Rondzending M-proteïne diagnostiek

SKML nabespreking, sectie HIM

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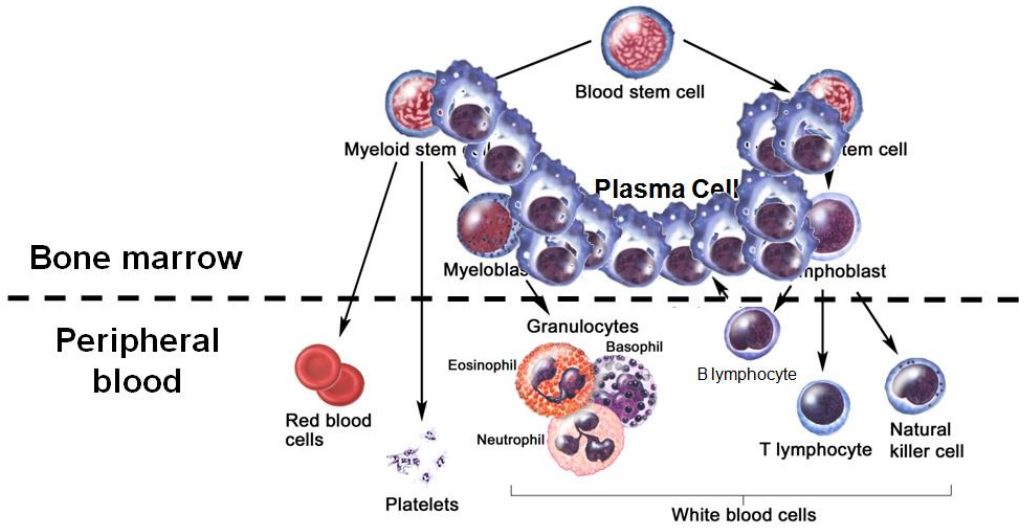
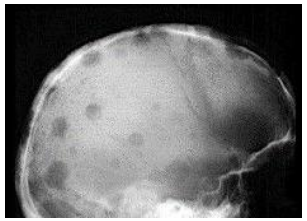
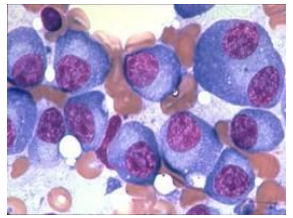
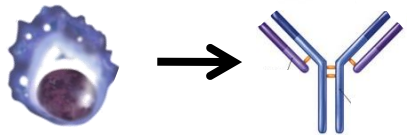
Monoclonal gammopathy; multiple myeloma

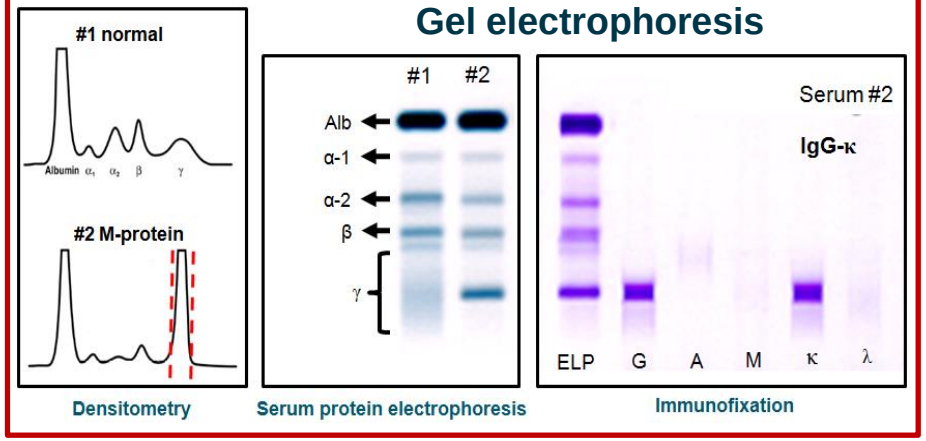




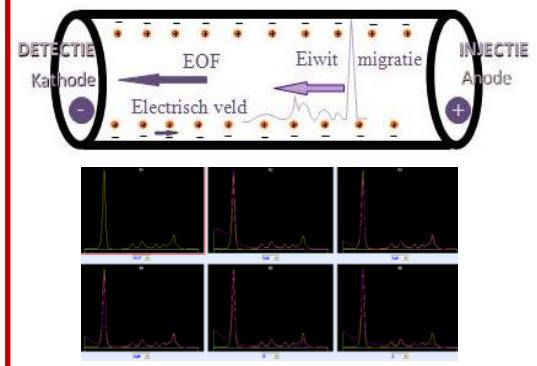




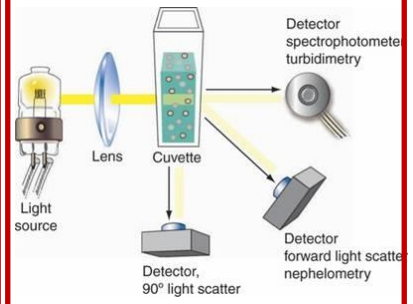
Gel electrophoresis



Capillary electrophoresis

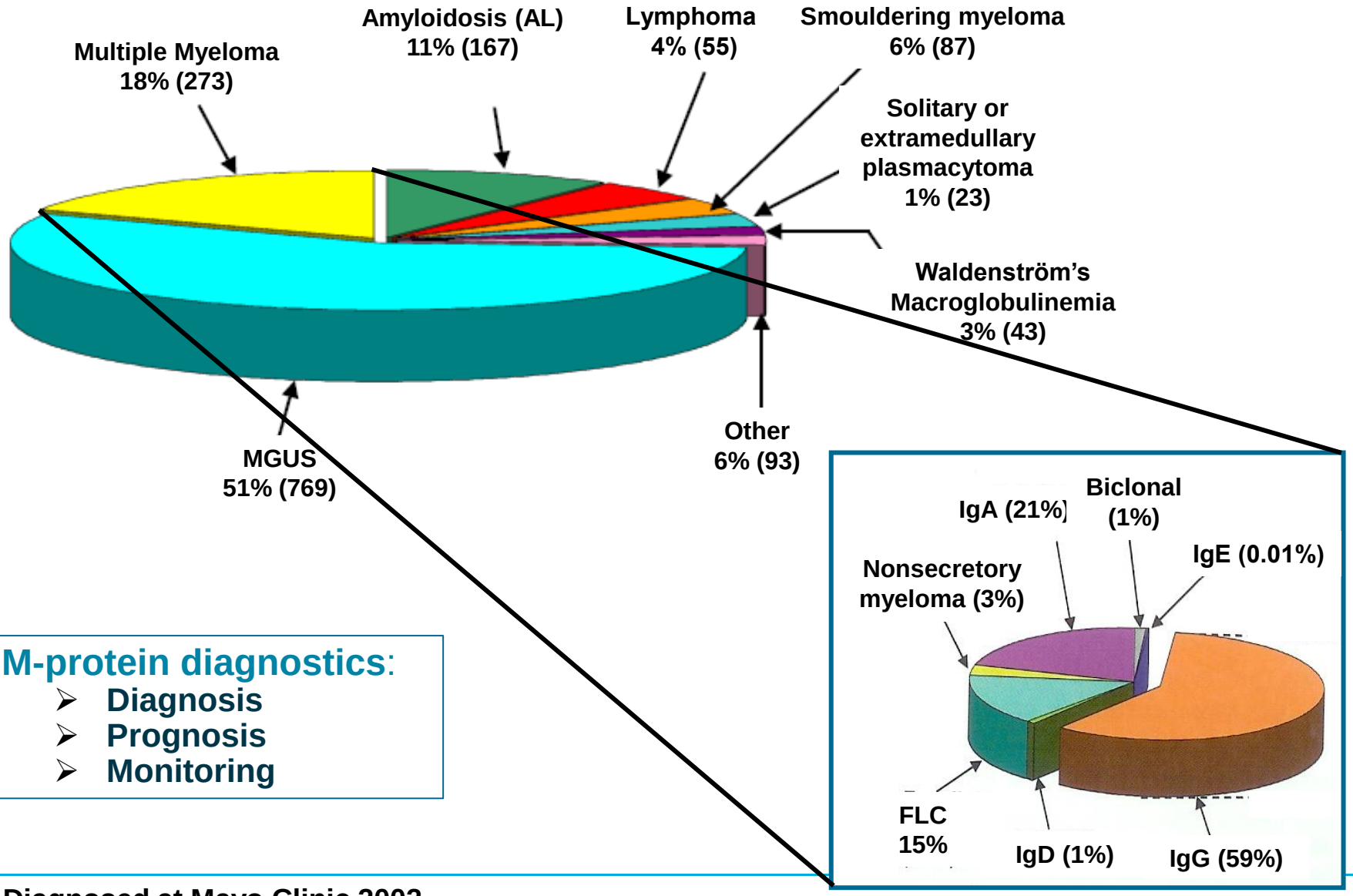


Immunochemistry



Nephelometry / Turbidimetry

Monoclonal gammopathies



M-protein diagnostics:

- Diagnosis
- Prognosis
- Monitoring

Diagnosed at Mayo Clinic 2002

M-proteine rondzendingen

Periode 2019.4 t/m 2023.4

- 4 rondzendingen per jaar, 75 deelnemers
- Elke rondzending 3 monsters (A,B,C), soms met casus
- Invoer en rapportage via Qbase

Inventarisatie:

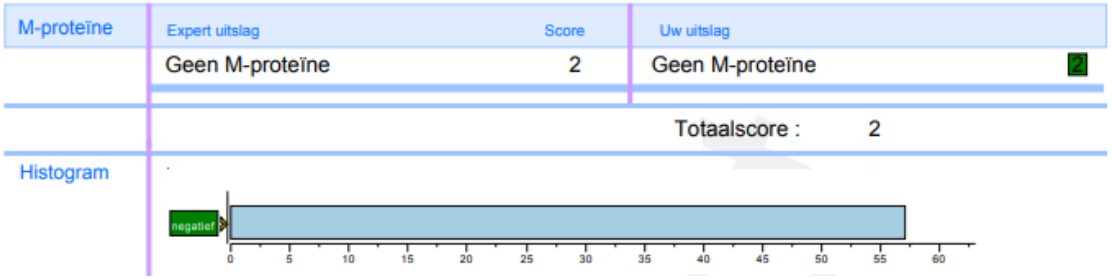
- Typering M proteine
- Kwantificering M proteine
- Kwantificering totaal eiwit, Ig's
- Soms kwantificering Vrije Lichte Ketens (VLK)
- Soms urine of cryobepaling
- Soms additionele vraag

Wat is er rondgestuurd in deze periode:

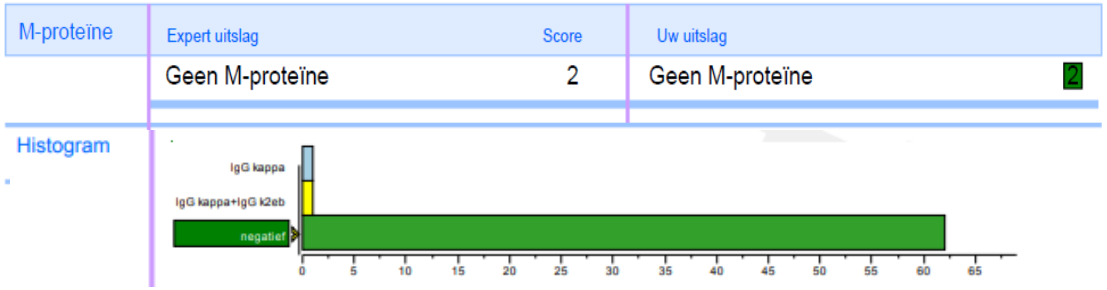
- IgG M-protein 13x
- IgM M-protein 9x
- IgA M-protein 7x
- IgD M-protein 1x
- VLK-K 1x
- VLK-L 3x
- Urine VLK-K 1x
- Urine VLK-L 1x
- Oligiklonaal 1x
- Geen MPR 14x
- Cryo 2x

Rapportage bij sera zonder M-proteine (n=14)

7x
Bijvoorbeeld 2022.3B



7x
Bijvoorbeeld 2019.1B



Hoe duidelijk moet een bandje zijn voordat je rapporteert?

Relevantie van kleine bandjes…?

