Zaburzenia białkowe u chorych ze szpiczakiem mnogim – znaczenie oznaczania wolnych łańcuchów lekkich w surowicy

Ryszard Drożdż
Zakład Diagnostyki Medycznej CMUJ
Zakład Diagnostyki Szpitala Uniwersyteckiego
r_drozdz@cm-uj.krakow.pl

Metody analizy białek

Elektroforeza białek-ogólna ocena

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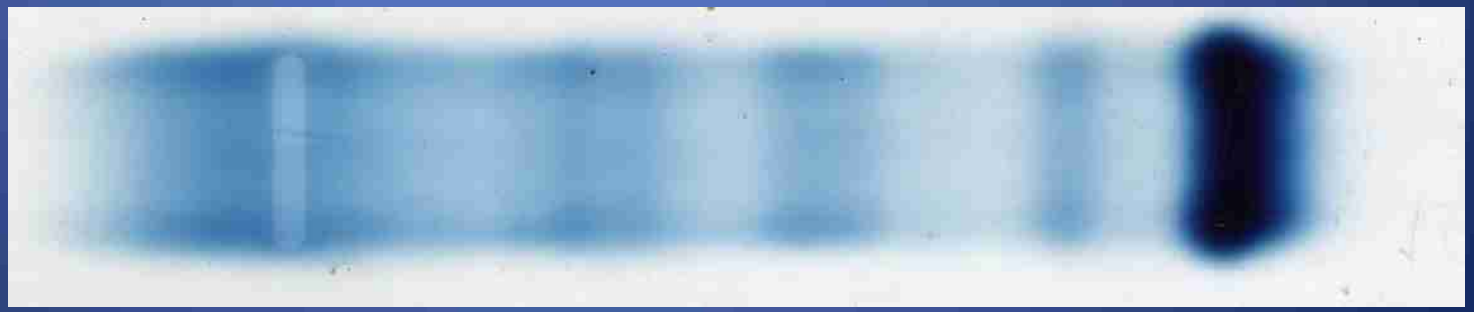
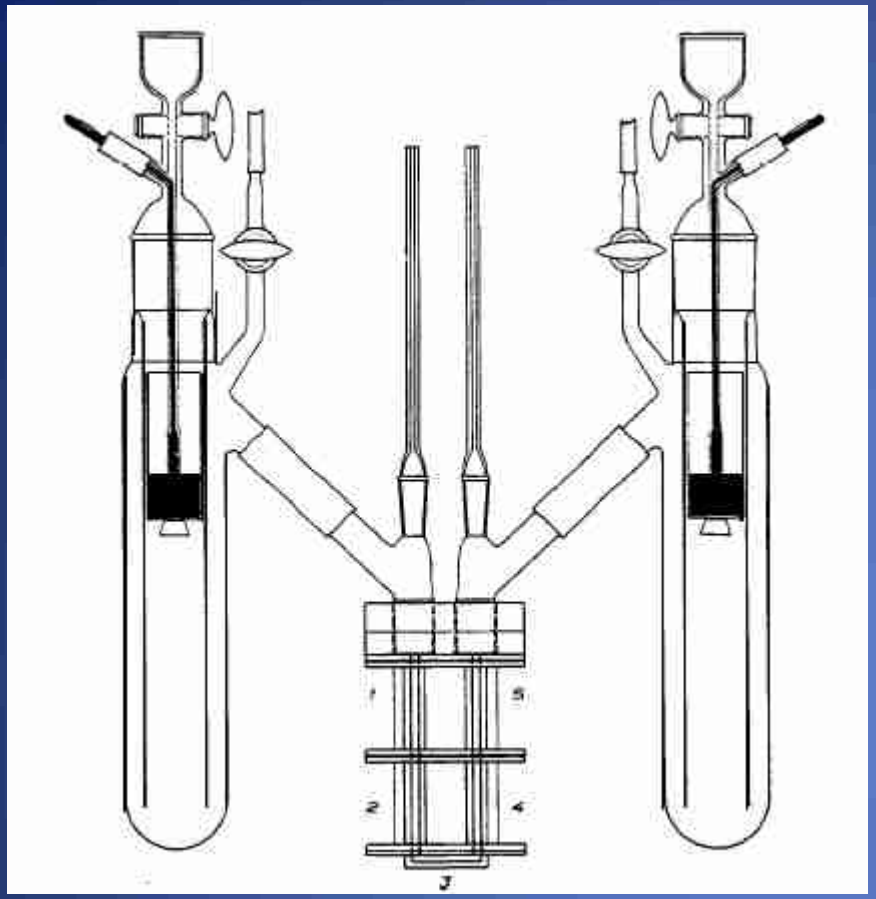




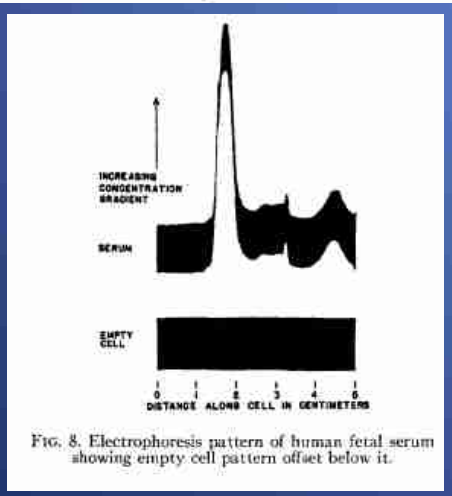
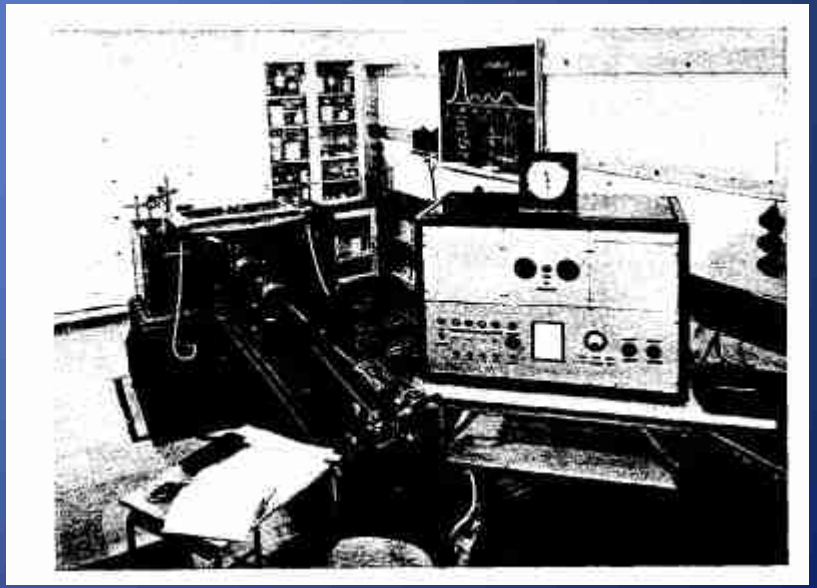


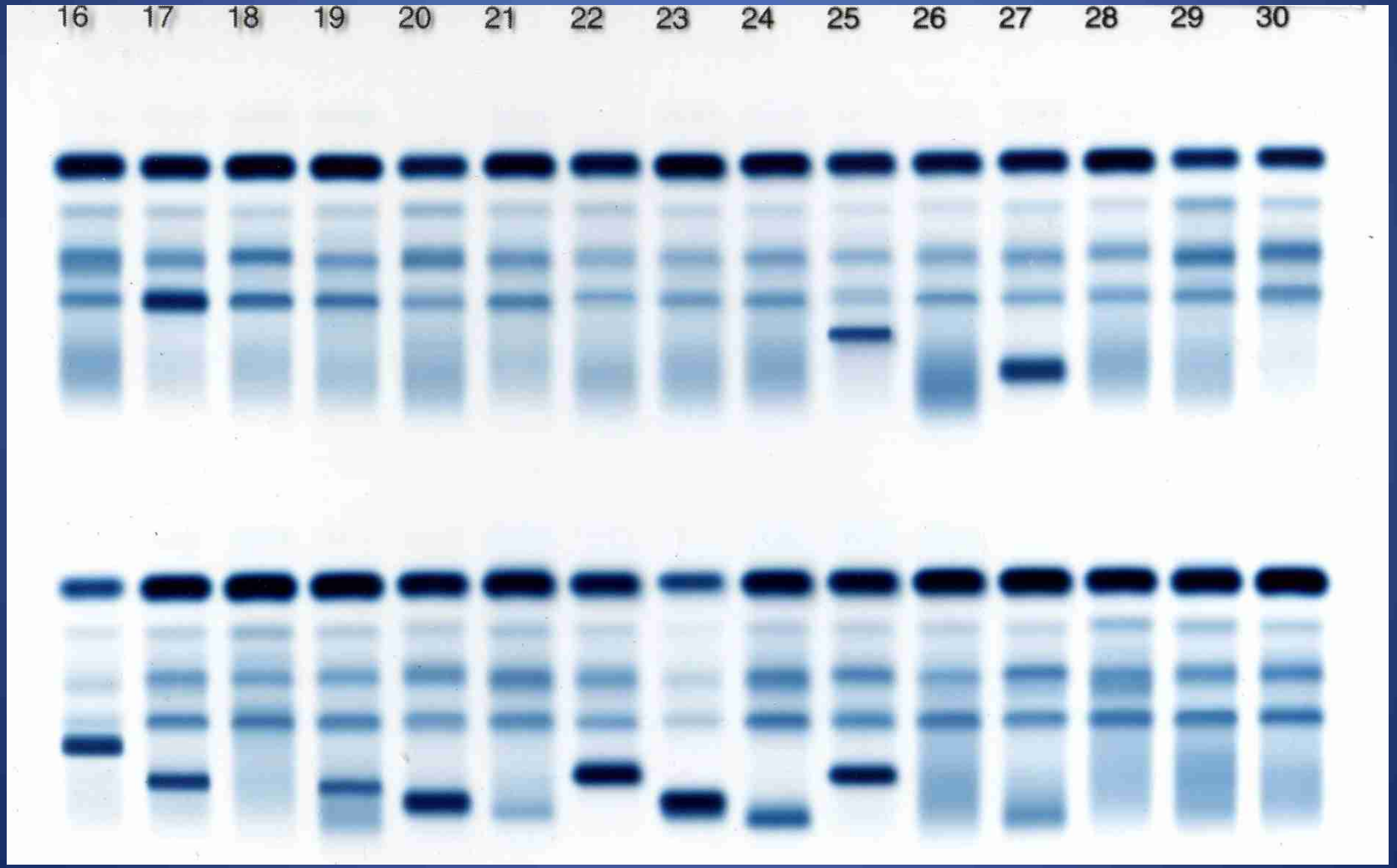
FIG. 8. Electrophoresis pattern of human fetal serum showing empty cell pattern offset below it.

A R N E W . K . TI S E L I U S

Electrophoresis and adsorption analysis as aids in investigations of large molecular weight substances and their breakdown products

Nobel Lecture, December 13, 1948

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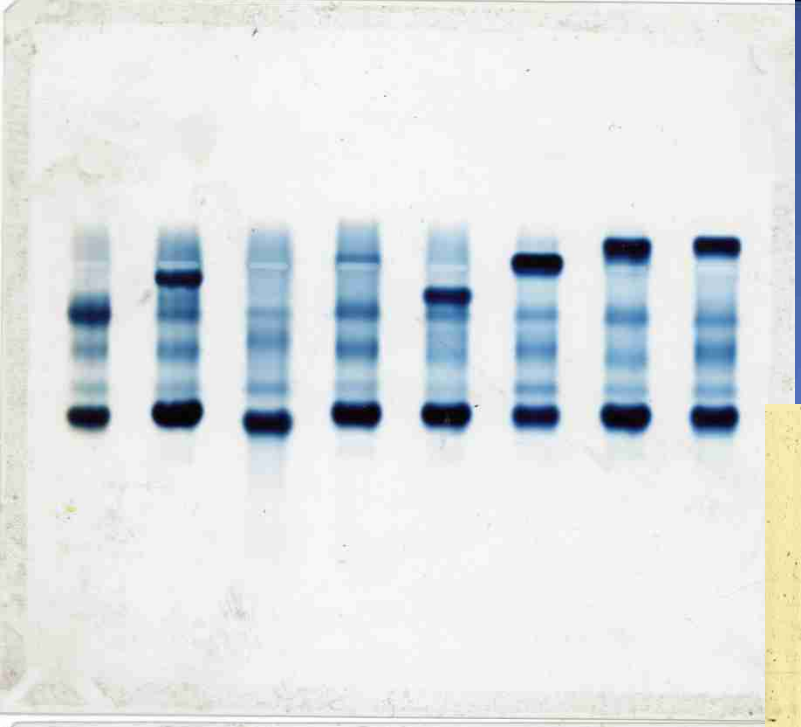
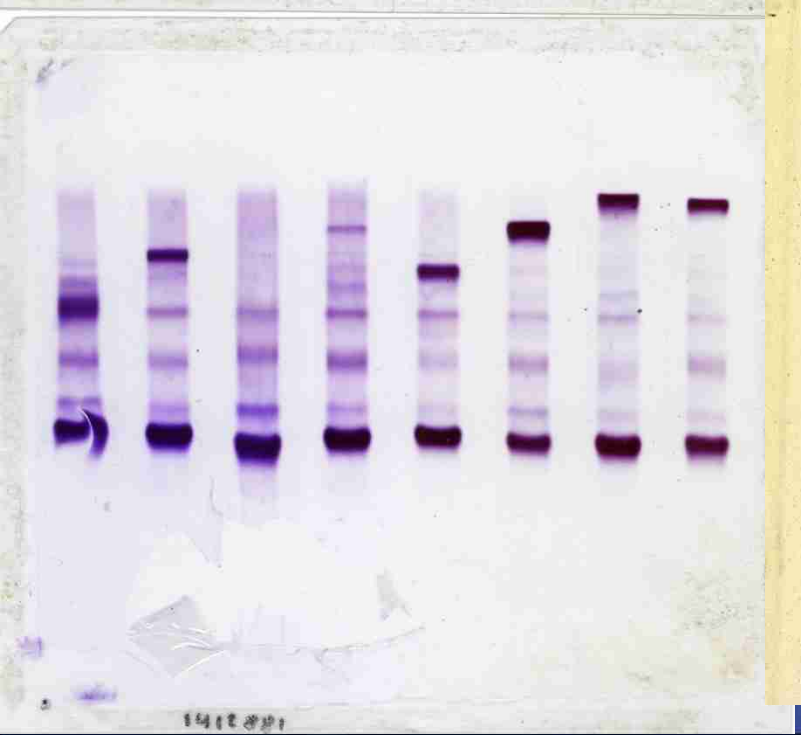
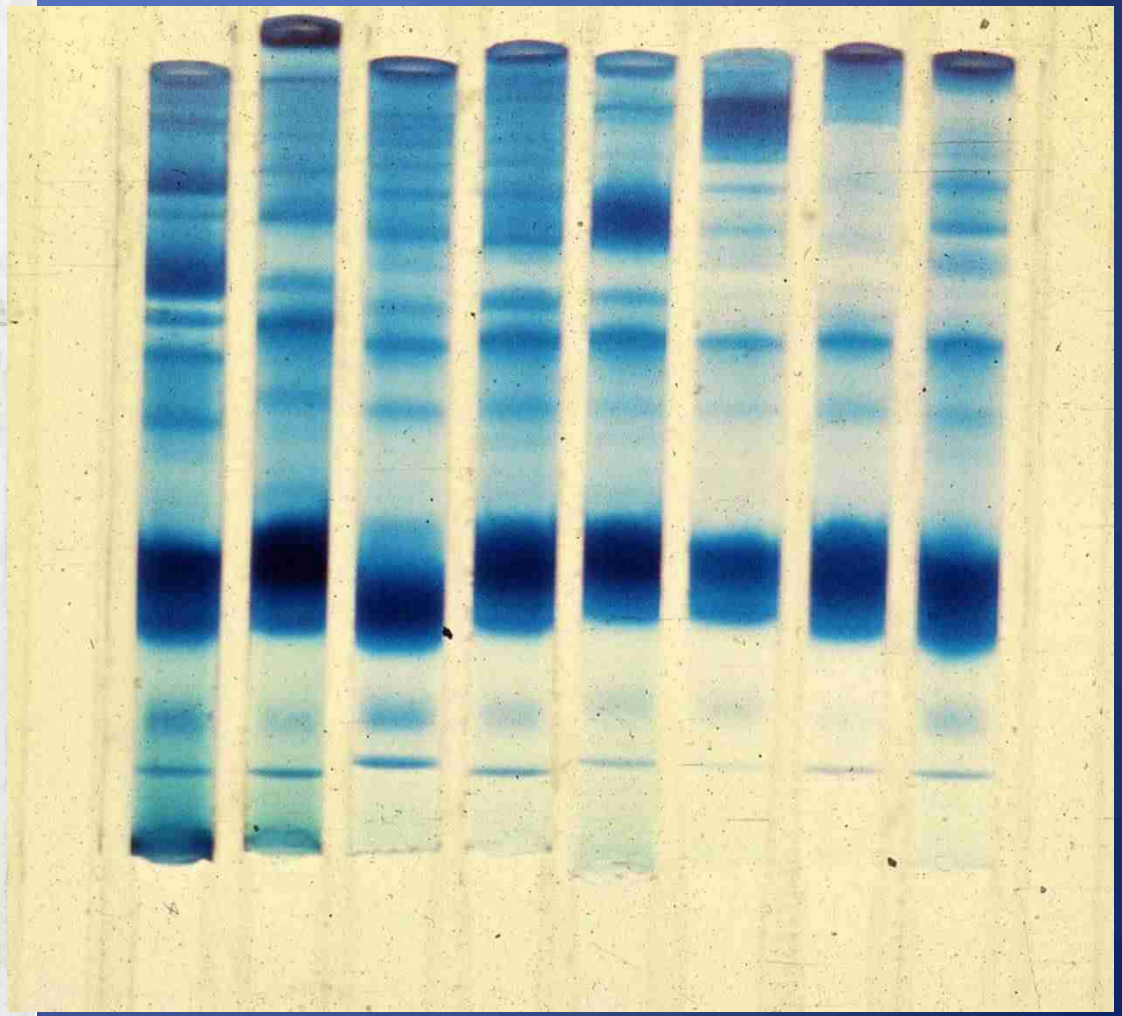
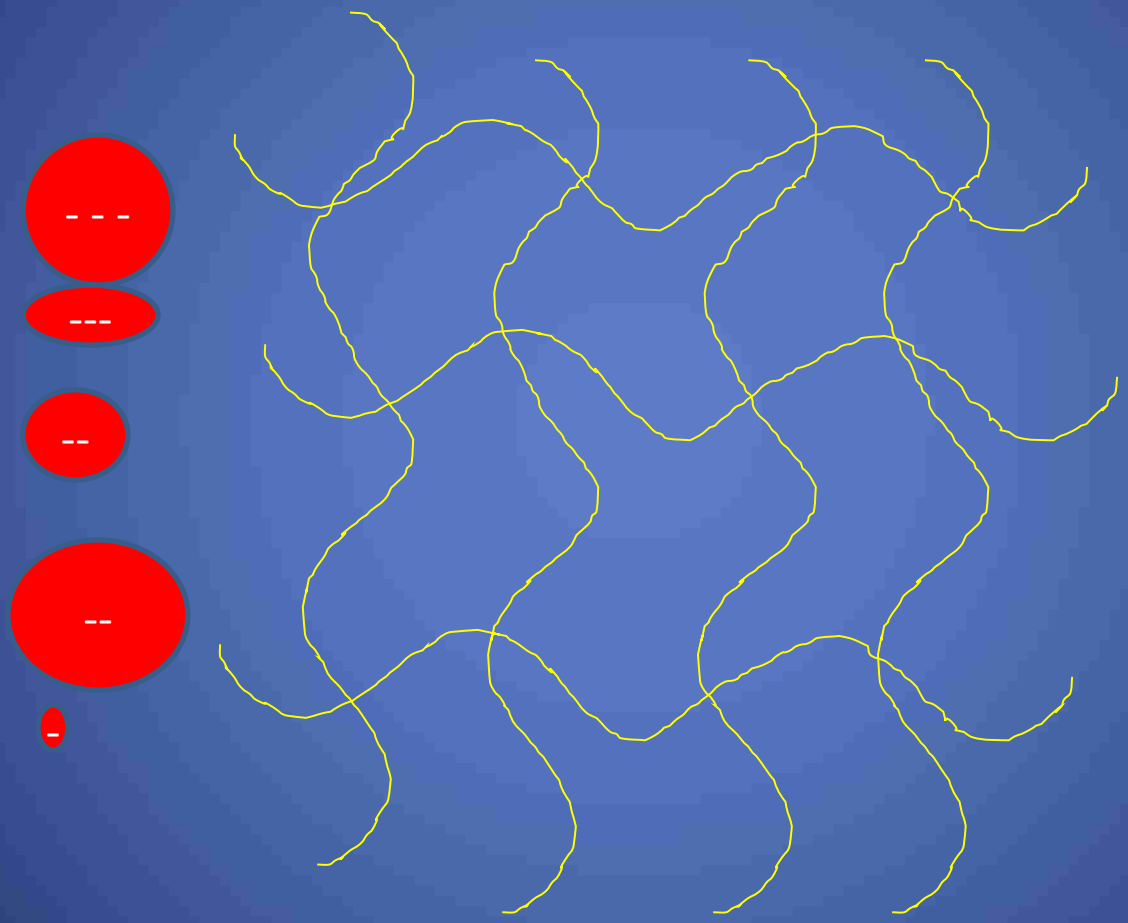
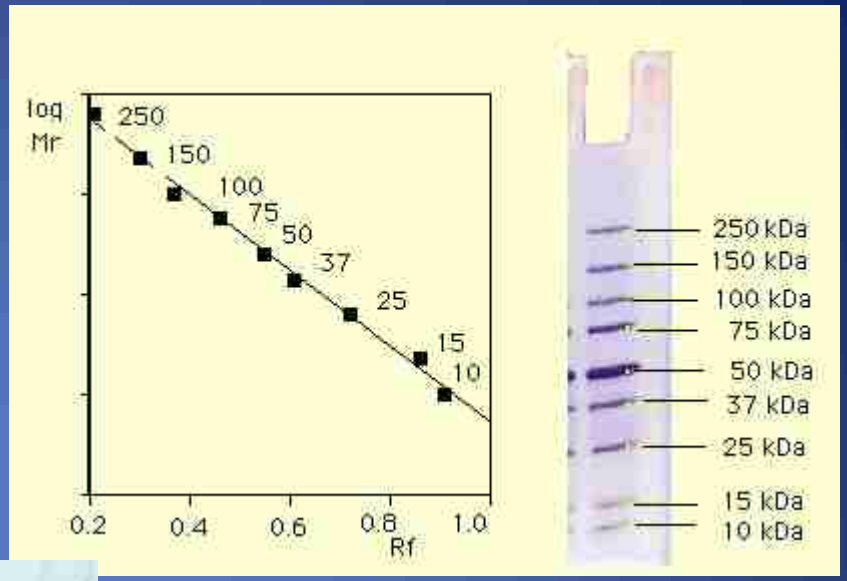






