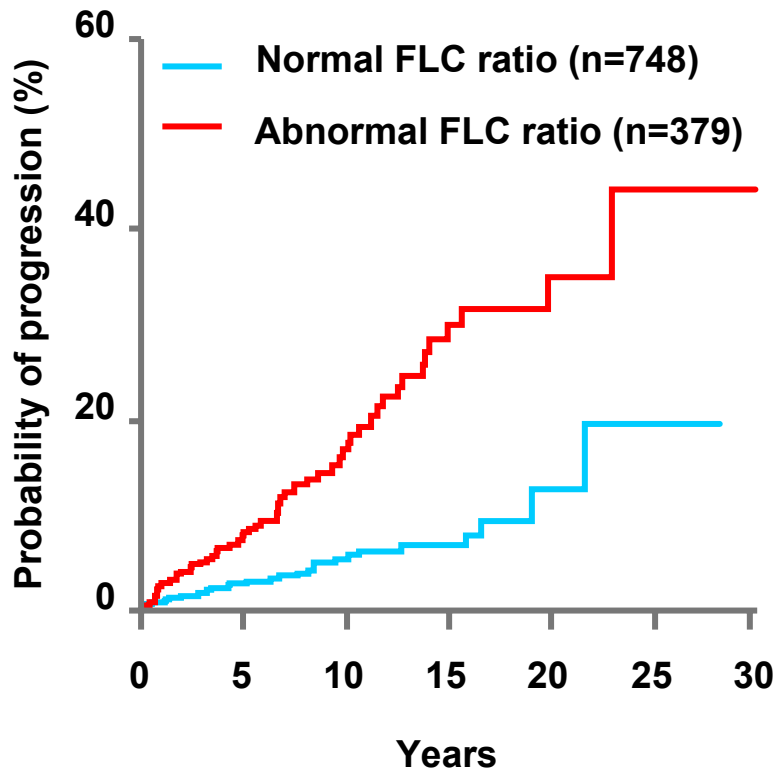
Monoclonal Gammopathies

- Myeloma is a part of the spectrum of plasma cell proliferative disorders

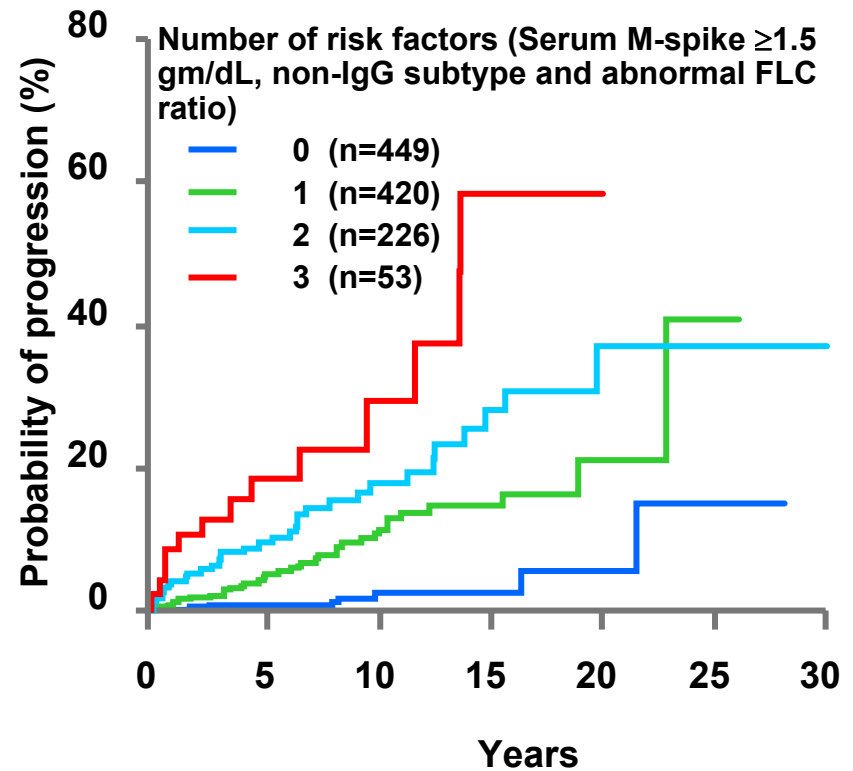


MGUS: Risk of Progression to Symptomatic Myeloma or Related Disorder

MGUS Progression



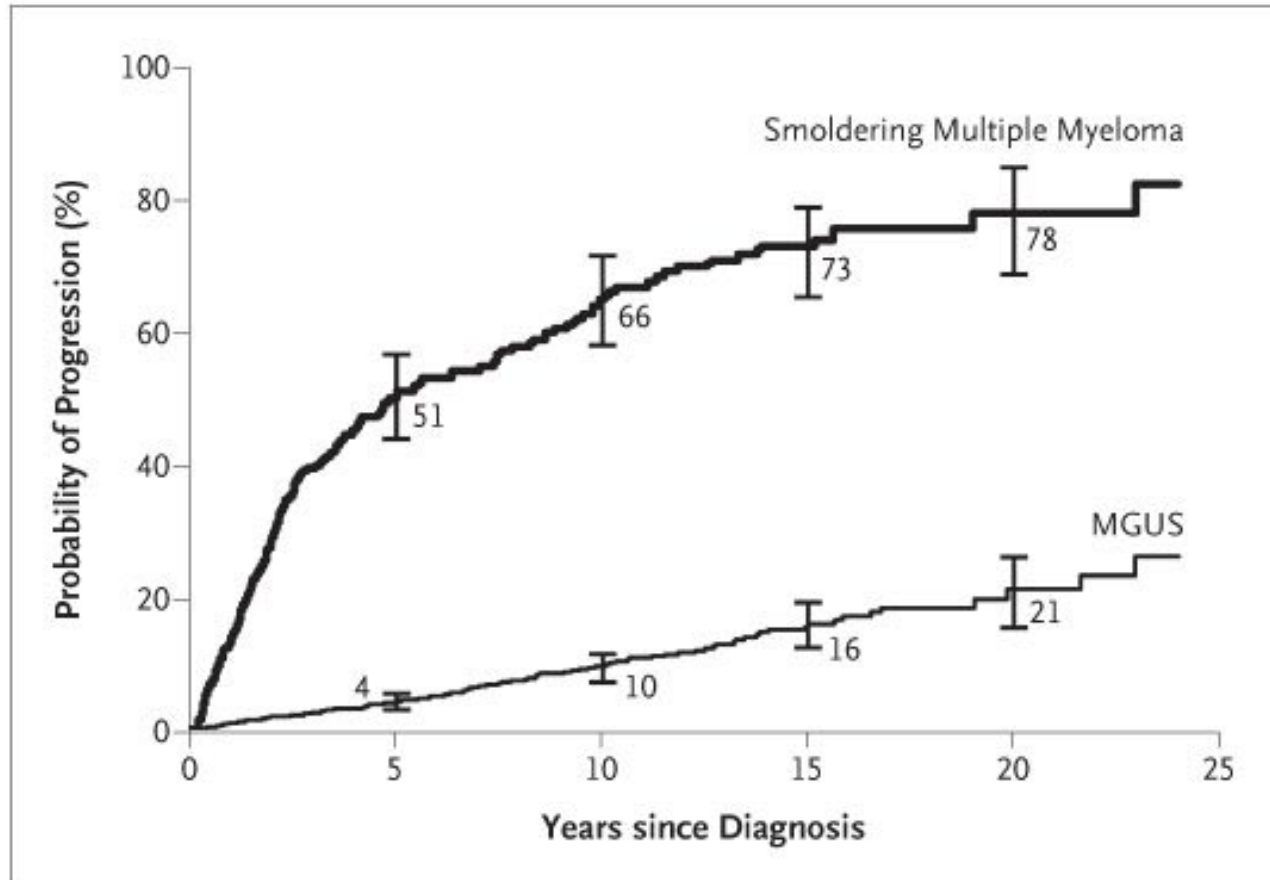
MGUS Progression



MGUS: Risk stratification model incorporating all 3 predictive factors

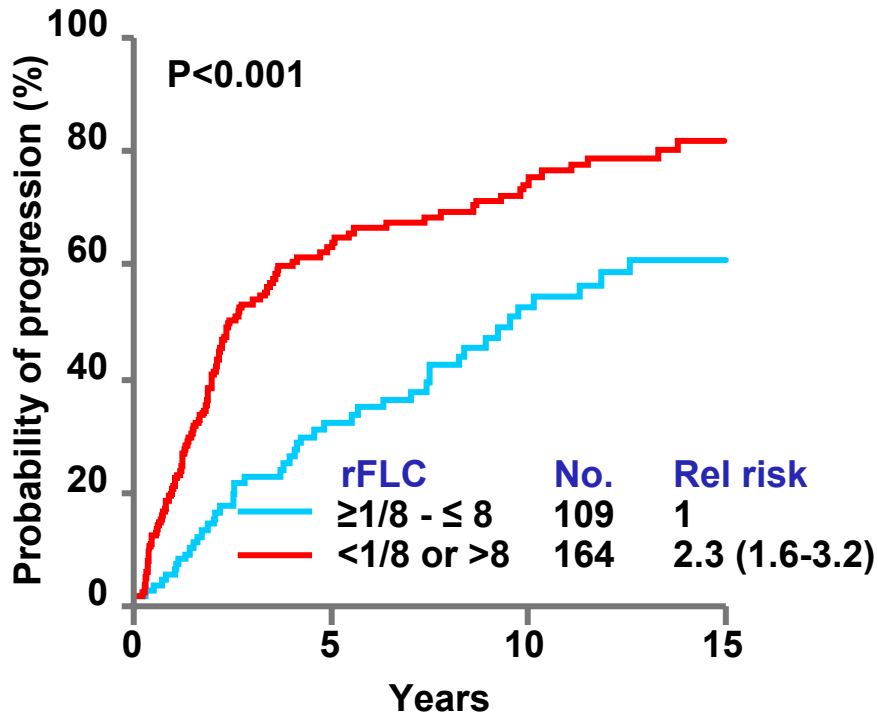
<u>Risk Group</u>	<u># of pts.</u>	<u>Absolute risk of progression at 20 yrs.</u>	<u>20 yr. risk of progression after other causes of death</u>
1. <u>Low-risk</u> Serum M protein <1.5 gm/dL, IgG subtype, normal FLC ratio	449	5%	2%
2. <u>Low/Intermediate-risk</u> <u>Any 1</u> factor abnormal	420	21%	10%
3. <u>Hi/Intermediate-risk</u> <u>Any 2</u> factors abnormal	226	37%	18%
4. <u>High-risk</u> <u>All 3</u> factors abnormal	53	58%	27%