Laboratory Persistence and Clinical Progression of Small Monoclonal Abnormalities AJCP, 138:609, 2012

David L. Murray, MD, PhD,[1] Justin L. Seningen, MD,[1] Angela Dispenzieri, MD,[1,2] Melissa R. Snyder, PhD,[1] Robert A. Kyle, MD,[1,2] S. Vincent Rajkumar, MD,[2] and Jerry A. Katzmann, PhD[1,2]

- Dysproteinemia Database
- Termed IFE M-proteins
- 439 patients at least one Follow-up
- Median follow-up 3.9 yrs (0.2-13 yrs)
- 3.2% progressed
- About 1% per year

Murray et al AJCP, 138:609, 2012

Type of Clinical Progression in Patients With IFE MGUS

Disease	Sex	Ig Class	Time to Progression, y
Multiple myeloma	M	IgA	1.0
	F	IgG	2.8
	M	IgA	9.9
	M	IgA	2.1
	M	IgG	3.5
	F	IgG	1.7
	F	IgA	1.3
	F	IgA	2.5
Smoldering myeloma	M	IgA	5.1
	M	IgA	4.5
Primary amyloidosis	M	IgG	4.5
Light chain deposition disease	F	IgG	8.9
Extramedullary myeloma	F	IgG	0.4
Lymphoplasmacytic lymphoma	F	IgA	5.6

IFE immunofixation electrophoresis; Ig, immunoglobulin; MGUS, monoclonal gammopathy of undetermined significance.

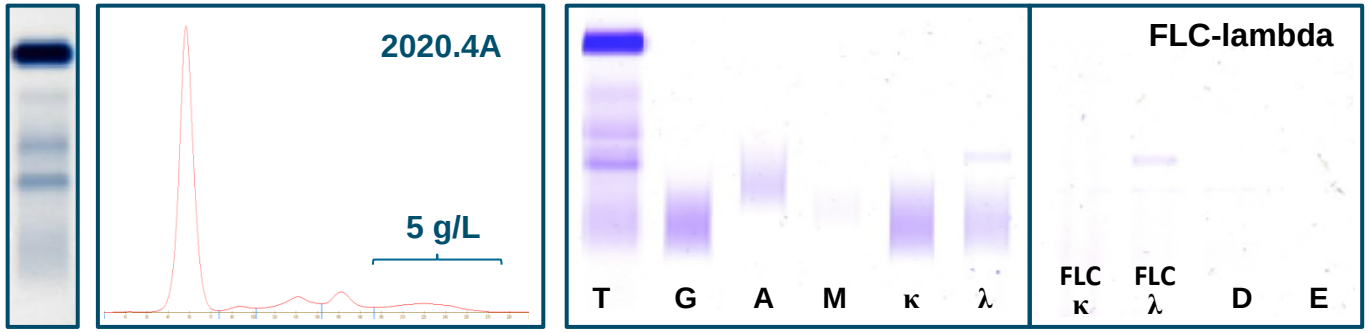
Samenvatting van dubieuze banden (NTK):

- **84% van patienten persisteert het M-proteine tijdens follow-up**
- **1% per jaar van de patienten vertoont klinische progressie**
- **8 ‘progressors’ zijn IgA**
- **6 ‘progressors’ zijn IgG**

Murray et al. American J Clin Pathol 2012

Radboudumc

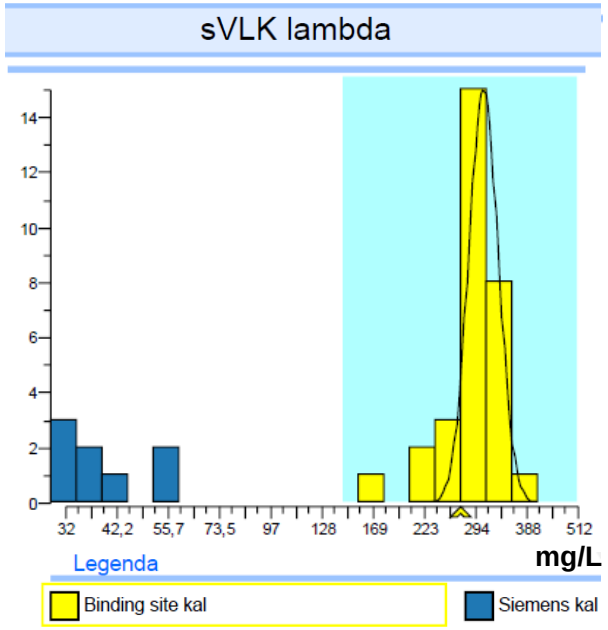
Small M-proteins can be clinically very relevant: monoclonal FLC

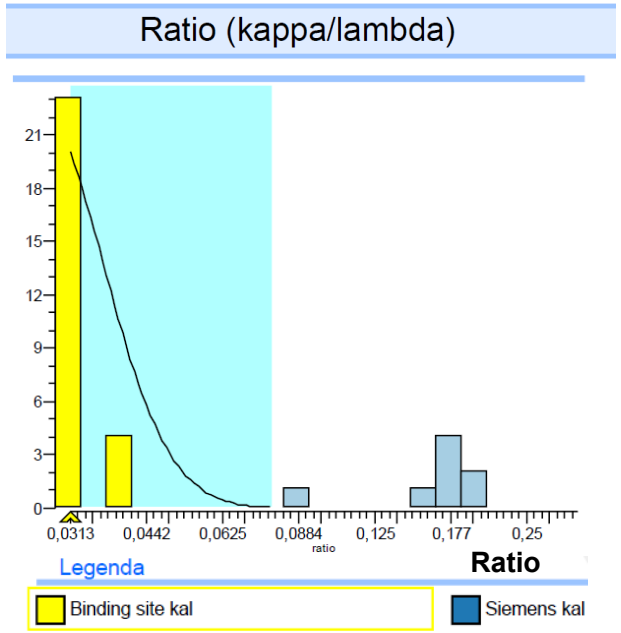


SPE Densitogram Immunofixatie elektrophorese (IFE)

Labs report:
60% FLC-Lambda
40% Negative

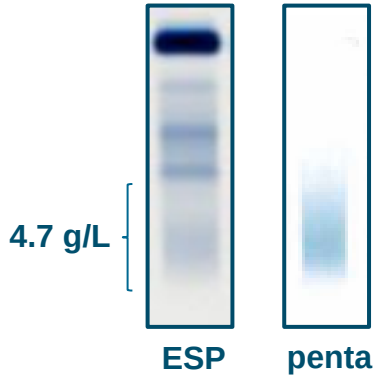
FLC quantification





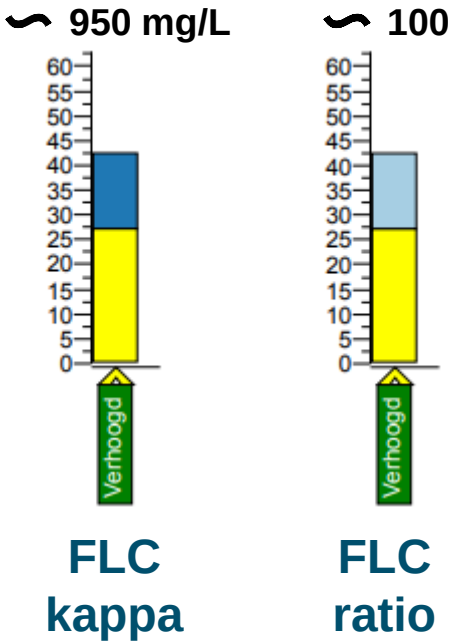
Monoclonal FLC_part 2

2022.1A



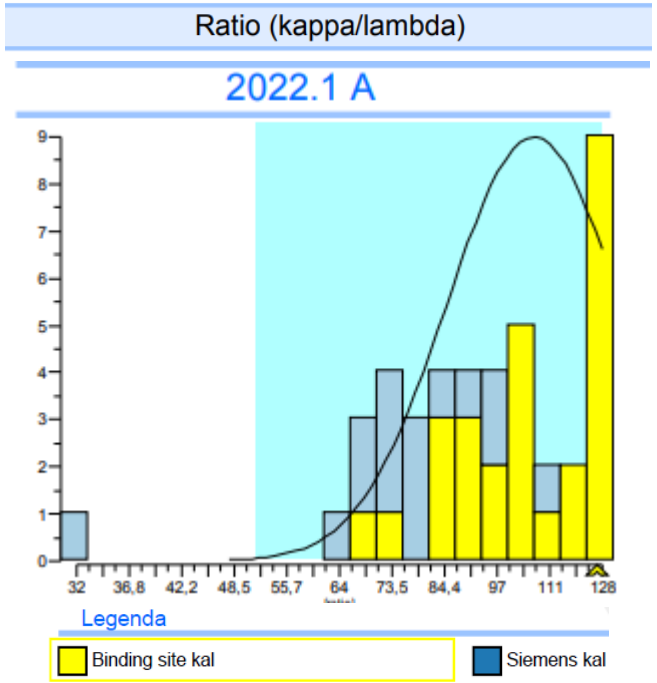
Labs report:
6 deelnemers detecteren FLC-Kappa op IFE