SDS elektroforeza

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75
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0.2
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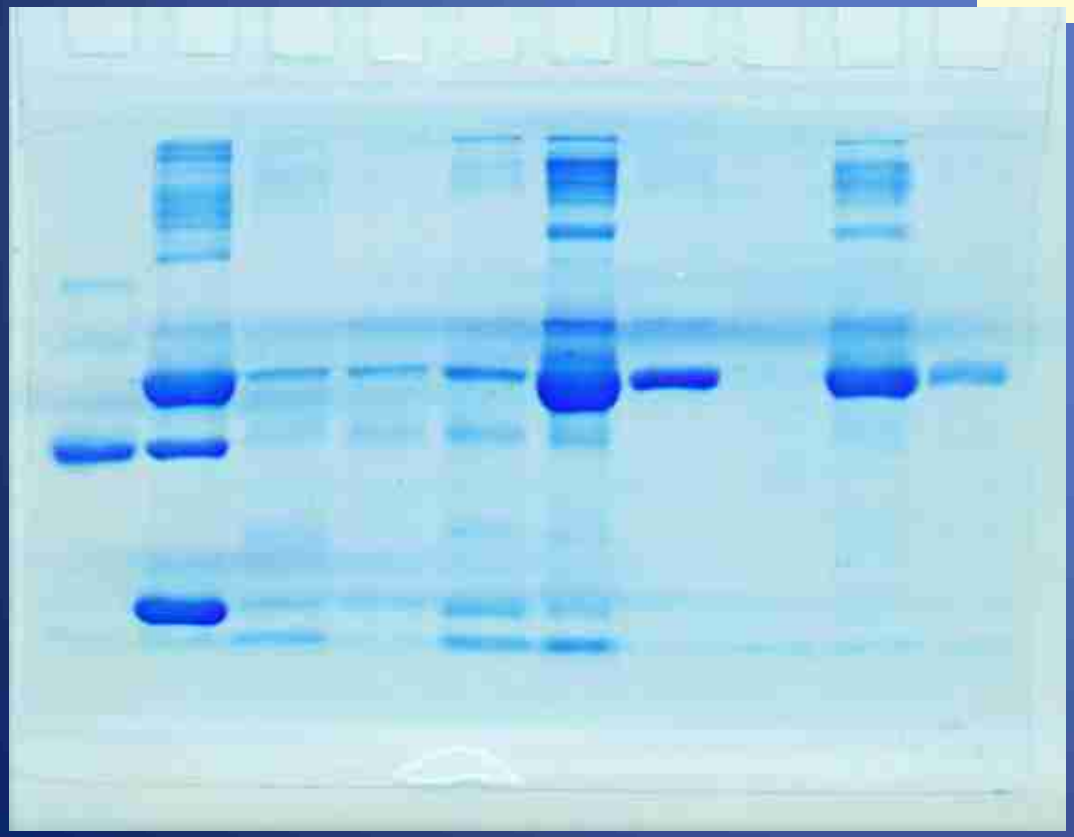
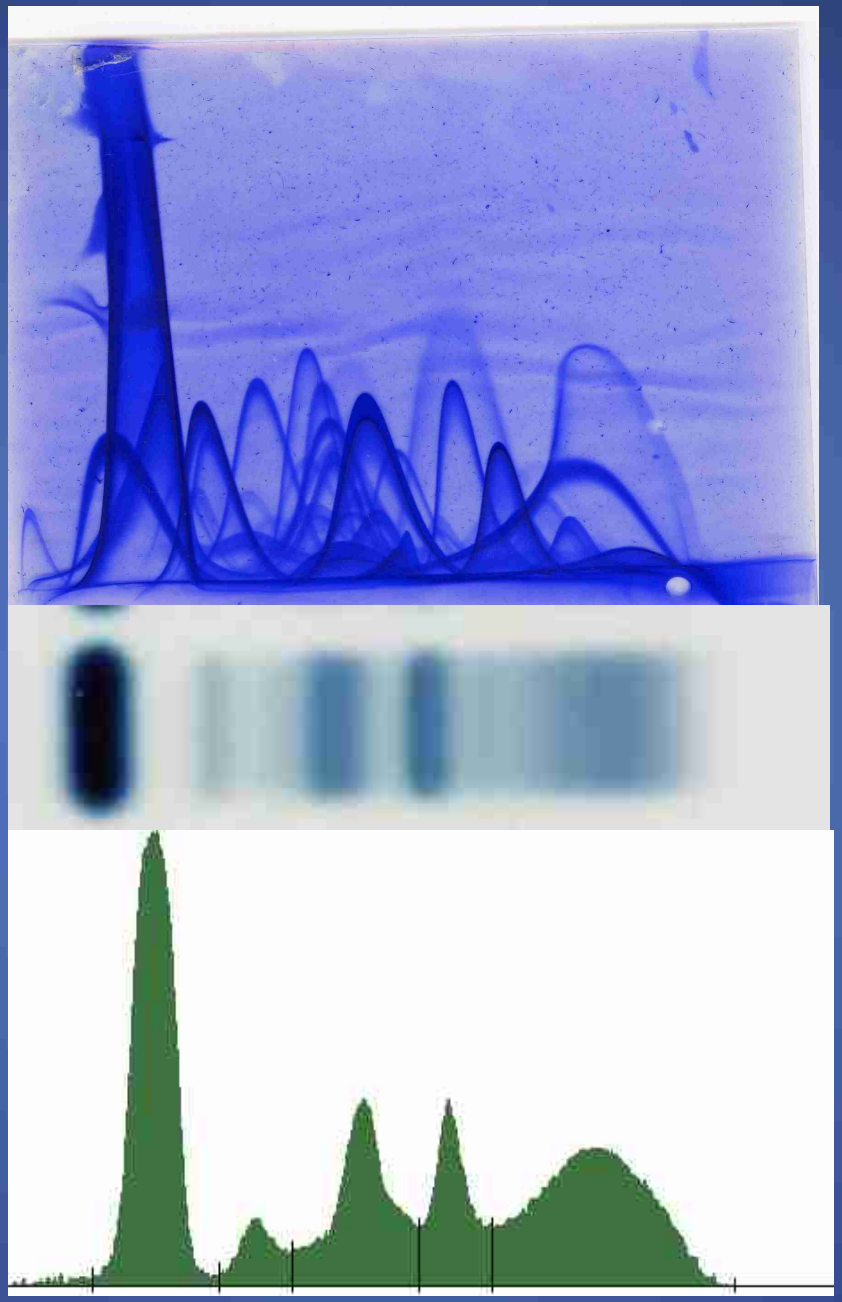
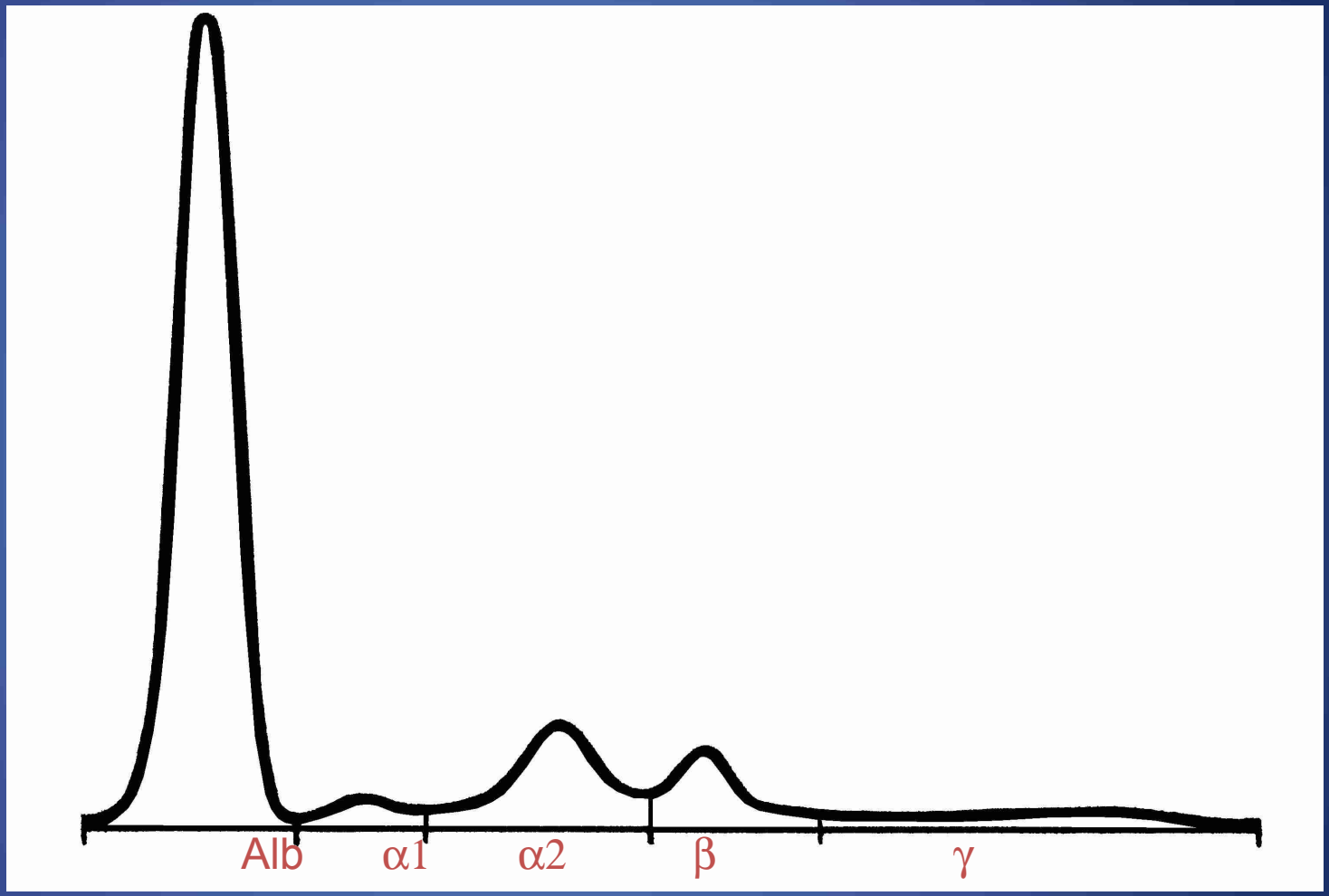


Frakcja	Ref %.
alb	60-71
α1	0,9-2,1
α2	5,0-7,9
β	5,7-7,9
γ	6,5-11,5
a/g	1,1-2,5



HIPOGAMMAGLOBULINEMIA



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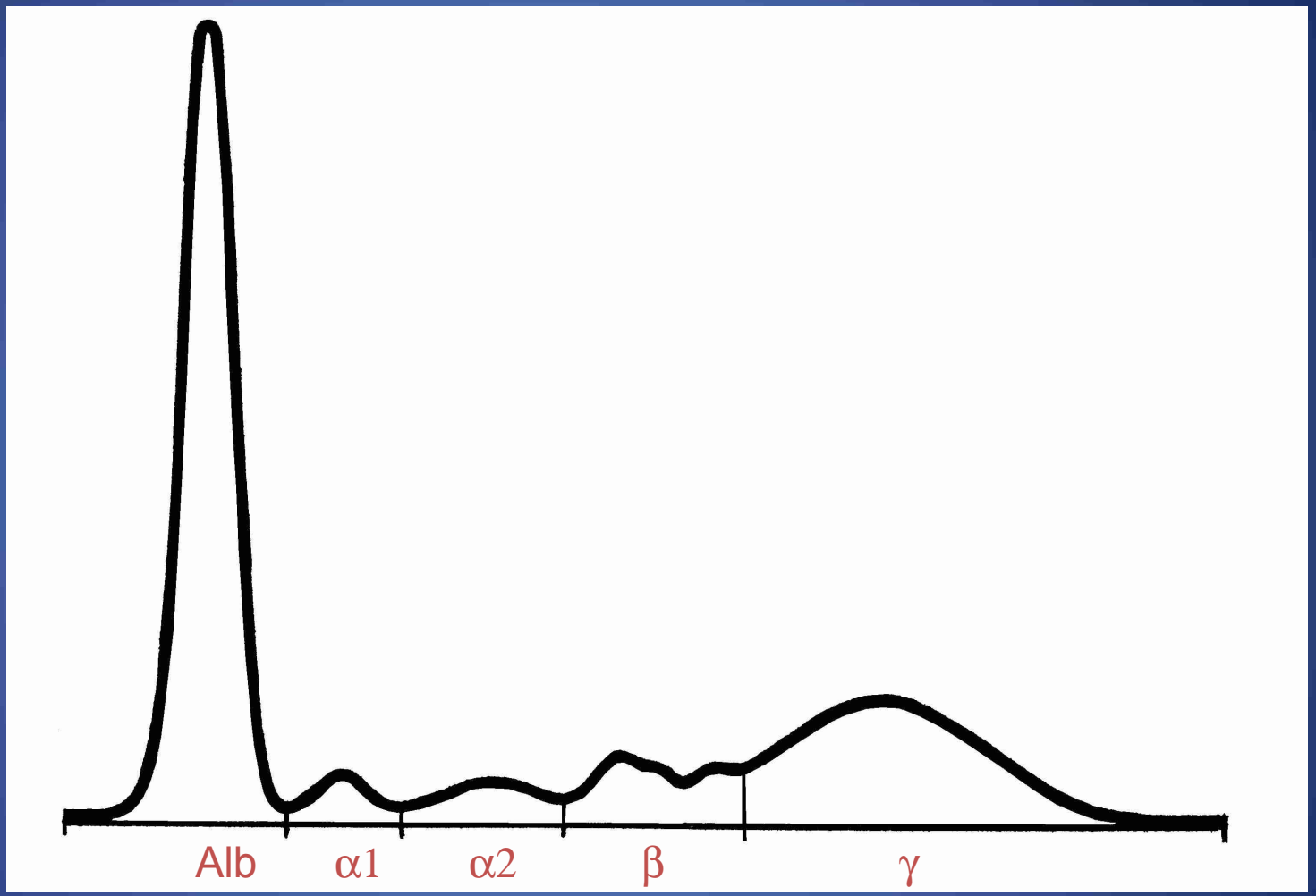

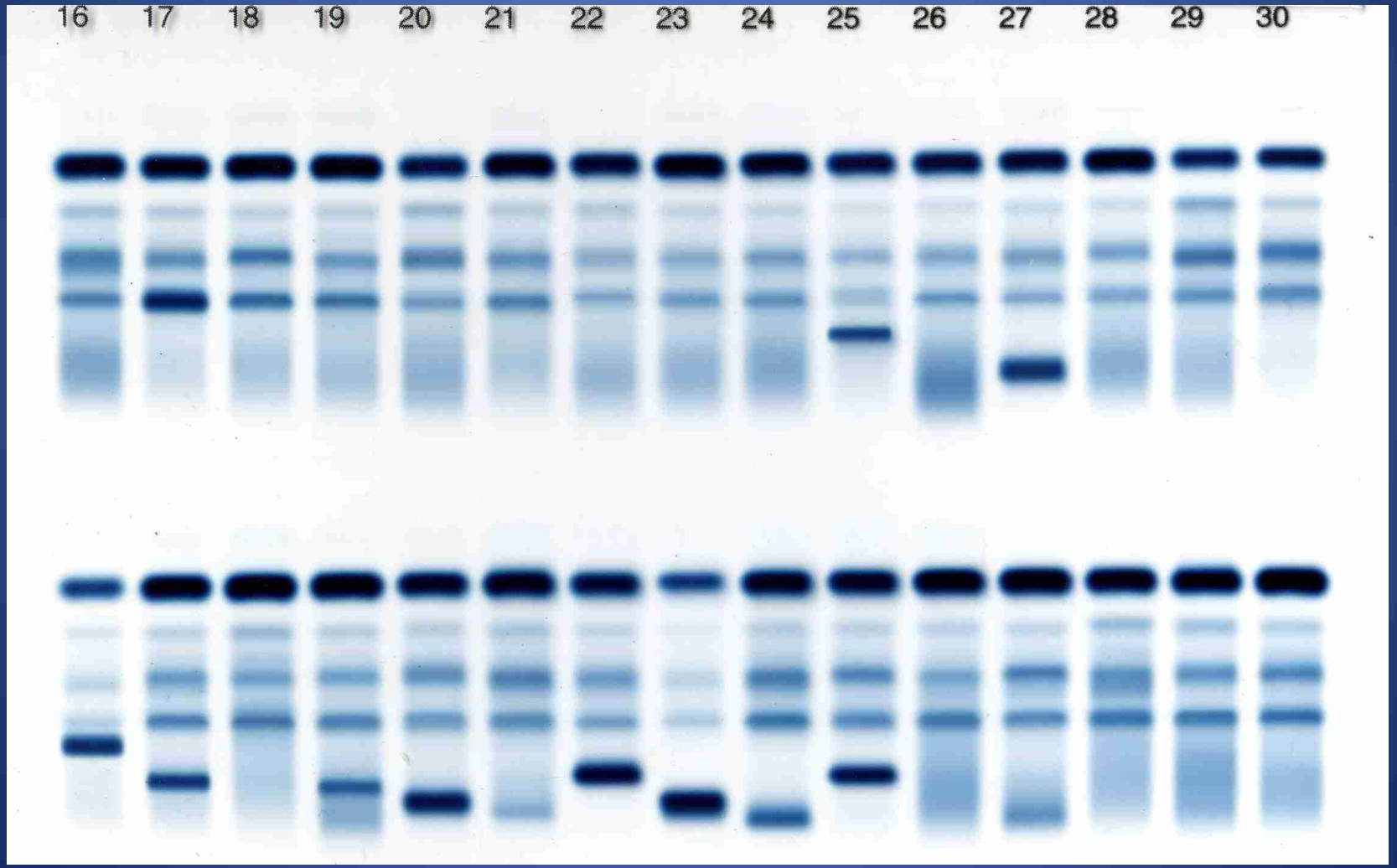
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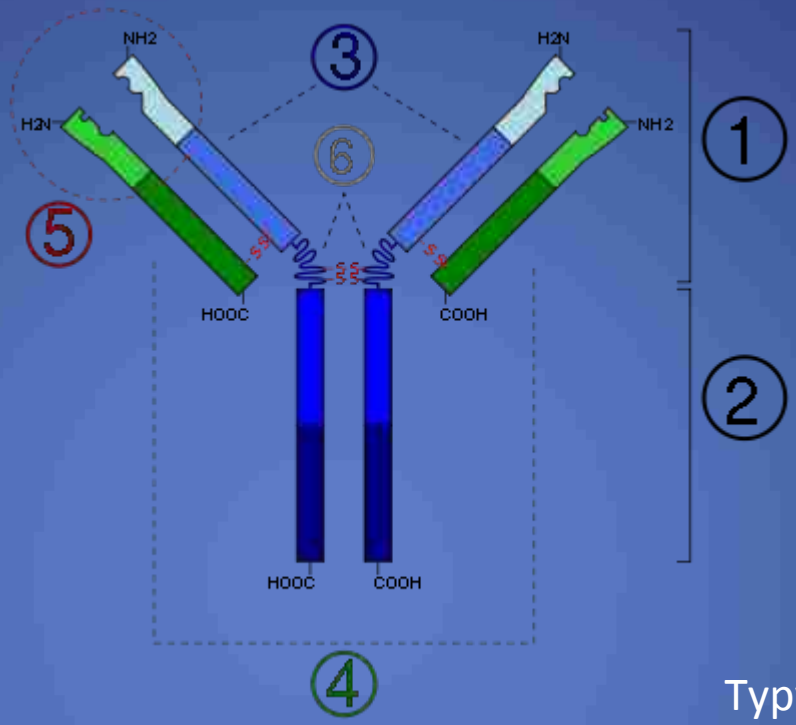
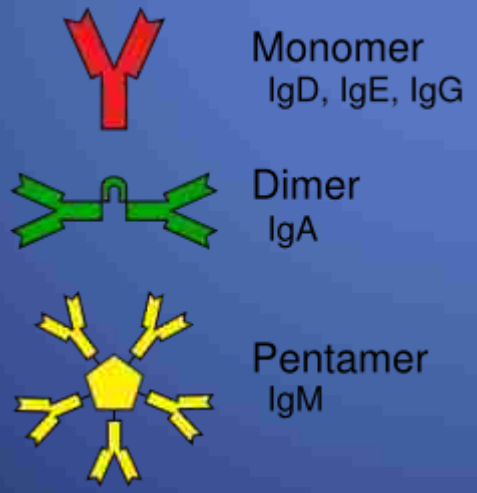
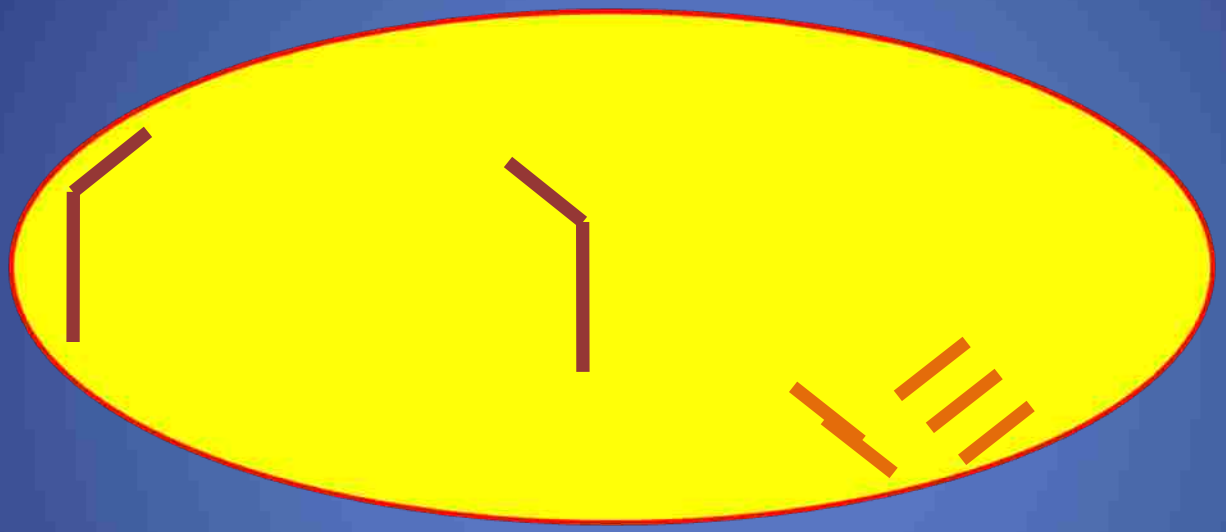
Klasa	Ref. (g/l)
IgG	9-15
IgA	1,1-1,8
IgM	0,39-1,17
IgD	0,0003-0,003
IgE	0,0005



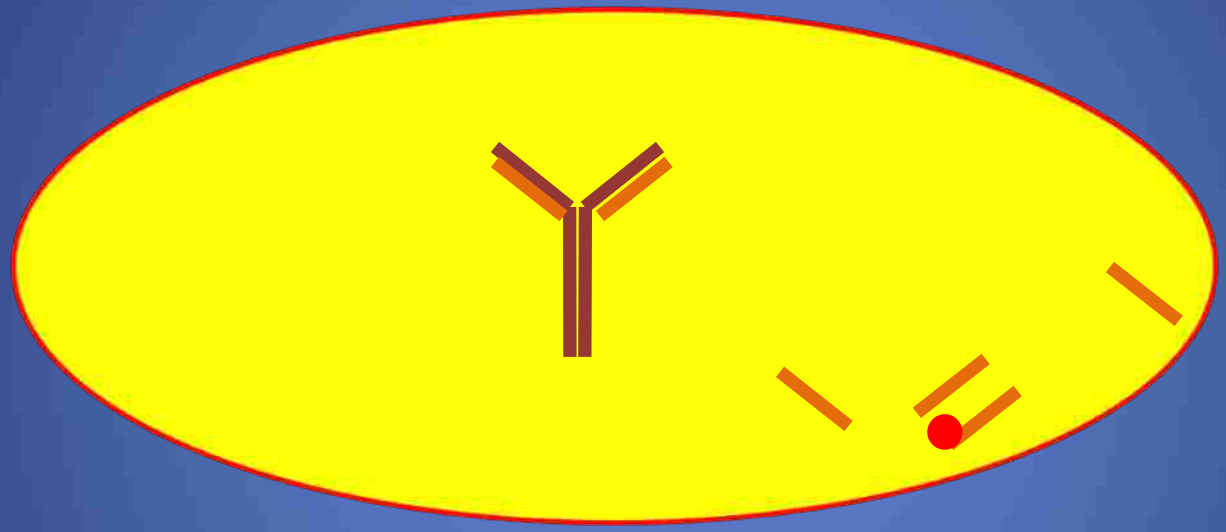


Typy immunoglobulin
kappa
lambda

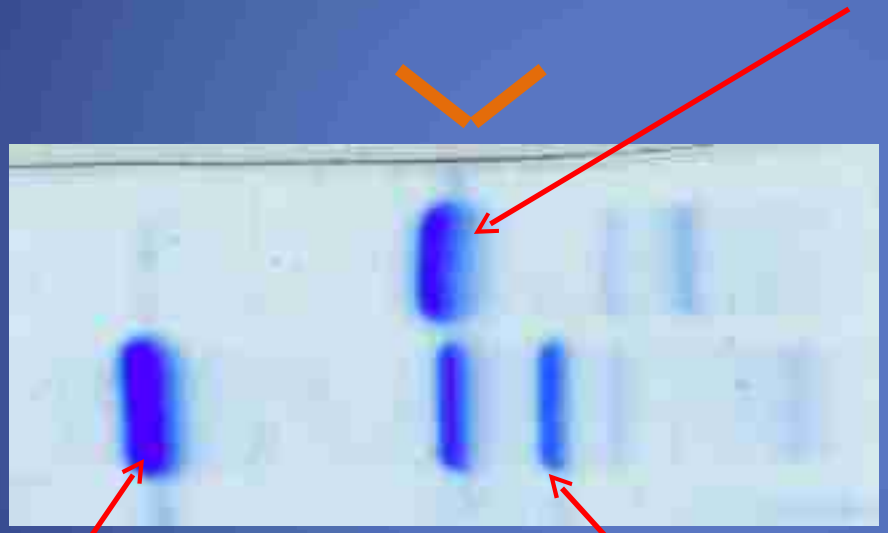
Synteza cząsteczek immunoglobulin



Wydzielanie cząsteczek immunoglobulin



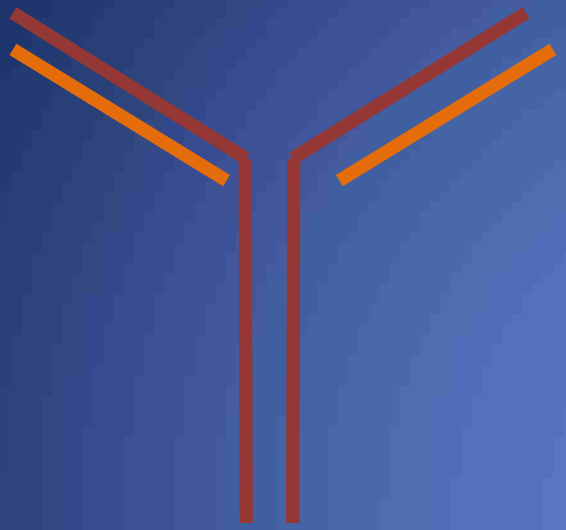
Postać niewydzielająca
Postać niewytwarzająca



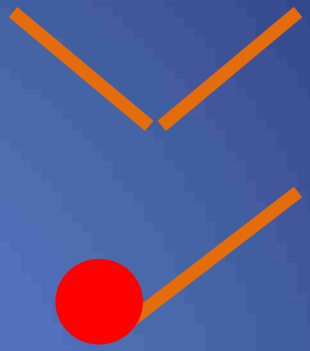
lmbda/kappa dimer

Kappa – tiolowany monomer

Albumina



Kappa lambda



Wolne kappa, wolne lambda

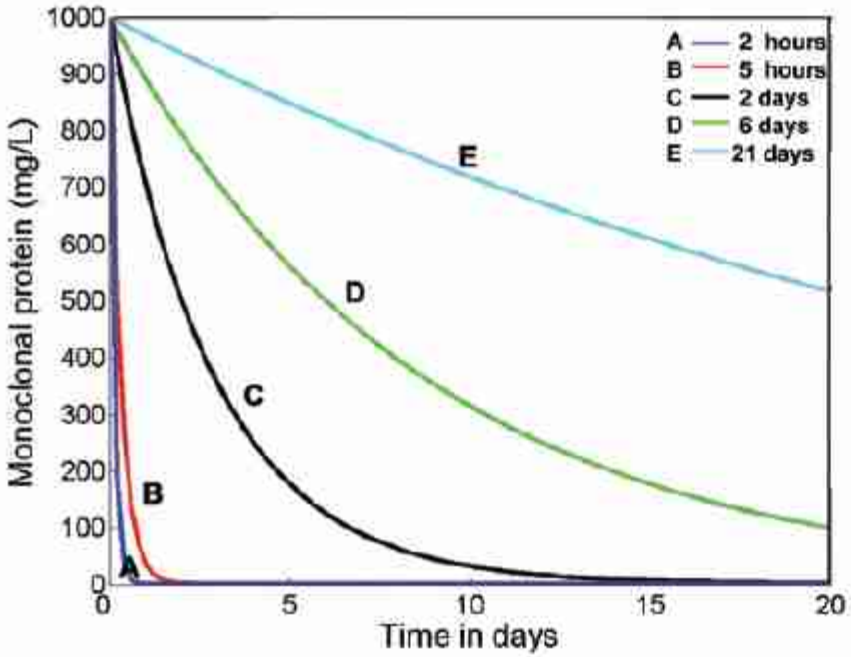


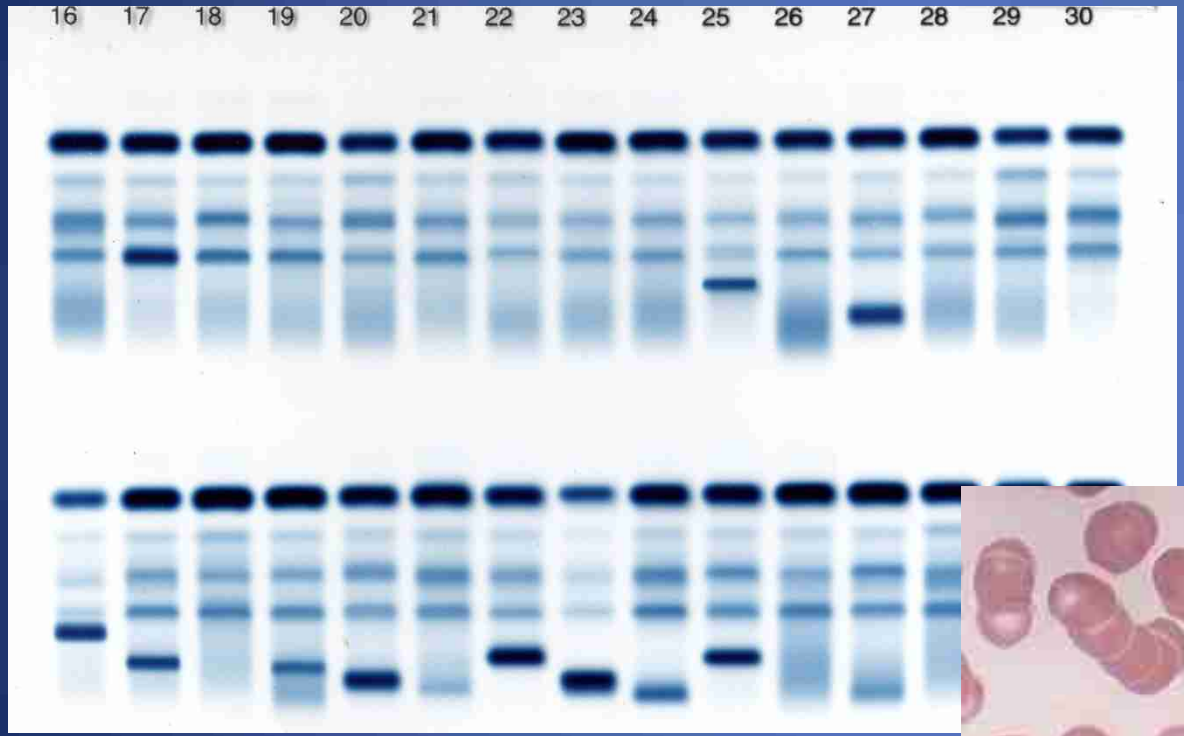
Figure 10.1
Calculated serum half-life curves for different immunoglobulin molecules.
(A) monomeric κ; (B) dimeric λ; (C) monomeric κ with renal failure; (D) IgA and (E) IgG. (Courtesy of N Evans and M Chappell).