SMM: Risk of progression

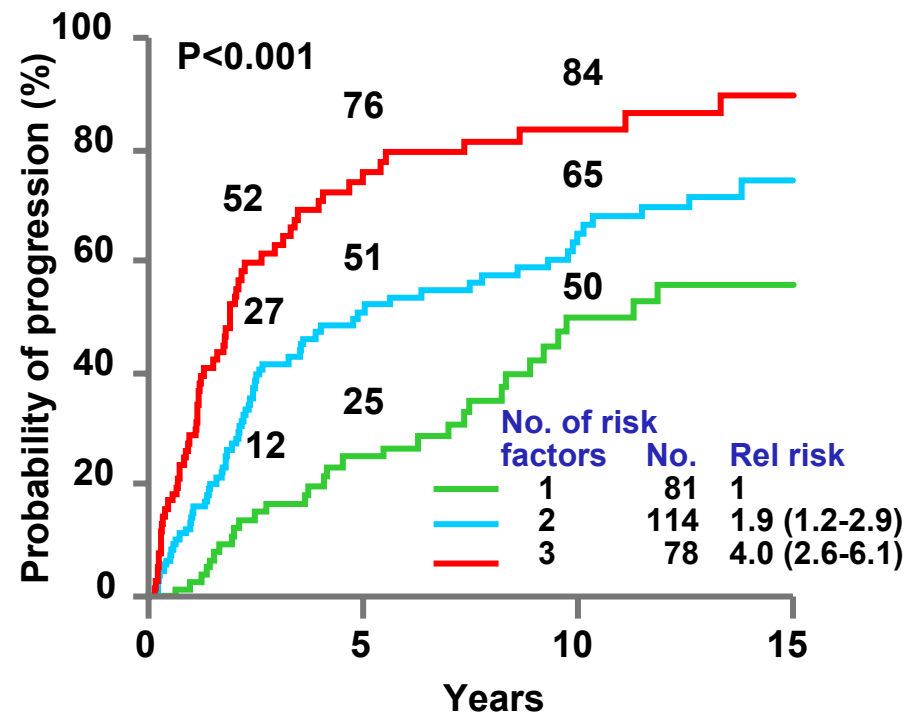


SMM: Risk of Progression to Symptomatic Myeloma or Related Disorder

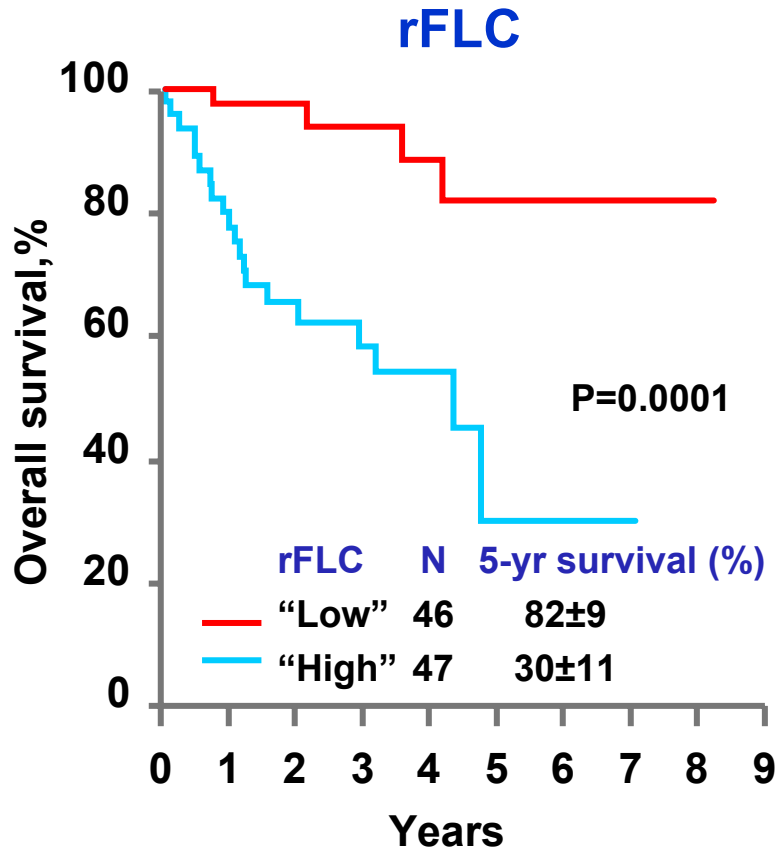
SMM Progression



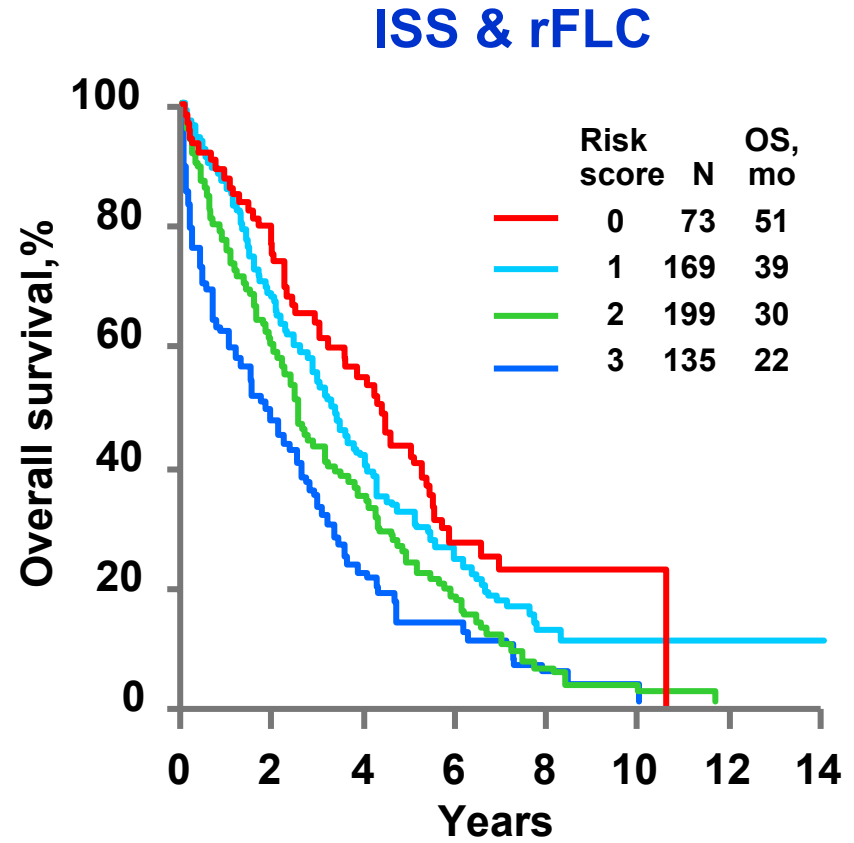
SMM Progression



Prognostic Value of FLC ratio at Diagnosis in Multiple Myeloma



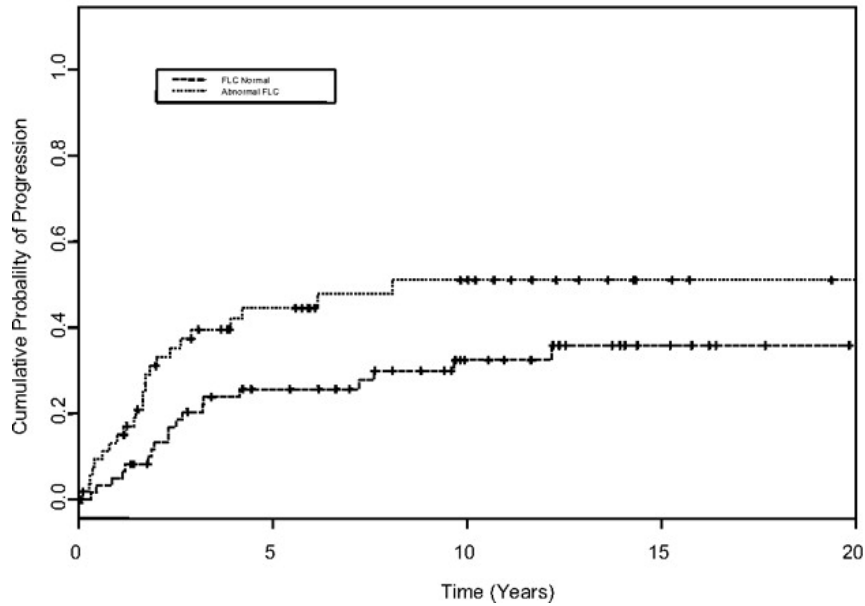
‘High rFLC’ >3.6 (κ) or <0.02 (λ)



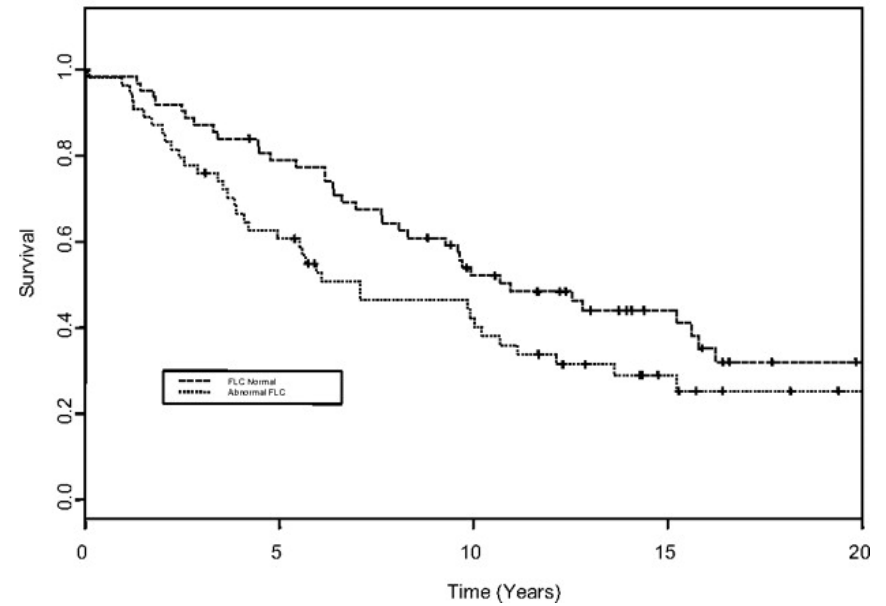
>32 (κ) or <0.03 (λ)