FLC metingen



FLC kappa

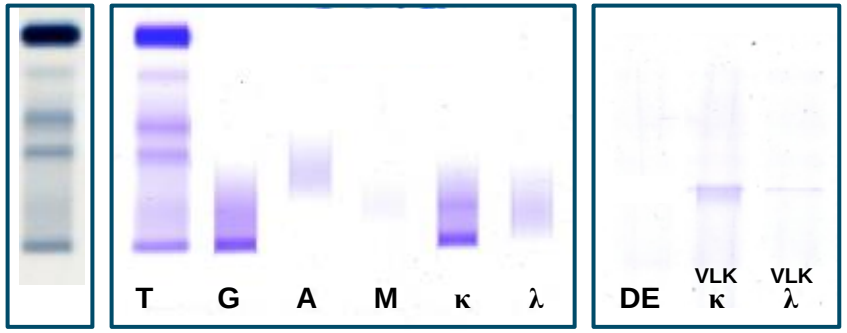
FLC ratio



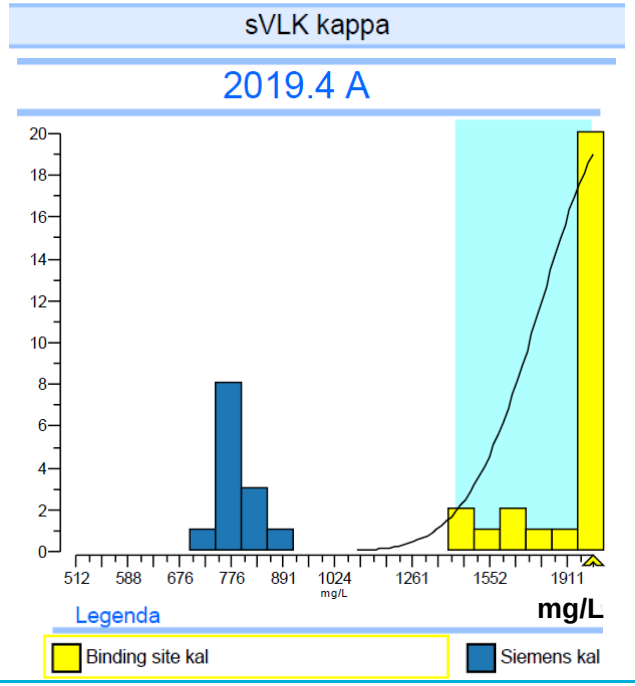
Monoclonal FLC_part 3

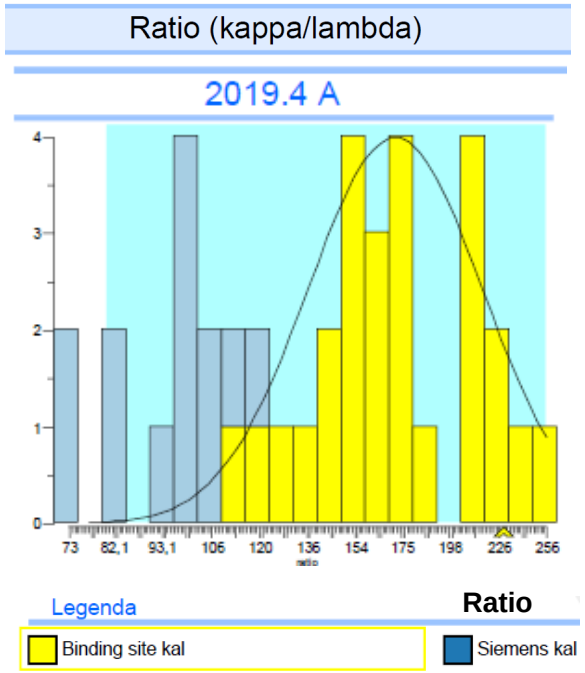
2019.4A

3.5 g/L

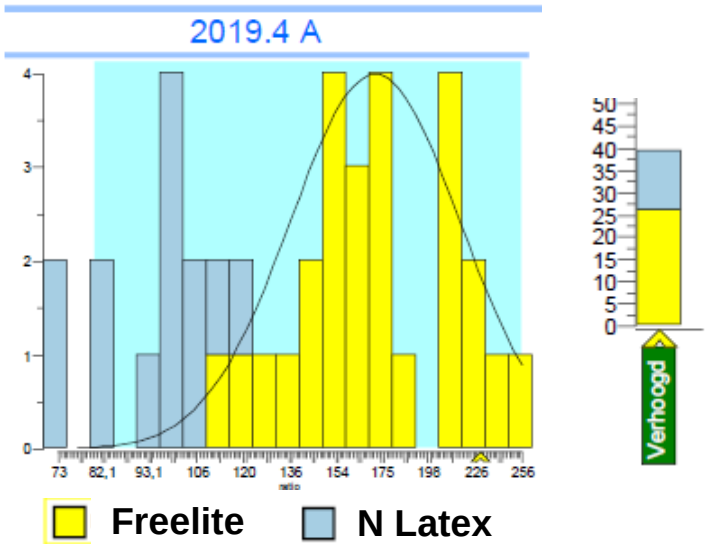


FLC quantification





The importance of FLC standardisation/harmonisation

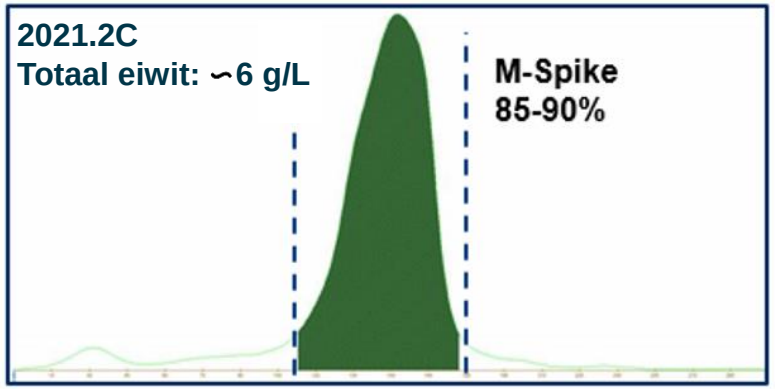


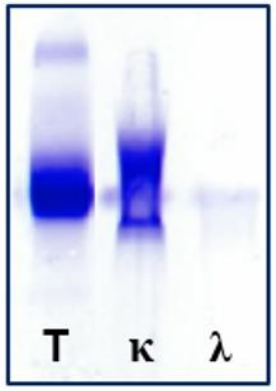
Newly Added Criteria To Diagnose MM

Clonal bone marrow plasma cells ≥10% or plasmacytoma plus one of these:	
	2-y Incidence of Organ Damage, %
Clonal marrow plasma cells ≥60%	95
Ratio of involved to uninvolved serum free light chain ≥100	80[a]
≥2 focal bone lesions ≥5 mm on MRI	70-80

Rajkumar et al. Lancet Oncology 2014
Jacobs, Tate & Merlini. Clin Chem Lab Med 2016

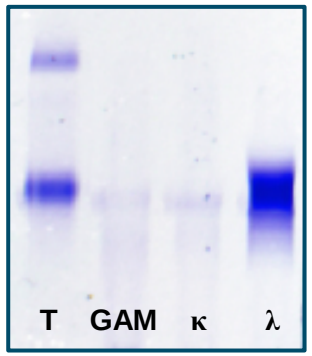
Bence Jones eiwitten.

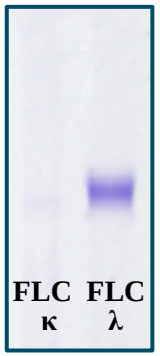




- Deze BJ als NEG gerapporteerd door 2 van de 50 deelnemers
- Circa 90% eiwitten = BJE
- BJ-kappa is circa 5-8 g/L
- Kwantificatie met Freelite/N-Latex overschat concentratie (range 58-177 g/L)
- Diagnose: light chain only MM

2023.2C
Totaal eiwit: ∽ 1 g/L

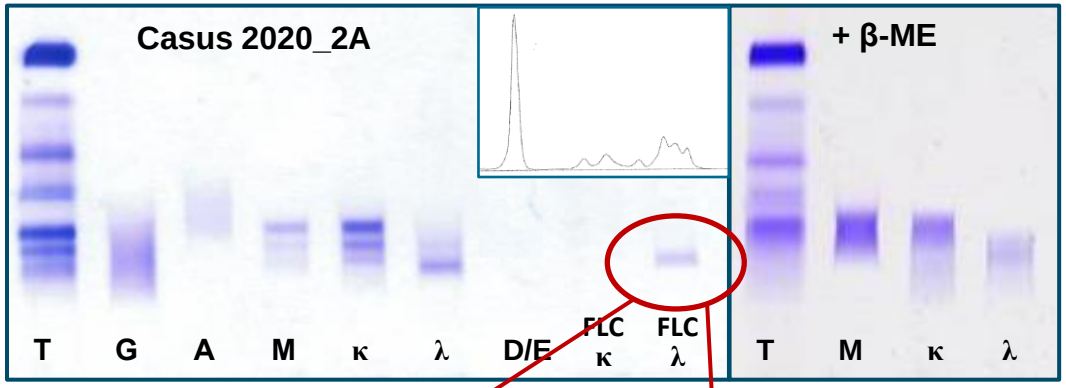




- 100% deelnemers beoordeelden als POS
- Circa 65% eiwitten = BJE
- BJ-lambda is circa 0.6 g/L
- Kwantificatie met Freelite/N-Latex overschat concentratie (range 1.2-7 g/L)
- Diagnose: light chain only MM

- BJE kunnen behoorlijk polymeriseren, ook in direct geanalyseerde urine.
- De elektroforese data kunnen daardoor ogenschijnlijk polyklonaal profiel geven.
- Echter de abnormale verhouding tussen VLK-kappa en VLK-lambda is dan suggestief voor klonale karakter.
- Kwantificeren middels totaal eiwit in urine en spike in elektroforese.

Bijzonder patroon…. (met dank aan dr. Gideon Lansbergen)



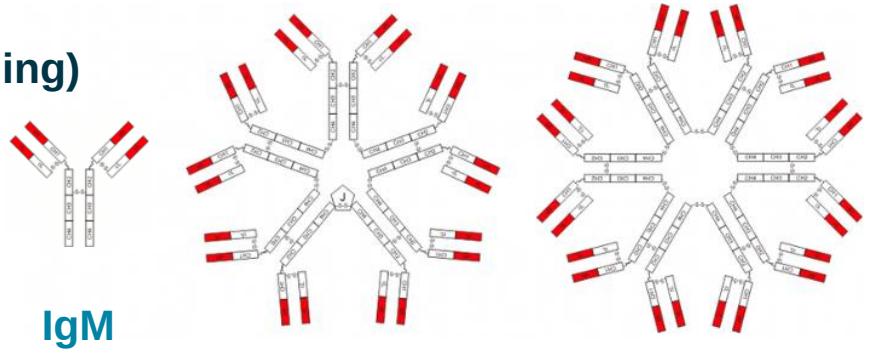
Immunofixatie elektroforese (IFE)

- 61 jarige vrouw
- Progressief doof gevoel in vingers + milde anaemie
- Beenmerg: 70-80% lymfoplasmacyt. prolif. (IgM-kappa)

- Diagnose: Morbus Waldenström
- Rx: dexameth., cyclofosf., Rituximab

- Terugkoppelen IgM-kappa M-proteïne (niet oligoklonale ontwikkeling)
- Kwantificering d.m.v. totaal IgM (niet met M-spike)

- FLC-kappa ranges 11-29 mg/L
- FLC-lambda ranges 6-17 mg/L
- FLC-ratio's normaal



IgM Monomer | Pentamer | Hexamer

Cryoglobuline

‘Cryoglobulinaemia: systemic inflammatory syndrome that generally involves small-to-medium vessel vasculitis due to CG-containing immune complexes’

Immuunglobulines die precipiteren bij koude

Komen vaak voor bij:

- Lymfoproliferatieve aandoeningen (Waldenström, MM, NHL)
- Systeemziekten (RA, SLE, syst sclerodermie, M. Sjögren)
- Leverziekten (Hepatitis B, C, AIH, cirrhose)
- Chronische infecties (HIV, ziekte v Lyme)
- Zonder aantoonbare onderliggende oorzaak

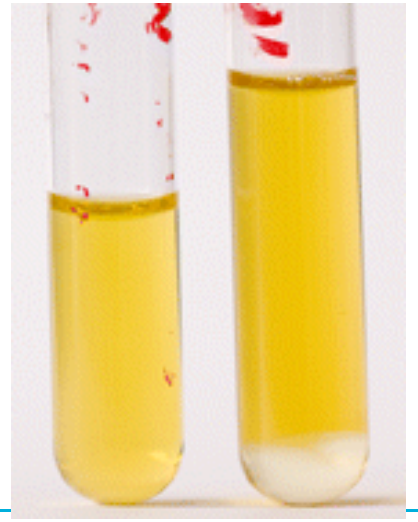
Kwantificering

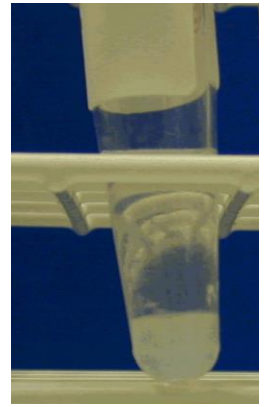
Typering

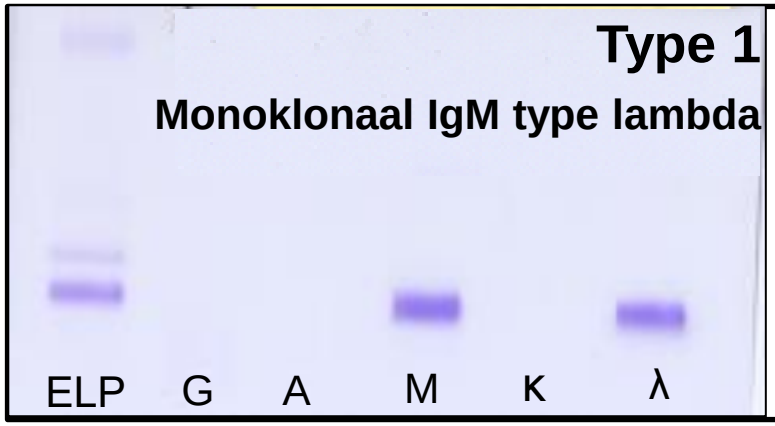
Eenmalig bij verhoogd cryoglobuline

Indeling volgens Brouet:

Type 1: monoklonaal Ig
Type 2: monoklonaal Ig met polyklonaal Ig
Type 3: polyclonaal Ig



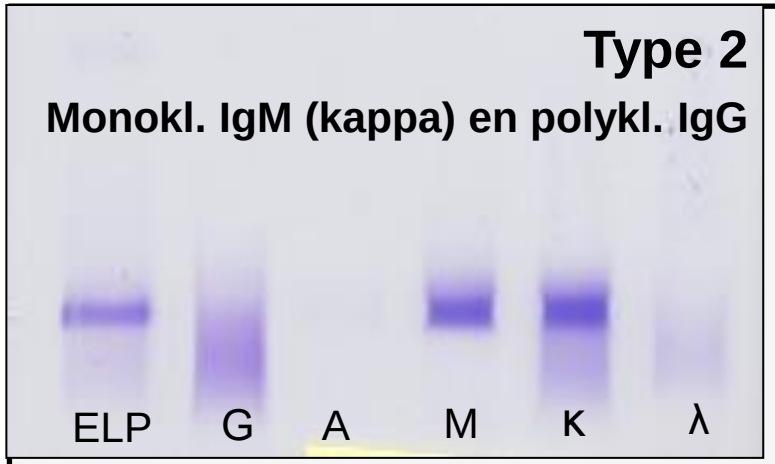


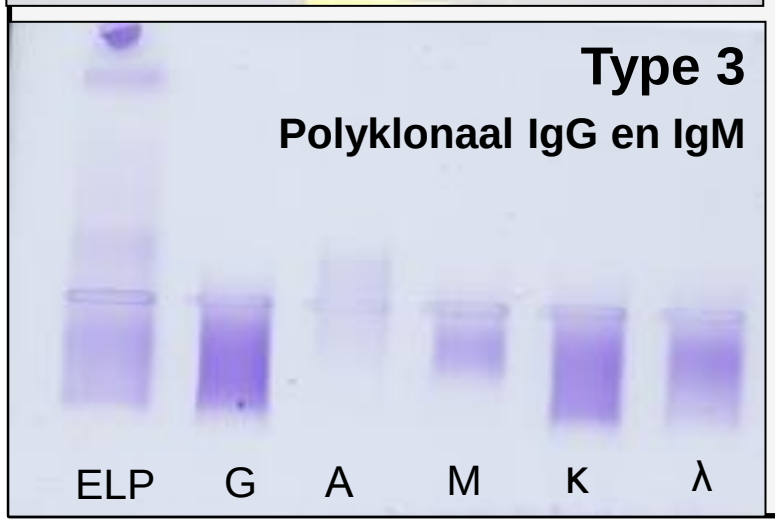


Oorzaak:
Immuno/lymfoproliferatieve aandoeningen

M. Waldenström, Multiple Myeloma, CLL,…

Symptomen m.n. door obstructie van perifere bloedvaten door hyperviscositeit vanwege het monoclonaal Ig + cryoactiviteit.