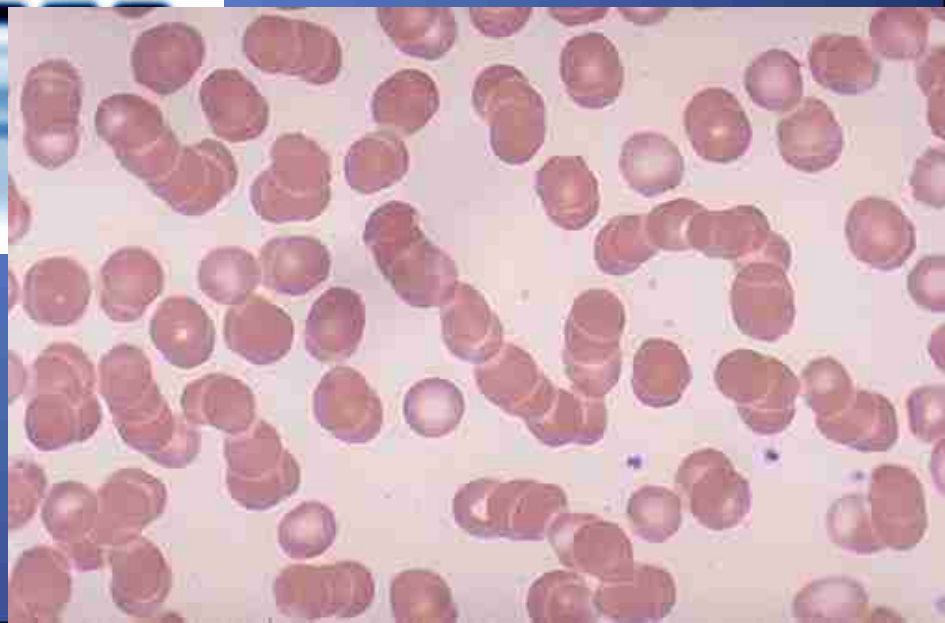
A.R. Bradwell: Serum Free Light Chain Analysis

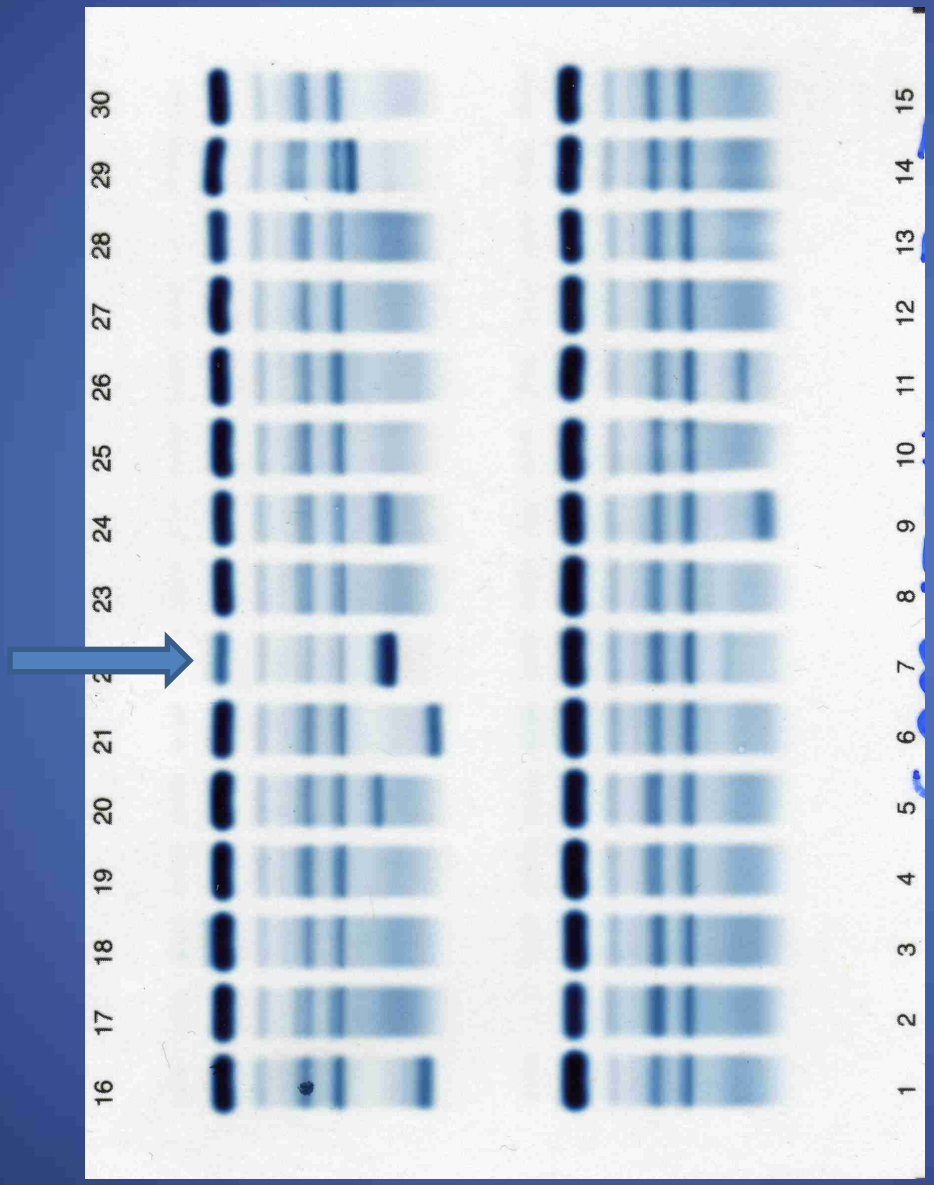
Pomiar białka całkowitego

Zakres referencyjny 60-85 g/l

Gammapatia monoklonalna często więcej niż 100 g/l

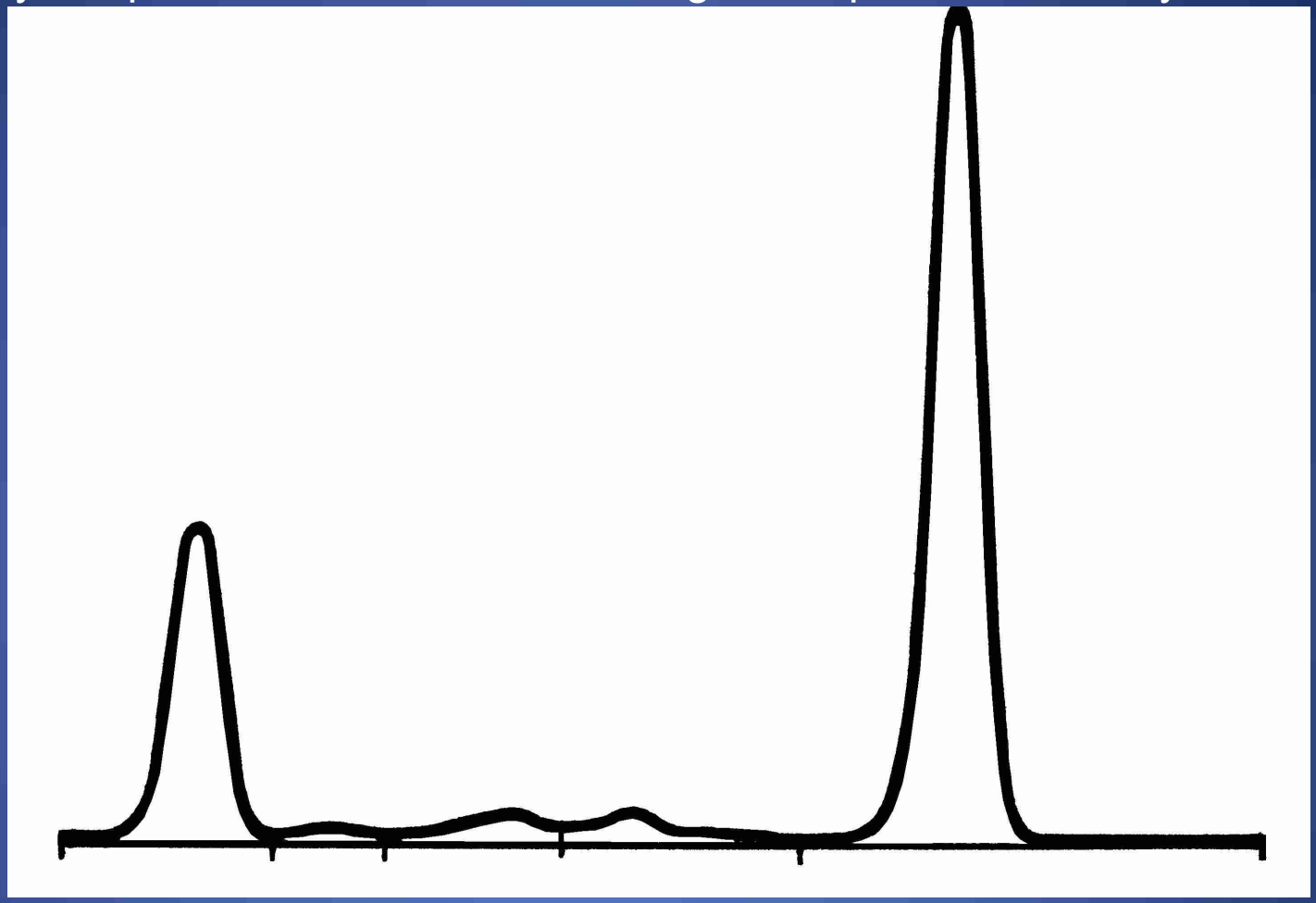






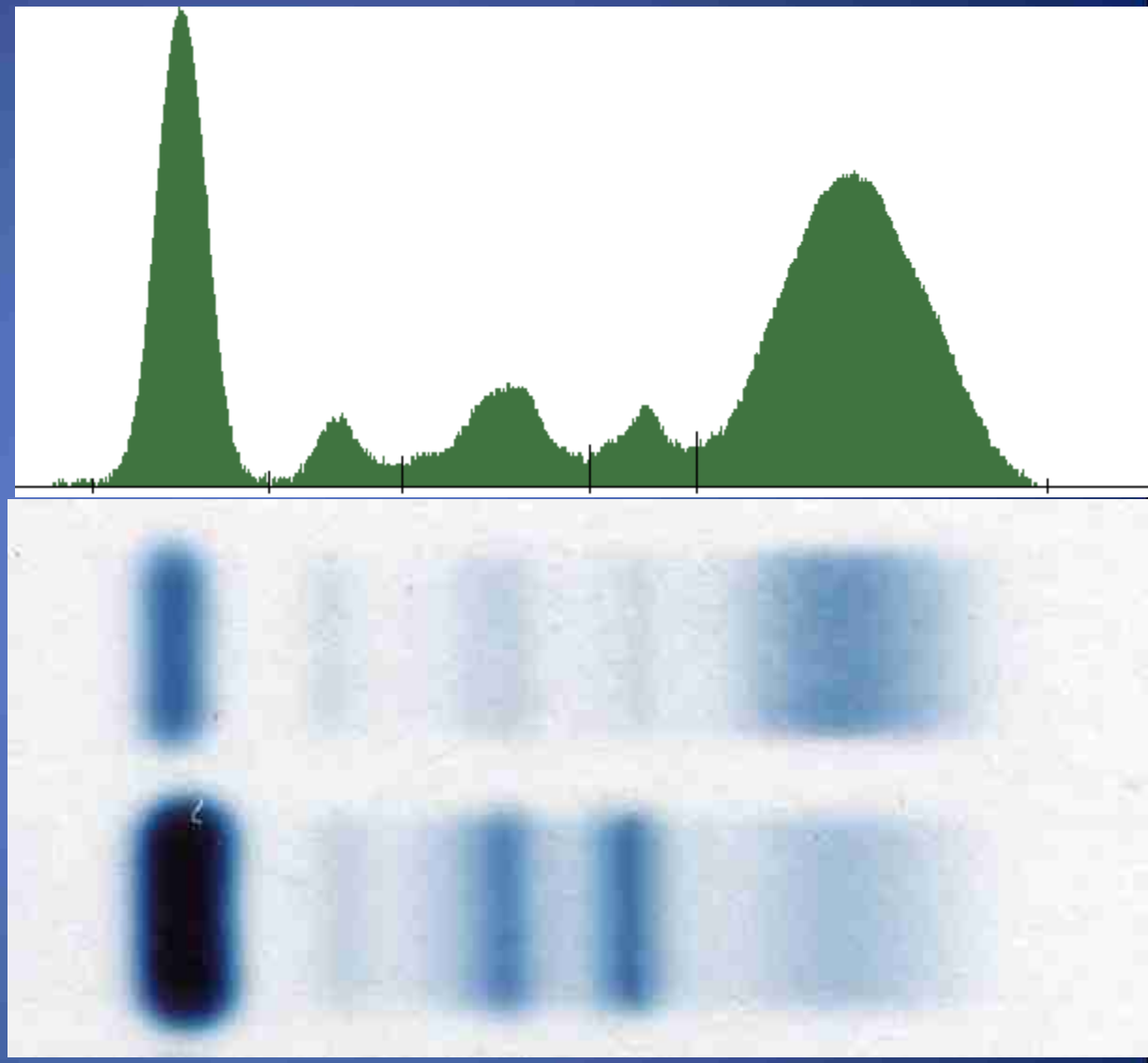
Stosunek albuminy do glob
Zakres referencyjny: 1.1-2.5

Wysoki poziom białka monoklonalnego niski poziom albuminy



TP 109 g/l

Frakcja	%	Ref.
alb	31,2	60-71
α1	3,4	0,9-2,1
α2	8,2	5,0-7,9
β	4,6	5,7-7,9
γ	52,6	6,5-11,5
a/g	0,45	1,1-2,5



Ilościowy pomiar białka monoklonalnego (na podstawie elektroforezy)

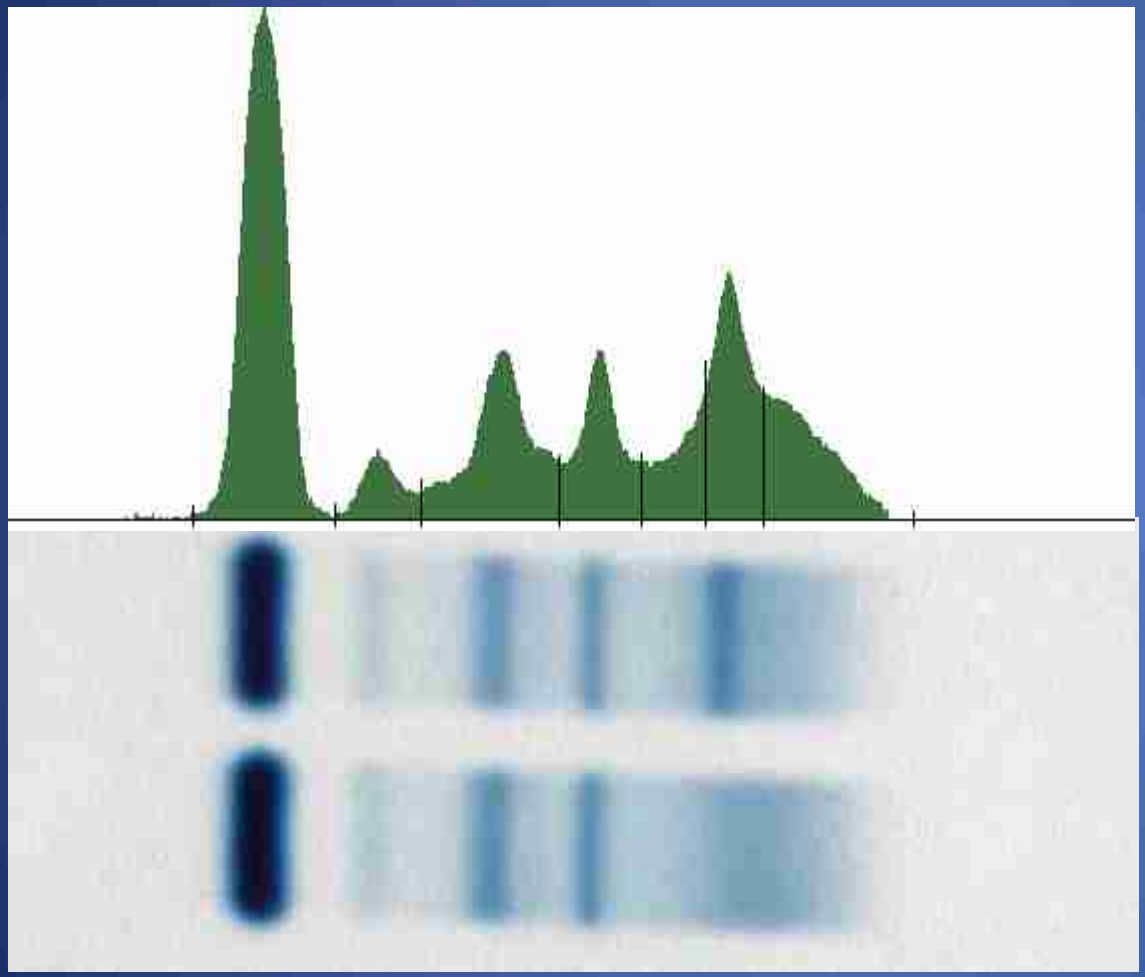


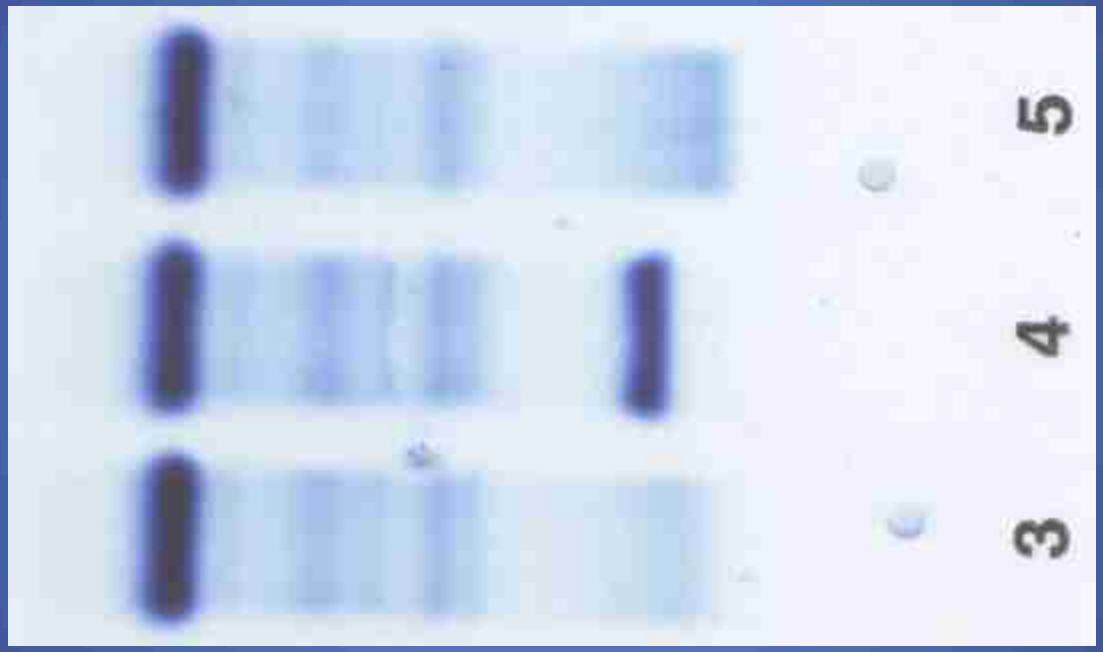
TABLE 44.14. CLINICAL STAGING OF PLASMA CELL MYELOMA (119)

Stage I	All of the following:
	Hg > 10 gm/dL
	Calcium ≤ 12 mg/dL
	Normal bone on radiograph or solitary lesion only
	Monoclonal protein
	IgG < 5 gm/dL
	IgA < 3 gm/dL
	Urine light chains < 4 g/24 h
Stage II	Fitting neither stage I nor III
Stage III	One or more of the following:
	Hg < 8.5 gm/dL
	Calcium > 12 mg/dL
	Advanced lytic bone lesions
	Monoclonal protein
	IgG > 7 gm/dL
	IgA > 5 gm/dL
	Urine light chains > 12 g/24 h
Renal Status A = creatinine < 2 mg/dL	
Renal Status B = creatinine > 2 mg/dL	

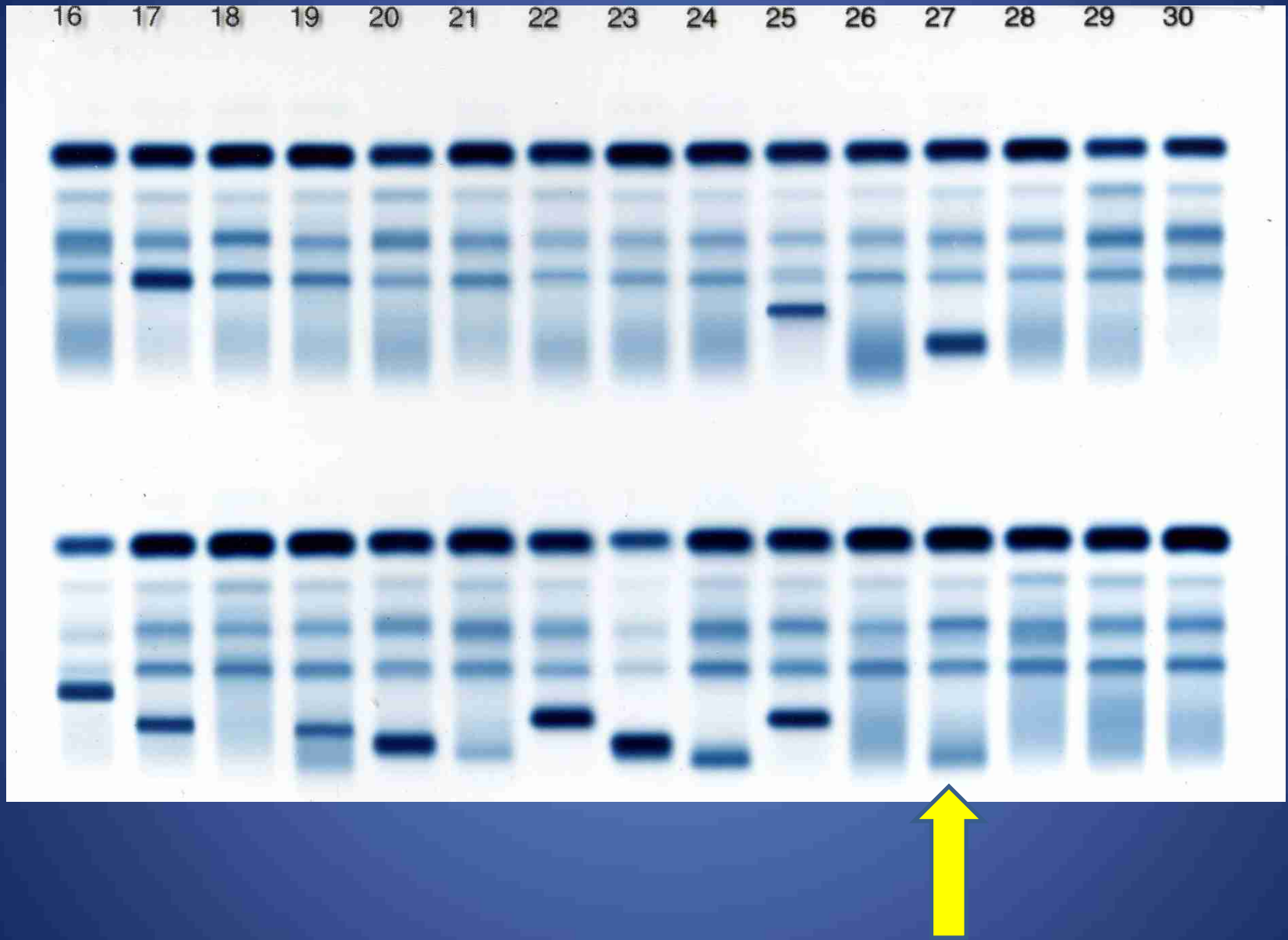
(From Dick FR. Chronic lymphocytic leukemia, prolymphocytic leukemia and leukemic non-Hodgkin's lymphoma. In: Koepke JA, ed. *Laboratory Hematology*. New York: Churchill Livingstone 1984;1:445–481.)