Solitary plasmacytoma

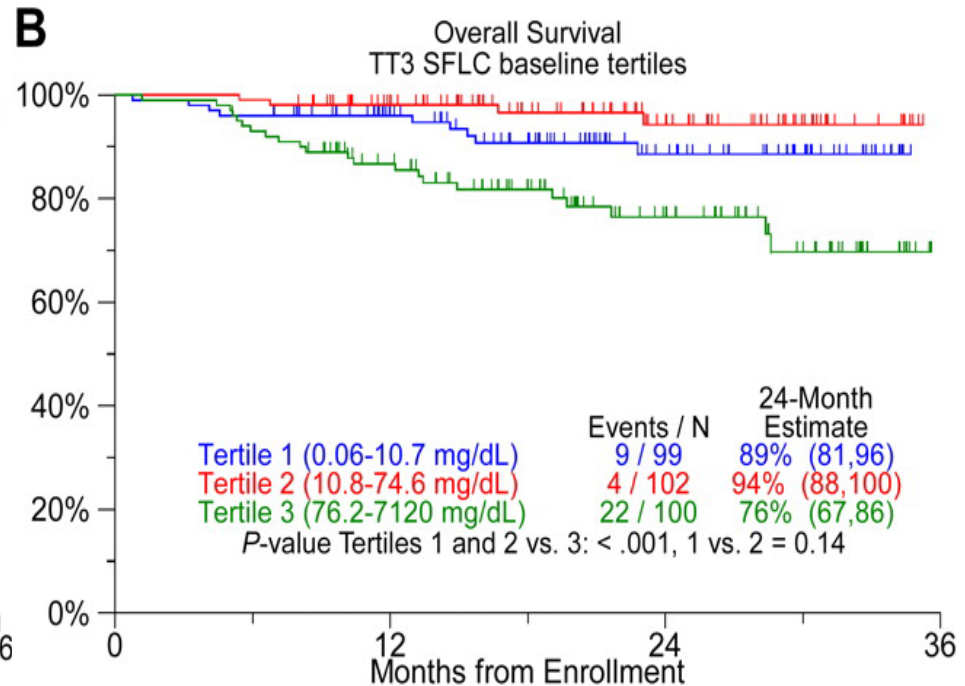
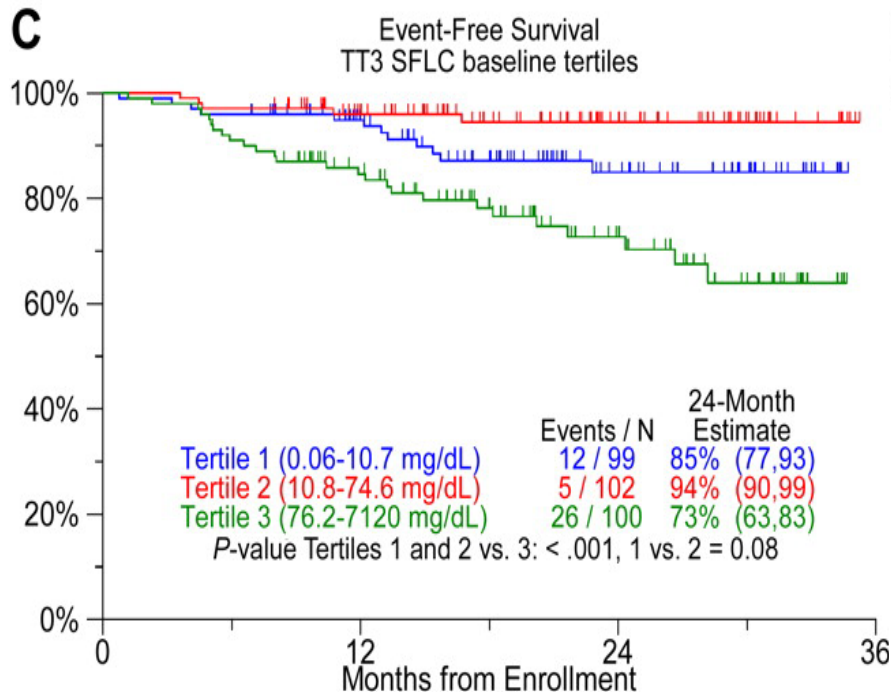
A



B



Prognostic Value of absolute value of involved FLC: untreated myeloma



Why is FLC abnormalities prognostic?

- We hypothesized that FLC abnormalities is associated with IgH translocations
- Studied 314 patients with newly diagnosed MM
- The median ratio and the difference between the FLC was higher with IgH translocations, especially t(14;16)
- Among those without the translocations FLC was prognostic, but did not add to risk assessment in those with the translocations
- The prognostic effect of FLC is only partially explained translocations

Prognostic value of FLC in AL amyloidosis

- We identified 730 patients with biopsy proven AL amyloidosis, who were seen at Mayo Clinic between Jan 1980 -July 2006.
- Patients seen within 90 days of their diagnosis.
- Proof of clonal process was required, either by monoclonal protein or clonal plasma cells in the marrow