Oorzaak:
Chronische B-cel stimulatie

- **Chronische infecties (HCV), met name type 2**
- **Soms lymfoproliferatieve aandoening (type 2)**
- **Autoimmuniteit (Sjögren, SLE, RA etc), met name type 3**

Gemengde CG slaan intravasculair neer bij koude en induceren een vooral immuuncomplex-gemediëerde inflammatie (leucoclastische vasculitis) en complementactivatie in de betroffen organen:

- **palpabele purpura**
- **arthralgieën**
- **Myalgia**

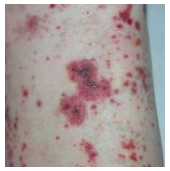
‘Meltzer’s triad’

- **Vaak RF positief**
- **Soms mengbeelden…**

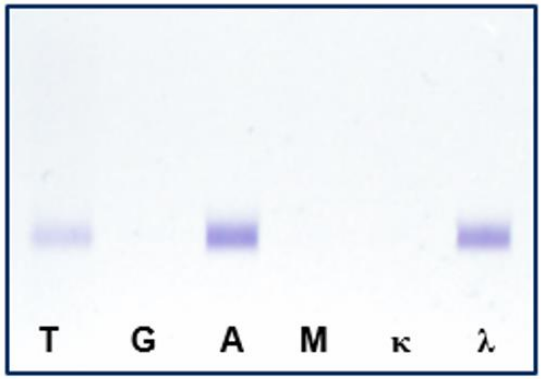
2020_4C, cryoglobuline bepaling

Casus

- Man, 65 jaar oud.
- Vasculitis van de huid



Cryo:



IFE van gewassen cryo

- 39/53 (=73%) deelnemers rapporteerden terecht pos.
- 31/33 (=94%) type 1
- 17 deelnemers kwantificeren: gem 860 mg/L (<100-2600 mg/L)

Type 1: Monoclonaal IgA-lambda

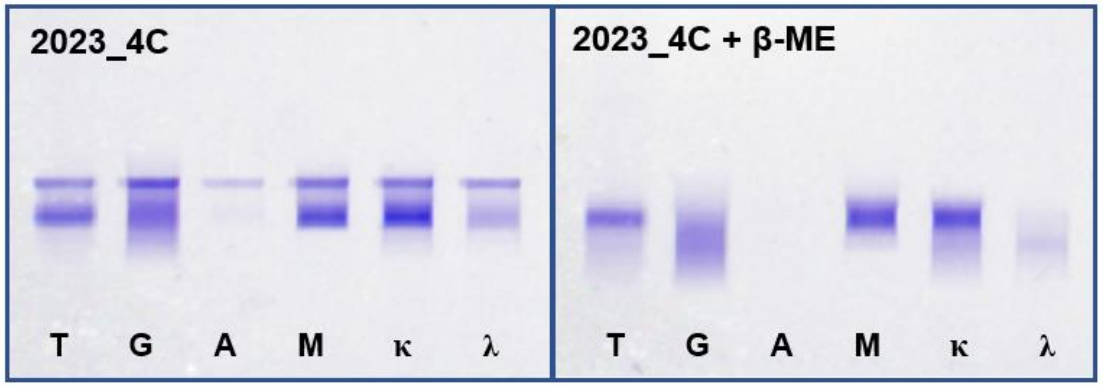
Vasculitis van de huid obv type 1 cryoglobulinaemie waarvoor kloongerichte therapie

2023_4C, cryoglobuline bepaling

Casus

- Vrouw, 70 jaar oud. Bekend met DM type 2, hypertensie.
- Toenemend moe. Lab: nierfunctiestoornis, CRP sterk pos

Cryo:



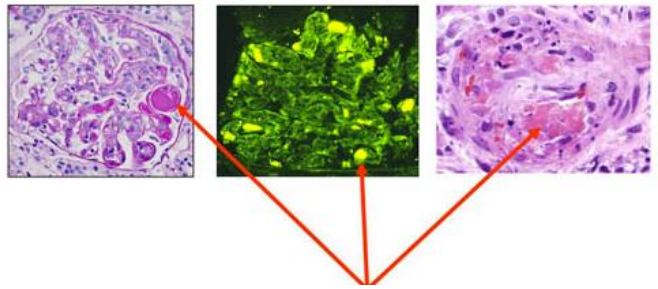
Figuur: Immunofixatie elektroforese (IFE) van cryo-precipitaat

- 45/47 deelnemers rapporteerden terecht pos.
- 60% type 2 (40% type 1)
- Forse spreiding in kwantificering (0,35 - 6 g/L)

Type 2: Monoclonaal IgM-kappa met polyclonaal IgG

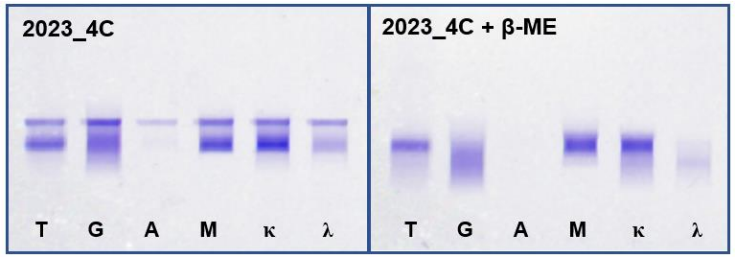
Achtergrond informatie bij deze patient:

- Sterk verlaagd C4 en licht verlaagd C3 tijdens diagnose, RF positief, CRP sterk verhoogd
- Glomerulonefritis obv cryoglobulinemie met kleine B-cel populatie in bloed + beenmerg
- Alle bloedwaarden normaliseren na (plasmaferese, prednison, cyclofosfamide, rituximab



Cryoglobulin deposits

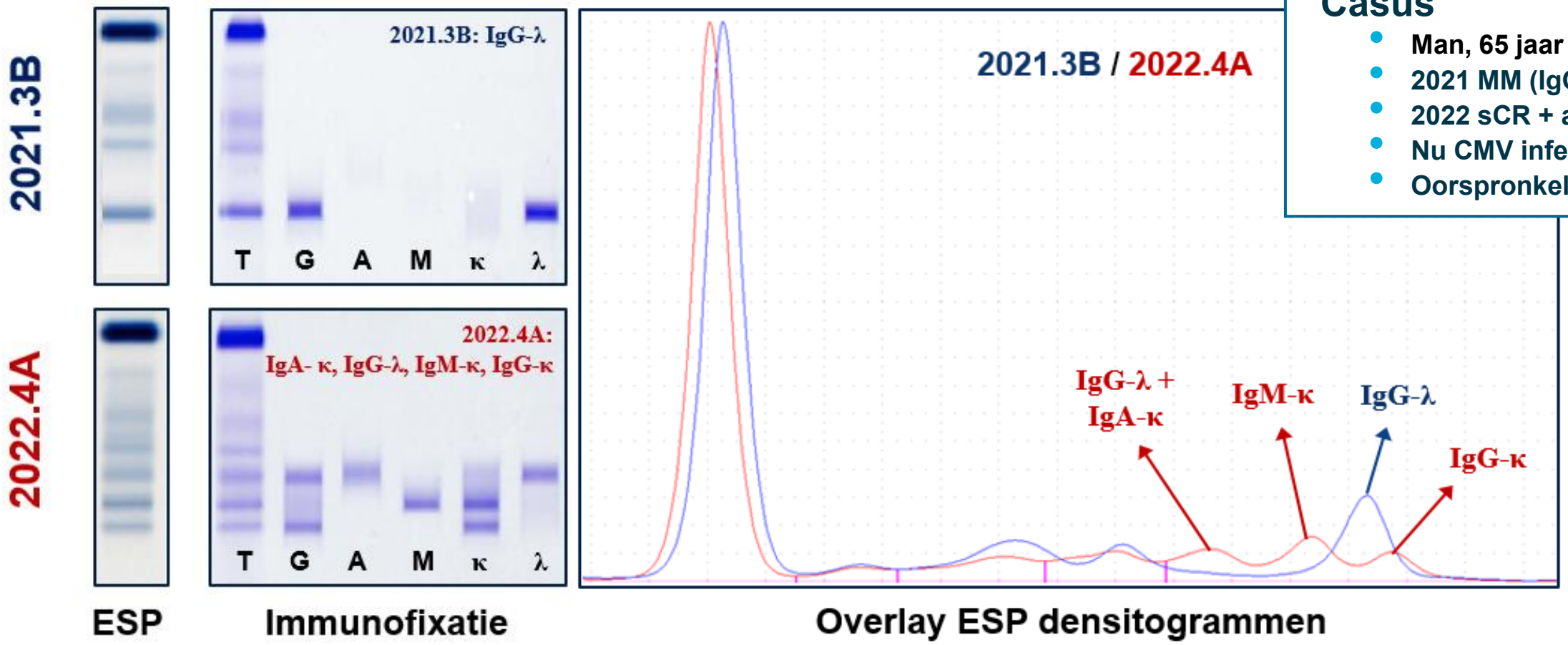
2023_4C, kwantificeren cryoglobuline



Methode	Aantal	Gerapporteerde range
Visueel waarbij de cryocrit wordt geschat als percentage van het totaal serumvolume	3	4-8 %
Immunoglobuline (IgG en/of IgM) meting in gewassen cryoprecipitaat	5	0,44 -2,0 g/l
Totaal eiwit meting in gewassen cryoprecipitaat (al dan niet met ESP correctie voor niet-immuunglobulines)	8	0,35 – 5,8 g/l
Ig (IgG en/of IgM) in serum vóór cryoprecipitatie minus Ig (IgG en/of IgM) in supernatant serum ná cryoprecipitatie	7	0,70 – 4,1 g/l
Anders of niet vermeld	4	0,72-4,0 g/l

Tabel 2. Overzicht van gerapporteerde methodes voor kwantificering van het cryoglobuline