Łańcuchy lekkie wolne i całkowite g/l

	Wolne	Całkowite
kappa	0,0073	2,500
lambda	0,0127	1,430
Kappa/lambda	0,6	1,78



k/l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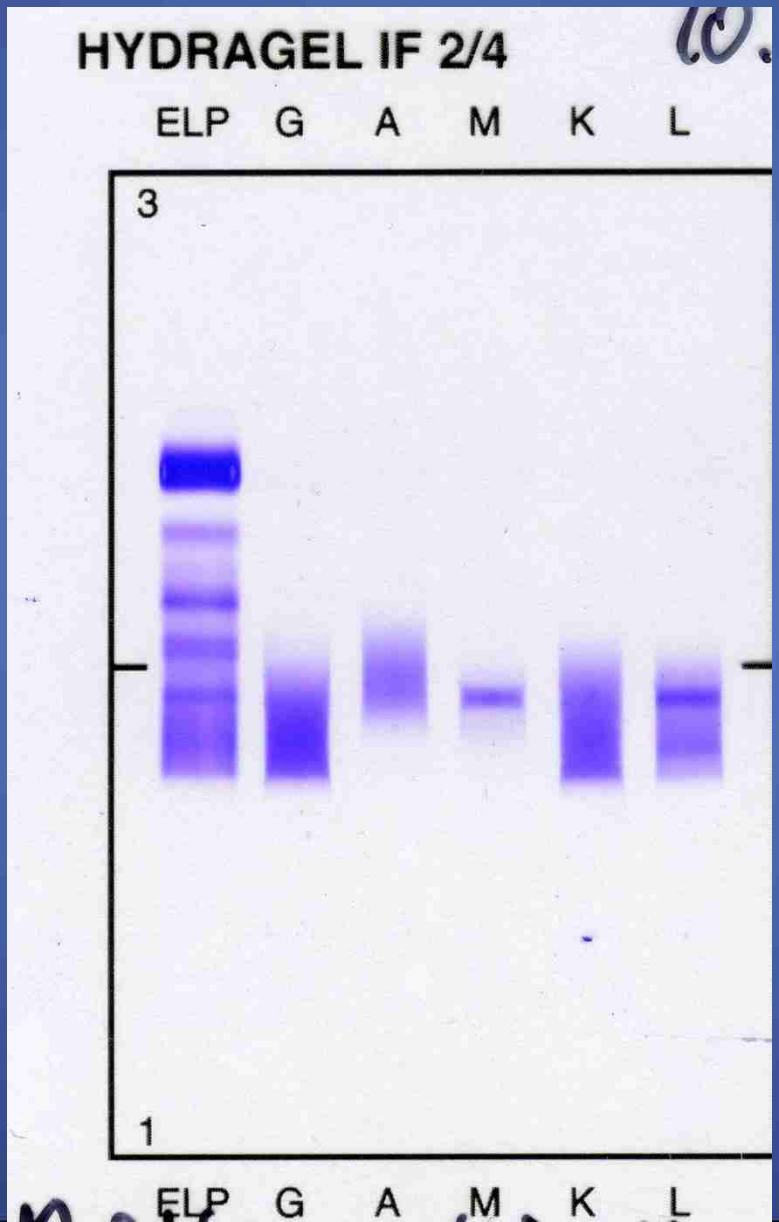
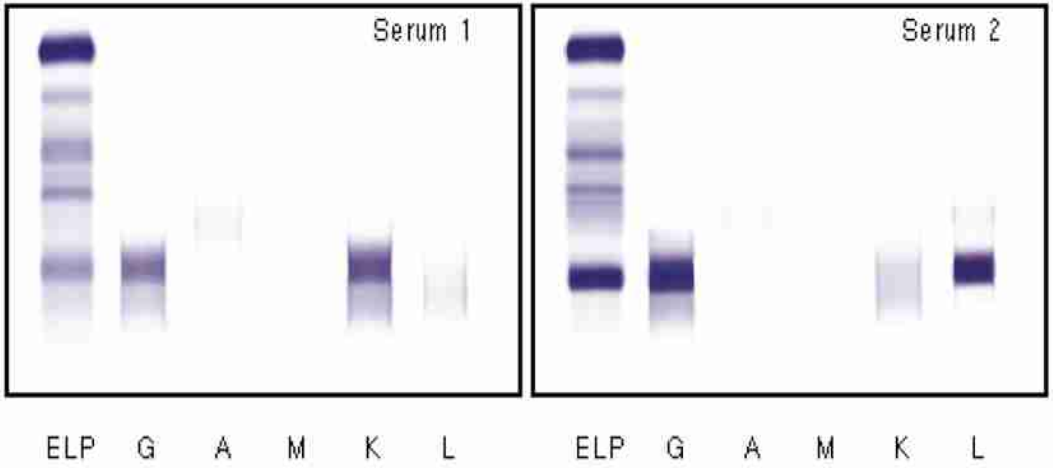
Kappa 5,26 g/l
Lambda 2,6 g/l
k/l 2,03


HYDRAGEL IF 2/4
ELP G A M K L
3
1
ELP G A M K L

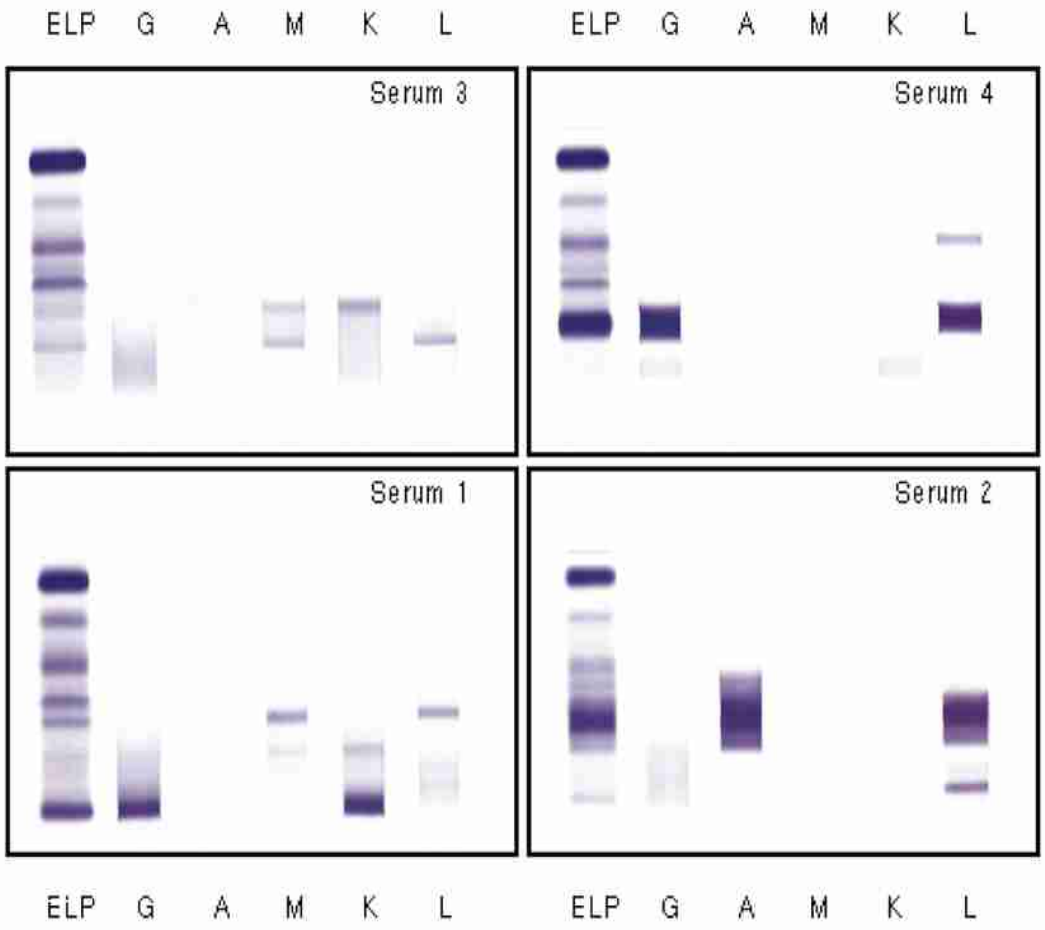
HYDRAGEL 2 IF

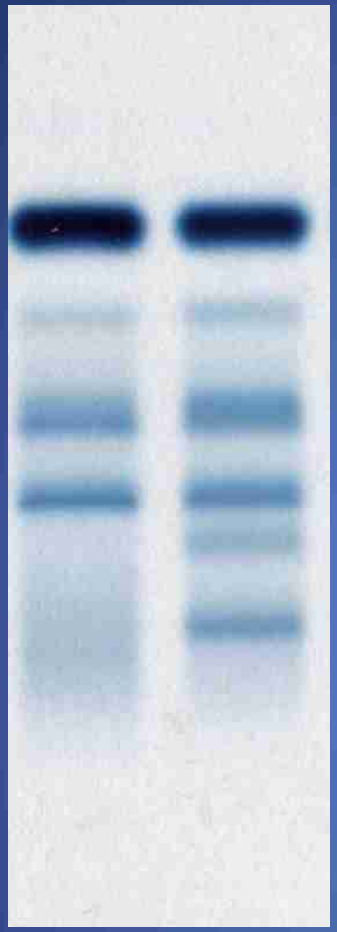
sebia

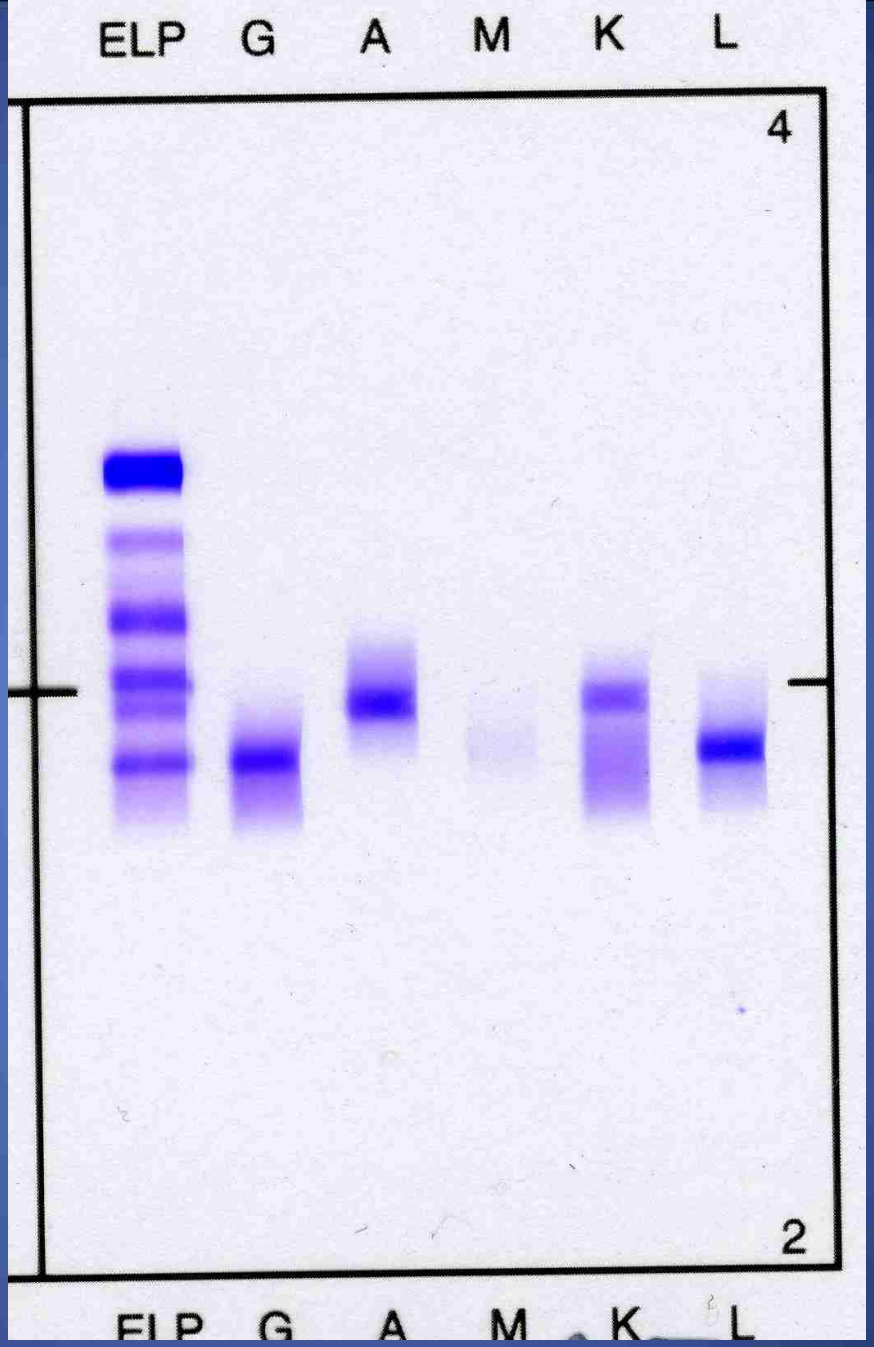


HYDRAGEL 4 IF

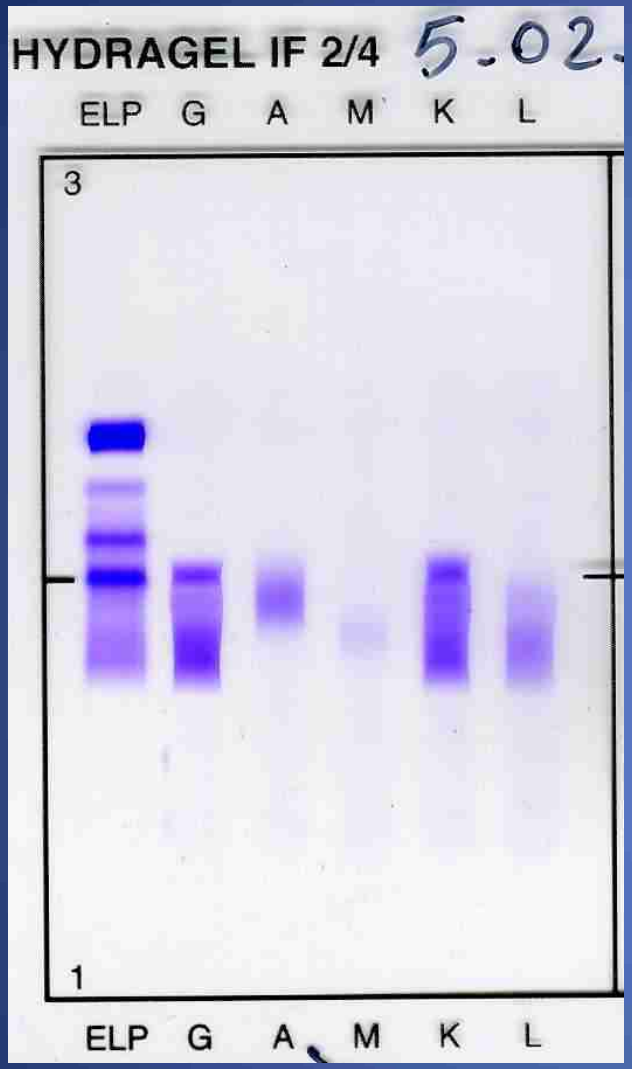
sebia

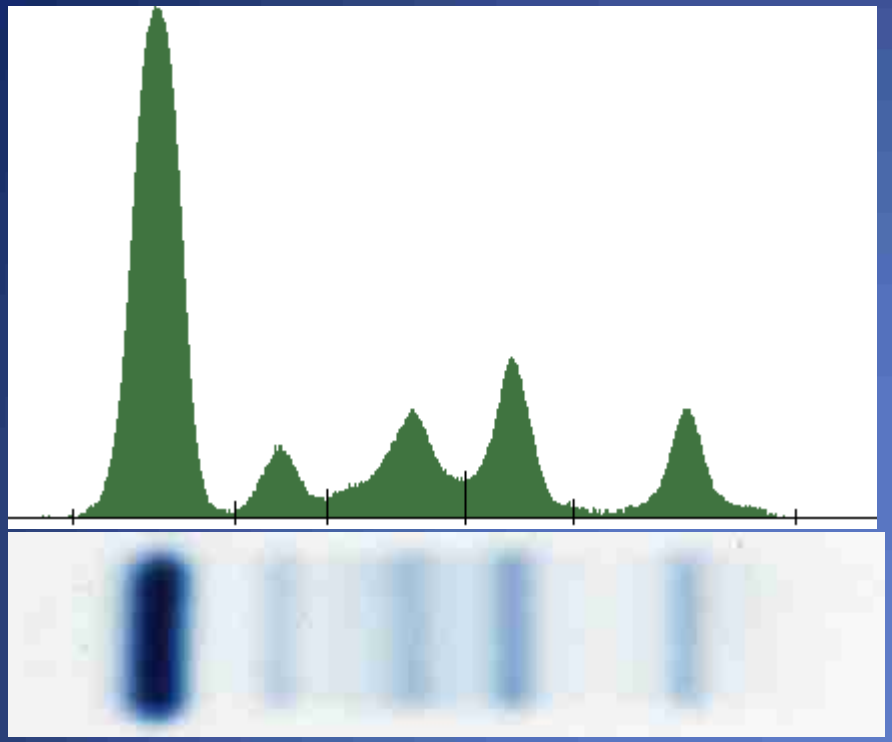


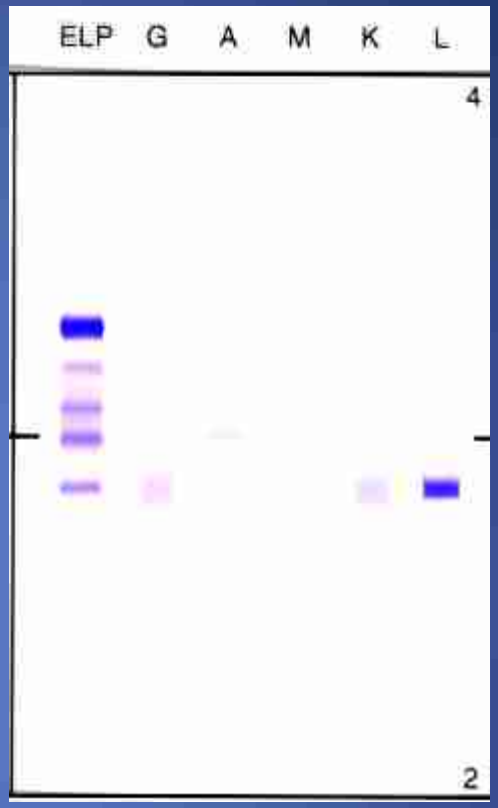




K 3,75 g/l ↑
L 2,64 g/l ↑
K/L 1,42 n
FK 44,7 mg/l ↑
FL 48,6 mg/l ↑
FK/FL 0,92 n


HYDRAGEL IF 2/4 5.02.
ELP G A M K L
3
1
ELP G A M K L

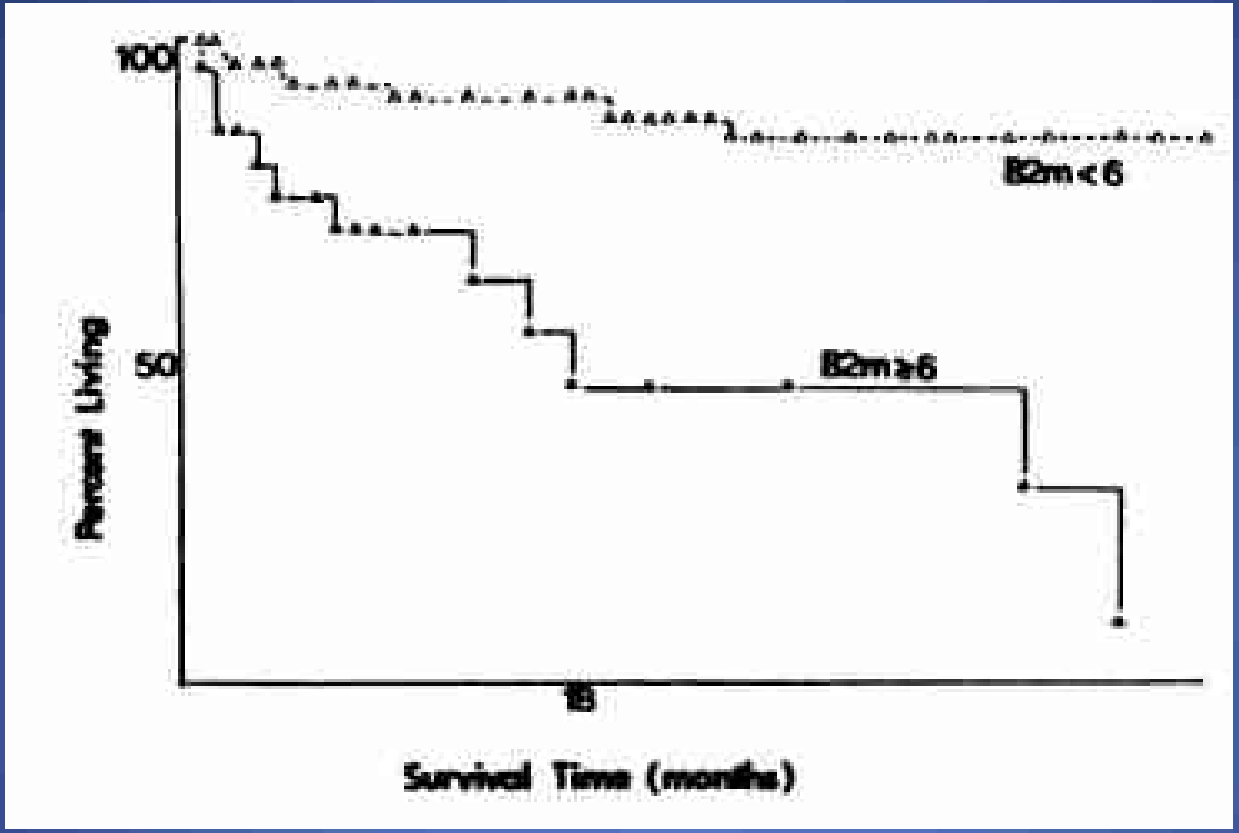



ELP
G
A
M
K
L
4
2

$\beta_2$mikroglobulina

Stopień	β-2-mikrolob.	albumina
I	<35mg/l	> 35 g/l
II	35-55 mg/l	<35g/l
II	>55 mg/l	<35g/l

International Staging System for Multiple Myeloma
Journal of Clinical Oncology, Vol 23, No 15 (May 20), 2005: pp. 3412-3420



R Bataille, J Grenier and J Sany **treatment--a prospective study of 160 patients**

Beta-2-microglobulin in myeloma: optimal use for staging, prognosis

Łańcuchy lekkie w moczu

"Dear Dr Bence Jones,
The tube contains urine of very high specific gravity. When boiled it becomes highly opaque. On the addition of nitric acid, it effervesces, assumes a reddish hue, and becomes quite clear; but as it cools assumes the consistence and appearance which you see. Heat reliquifies it. What is it?"

Historia

- Rok 1845 – dr William MacIntyre i dr Thomas Watson badając mocz pacjenta z podejrzeniem zespołu nefrotycznego odnotował przypadek nietypowego zachowania osadu białkowego – rozpuszczenia podczas ogrzewania i ponownej precypitacji po oziębieniu próbki.

- Mocz pacjenta został wysłany do dr Bence-Jonesa – patologa pracującego w londyńskim Szpitalu Św. Grzegorza w celu dokładniejszego zbadania tego zjawiska.

...

- **1850** MacIntyre po śmierci pacjenta publikuje swoje obserwacje
- **1848** r – dr Bece-Jones publikuje swoje obserwacje związane z występowaniem nietypowego białka
- Do r. 1909 opublikowano 40 przypadków wyst. Białka B-J
- 1922 Bayne-Jones i Wilson charakteryzują dwa typy białek B-J (typ I i typ II)
- 1956 Korngold i Lapiri - przeciwciała przeciwko różnym grupom białka B-J reagują z białkami szpiczakowymi.
- 1962 Edelman, Gally FLCs z cząsteczki immunoglobuliny to cząsteczki białka B-J

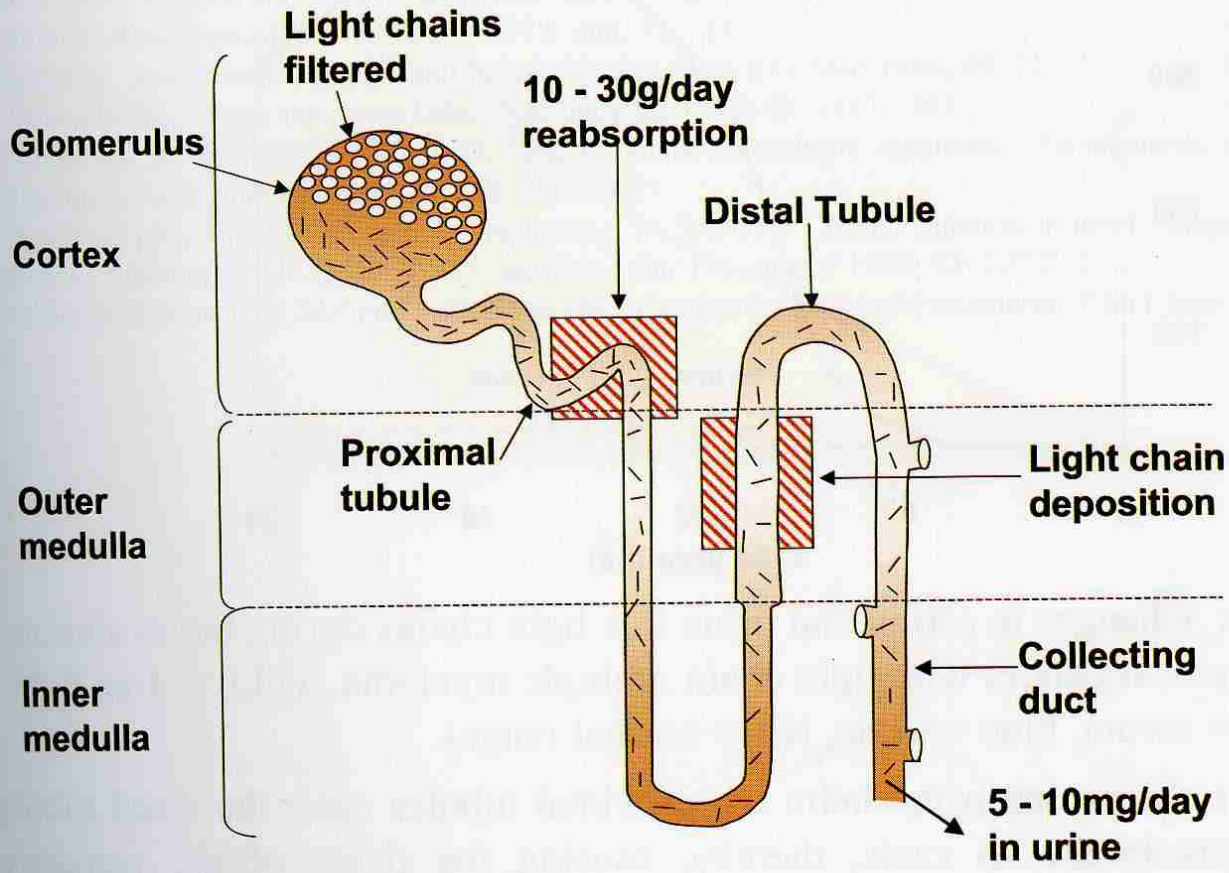


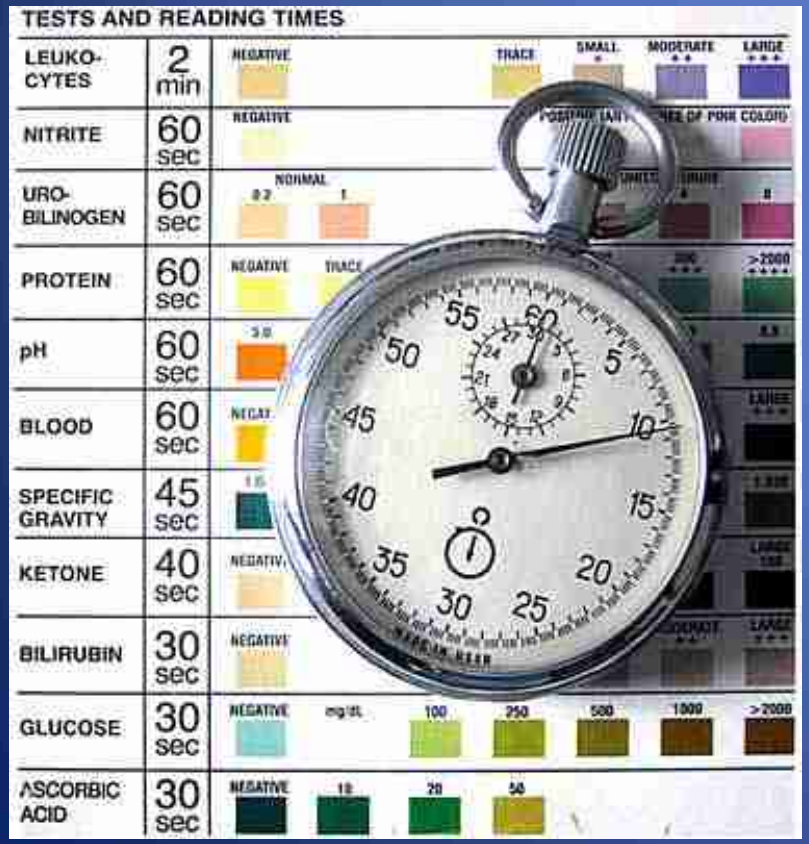
Figure 3.7. Nephron showing filtration, metabolism and excretion of free light chains.

