Proteins, kidneys, free radicals etc…..

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Dif.dg.

Causes of acute kidney injury include:

1. decreased renal blood flow - prerenal uremia
2. parenchymal damage – renal uremia
3. obstruction in the urinary tract – postrenal uremia

Acute kidney injury - sudden, often transient reduction in glomerular filtration rate. It is characterized by a rapid increase in urea and creatinine in plasma and is usually (but not always) associated with oliguria (less than 400 ml / day). RIFLE and AKIN classification.

New marker of AKI – NGAL (neutrophil gelatinase associated lipocalin).

correct resolution is necessary → adequate therapy

Laboratory tests :

S-urea a S-creatinine

S- K^+ , S-Na^+, S-Cl^-, S-osmolality

examination of urine and urinary sediment

acid base balance

electrolytes in urine, urine osmolality

blood count – anemia

calcium and anorganic phosphates

Dif.dg.

Test	Unit	Values	
		Prerenal uraemia	Renal uraemia
U-Na^+	mmol/l	5-35	20 - 130
FE_{Na+}	%	0.2-1.0	1,0 - 8,0
U-Cl^-	mmol/l	5-25	25 - 140
FE_{Cl-}	%	0.10-1.0	1.0 – 9.0
U/S osmolality		1.0-3.0	0.9 – 2.6
U/S urea		0.5-90 (depends on protein intake, eventually on breakdown of tissue proteins)	0.5-30
U/S creatinine		20-130	1-55
S-urea/creatinine		30-140	15-140

Transferrin

- 676 AA, Fe3+, Mr 81 000, glykoprotein
- Synthesis in liver, bone marrow, nodes,
- 2-3,7 g/l
- transport Fe - anemia, malnutrition, hemochromatosis
- negative acute phase reactant
- *CDT transferrin*

Ferritin

- resources Fe, Mr 450 000,
- increase with age

♂ 20-300 µg/l ♀ 4-200 µg/l

↓ liver diseases, liver carcinoma, obstructive jaundice,

↓ anemia Fe

↑ leukemia, inflammation, hemochromatosis

Fancony syndrome

global functional impairment of proximal tubule →

losses of aminoacids, glucose, phosphates, bicarbonate and other solutes which are transported in proximal tubule →

acidosis, dehydratation, electrolyte imbalance, rhachitis, osteomalacia and growth disturbances

causes - hereditary and acquired

Examination – multiple myeloma

a) Detection and typing of paraprotein

   - M-component at ELFO of proteins (paraprotein)

   - immunofixace

   - detection of Bence - Jones´ protein in the urine

   - free light chains kappa a lambda in serum

b) more than 10% plasma cells in the bone marrow

c) X-ray of large bones shows osteolytic lesions

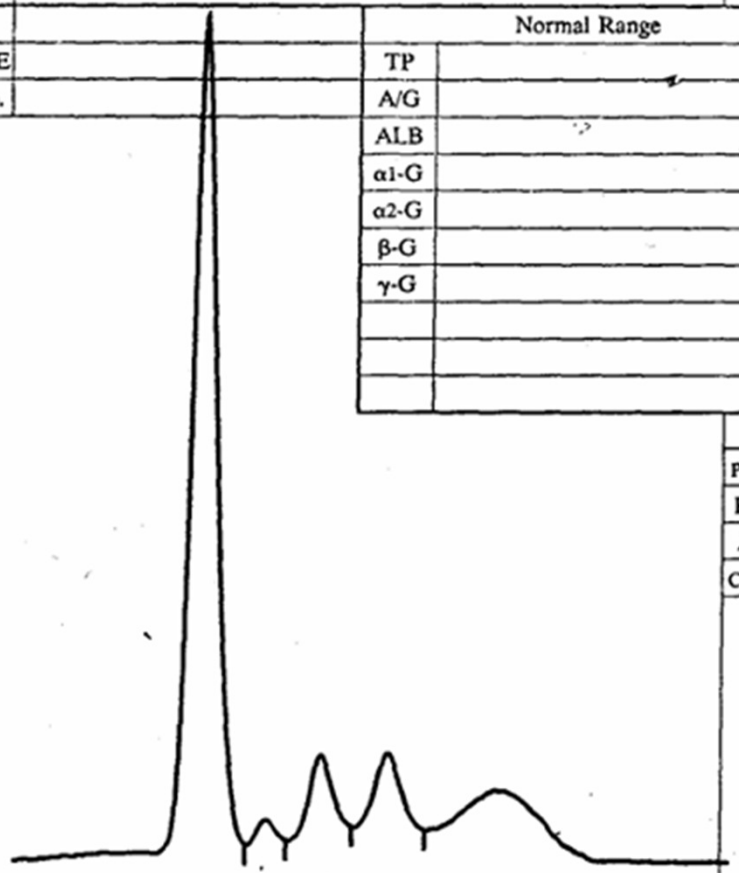
d) progression of a single clone of immunocompetent cells suppresses other systems of hematopoiesis (tendency to anemia)