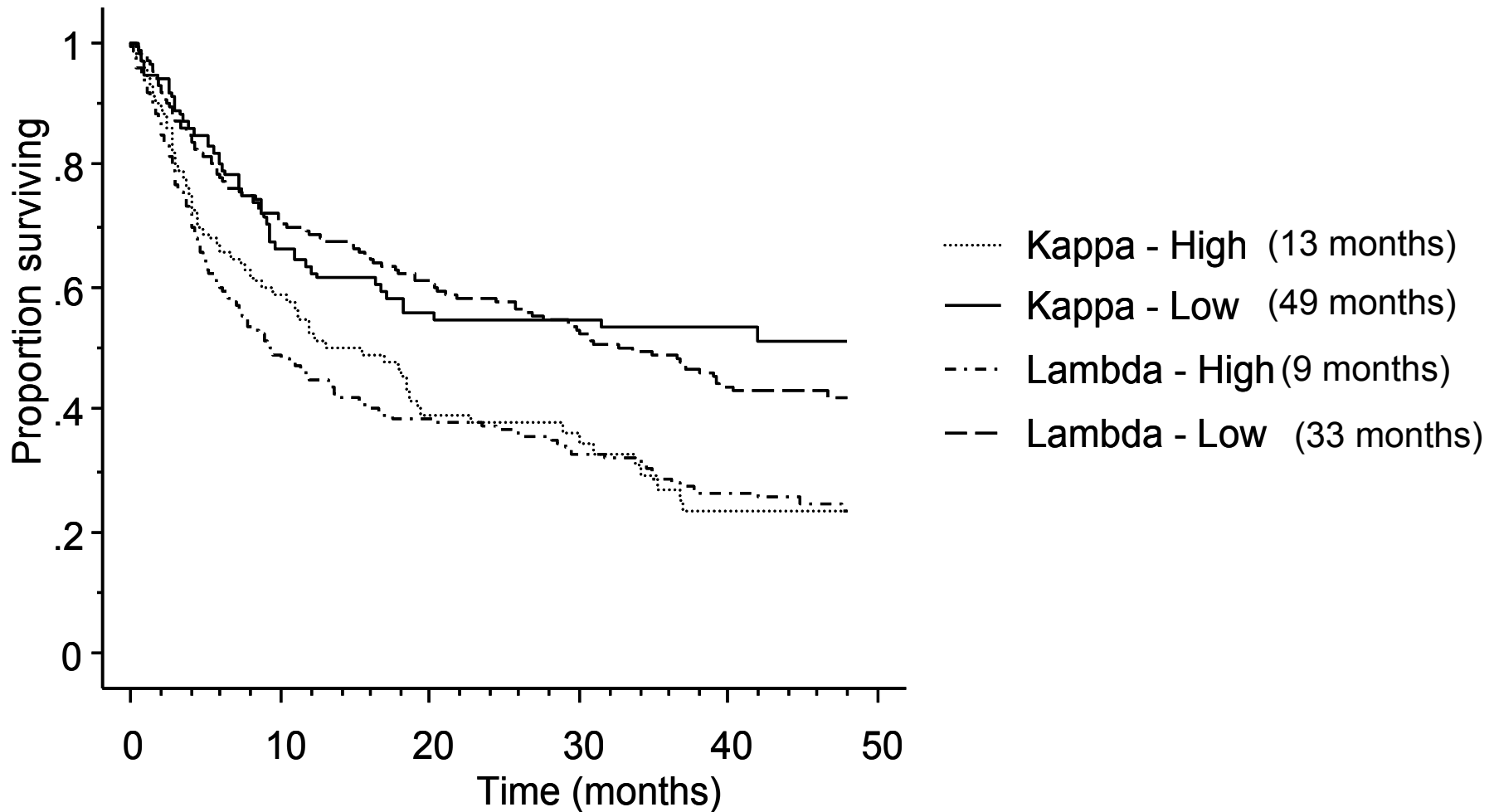
FLC type and measurement

- The clone was lambda in 528 patients (72%) and kappa in 202 (28%).
- The median absolute difference between the FLCs was 19.6 mg/dL for the entire group (range; 0.01-2478)
- For kappa patients it was 29.4 mg/dL (range; 0.01-1359)
- For lambda patients it was 18.2 mg/dL (range; 0.03-2478).

Organ involvement

- Kappa LC was associated with more involvement of the GI tract and liver
 - higher alkaline phosphatase (1.8 vs. 1.3 fold; $P=0.03$),
 - higher total bilirubin (0.95 vs. 0.78 mg/dL, $P = 0.04$)
 - lower serum carotene (119 vs. 155 ug/dL; $P < 0.002$).
- Renal involvement was more in the lambda LC
 - 24 hr urinary albumin (2.4 gm vs. 1.4 gm; $P < 0.002$)
 - lower serum albumin (2.8 vs. 3.0 mg/dL; $P = 0.001$).
- High FLC difference correlated with degree of organ involvement, especially cardiac.

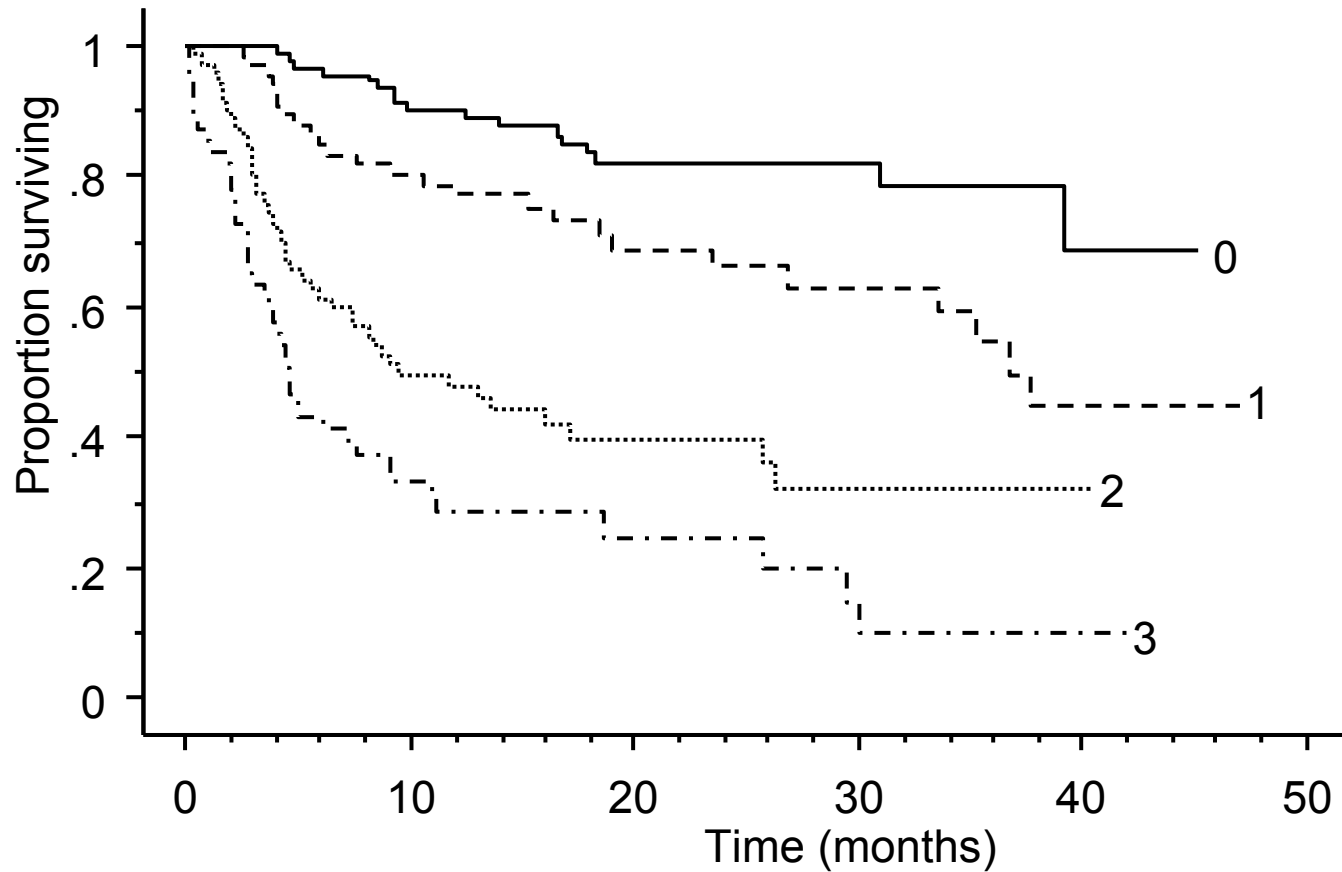
Survival



Multivariate analysis

- The FLC difference was independent of the cTnT, NT Pro BNP, uric acid and fold-increase in alkaline phosphatase, all of which were significant in the multivariable analysis.