A.R. Bradwell: Serum Free Light Chain Analysis

1

2

3

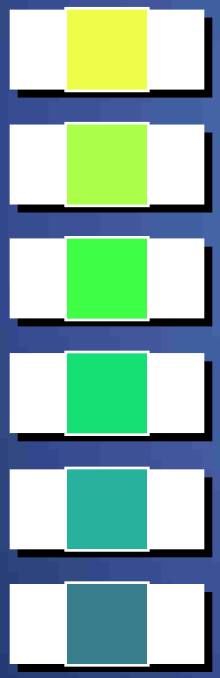

TESTS AND READING TIMES
LEUKO-CYTES
2 min
NITRITE
60 sec
URO-BILINOGEN
60 sec
PROTEIN
60 sec
pH
60 sec
BLOOD
60 sec
SPECIFIC GRAVITY
45 sec
KETONE
40 sec
BILIRUBIN
30 sec
GLUCOSE
30 sec
ASCORBIC ACID
30 sec
NEGATIVE
TRACE
SMALL
MODERATE
LARGE
NORMAL
mg/dL
100
250
500
1000
>2000

4

5

Testy paskowe :

Białko



Negatywny

Ślad

\+ (50 mg/dL)

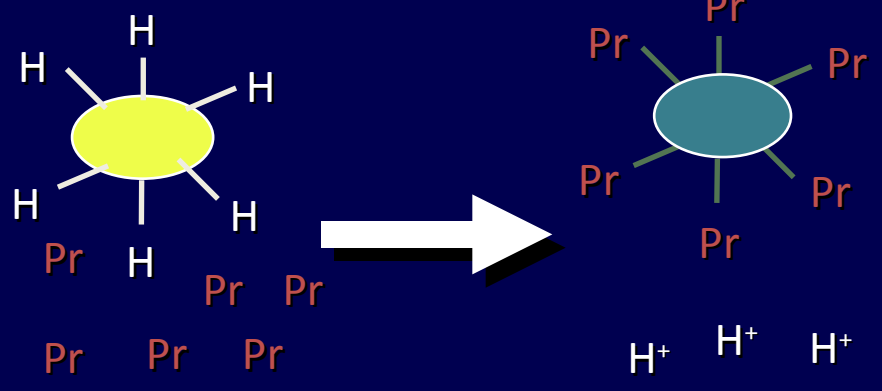
++ (100 mg/dL)

+++ (300 mg/dL)

++++ (1000 mg/dL)

Zasada testu

"Błąd białkowy wskaźnika pH"



Błękit tetrabromofenolowy
(buforowany do pH 3.0)

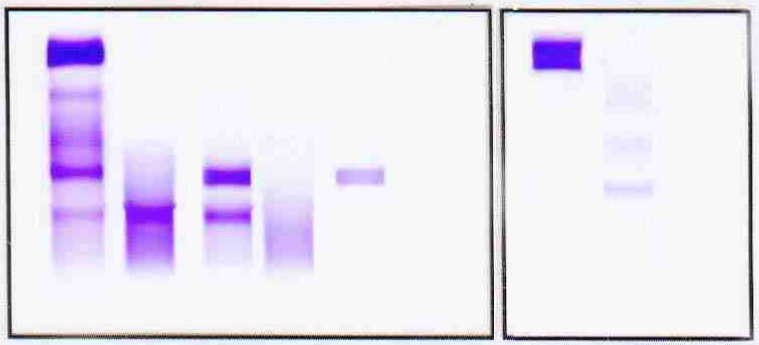
Odczyt w ciągu 60 sekund
Prawidłowo: Negatywny

HYDRAGEL SERUM / URINE PROFIL(E)

sebia

BENCE JONES

TYPAGE

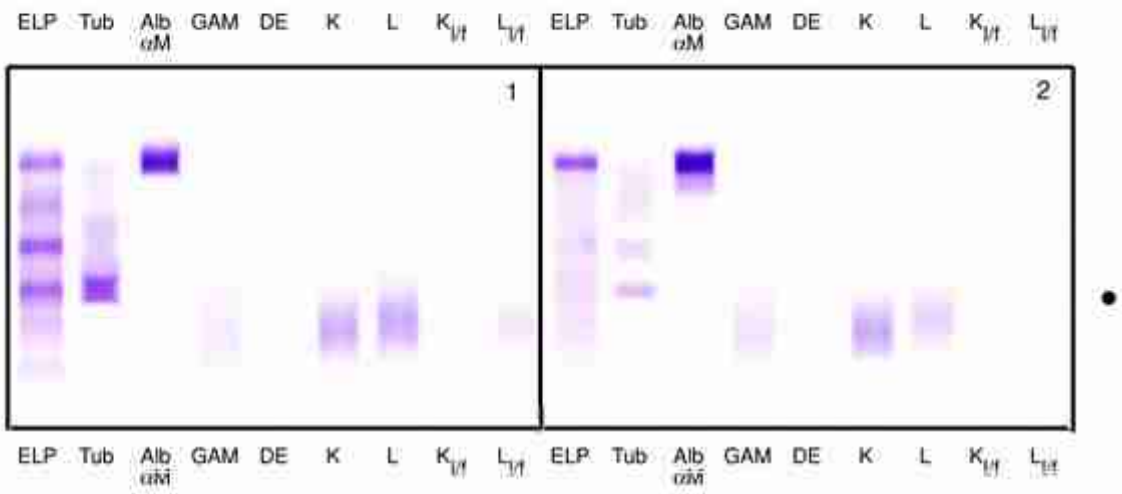


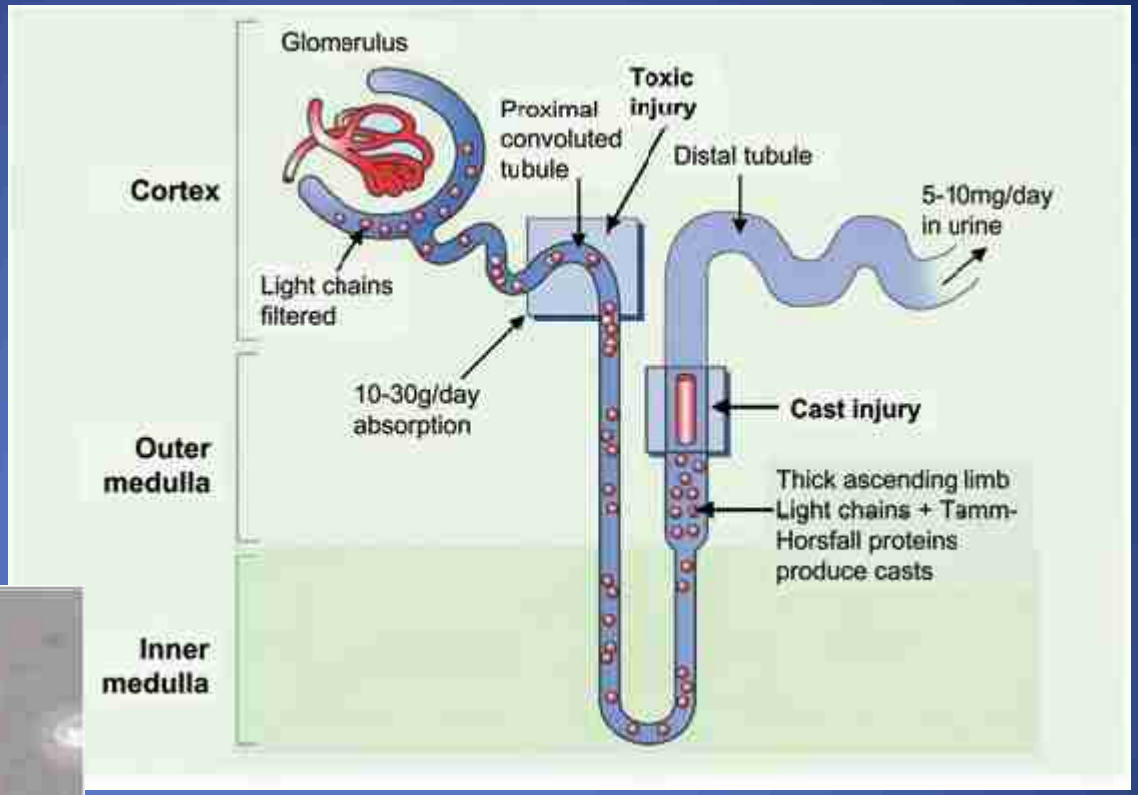
Patient:

. . . . . .

HYDRAGEL 2 URINE PROFIL(E)

sebia





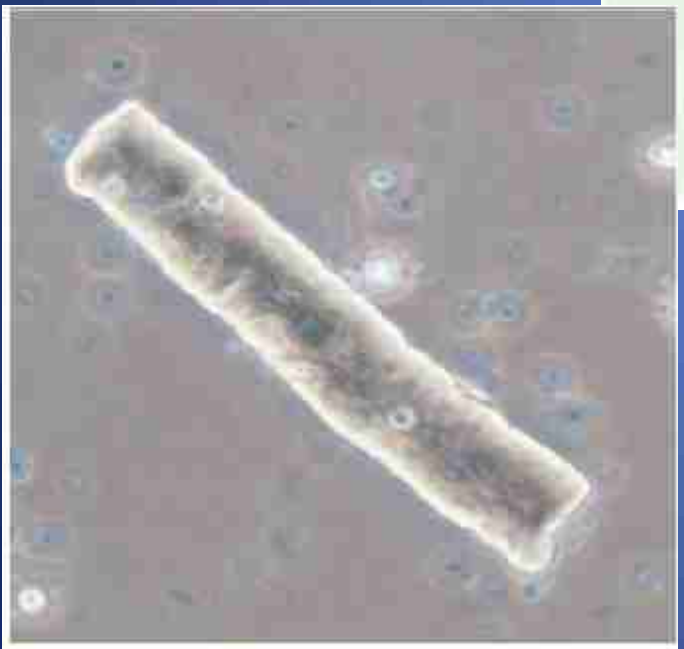


Figure 3.8. Waxy cast from the urine of a patient with multiple myeloma.

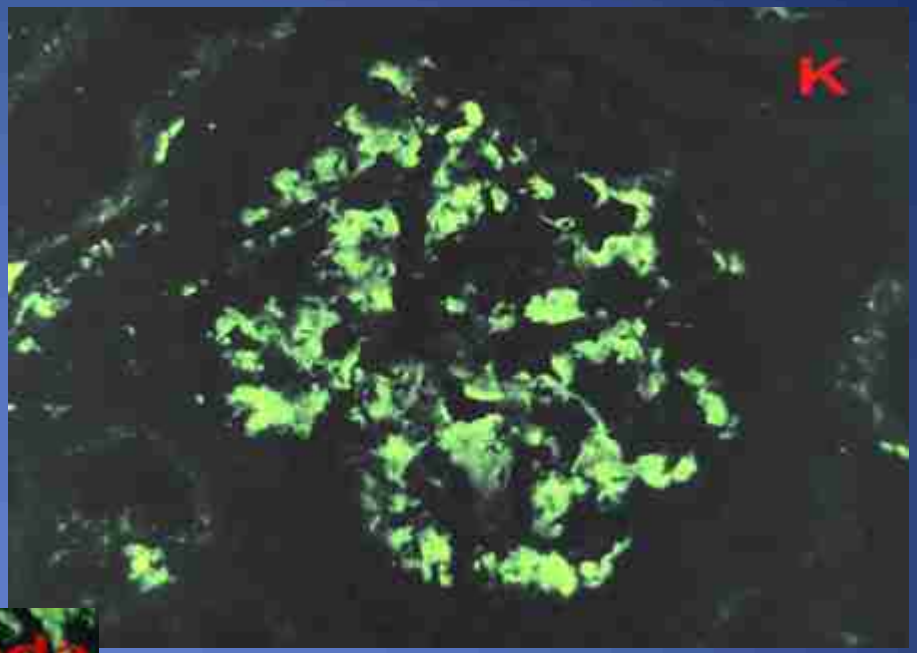
Nerka szpiczakowa

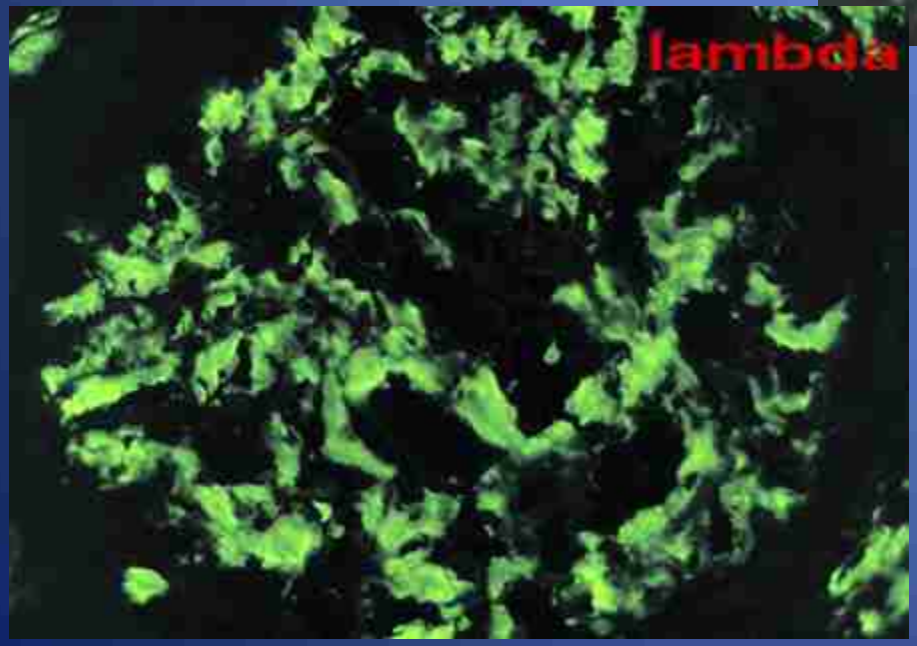
A.R. Bradwell: Serum Free Light Chain Analysis

LCDD

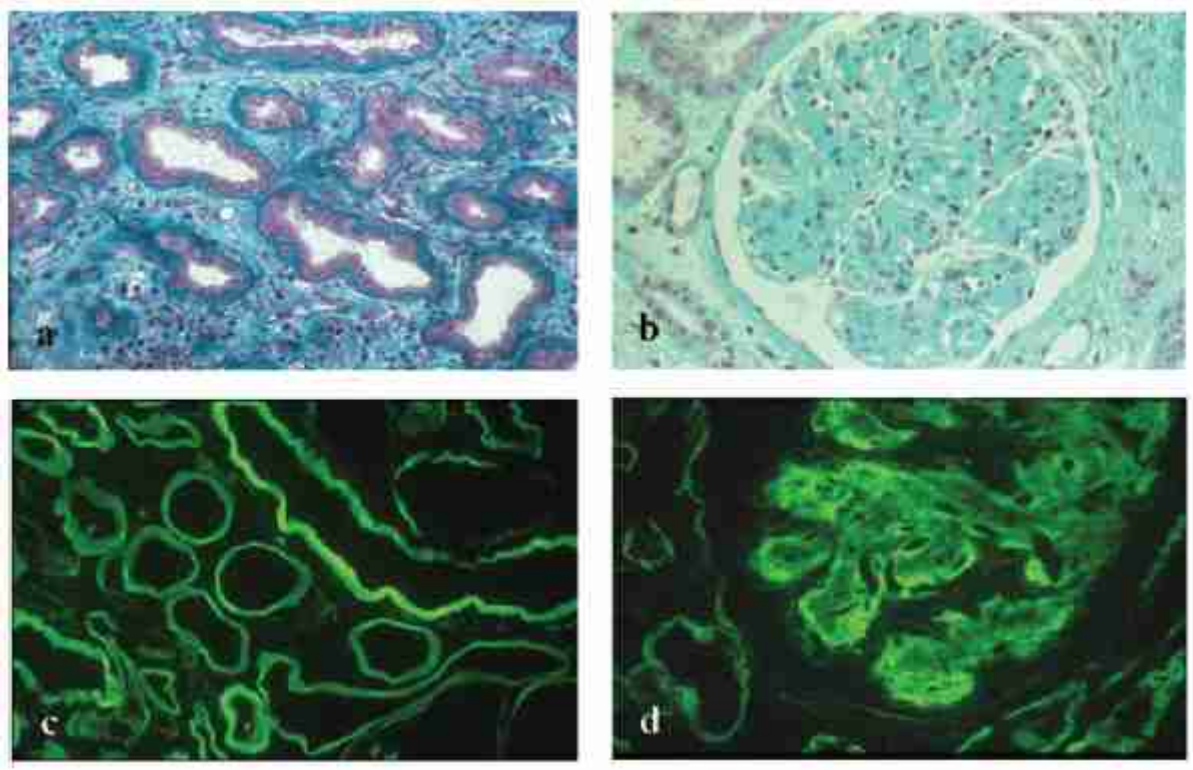
- LCDD Powstawanie monoklonalnych, amorficznych depozytów białkowych
- Zawsze w nerkach
- Częściej Kappa 85%
- 25% pacjentów nie wykazuje obecności białka monoklonalnego (elektroforeza, immunofiksacja, obserwowany jest wzrost ilości komórek plazmatycznych w szpiku)

LCDD





Light chain deposition disease
Dr Franco Ferrario

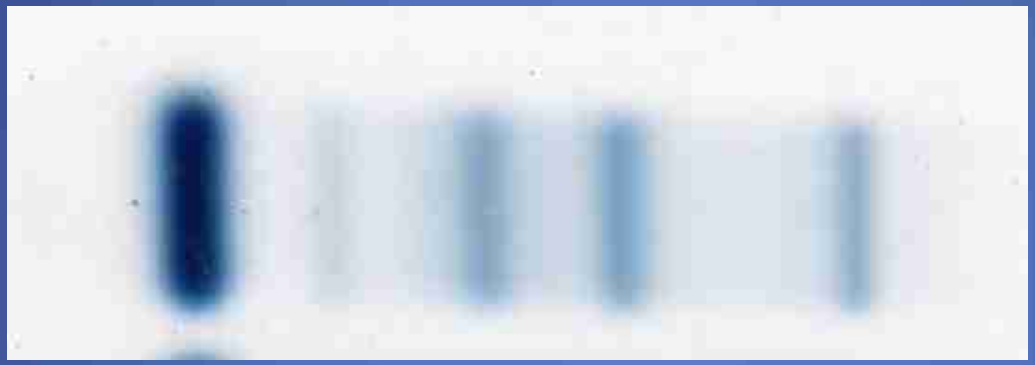


Immunoglobulin Light (Heavy)-Chain Deposition Disease: From Molecular Medicine to Pathophysiology-Driven Therapy

Pierre Ronco,* Emmanuelle Plaisier,* Béatrice Mougenot,* and Pierre Aucouturier†

Clin J Am Soc Nephrol 1: 1342–1350, 2006.

Krioglobuliny



Po ogrzaniu

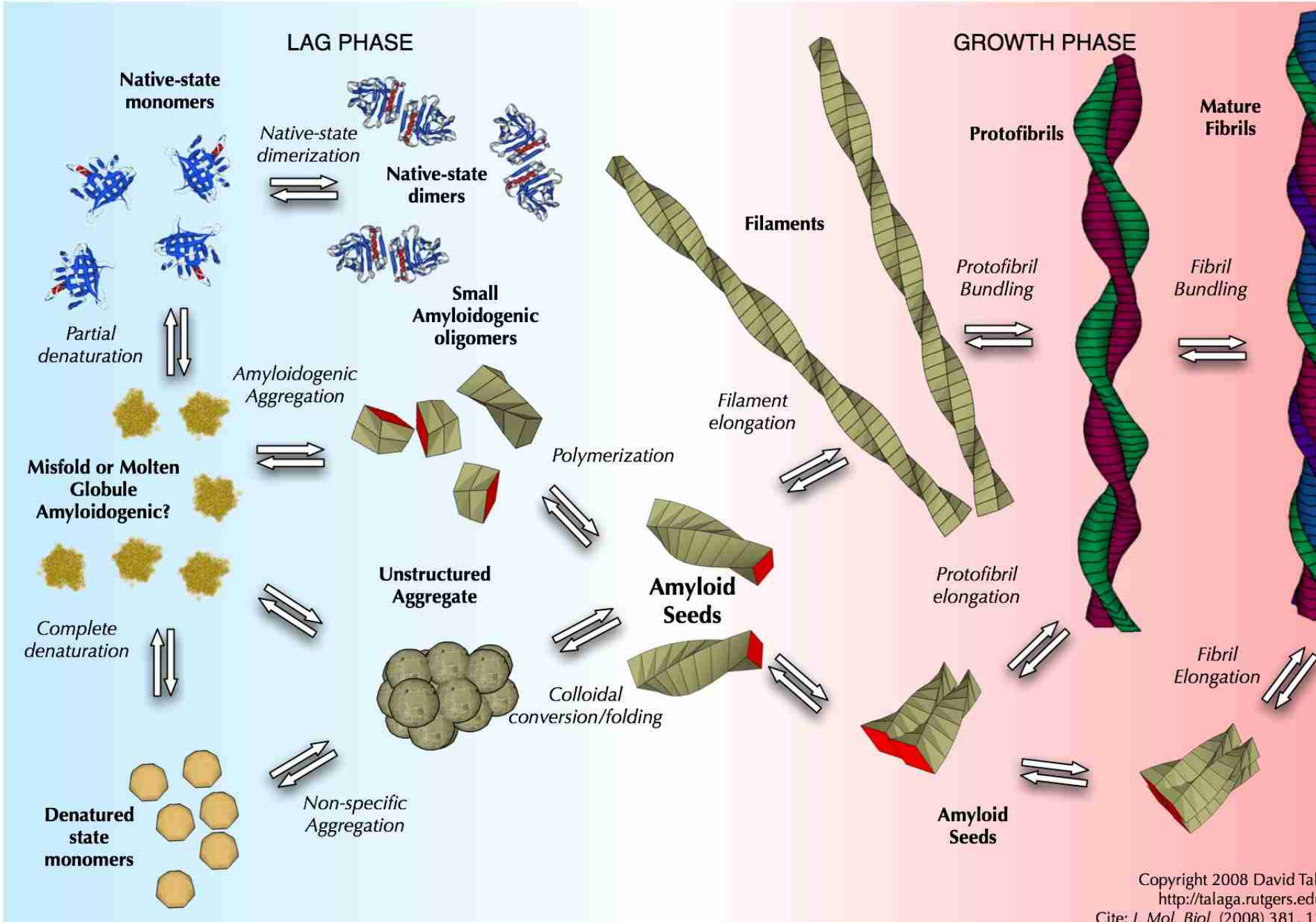
Amyloidoza

Heterogenna grupa chorób charakteryzujących się pozakomórkowym odkładaniem się pierwotnie rozpuszczalnych białek

Częstość występowania amyloidozy
8 zachorowań / milion / rok
92% pacjentów AL ma BJP