Normal sample

Electrophoresis with high resolution

Zones	Serum Proteins Found in Zones
1. PREALBUMIN ZONE	-Prealbumin
2. ALBUMIN ZONE	-Albumin
3. ALBUMIN-ALPHA-1 INTERZONE	-Alpha-lipoprotein (Alpha-fetoprotein)
4. ALPHA-1 ZONE	-Alpha 1-antitrypsin, Alpha-1-acid glycoprotein
5. ALPHA-1-ALPHA-2 INTERZONE	-Gc-globulin, Inter-alpha-trypsin inhibitor, Alpha-1-antichymotrypsin
6. ALPHA-2 ZONE	-Alpha-2-macroglobulin, Haptoglobin
7. ALPHA-2-BETA-1 INTERZONE	-Cold insoluble globulin, (Hemoglobin)
8. BETA-1 ZONE	-Transferrin
9. BETA-1-BETA-2 INTERZONE	-Beta-lipoprotein
10. BETA-2 ZONE	-C3
11. GAMMA-1 ZONE	-IgA, (Fibrinogen), IgM (Monoclonal Ig's, light chains)
12. GAMMA-2 ZONE	-IgG, (C-reactive protein) (Monoclonal Ig's, light chains)

CUST.		P-COM.	
		Normal Range	L/G 11 17. Result
DATE		TP	g/l
S-No.		A/G	18.01.2001
		ALB	0109 % g/l
		α1-G	1.55 % g/l
		α2-G	60.8 % g/l
		β-G	2.9 % g/l
		γ-G	8.5 % g/l
			10.1 % g/l
			17.7 % g/l
			% g/l



P-Name			
P-ID			
AGE		SEX	
COMM.			

Electrophoresis of serum proteins

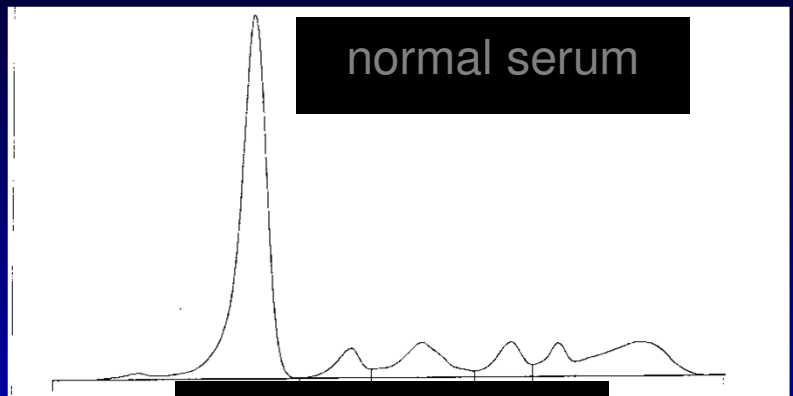
Fractions:

albumin	55 - 68
α-1 glob.	1,6 - 5,6
α-2 glob.	5.9 - 11.1
β glob.	7.9 - 13.9
γ glob.	11.4 - 18.4