Follow-up of an M-protein

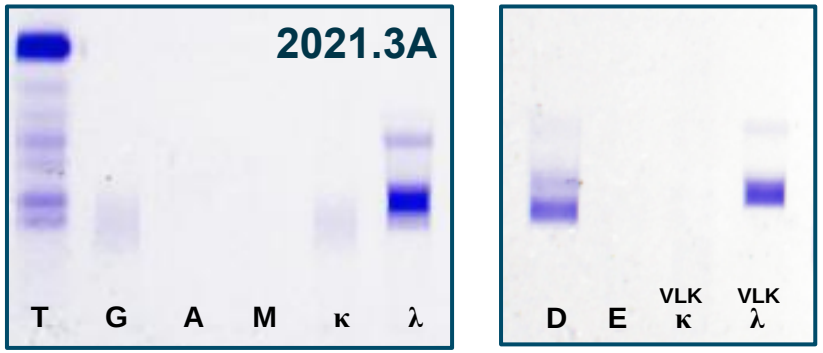


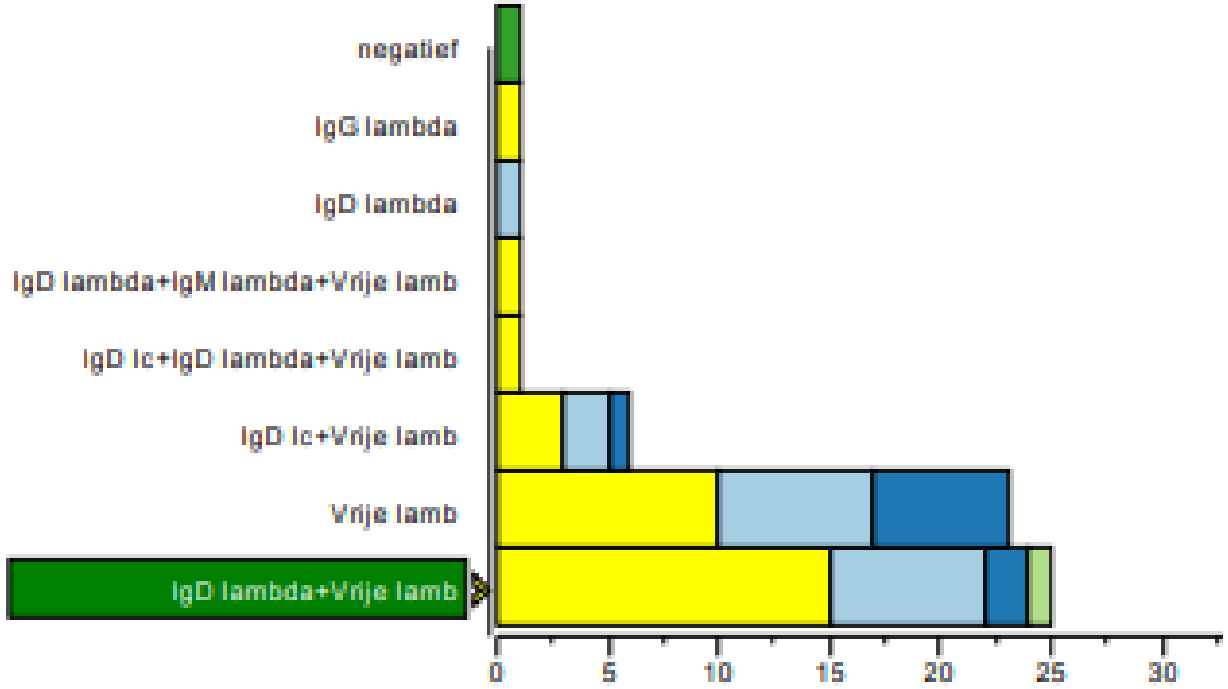
Casus

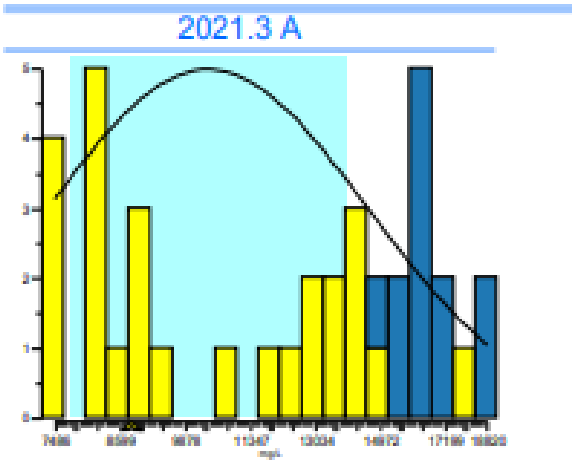
- Man, 65 jaar oud
- 2021 MM (IgG-lambda)
- 2022 sCR + autologe SCT
- Nu CMV infectie
- Oorspronkelijke M-prot?

- Aan of afwezigheid van oorspronkelijke M-proteine prognostisch belangrijk
- Goed archief is belangrijk voor vervolgen van een bekend M-proteine

IgD M-proteïne…



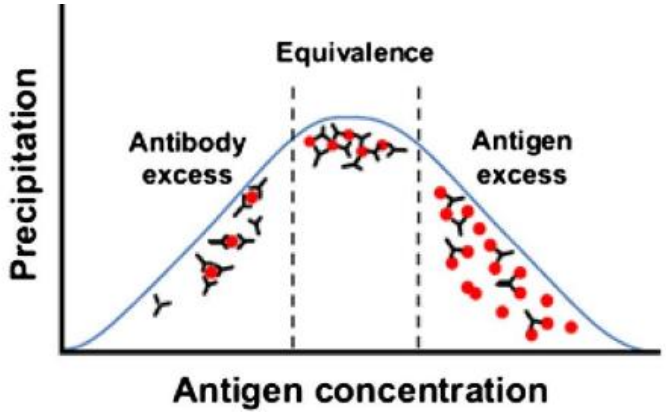




	cons.	meth.	ALTM	lab
gem.	10355	10355	12269	8790
SD	4180	4180	5829	
n	26	26	38	
nu	1	1	2	
rec.	85%	85%	72%	

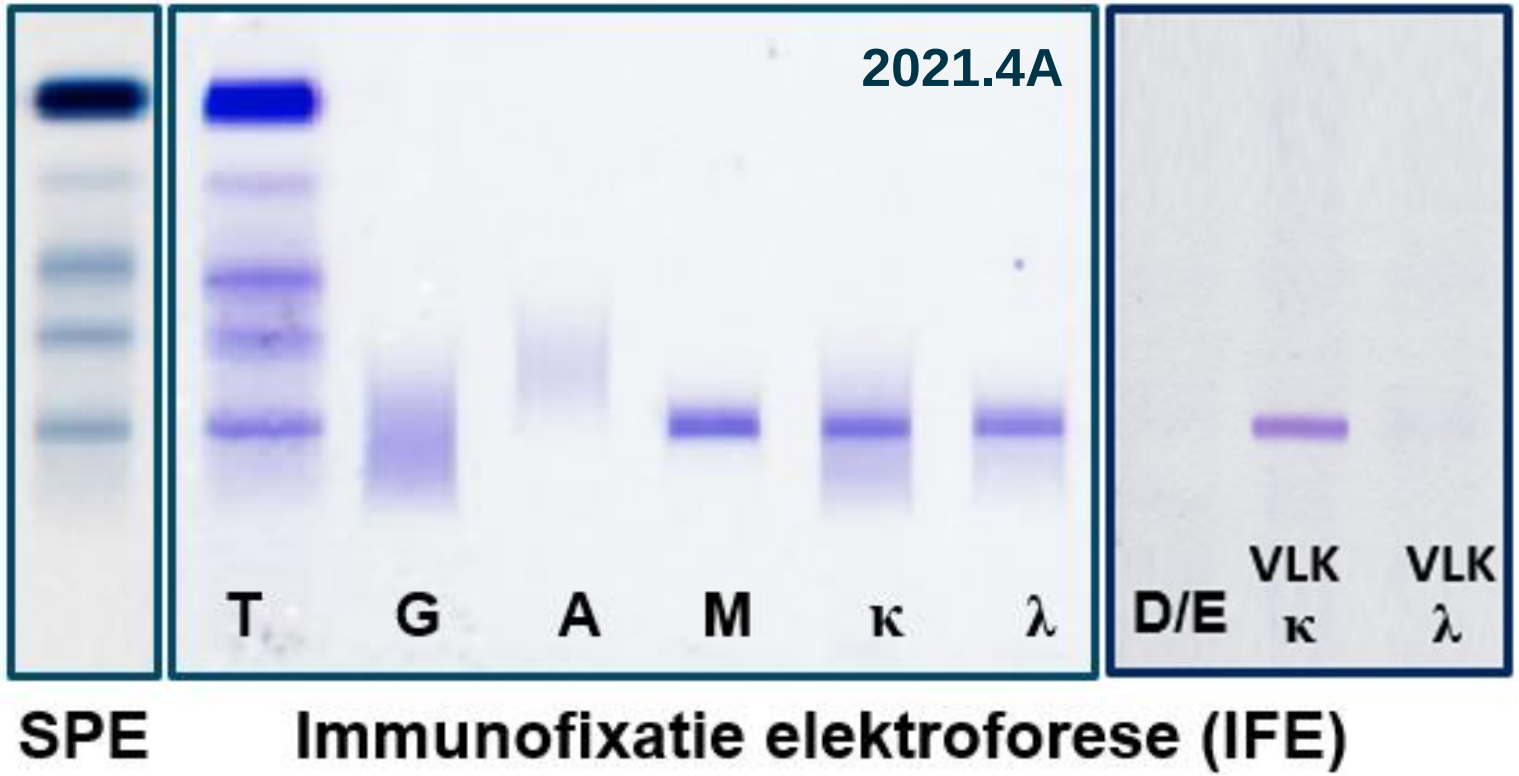
VLK lambda uitslagen range: 6.000 tot 21.000 mg/L (M-sprike <2 g/L)

1 deelnemer rapporteert VLK-lambda 67 mg/L met een normale VLK-ratio (!)



Jacobs et al. Autoimmun Rev. 2015

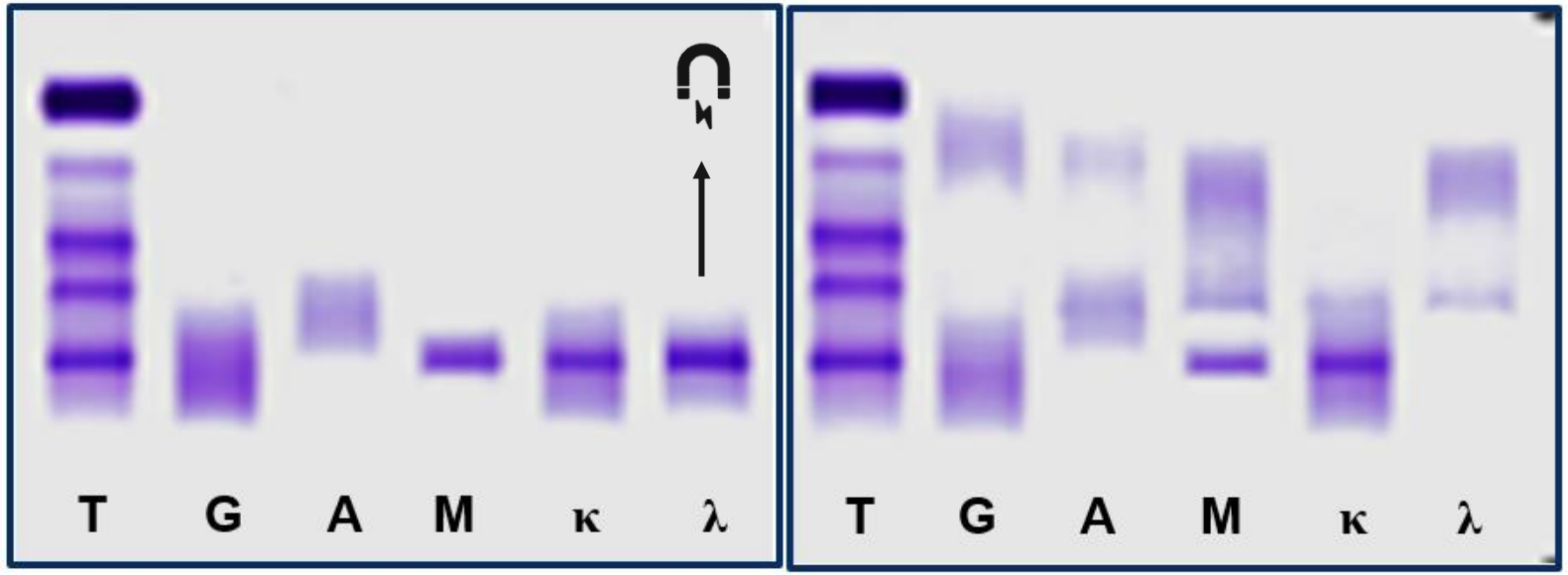
Pittige typering…. (met dank aan dr. Gideon Lansbergen)



SPE Immunofixatie elektroforese (IFE)

2 banden… maar welke isotypes?