- Częstość występowania amyloidozy

  8 zachorowań / milion / rok


LAG PHASE
GROWTH PHASE
Native-state monomers
Native-state dimerization
Native-state dimers
Partial denaturation
Small Amyloidogenic oligomers
Amyloidogenic Aggregation
Misfold or Molten Globule Amyloidogenic?
Polymerization
Filaments
Filament elongation
Protofibrils
Protofibril Bundling
Mature Fibrils
Fibril Bundling
Unstructured Aggregate
Amyloid Seeds
Protofibril elongation
Complete denaturation
Colloidal conversion/folding
Fibril Elongation
Denatured state monomers
Non-specific Aggregation
Amyloid Seeds
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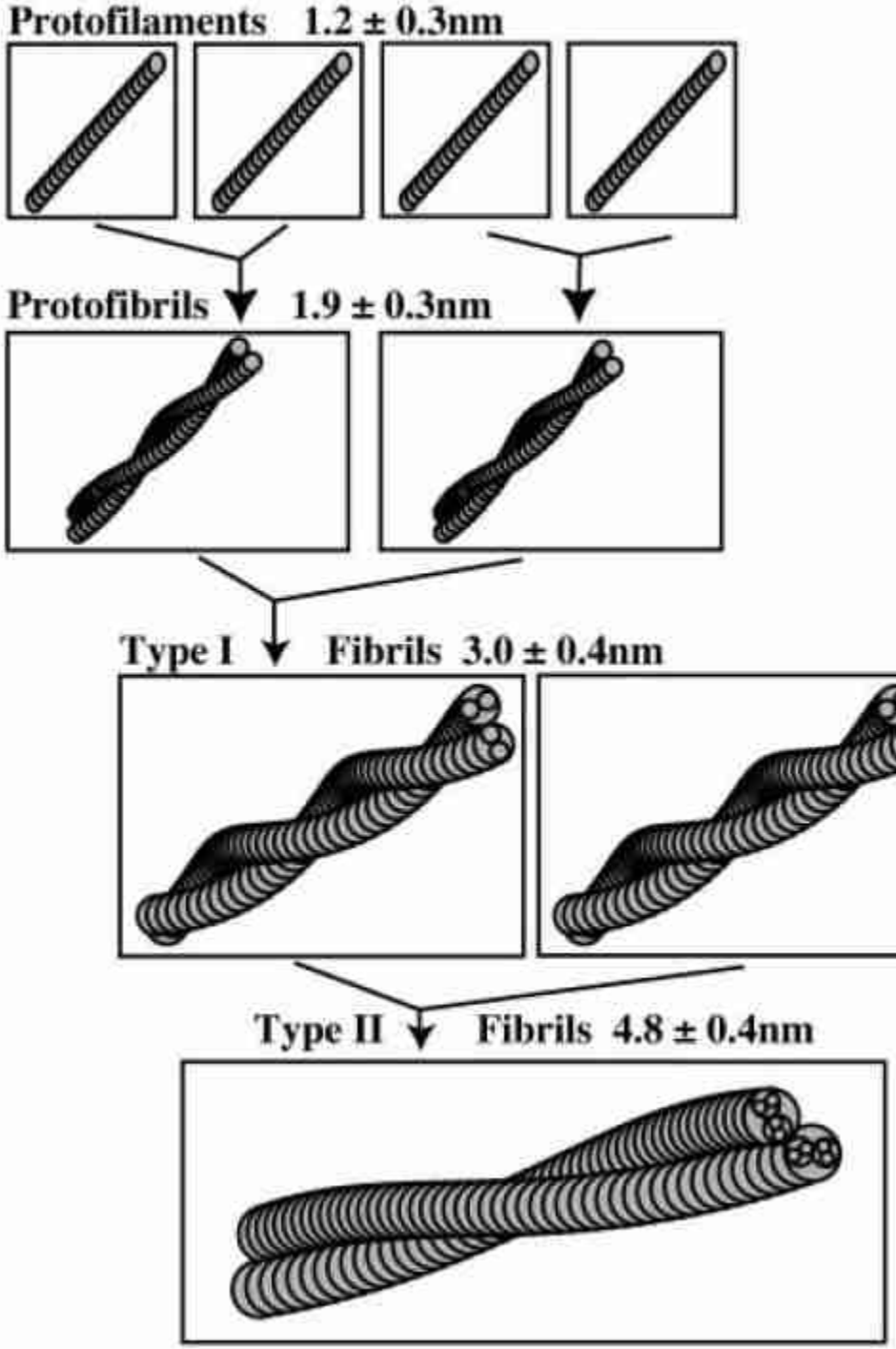
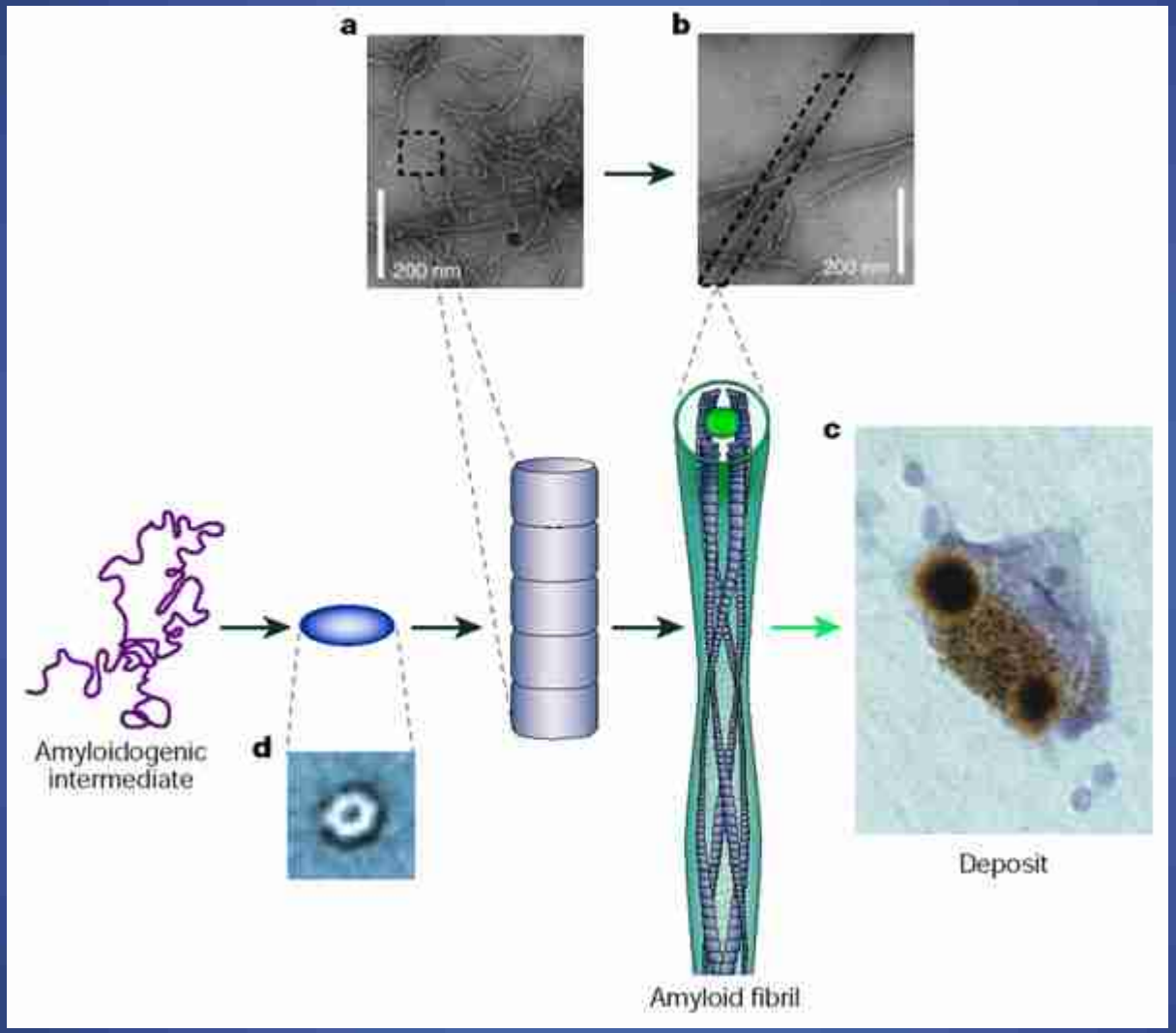
http://talaga.rutgers.ed
Cite: J. Mol. Biol. (2008) 381, 1

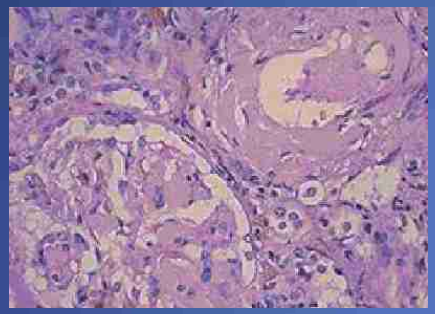

JMB


Protofilaments 1.2 ± 0.3nm
Protofibrils 1.9 ± 0.3nm
Type I Fibrils 3.0 ± 0.4nm
Type II Fibrils 4.8 ± 0.4nm

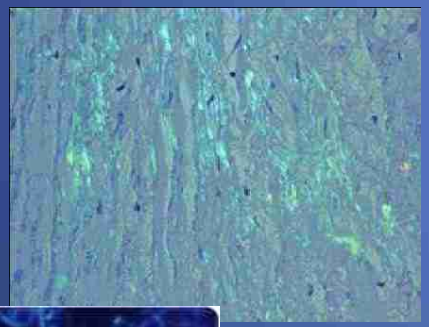


Christopher M. Dobson
Nature **426**, 884-890(18 December 2003)

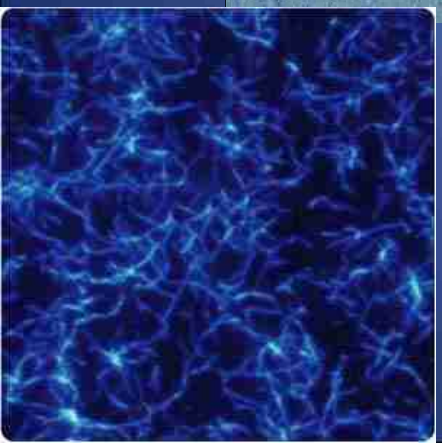
Barwienie złogów amyloidowych



Amorphous eosinophilic interstitial amyloid observed on a renal biopsy.



Congo red staining of a cardiac biopsy specimen containing amyloid, viewed under polarized light.

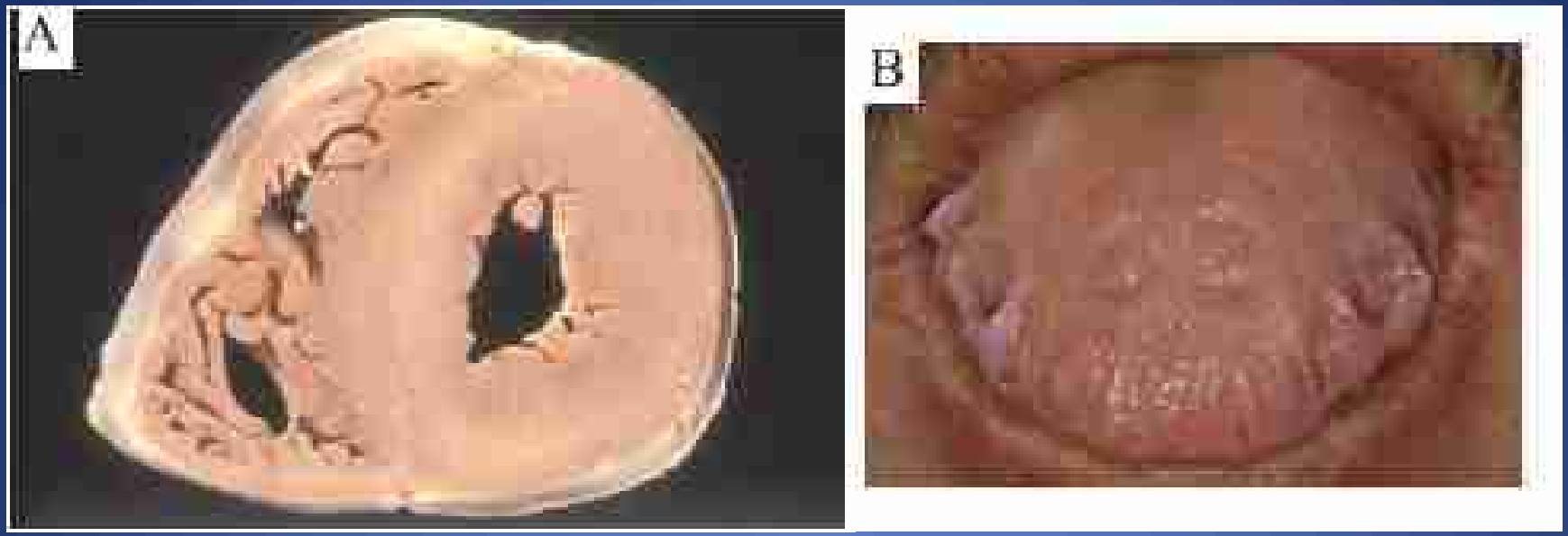


Amyloidosis, Overview

Author: Bruce A Baethge,

Table I. Amyloid fibril proteins and their precursors in human[#].

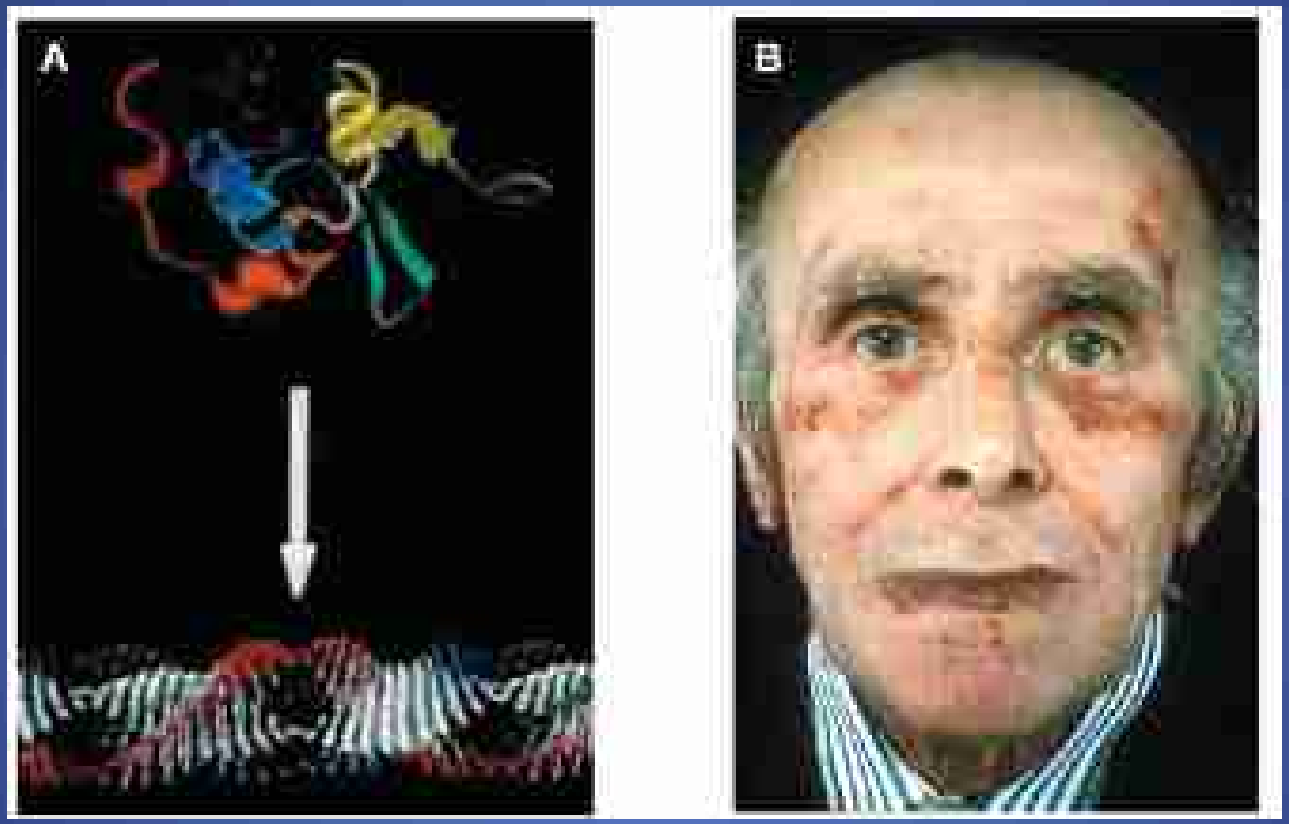
Amyloid protein	Precursor	Systemic (S) or localized, organ restricted (L)	Syndrome or involved tissues
AL	Immunoglobulin light chain	S, L	Primary Myeloma-associated
AH	Immunoglobulin heavy chain	S, L	Primary Myeloma-associated
Aβ_2M	β_2-microglobulin	S L?	Hemodialysis-associated Joints
ATTR	Transthyretin	S L?	Familial Senile systemic Tenosynovium
AA	(Apo)serum AA	S	Secondary, reactive
AApoAI	Apolipoprotein AI	S L	Familial Aorta, meniscus
AApoAII	Apolipoprotein AII	S	Familial
AApoAIV	Apolipoprotein AIV	S	Sporadic, associated with aging
AGel	Gelsolin	S	Familial (Finnish)
ALys	Lysozyme	S	Familial
AFib	Fibrinogen α-chain	S	Familial
ACys	Cystatin C	S	Familial
ABri	ABriPP	S	Familial dementia, British
ADan*	ADanPP	L	Familial dementia, Danish
Aβ	Aβ protein precursor (AβPP)	L	Alzheimer's disease, aging
APrP	Prion protein	L	Spongiform encephalopathies
ACal	(Pro)calcitonin	L	C-cell thyroid tumors
AIAPP	Islet amyloid polypeptide**	L	Islets of Langerhans Insulinomas
AANF	Atrial natriuretic factor	L	Cardiac atria
APro	Prolactin	L	Aging pituitary Prolactinomas
AIns	Insulin	L	Iatrogenic
AMed	Lactadherin	L	Senile aortic, arterial media
AKer	Kerato-epithelin	L	Cornea, familial
ALac	Lactoferrin	L	Cornea
AOaap	Odontogenic ameloblast-associated protein	L	Odontogenic tumors
ASemI	Semenogelin I	L	Vesicula seminalis
ATau	Tau	L	Alzheimer's disease, fronto-temporal dementia, aging, other cerebral conditions



A B

A.R. Bradwell: Serum Free Light Chain Analysis

A. AL amyloidoza powigrubienie lewej komory serca.
B. AL amyloidoza powiększenie języka(20% pacjentów).



A.R. Bradwell: Serum Free Light Chain Analysis

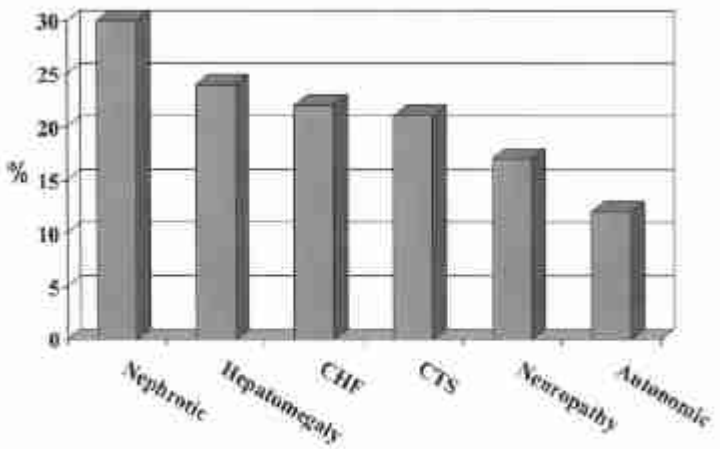


Figure 1. Clinical syndromes in amyloidosis.

Abbreviations: CHF, congestive heart failure; CTS, carpal tunnel syndrome

Morie A. Gertz, Giampaolo Merlini, and Steven P. Treon

Hematology 2004

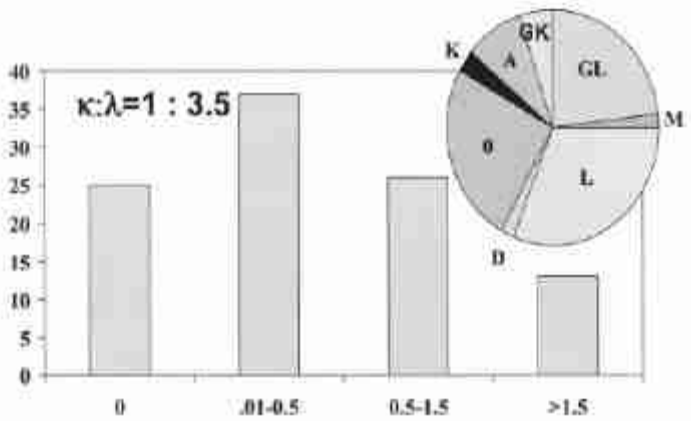


Figure 2. Serum M proteins in amyloidosis.

The pie chart gives the immunoglobulin heavy (A, G, D, M) and light chain seen at diagnosis (K = kappa, L = Lambda, 0 = none)

Morie A. Gertz, Giampaolo Merlini, and Steven P. Treon

Hematology 2004

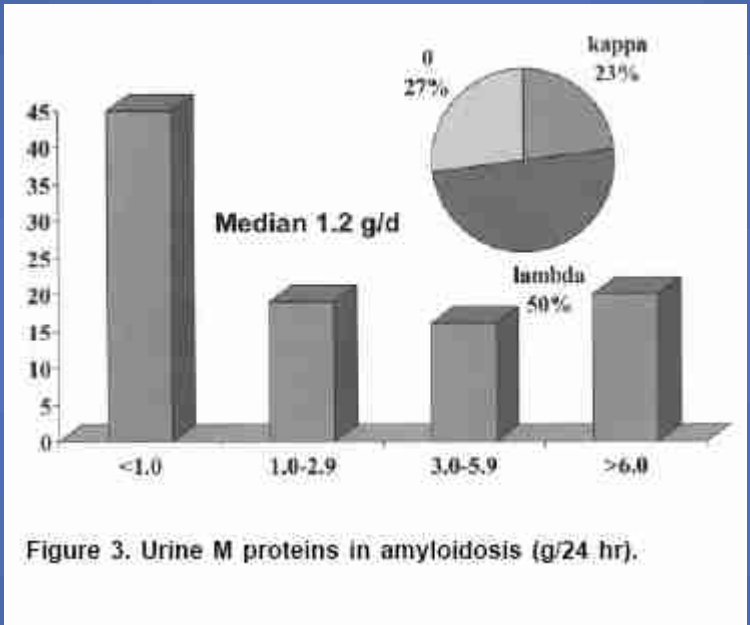
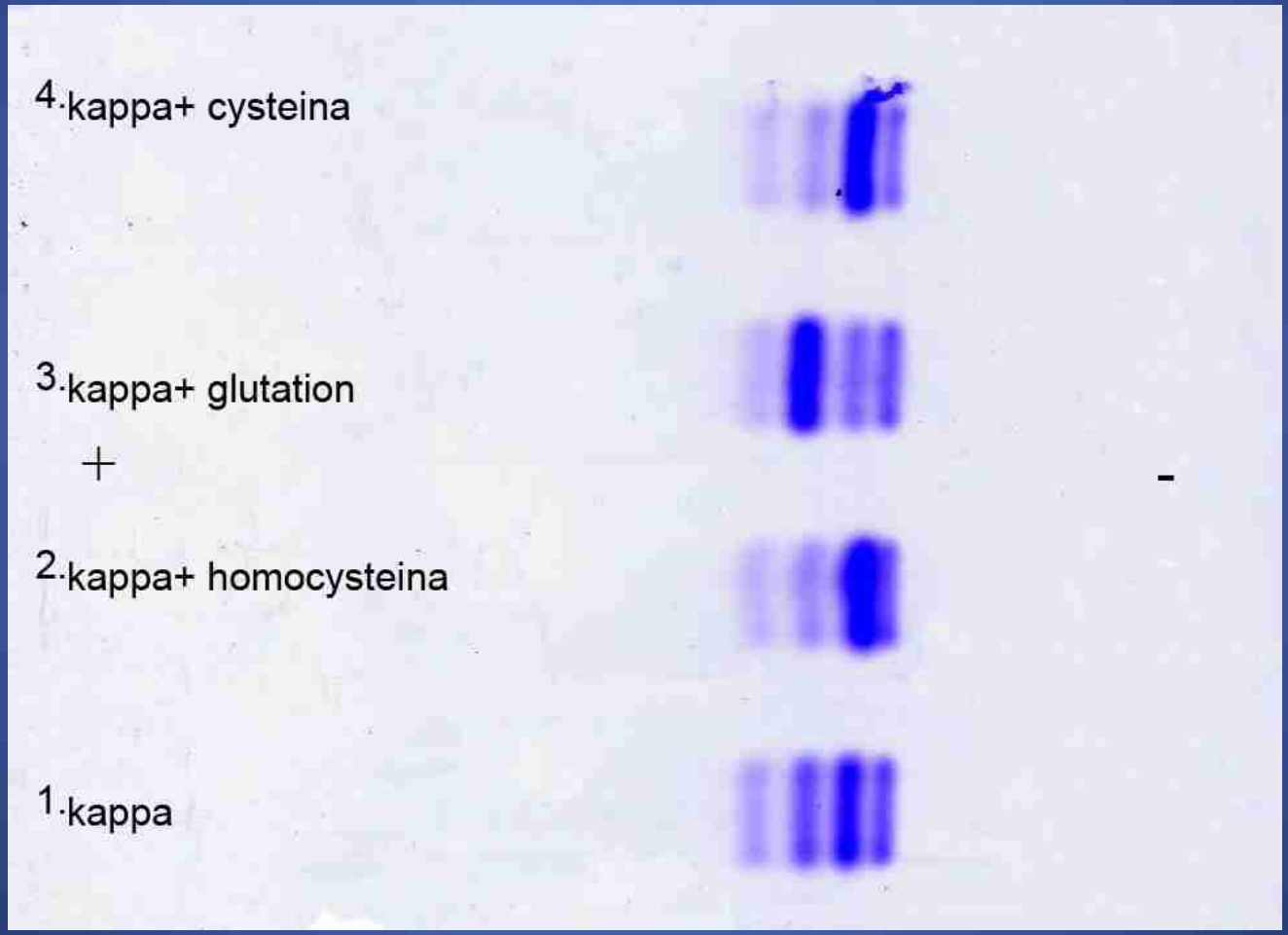
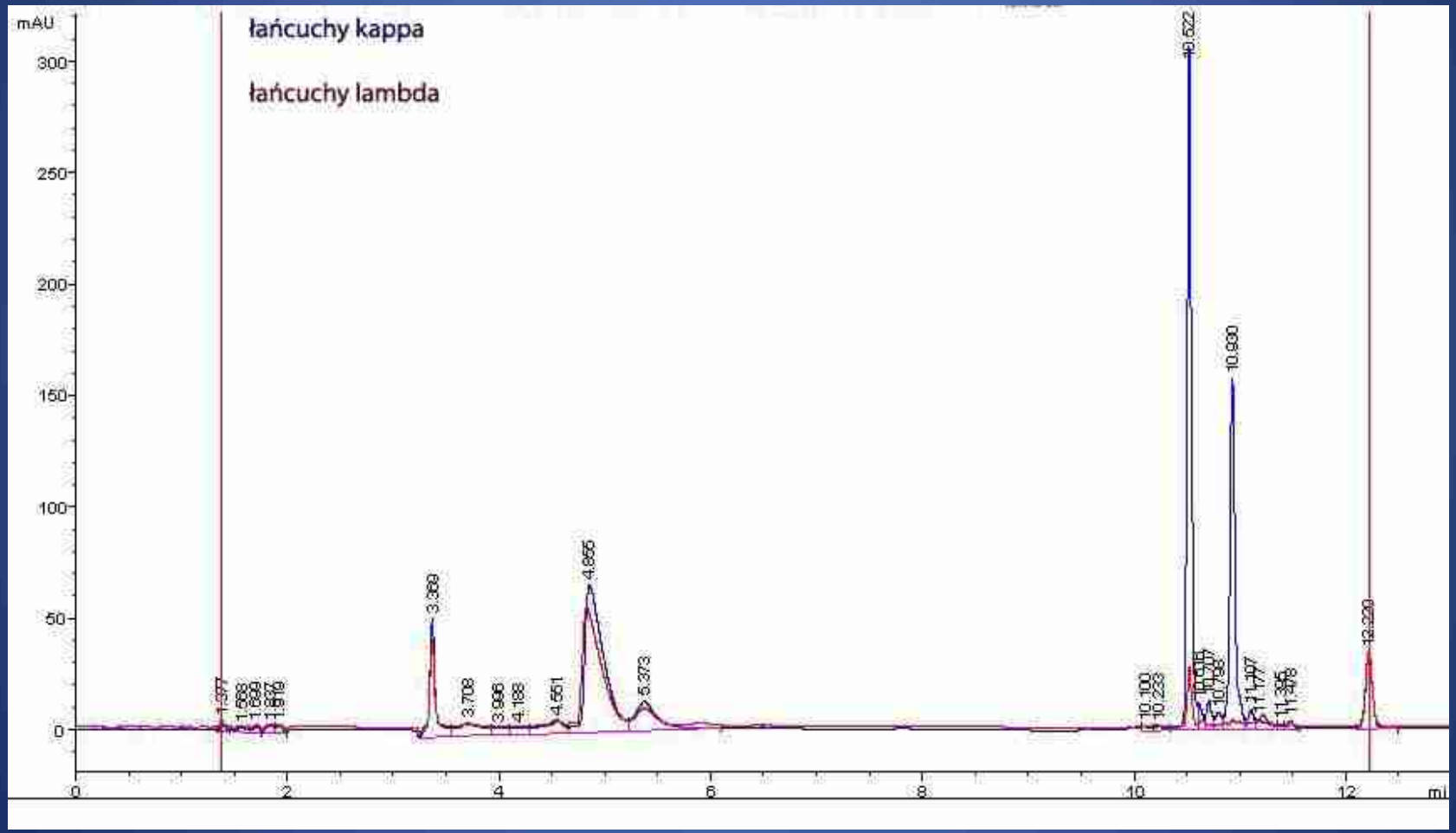
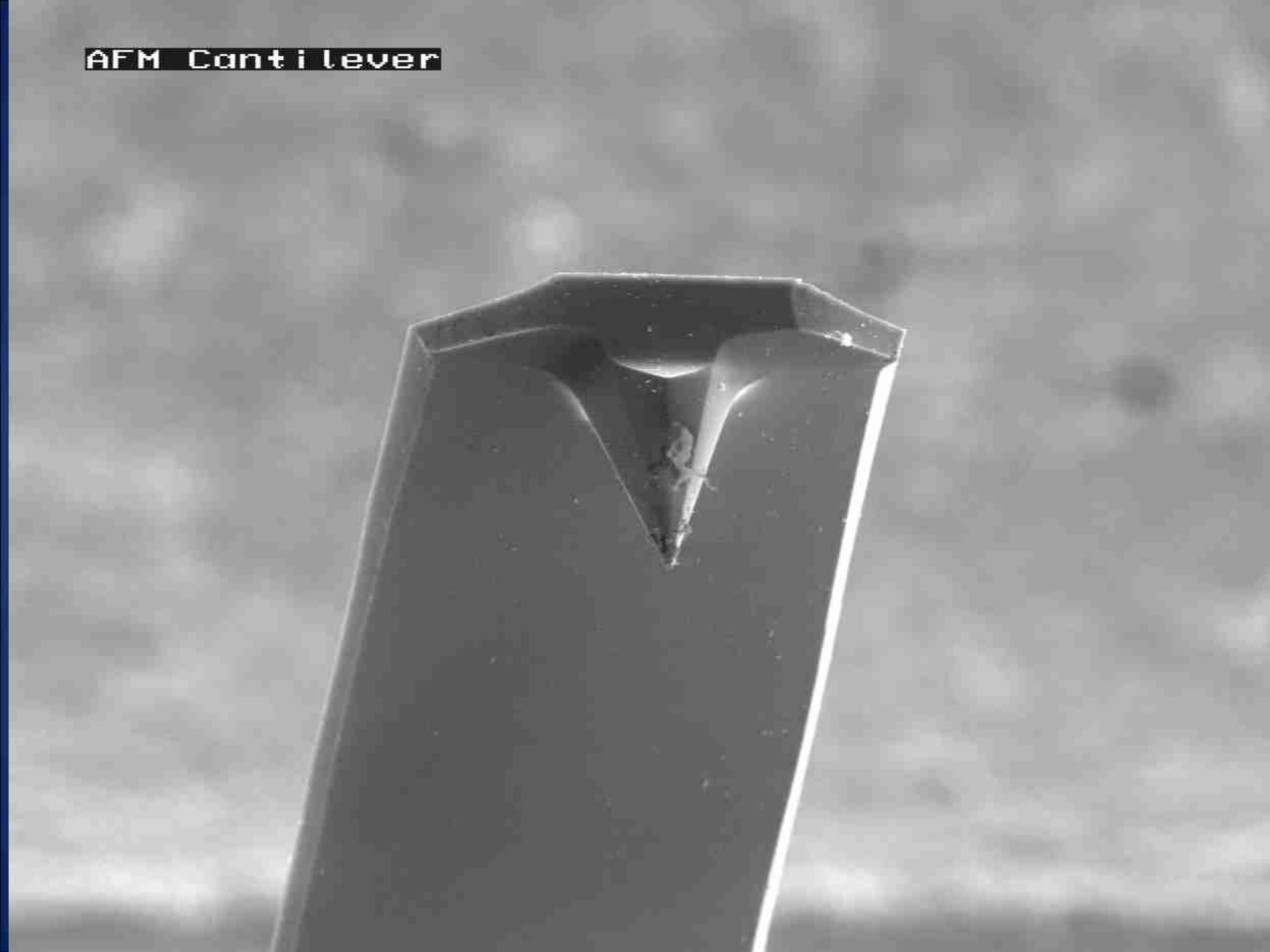