Improved staging system



FLC difference > 35 mg/dL, Troponin-T > 0.035, BNP > 350

FLC measurements for response assessment

- As a measure of tumor burden
- Marker of minimal residual disease
- As a prognostic marker: kinetics of response

Response Criteria in Myeloma

	PR	VGPR	nCR	CR
Serum Protein Electrophoresis	>50%	>90%	0	0
Urine Protein Electrophoresis	>90%	<100 mg/ 24 hrs	0	0
Serum/Urine Immunofixation	—	—	Positive	Negative
Bone Marrow PC	—	—	<5%	<5%
Bone Marrow Immunofluorescence	—	—	—	—

Appraisal of Immunoglobulin Free Light Chain as a Marker of Response

- Data and stored samples from a mature ECOG clinical trial (E9486) data set to assess serum FLC at baseline and after 2 months of alkylator based therapy in 399 patients
- FLC response after 2 months of therapy was superior to early M-protein measurement to predict overall response, but did not predict for overall or progression free survival

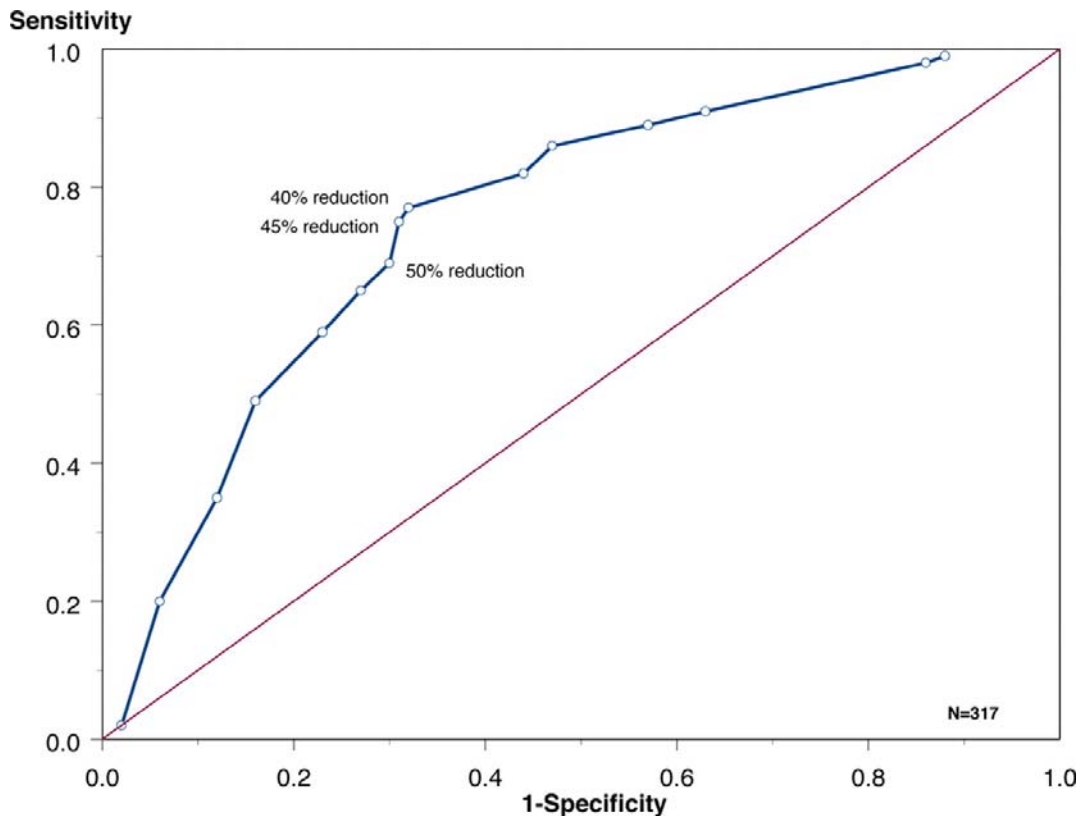
Do early changes in FLC predict for response?

FLC response	No. of patients	2-month FLC response			2-month paraprotein response			P
		Sensitivity %	Specificity %	Risk	Sensitivity %	Specificity %	Risk	
Overall population	181	69	73	0.30	34	98	0.48	<.001
Patients with iFLC more than 10 mg/dL	120	83	67	0.24	36	100	0.43	.001
Patients with abnormal rFLC and dFLC 5 mg/dL or more	139	77	69	0.26	34	100	0.45	<.001

* Among patients with both paraprotein and FLC data after 2 months of therapy.

FLC response performed better than the paraprotein response at 2 months for the overall population for predicting eventual response (risk value = 0.30 and 0.48, respectively, $P = .001$)

What is the best definition for free light chain response?



- A dFLC reduction of 40% to 50% had the best performance.
- A 50% reduction had a sensitivity of 69% and specificity of 70%.

Which Value Best to Use for Response Estimation

Comments

- iFLC Confounded by renal failure
- rFLC Confounded by suppression of uninvolved FLC: 80/1 vs 80/.05
- dFLC Provides some correction for renal failure

Can serum FLC replace 24 hour Urine Electrophoresis?

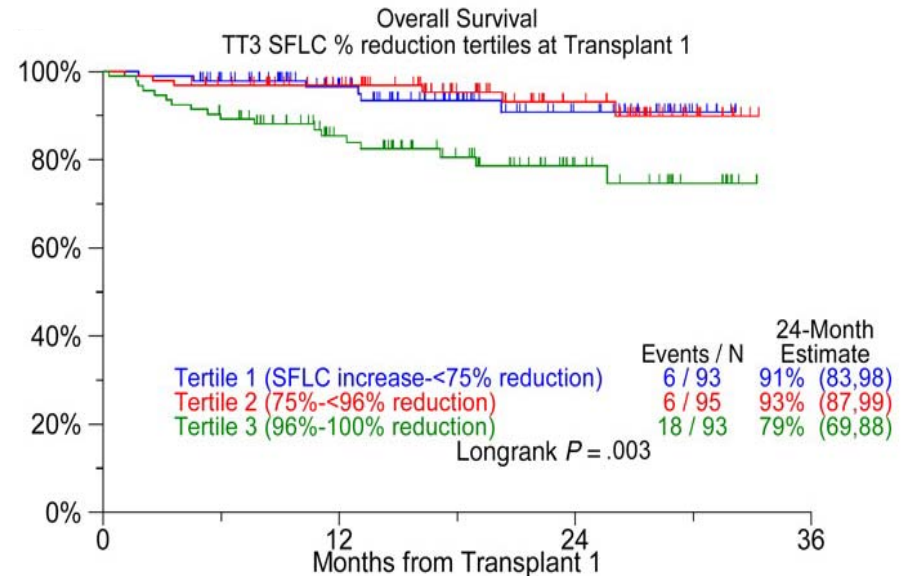
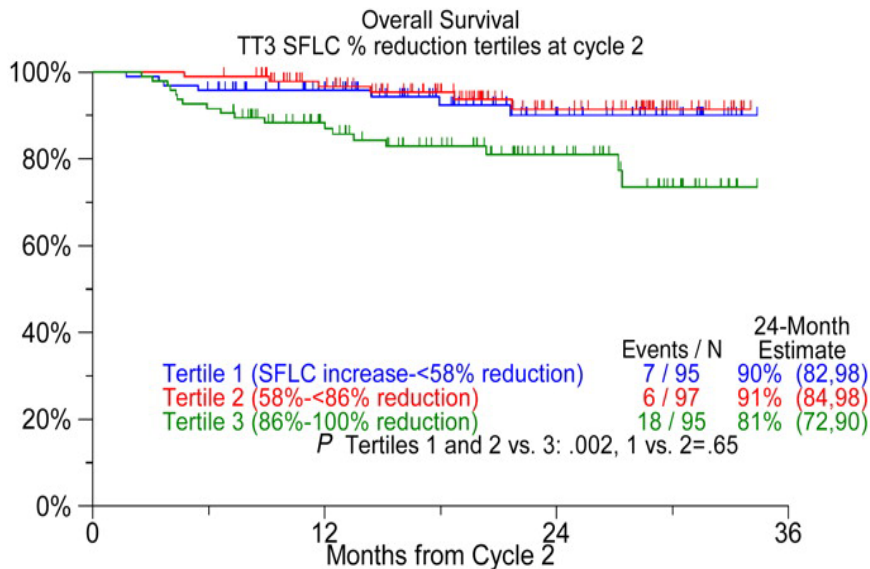
	Overall Population	Normal Renal Function	Abn. Renal Function
	Urine M-spike		
Urine only pts + Serum & Urine pts	N=101	n=81	n=20
iFLC	0.60 (<0.0001)	0.67 (<0.0001)	0.55 (0.0129)
dFLC	0.60 (<0.0001)	0.66 (<0.0001)	0.61 (0.0044)
rFLC	0.40 (0.0011)	0.43 (<0.0001)	0.27 (0.2585)
Urine only patients	N=32	n=26	n=6
iFLC	0.58 (0.0005)	0.69 (0.0001)	0.49 (0.3287)
dFLC	0.58 (0.0005)	0.69 (0.0001)	0.49 (0.3287)
rFLC	0.54 (0.0016)	0.61 (0.0010)	0.31 (0.5441)