- ✓ inflammation : ↑ α-fraction
- ✓ chron. inflam, DM : ↑ β-fraction
- ✓ age: ↓ albumin, ↑ α-2 fraction, ↑ β-fraction

Results of capillary electrophoresis

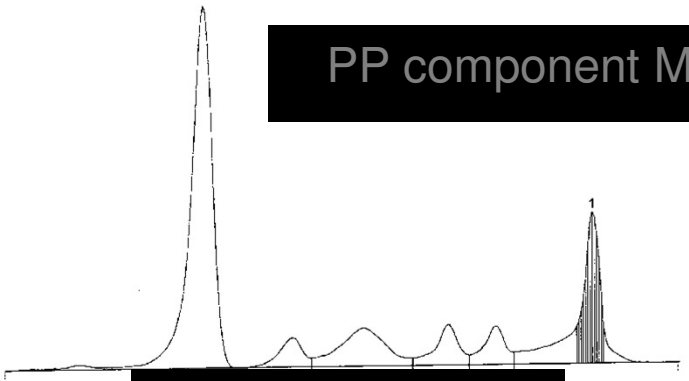
normal serum



Electrophoresis of serum protein

Fractions	%	Range [%]	Range g/l
Albumin	60,7	55,1 - 65,7	40,1 - 47,8
Alpha 1	4,3	3,1 - 5,6	2,2 - 4,1
	10,2	8,0 - 12,7	5,8 - 9,2
	6,0	4,9 - 7,2	
	4,8	3,1 - 6,1	
	14,0	10,3 - 18,2	
	1,54	TP :	g/l

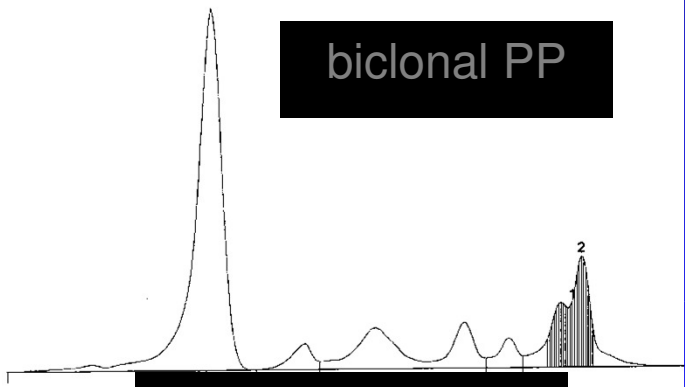
PP component M



Electrophoresis of serum protein

Fractions	%		Range [%]	Range g/l
Albumin	51,1	<	55,1 - 65,7	40,1 - 47,8
Alpha 1	4,2		3,1 - 5,6	2,2 - 4,1
Alpha 2	11,6		8,0 - 12,7	5,8 - 9,2
Beta 1	5,9		4,9 - 7,2	3,6 - 5,2
Beta 2	5,2		3,1 - 6,1	2,2 - 4,5
Gamma	22,0	>	10,3 - 18,2	7,5 - 13,2
1	14,3		TP :	g/l
pp				