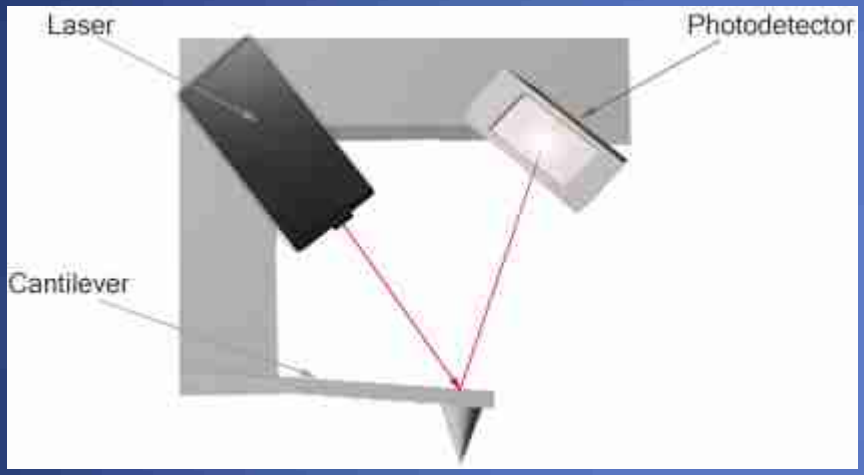
Figure 3. Urine M proteins in amyloidosis (g/24 hr).

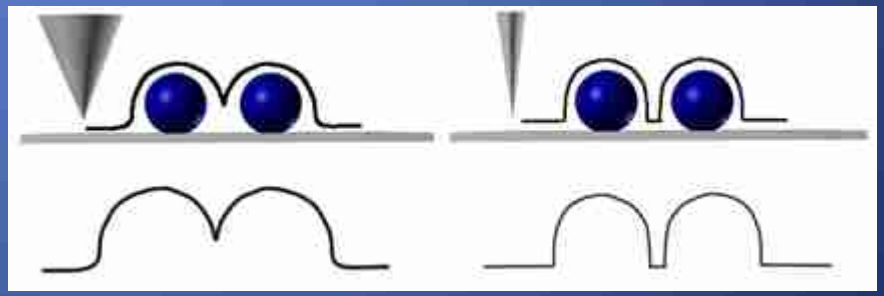
Morie A. Gertz, Giampaolo Merlini, and Steven P. Treon — *Hematology 2004*

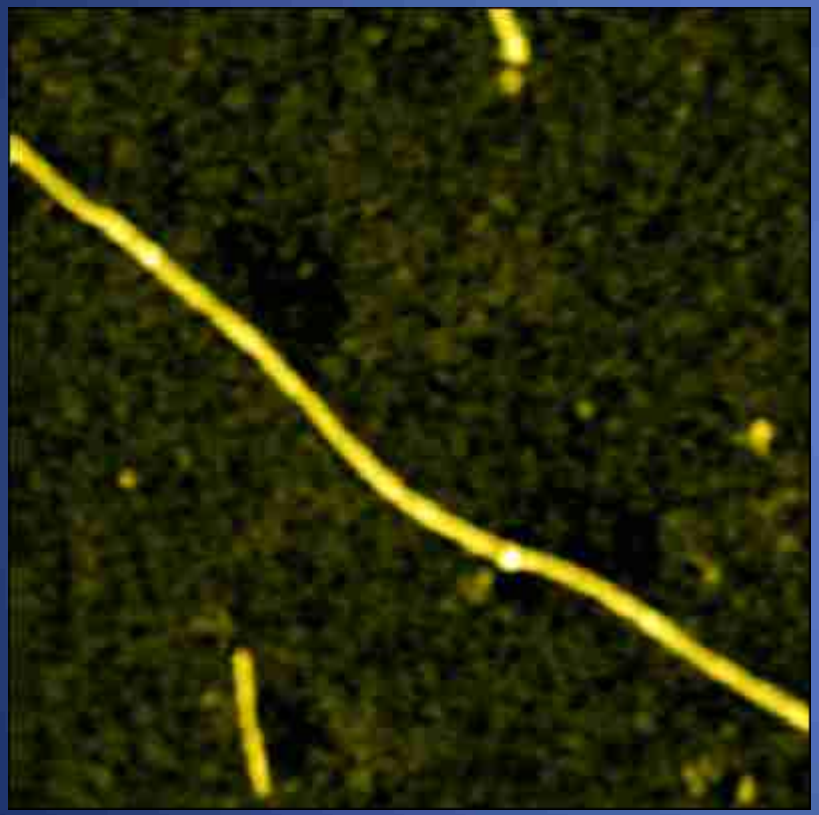

4. kappa+ cysteina
3. kappa+ glutation
+
2. kappa+ homocysteina
1. kappa
-

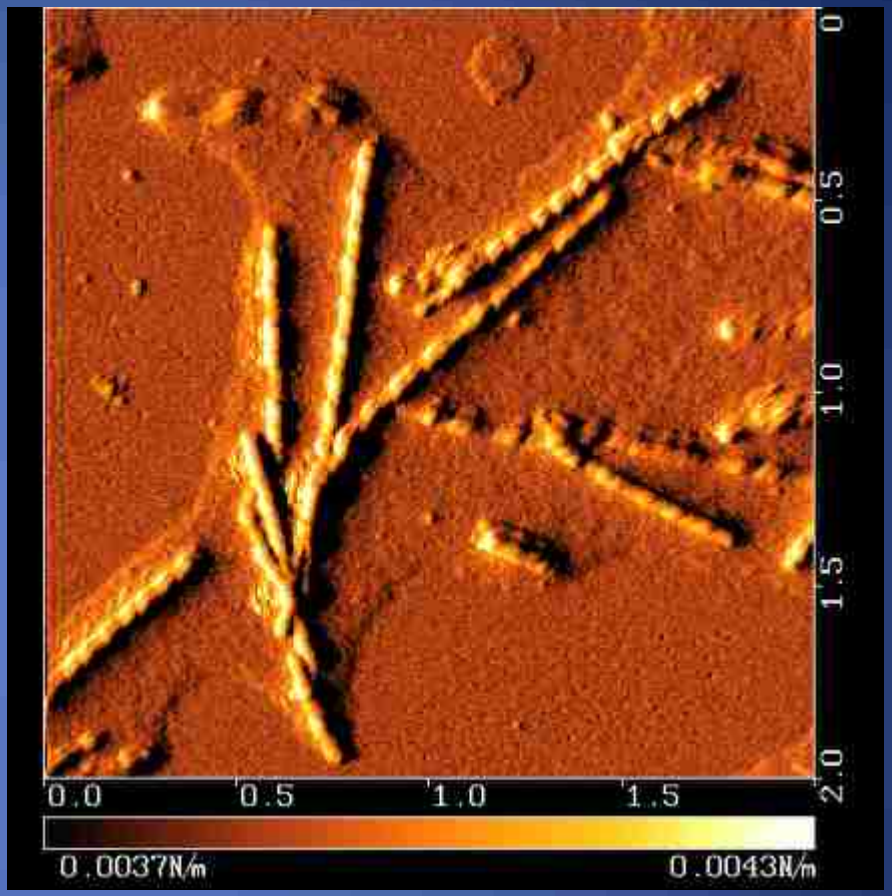

łańcuchy kappa
łańcuchy lambda
mAU
300
250
200
150
100
50
0
1.377
3.389
3.708
3.996
4.188
4.551
4.855
5.373
10.100
10.233
10.622
10.930
11.707
11.978
12.220
0
2
4
6
8
10
12
mi

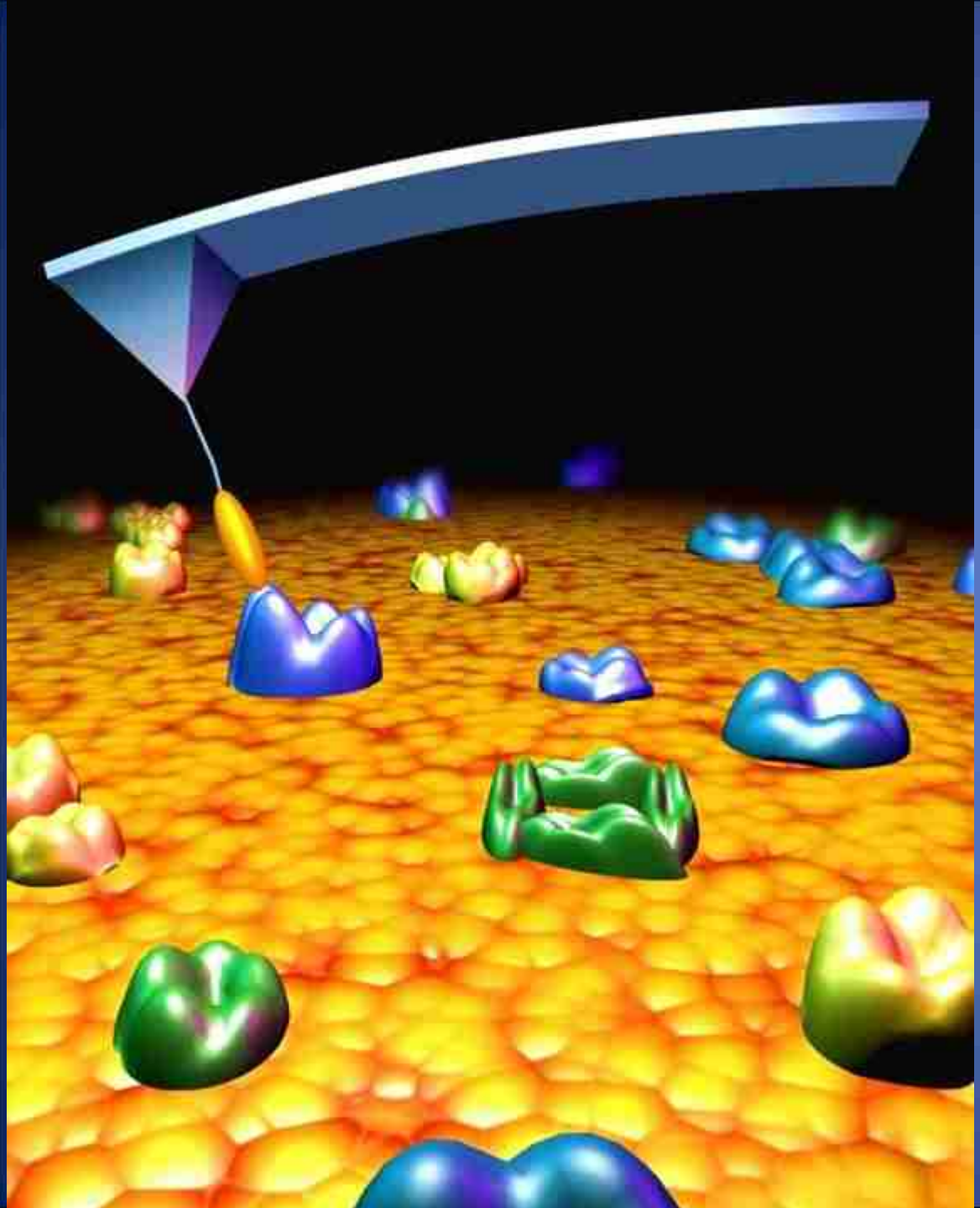

AFM Cantilever


Laser
Photodetector
Cantilever

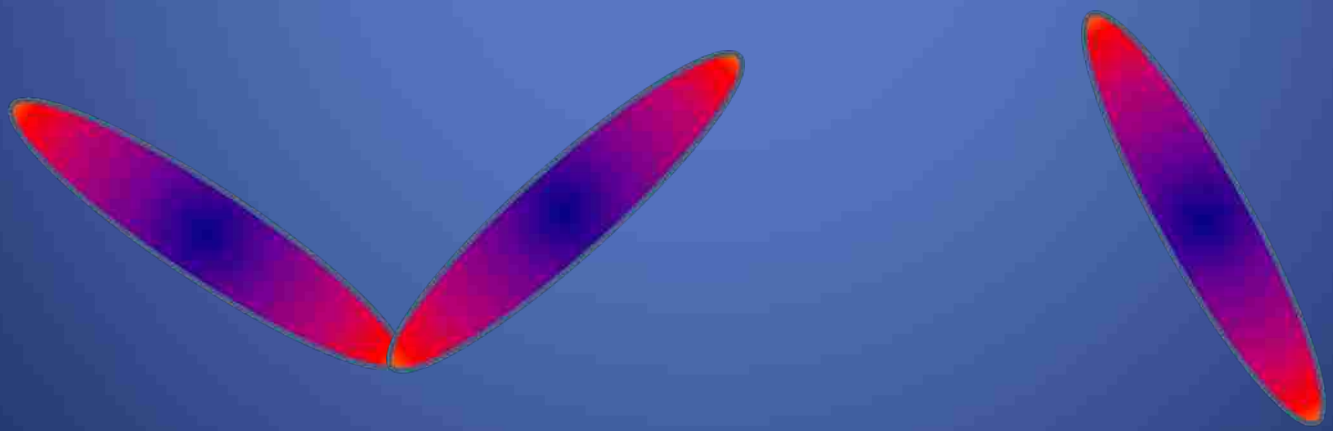




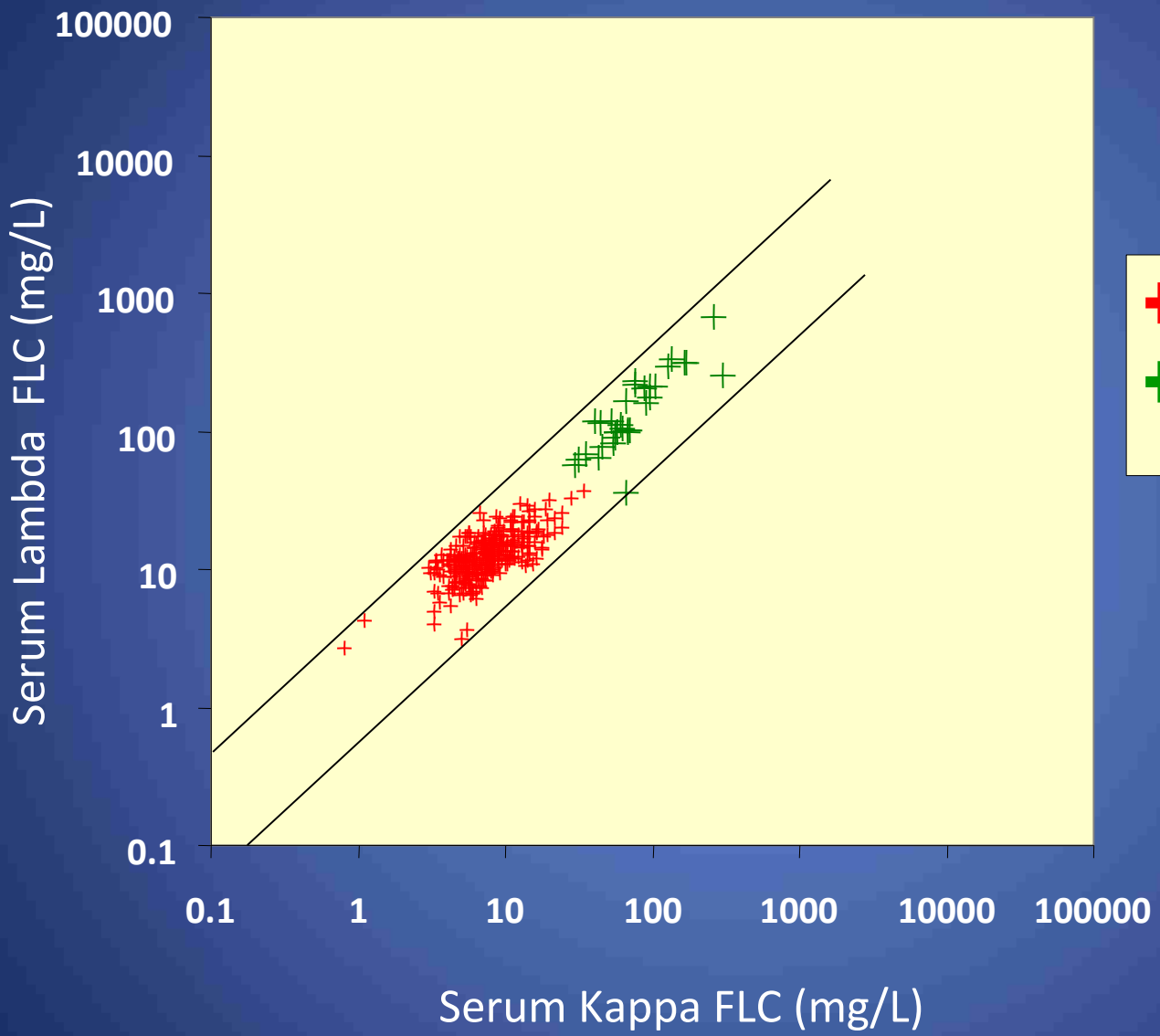

0.0
0.5
1.0
1.5
0
0.5
1.0
1.5
2.0
0.0037N/m
0.0043N/m



Wolne łańcuchy lekkie FLC’s nowe narzędzie do diagnostyki i monitorowania leczenia szpiczaka



sFLCs published normal range

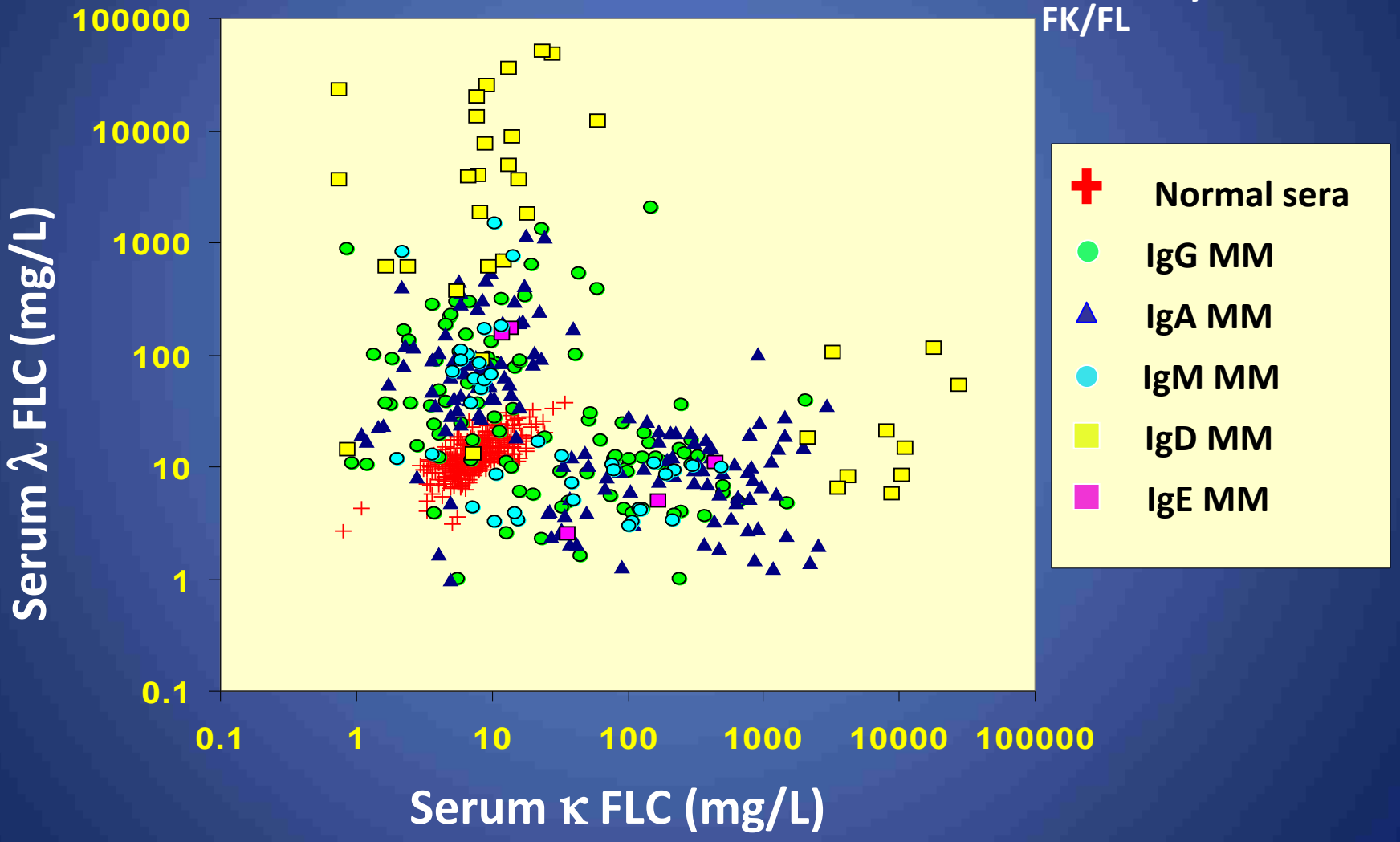


Bradwell, *Lancet* 2003; 361: 489-491

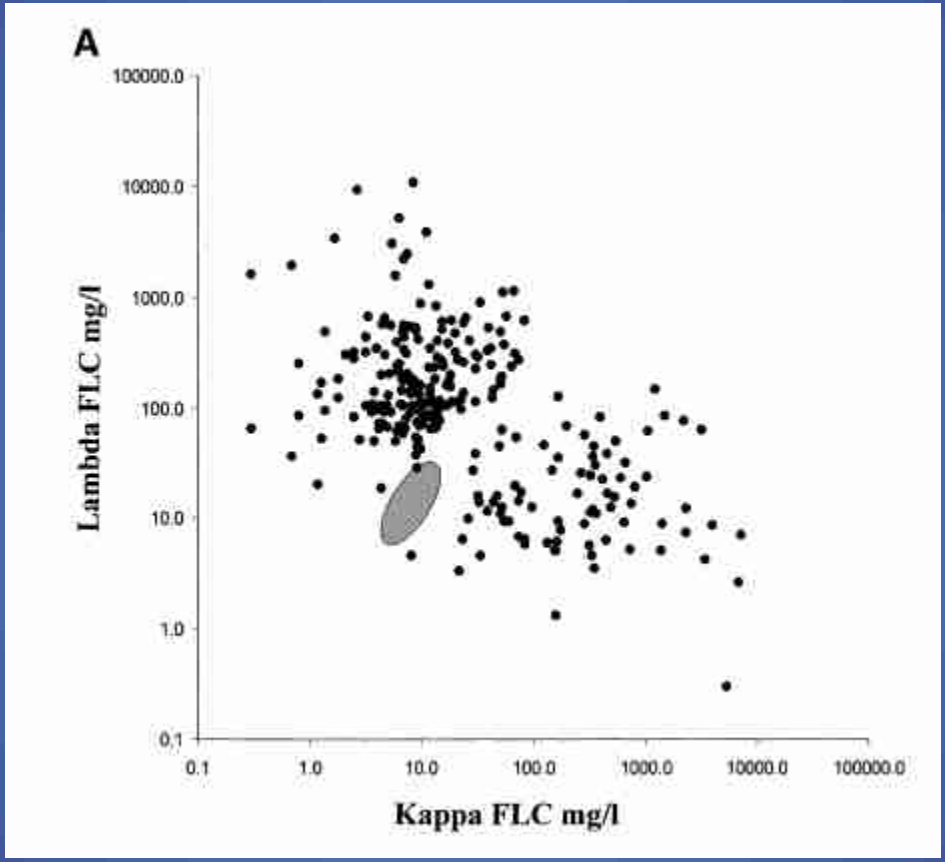
Katzmann, *Clin Chem* 2002; 48: 1437 - 1444