The correlation coefficients between % change of serum FLCs and urine M-protein ranged between 0.37 and 0.62.

Do changes of FLC predict for overall or progression-free survival?

- Although FLC response after 2 cycles of therapy predicted for eventual overall response, FLC response at 2 months did not predict for OS or PFS

Kinetics of response and outcome



Marker of minimal residual disease

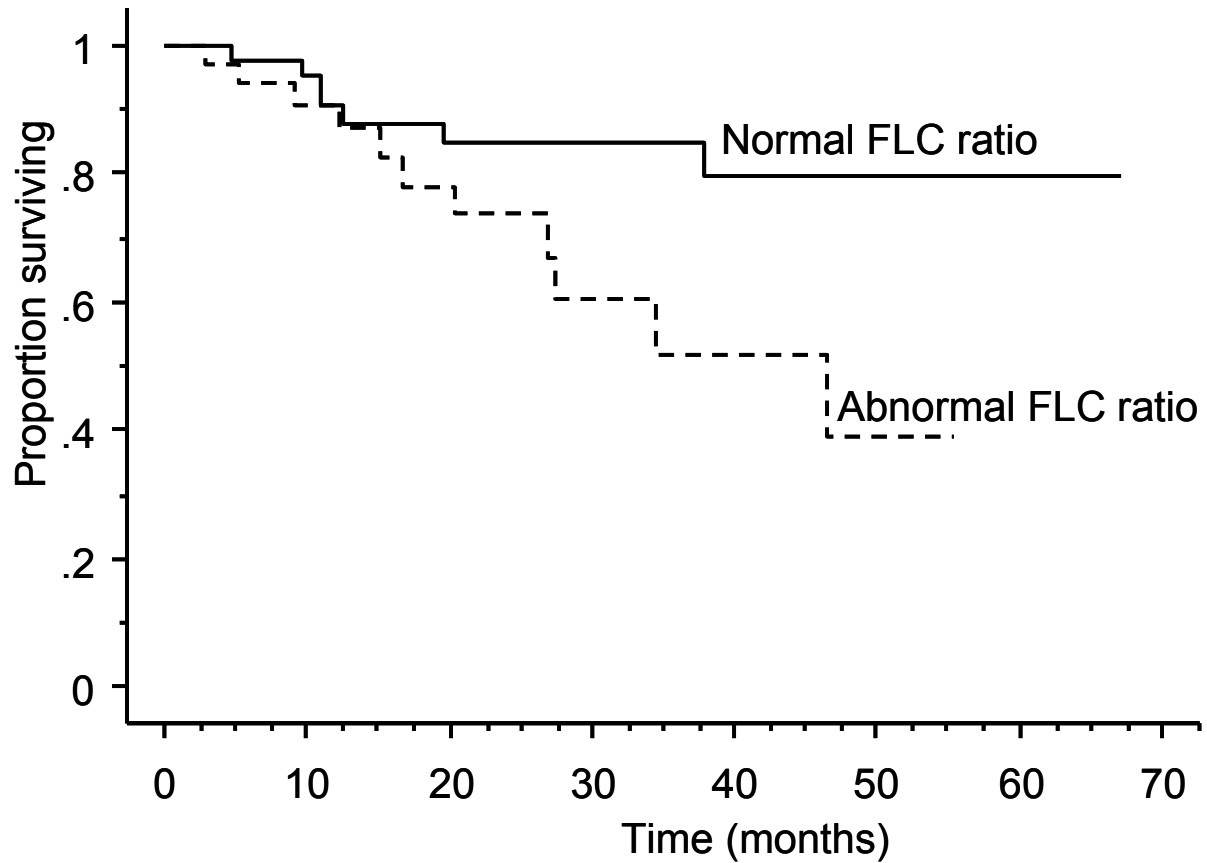
	PR	VGPR	nCR	CR	sCR
Serum Protein Electrophoresis	>50%	>90%	0	0	0
Urine Protein Electrophoresis	>90%	<100 mg/24 hrs	0	0	0
Serum/Urine Immunofixation	—	—	Positive	Negative	Negative
Bone Marrow PC	—	—	<5%	<5%	<5%
Bone Marrow Immunofluorescence	—	—	—	—	Negative
Serum Free Light Chain Ratio	—	—	—	—	Normal

Durie et al. Leukemia. 2006;20(9):1467-1473.

FLC as marker of MRD

- From among patients seen since 1995
 - We selected patients with MM who had measurable monoclonal (M) protein levels at baseline
 - Then identified patients who had a negative immunofixation in the serum and urine (within 30 days of each other)
 - Examined the outcome depending on whether they had a normal FLC at that time