λ-shift:
add anti-λ-antibodies…



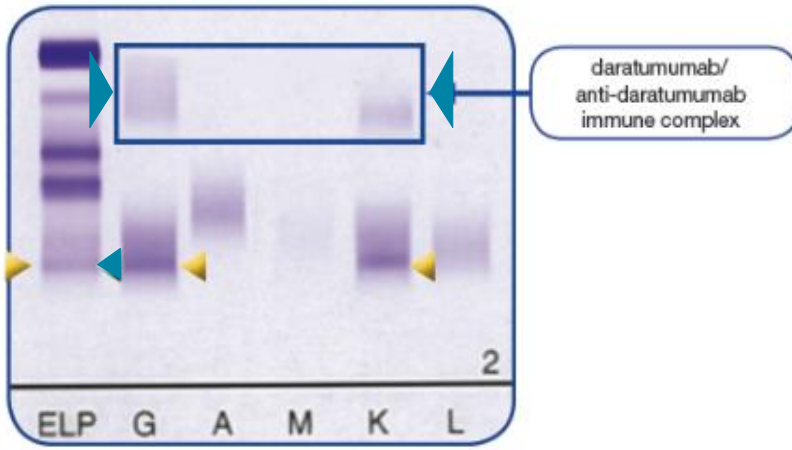
IFE zonder shift

IFE met λ-shift

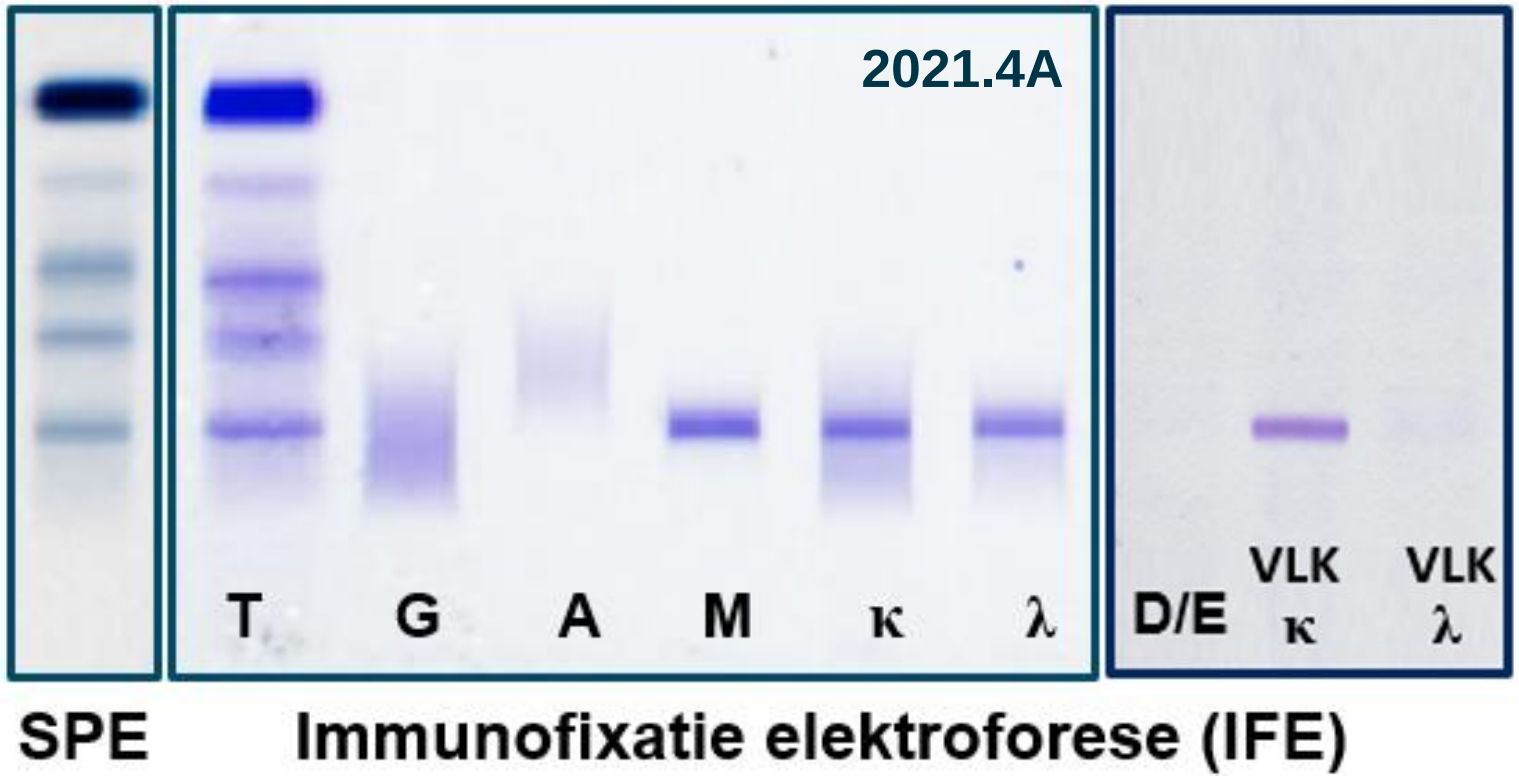
Conclusie:
IgM-kappa en IgM-lambda M-proteine

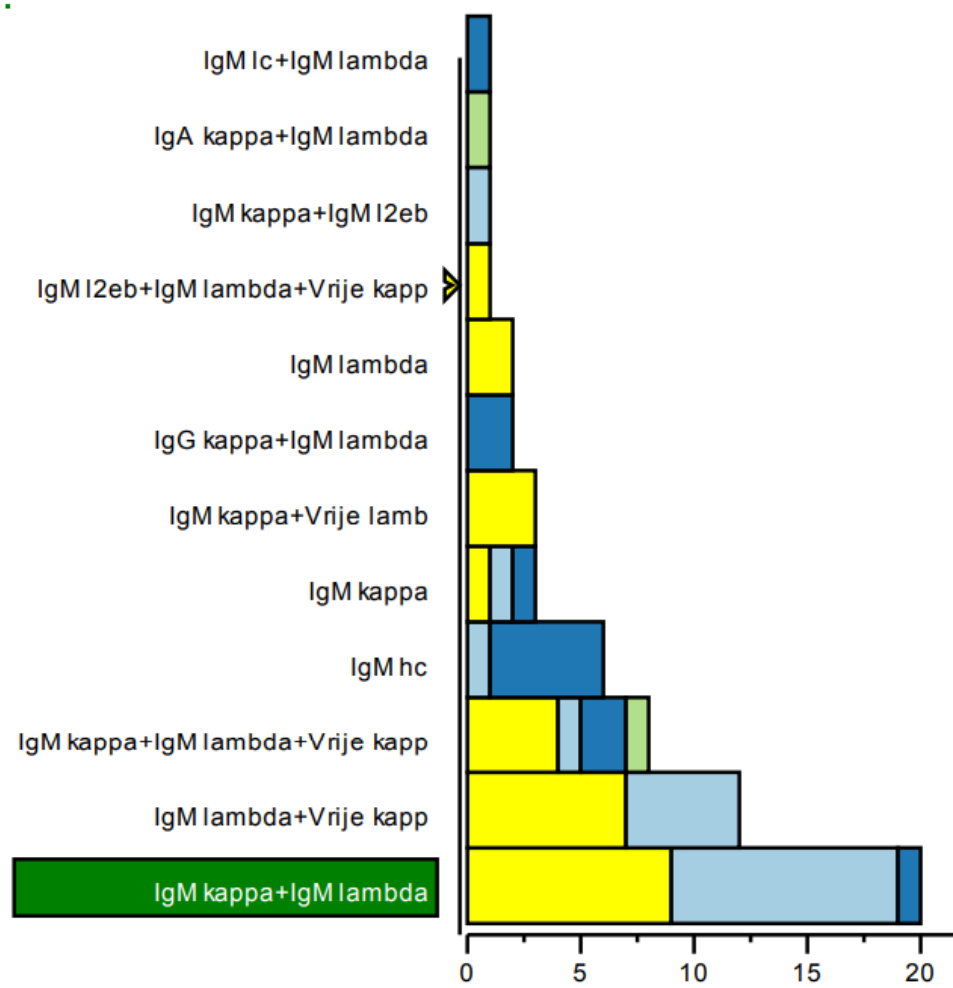
(met dank aan Sebia (geen reguliere reagentia)

Daratumumab Hydrashift assay (=pos)



Pittige typering…. (met dank aan dr. Gideon Lansbergen)





Reagentia	sVLK-kappa	sVLK-lambda	sVLK-ratio
Freelite (TBS)	14,7 mg/L	14,4 mg/L	1,01
N-Latex FLC (Siemens)	13,6 mg/L	14,5 mg/L	0,87
Sebia-FLC (Sebia)	14,7 mg/L	34,2 mg/L	0,43

Tevens een interessant sample geschikt voor de M-proteine rondzending?

Zeer welkom!

- **70 ml serum nodig per monster (!)**
- **Echter, samples kunnen ook gespiked worden in normaal serum. In dat geval minder serum nodig.**
- **Graag contact:**

H.Jacobs@Radboudumc.nl

Dank je wel

Collega's Lab Medische Immunologie
Corrie de Kat Angelino

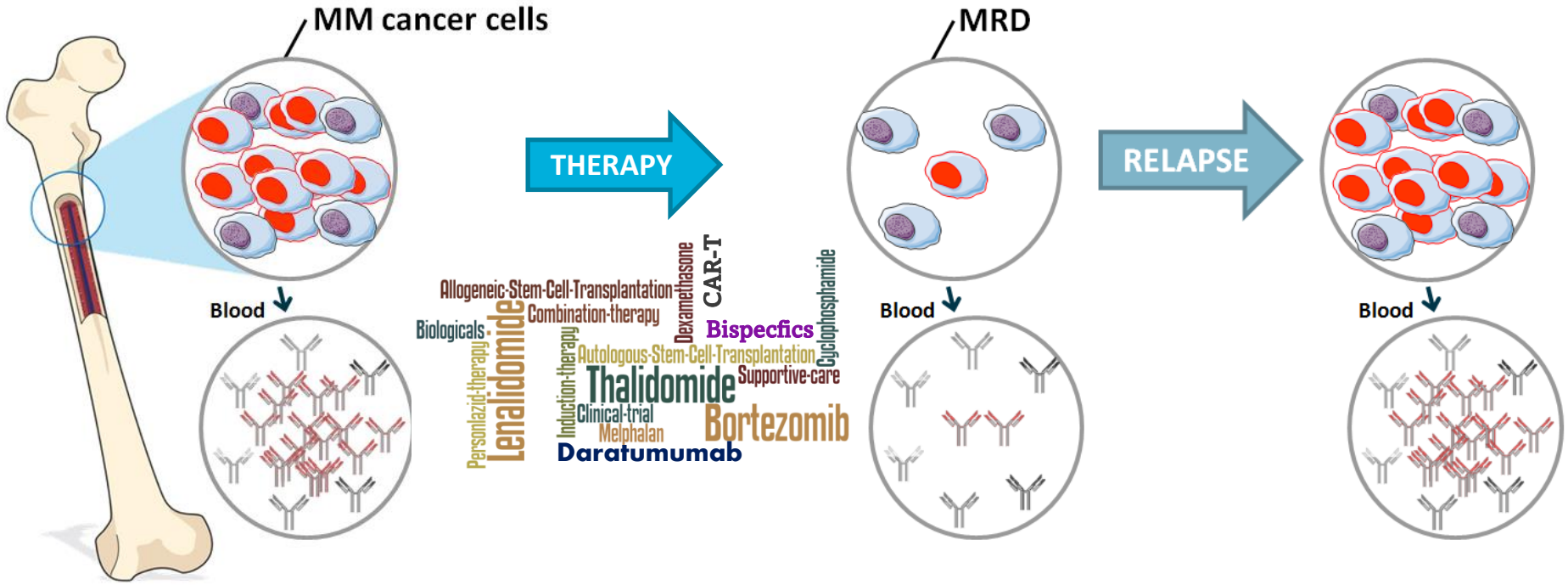
Radboudumc

Ondersteuning SKML bureau
SKML Sectie Humorale Immunologie

skml

M-protein diagnostics = Personalized diagnostics

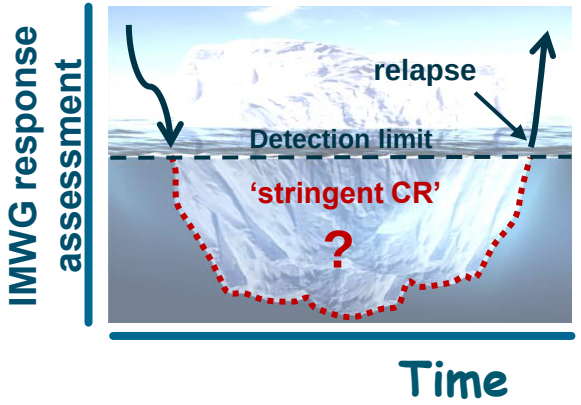
Minimal Residual Disease (MRD) in multiple myeloma



International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma

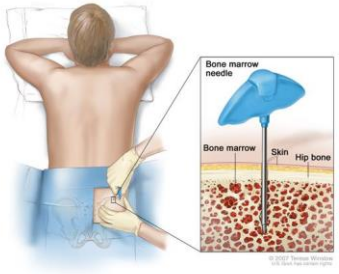
Lancet Oncol 2016; 17: e328–46

"…>70% of patients achieve sCR…"



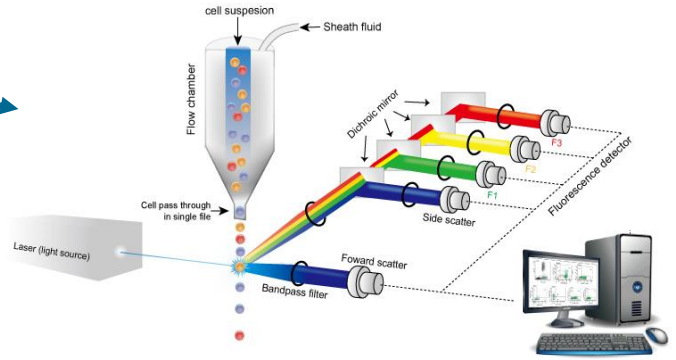
MRD-evaluation in bone marrow aspirates

Next Generation Sequencing



Bone Marrow aspirate

Next Generation Flow Cytometry



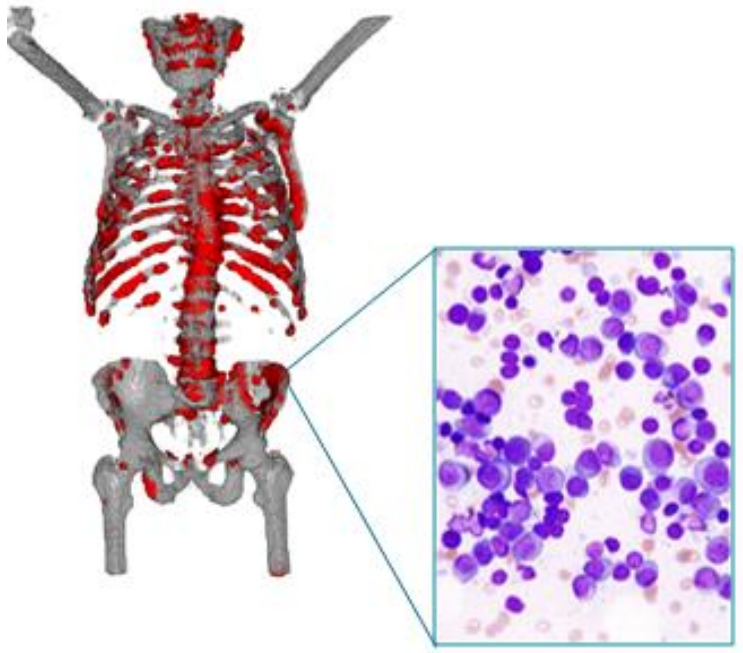
Why evaluate MRD?