Intact Immunoglobulin Multiple Myeloma

89% of pacjentów ma zaburzony stosunek FK/FL

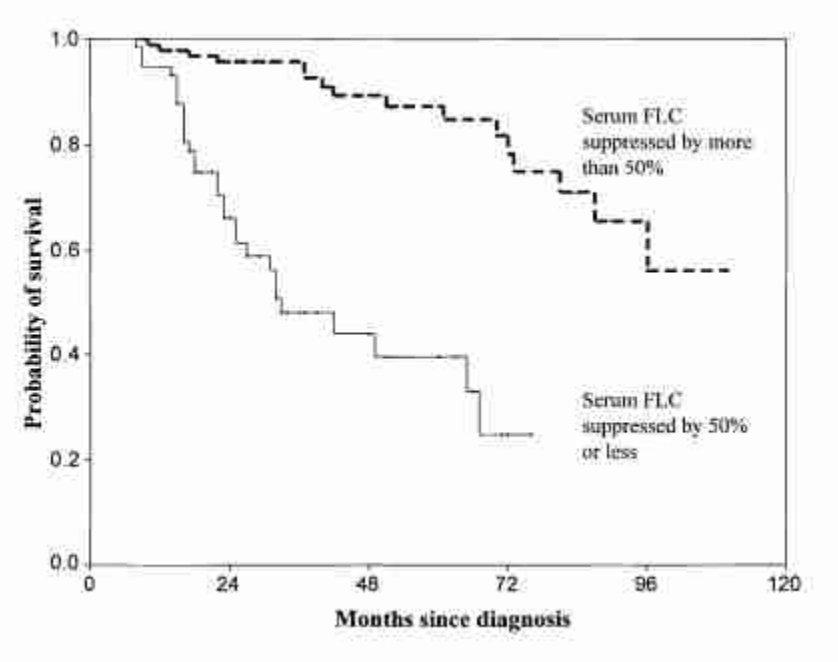


Mead *et al. Br J Haem.2003:* ***122****: 78-84*



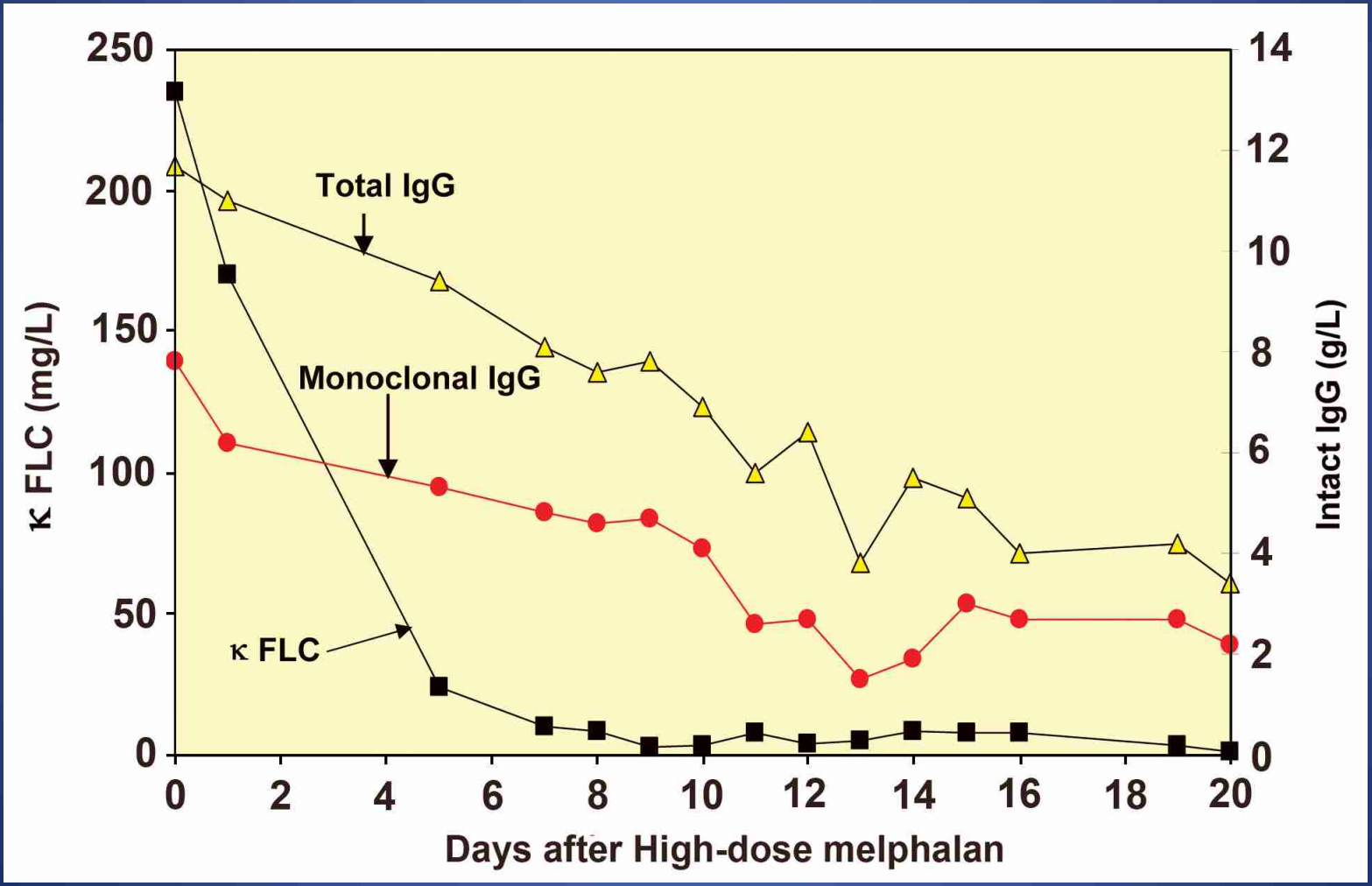
British Journal of Haematology, 2003, 122, 78–84

Odpowiedź na leczenie amyloidozy

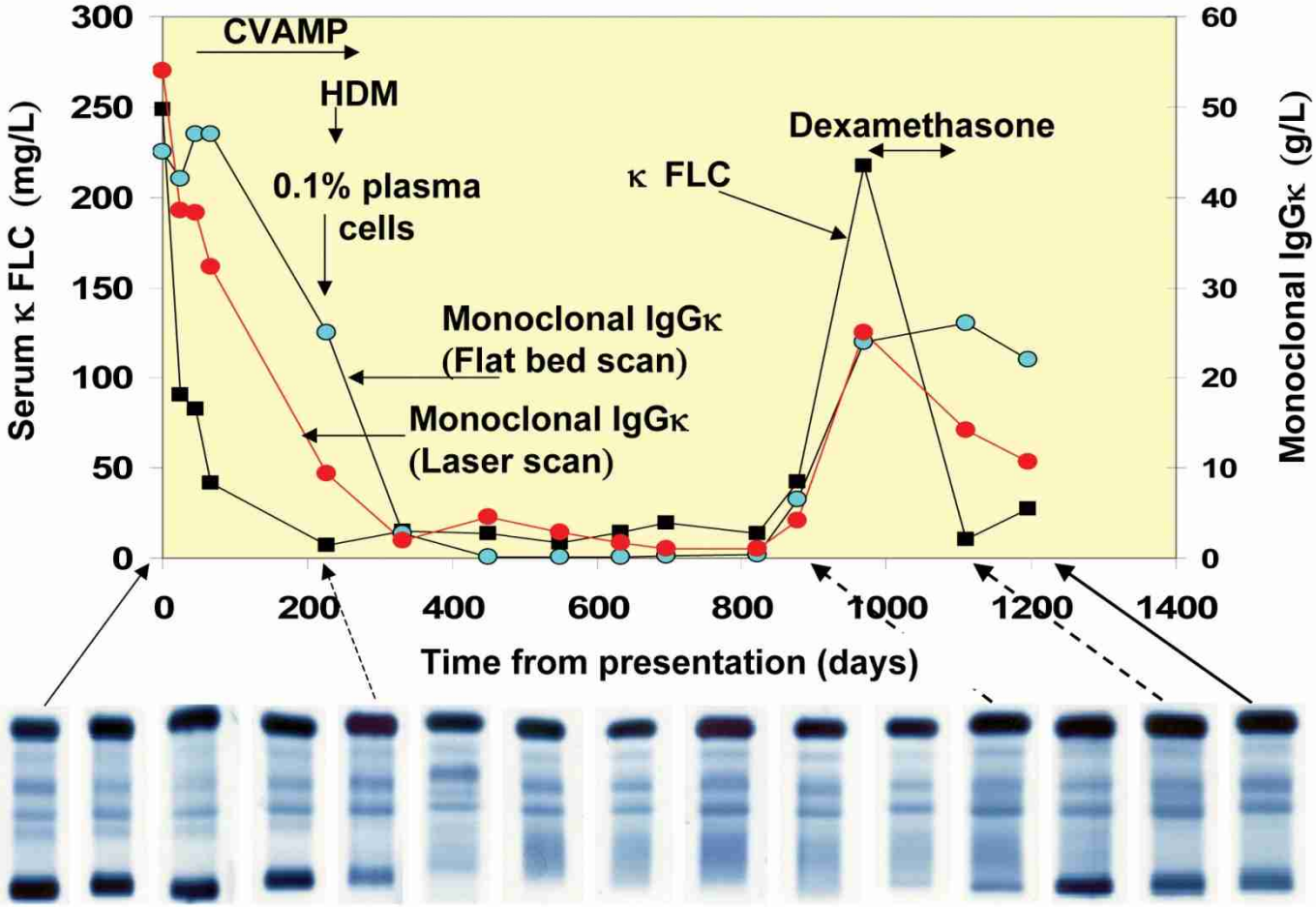


British Journal of Haematology, 2003, **122**, 78–84

Odpowiedź na leczenie szpiczaka



A.R. Bradwell: Serum Free Light Chain Analysis

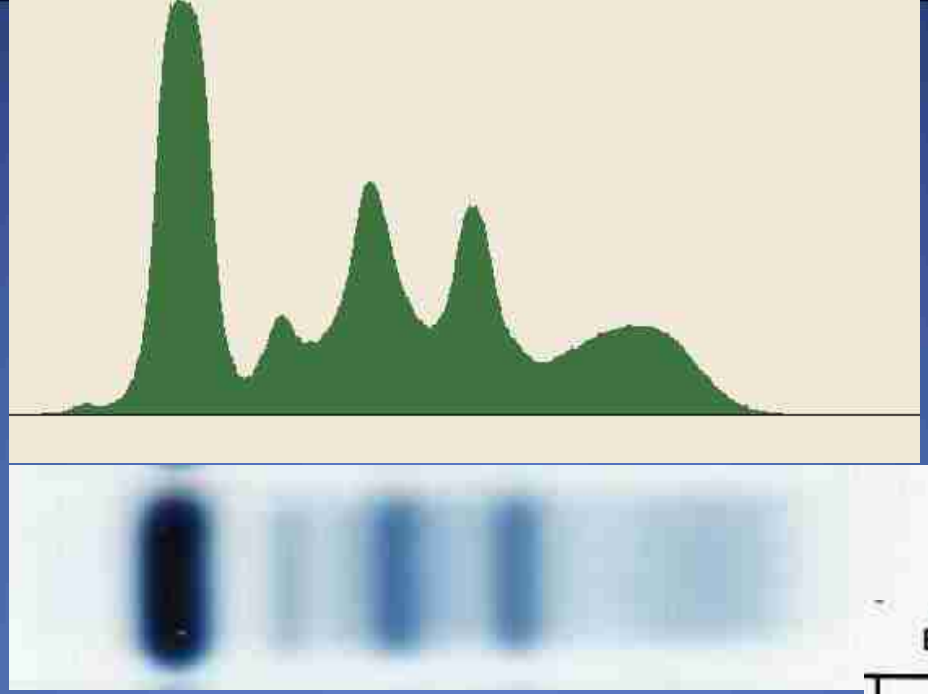


A.R. Bradwell: Serum Free Light Chain Analysis

Pacjent 005

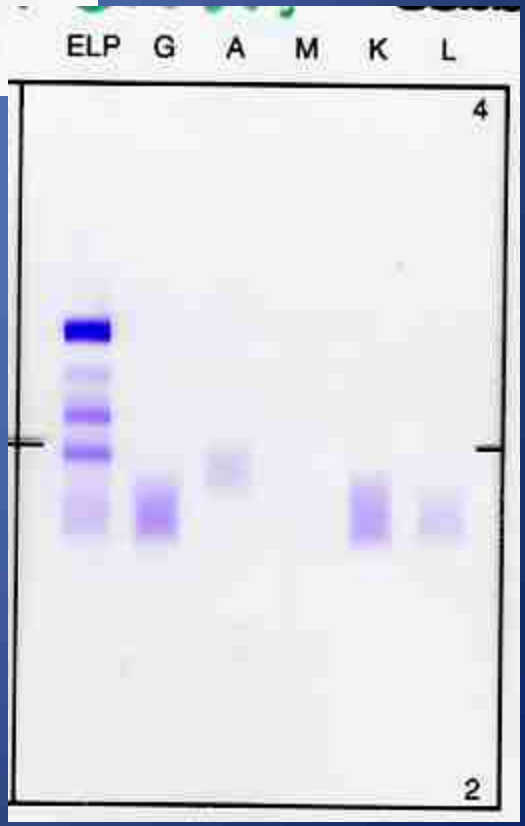
•wynik elektroforezy białek surowicy

- Białko całkowite: 76 g/l

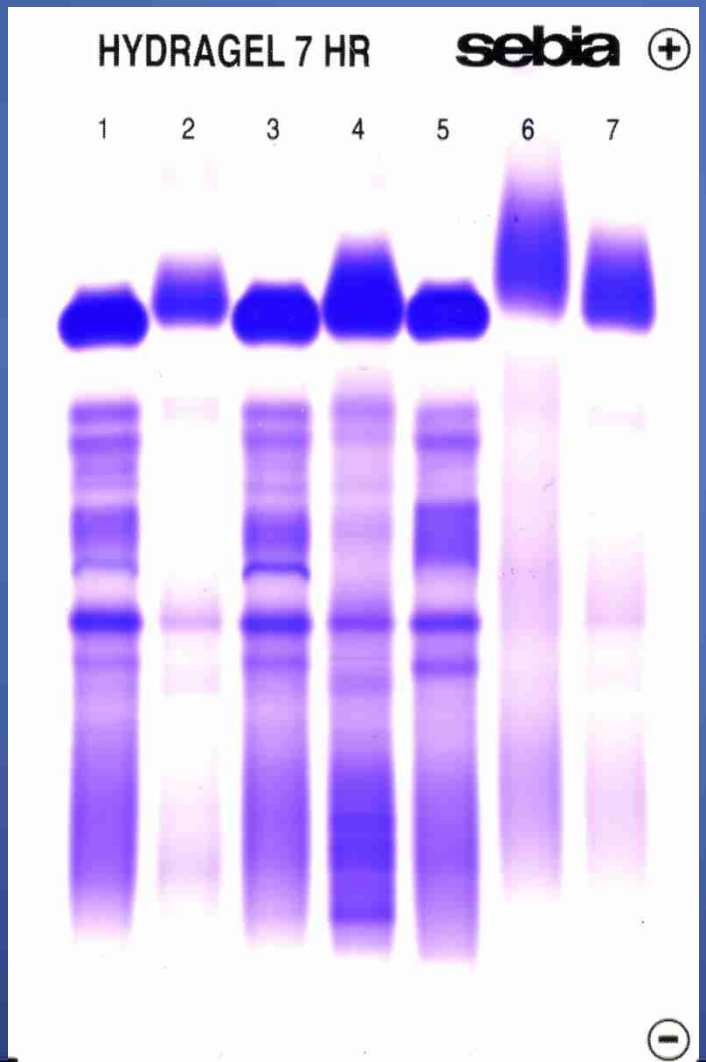


Albumina	64.9 %	49.2 g/l
Alfa1	3.1 %	2.4 g/l
Alfa2	12.9 %	9.8 g/l
beta	10.6 %	8.1 g/l
Gamma	8.5 %	6.5 g/l

KF	447 mg/l
LF	16.1 mg/l
KF/LF	27.9



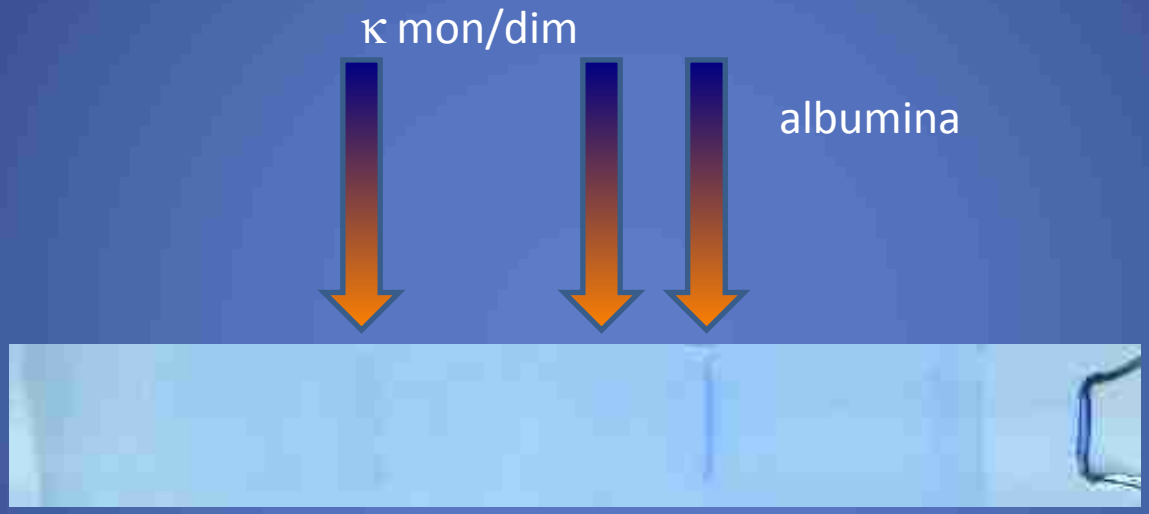
PMR prążki oligoklonalne



Elevated levels of kappa free light chains in CSF support the diagnosis of multiple sclerosis

J Neurol (2008) 255:1508–1514

wynik elektroforezy białek moczu:
W moczu stwierdzono obecność słabej strefy łańcuchów lekkich immunoglobulin oraz słabej strefy albuminy.



Typ l.	St. mg/l
Fk	123
Fl	3,91
Fk/Fl	94,5 (1,7-3,5)

Wolne łańcuchy w moczu

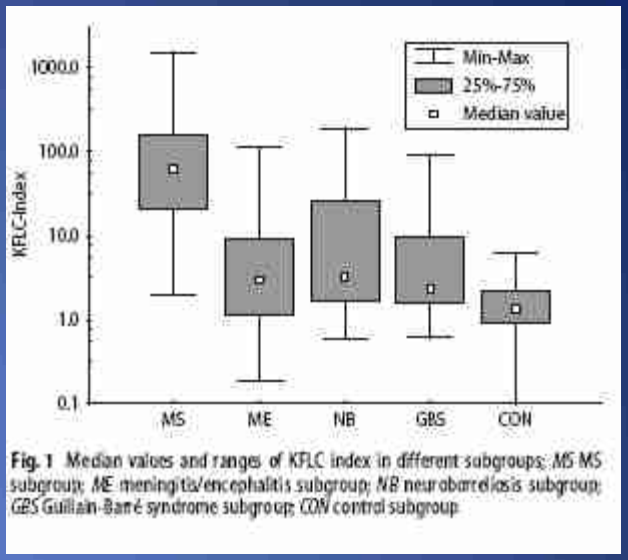


Fig. 1 Median values and ranges of KFLC index in different subgroups; *MS* MS subgroup; *ME* meningitis/encephalitis subgroup; *NB* neuroborreliosis subgroup; *GBS* Guillain-Barré syndrome subgroup; *CON* control subgroup

	KFLC (mg/l) in CSF	LFLC (mg/l) in CSF	KFLC index	LFLC index
MS group (n = 70)	4.12 (1.4–8.77)	0.67 (0.25–1.54)	65.74 (22.25–159.42)	9.62 (4.27–37.25)
Definite MS (n = 41)	4.62 (1.41–10)	0.72 (0.29–1.46)	78.97 (19.58–189.39)	9.34 (4.49–29.24)
CISS MS (n = 29)	2.95 (1.39–8.65)	0.53 (0.24–1.57)	63.03 (29.91–95.19)	12.24 (3.31–42.3)
Control group (n = 45)	0.18 (0.13–0.22)	0.16 (0.13–0.2)	1.35 (0.94–2.21)	1.23 (0.91–1.54)
Encephalitis/meningitis (n = 41)	0.55 (0.32–1.27)	0.57 (0.27–1.27)	3.01 (1.13–9.03)	2.1 (1.07–5.27)
Guillain-Barré syndrome (n = 15)	0.8 (0.25–1.44)	0.35 (0.27–0.69)	2.33 (1.63–9.39)	1.88 (1.27–3.48)
Neuroborreliosis (n = 15)	0.59 (0.14–5.94)	0.82 (0.14–5.48)	3.3 (1.74–24.65)	2.19 (1.33–30.17)
CIDP (n = 7)	0.34 (0.22–2.03)	0.25 (0.22–0.43)	1.34 (1.1–8.09)	1.44 (1.02–2.03)

Elevated levels of kappa free light chains in CSF support the diagnosis of multiple sclerosis

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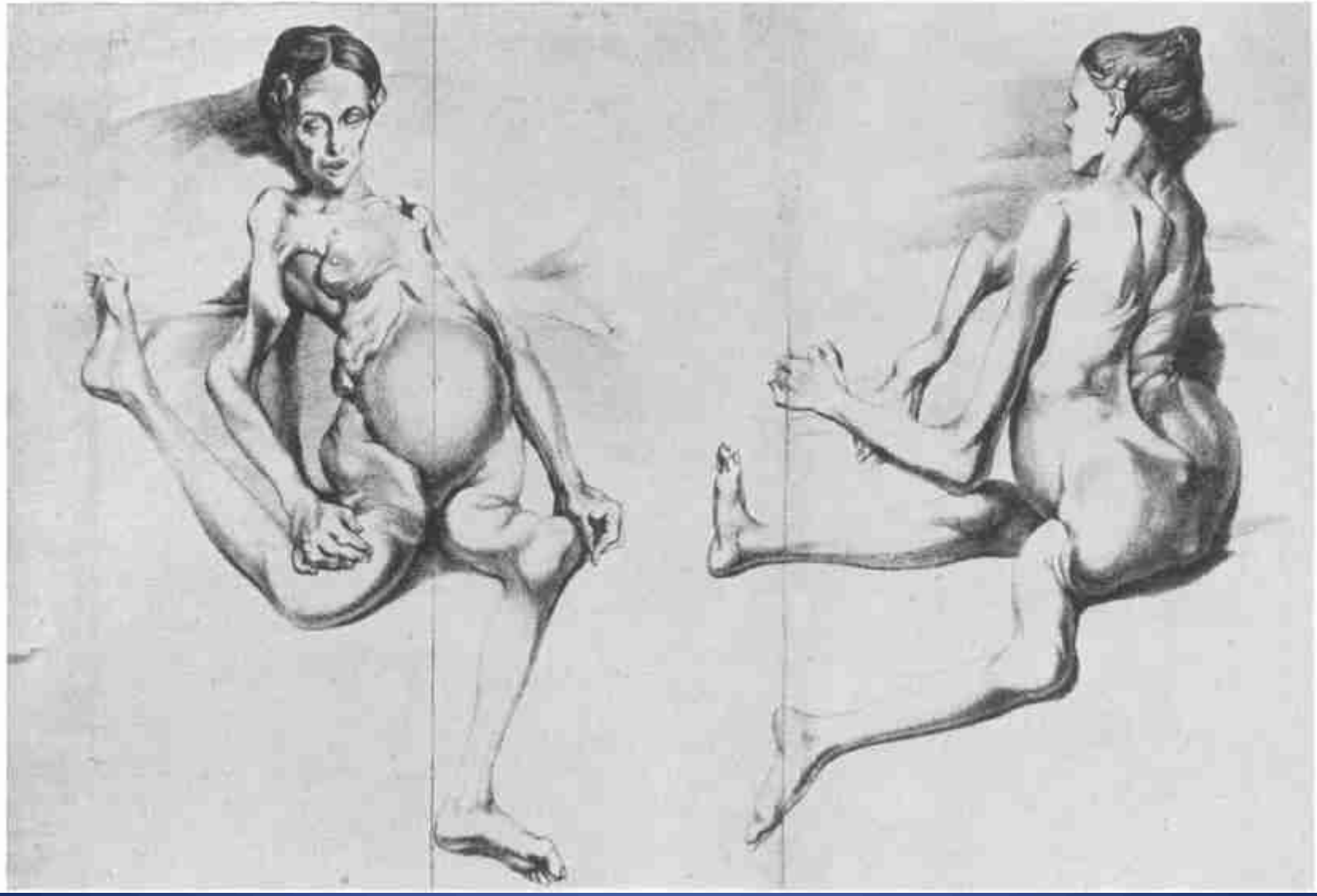

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