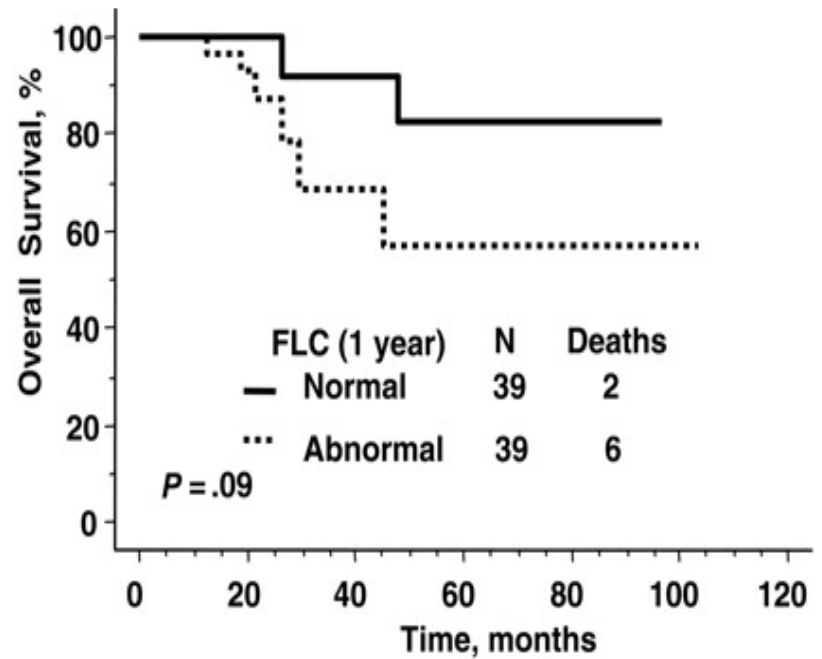
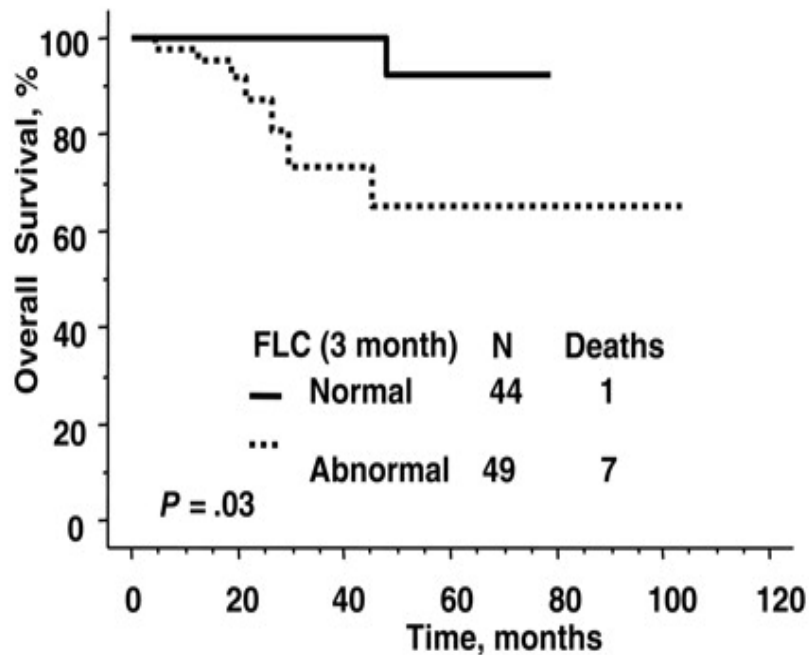
Overall survival



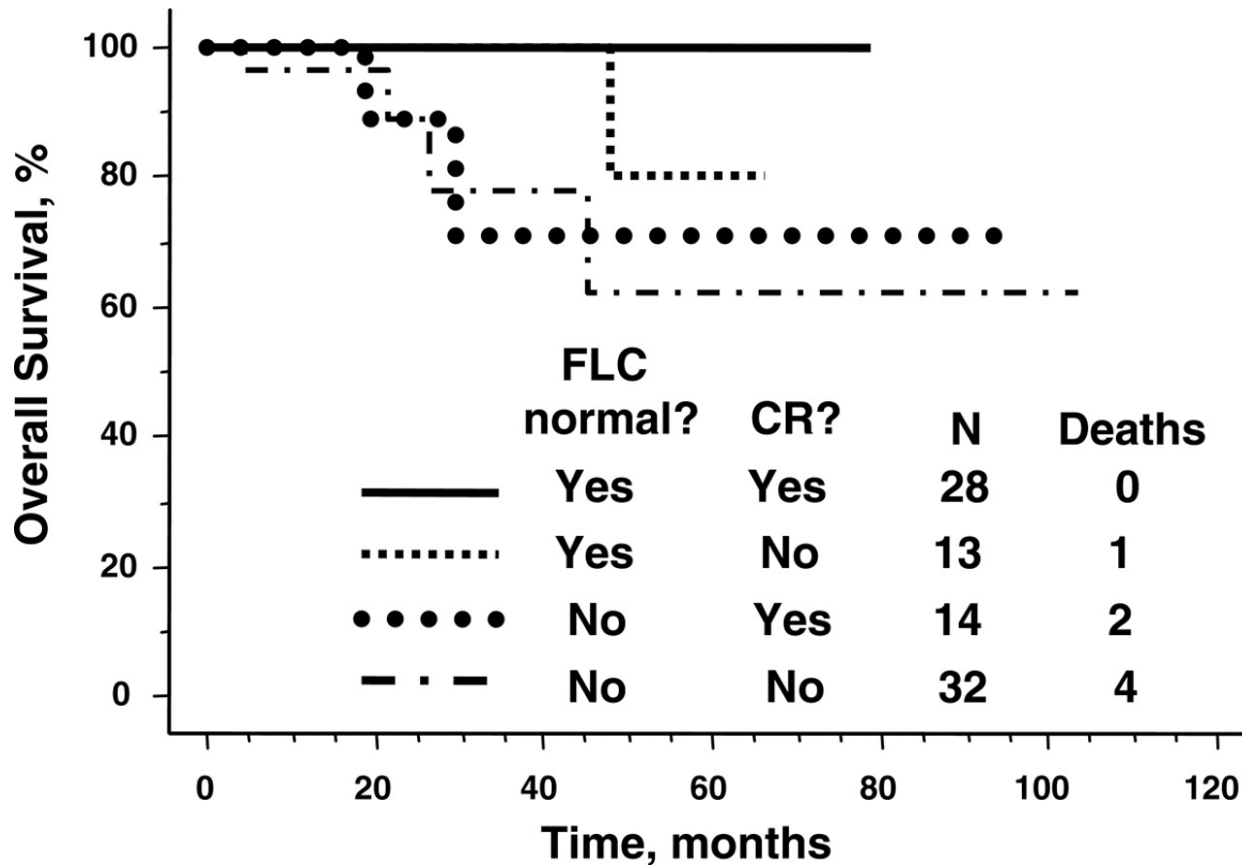
Assessing response to treatment in AL amyloidosis

- Free light chain is the substrate for amyloid protein
- Theoretically, FLC estimation may be a better marker to follow for response

Relevance of absolute value of serum FLC after PBSCT for AL



FLC response is better predictor of OS than is IFE response in AL



Response Criteria for FLC

	PR	CR	sCR	Prog
No measurable disease				
MM	FLC Difference ↓ 50%		Normal FLC ratio + CR	↑ 50% FLC difference (> 100 mg/L)
AL	Involved FLC ↓ 50%	Normal FLC ratio + CR	ND	Involved FLC ↑ 50% (> 100 mg/L)
Measurable disease				
MM			Normal FLC ratio + CR	

Measurable disease :

- Serum M-protein ≥ 10 g/L or
- Urine M-protein ≥ 200 mg/day for myeloma patients

Technical Limitations of FLC Assay

- Assay imprecision, especially with different lots of FLC reagent, may have a significant effect on changes in the FLC concentration and κ/λ ratio
- Antigen excess can cause falsely low serum FLC results with nephelometric techniques, and manual dilution may be required for suspicious samples
- Some studies suggest that there might be cross reactivity with light chain bound to intact immunoglobulin

Tate JR et al. Clin Chim Acta. 2007;376:30-36.

Daval S et al. Clin Chem. 2007;53:1985-1986.

Conclusions

- Clear value in screening for plasma cell disorders: can replace 24 hour urine testing
- As a prognostic marker at diagnosis in various plasma cell disorders
- As a measure of tumor burden in patients with myeloma without measurable disease
- For defining stringent CR
- Probably as a marker for hematologic response in amyloidosis

Thank you!

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Questions?