- To be informed beyond Complete Remission (sCR)
- Best MM prognostic marker
- As (primary!) endpoint of treatment in clinical trials
- MRD-guided therapy

IMWG MRD criteria (requires a complete response as defined below)	
Sustained MRD-negative	MRD negativity in the marrow (NGF or NGS, or both) and by imaging as defined below, confirmed minimum of 1 year apart. Subsequent evaluations can be used to further specify the duration of negativity (eg, MRD-negative at 5 years)†
Flow MRD-negative	Absence of phenotypically aberrant clonal plasma cells by NGF‡ on bone marrow aspirates using the EuroFlow standard operation procedure for MRD detection in multiple myeloma (or validated equivalent method) with a minimum sensitivity of 1 in 10^5 nucleated cells or higher
Sequencing MRD-negative	Absence of clonal plasma cells by NGS on bone marrow aspirate in which presence of a clone is defined as less than two identical sequencing reads obtained after DNA sequencing of bone marrow aspirates using the LymphoSIGHT platform (or validated equivalent method) with a minimum sensitivity of 1 in 10^5 nucleated cells§ or higher
Imaging plus MRD-negative	MRD negativity as defined by NGF or NGS plus disappearance of every area of increased tracer uptake found at baseline or a preceding PET/CT or decrease to less mediastinal blood pool SUV or decrease to less than that of surrounding normal tissue¶

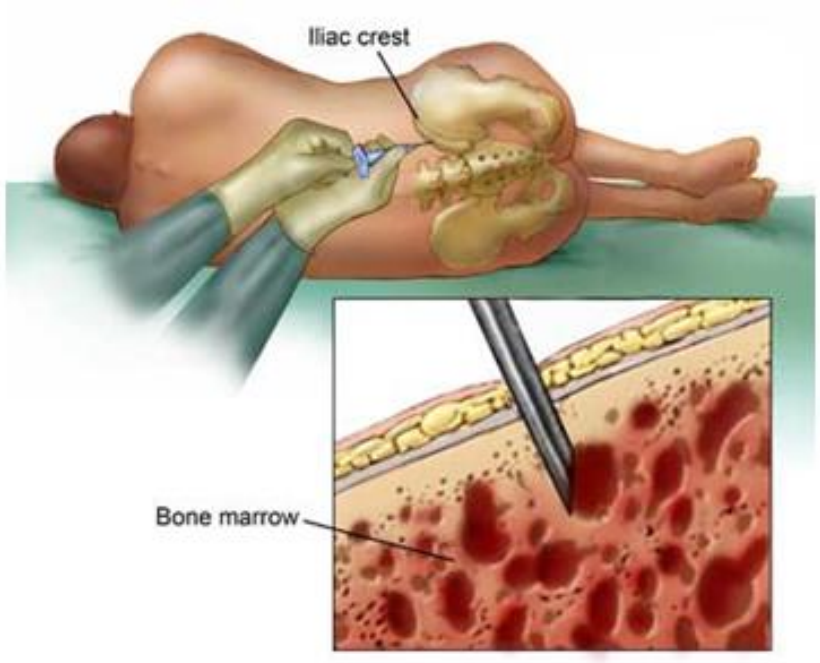
Kumar et al. Lancet Oncology 2016 'IMWG MRD consensus criteria'

Bone marrow not preferred for monitoring MM



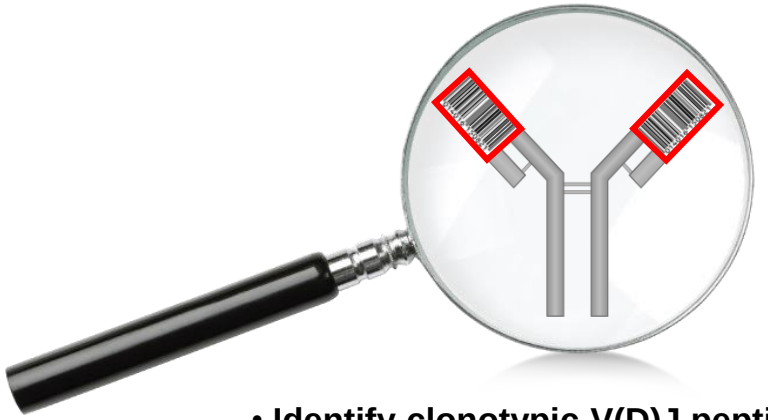
Sampling bias:

- Patchy disease
- Hemodilution
- Extramedullary growth



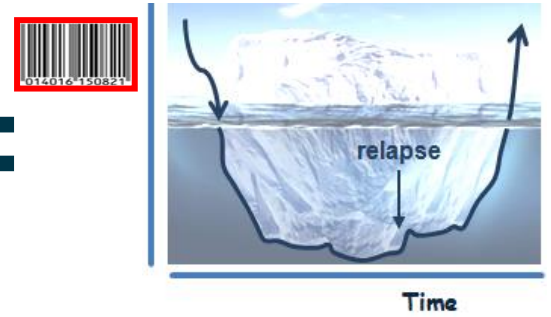
Invasive procedure for repetitive monitoring

Targeted mass spectrometry to measure clonotypic M-protein peptides: MS-MRD



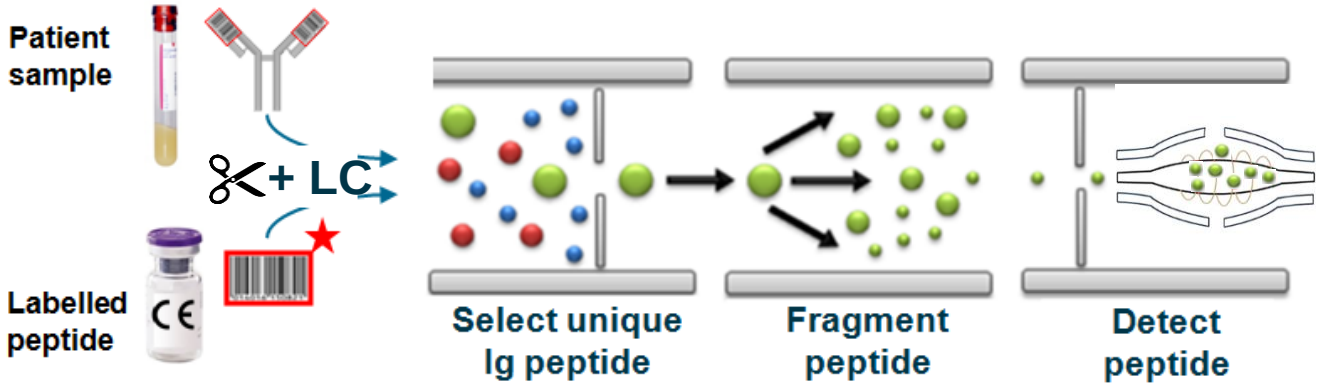
• Identify clonotypic V(D)J peptides

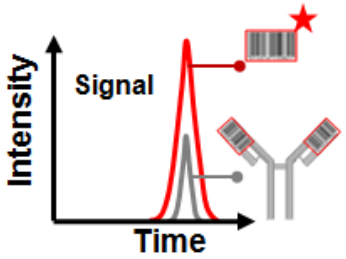
• Targeted MS of V(D)J peptides

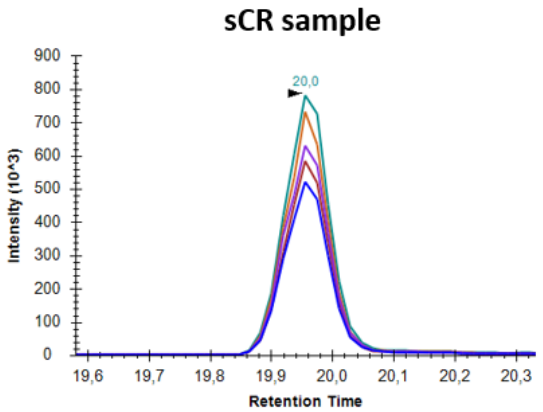


• monitor deep remission & early relapse

Parallel Reaction Monitoring (PRM)





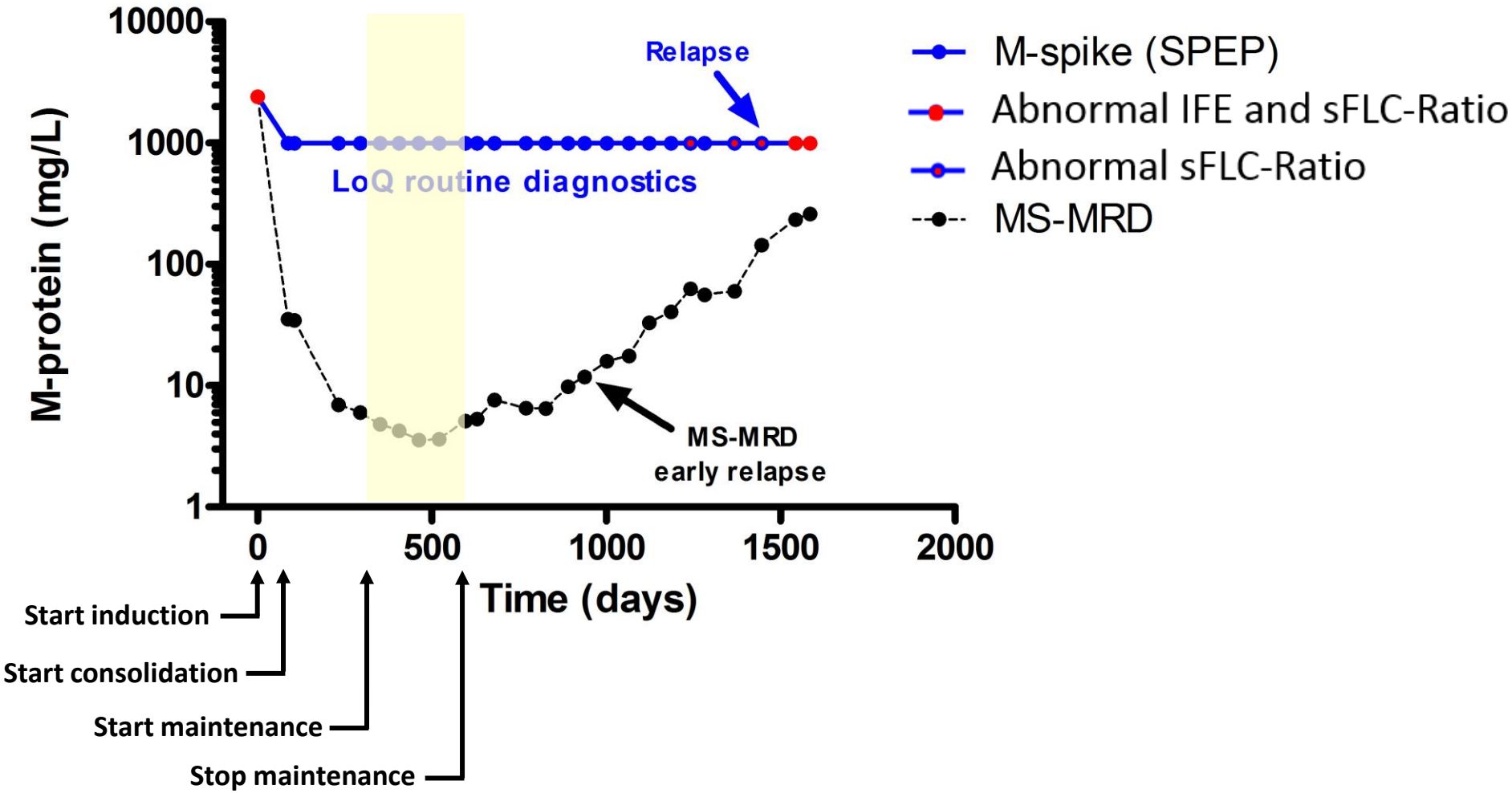


Zajec et al. J Proteome Res. 2018
Zajec et al. Clin Chem 2020

Langerhorst et al. Clin Chem 2021

Radboudumc

Dynamic MRD monitoring using MS-MRD.



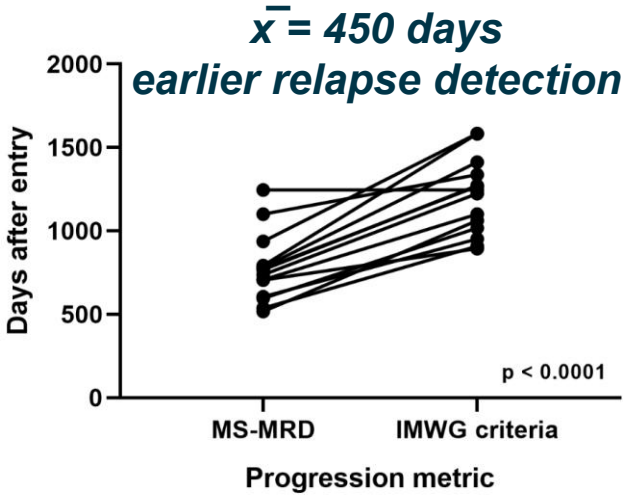
Dynamic MRD provides unique information on individual therapy-responses.

N=41 MM pts
- IFM 2009 study

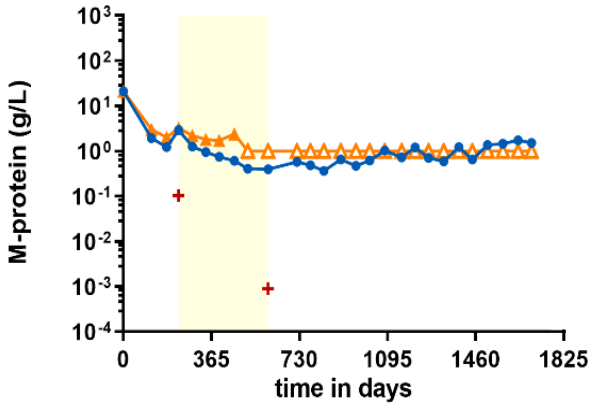
926 follow-up sera
(~22 sera per pt)

M-spike (ESP/IFE)

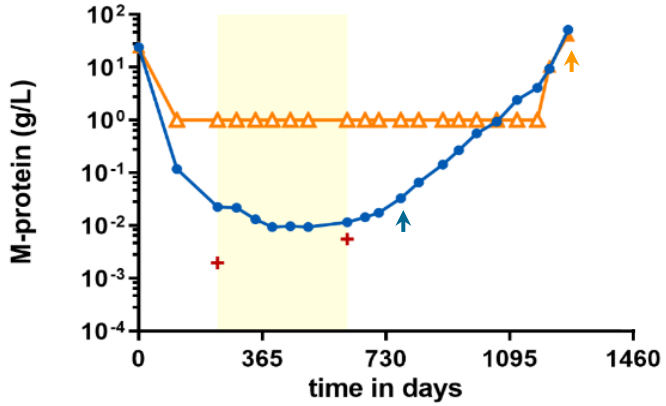
MS-MRD



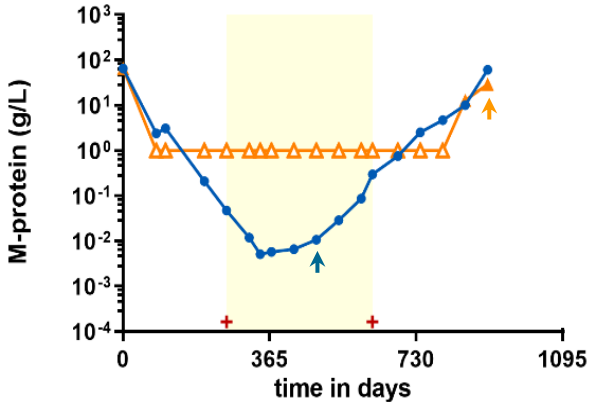
No deep response



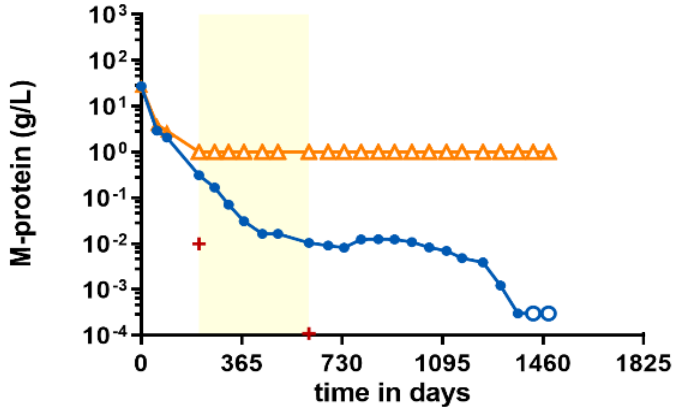
Disease activity ↑ soon after stop maintenance therapy



Disease activity ↑ during maintenance therapy



Deep and lasting responses



Noori et al. Blood Cancer J 2023

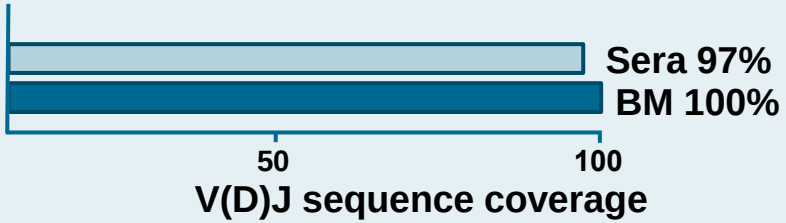
Erasmus MC University Medical Center Rotterdam

ifm

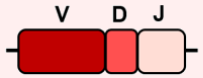
Radboudumc

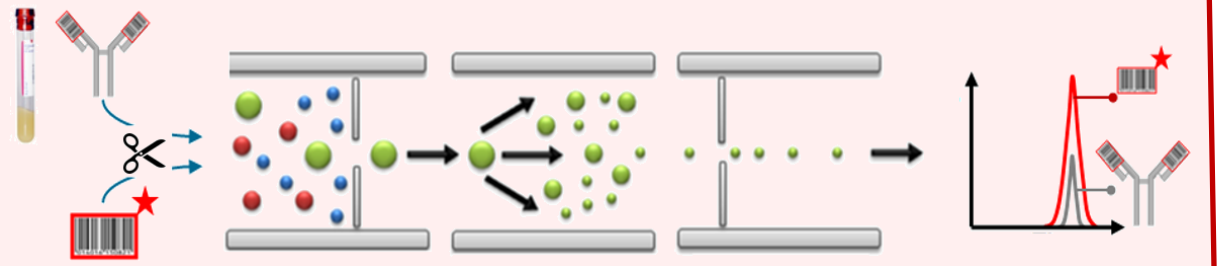
MS-MRD towards clinical implementation: M-InSight

1. V(D)J *De Novo* sequencing



Bonifay et al abstract @EMN 2023
Noori et al Hemasphere 2022



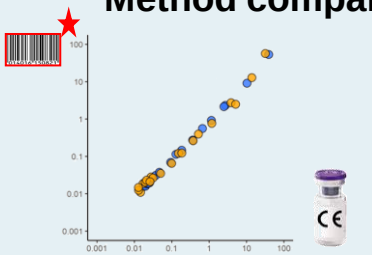


3. Automation

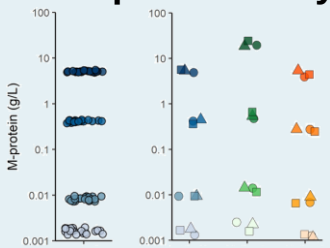
2. Off-the-shelf Calibrator

Wijnands et al CCLM 2023

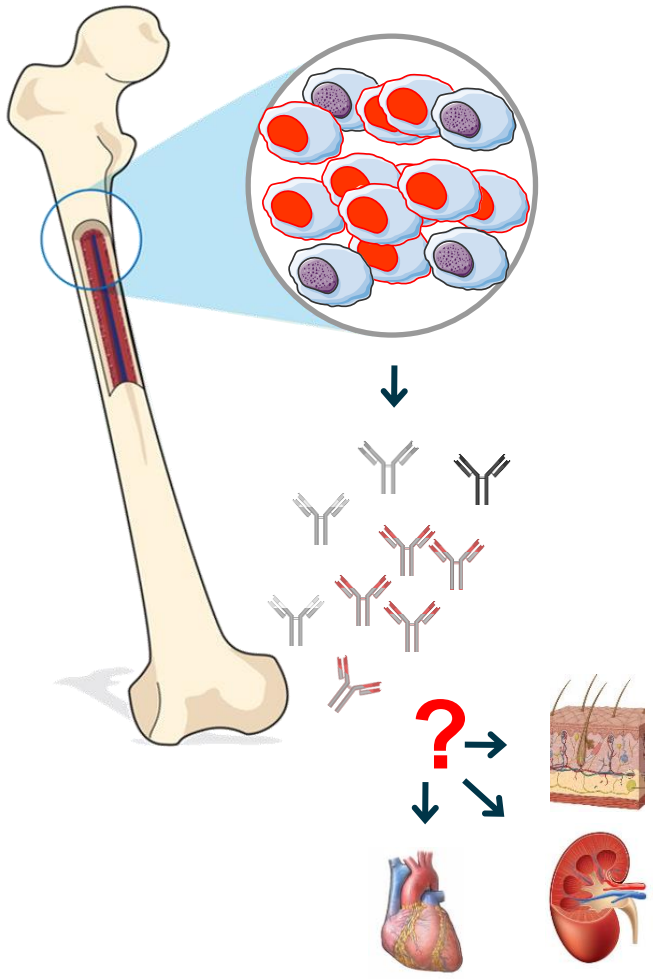
Method comparison



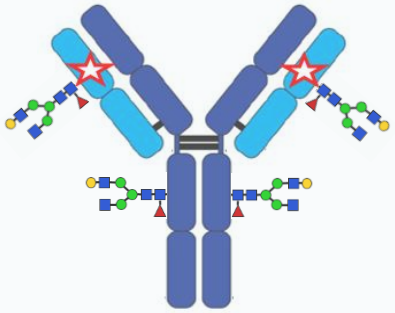
Reproducibility

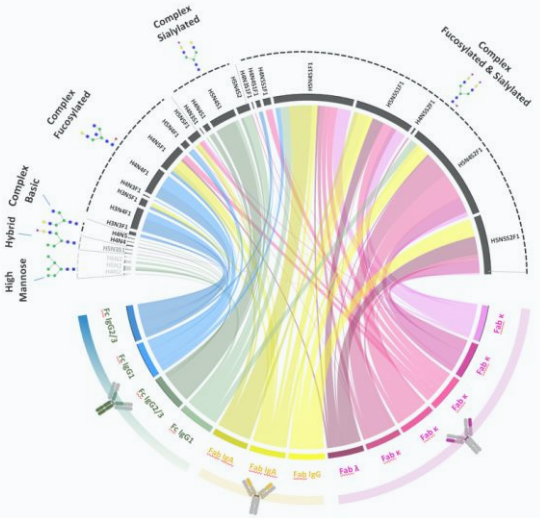


Future perspective: predict M-protein pathogenicity?



M-protein glycosylation





Langerhorst et al. CCLM 2024.

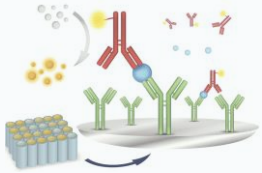
Amyloidogenicity

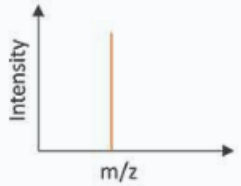
Process of detecting amyloidogenic λ-FLC

Limited proteolysis

dLCCD
λ-FLC epitope

Biomarker Detection





Jiang et al. Presented @ASH 2023.