biclonal PP

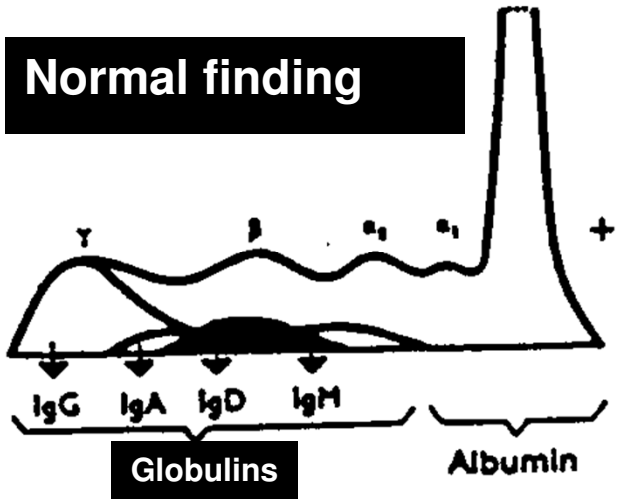


Electrophoresis of serum protein

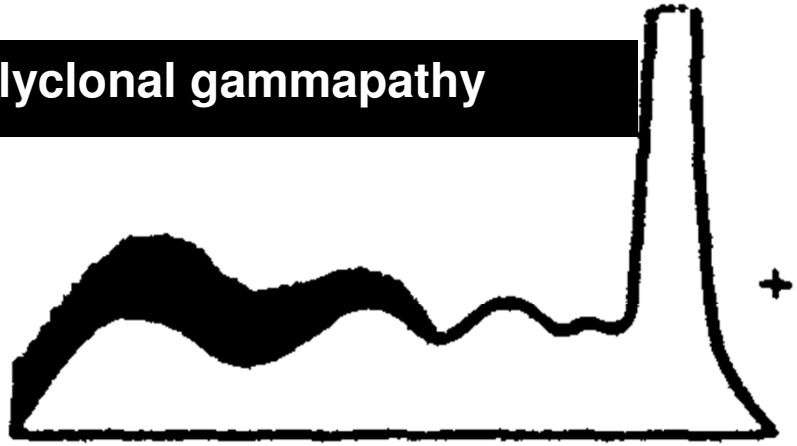
Fractions	%		Range [%]	Range
Albumin	56,4		55,1 - 65,7	40,1 -
Alpha 1	3,1		3,1 - 5,6	2,2 -
Alpha 2	10,7		8,0 - 12,7	5,8 -
Beta 1	6,2		4,9 - 7,2	3,6 -
Beta 2	3,3		3,1 - 6,1	2,2 -
Gamma	20,3	>	10,3 - 18,2	7,5 -
1	17,6		TP :	g/l
2	9,5			
pp				

The most important gammapathies

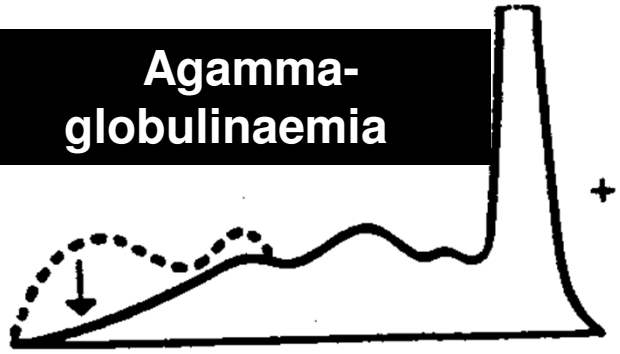
Normal finding



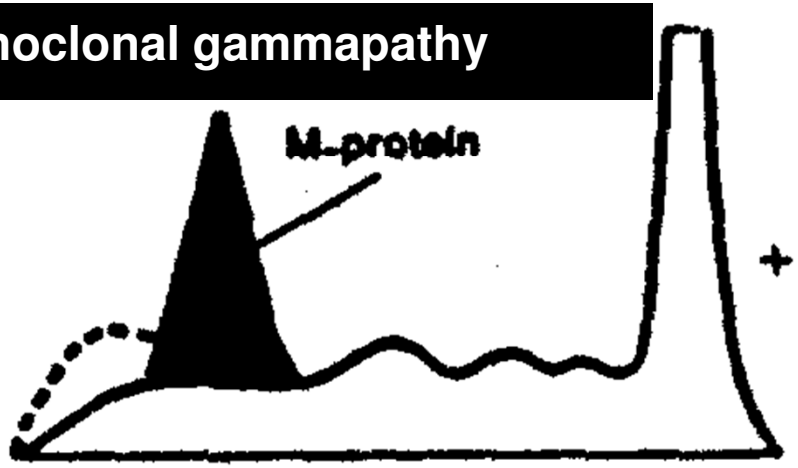
Polyclonal gammapathy



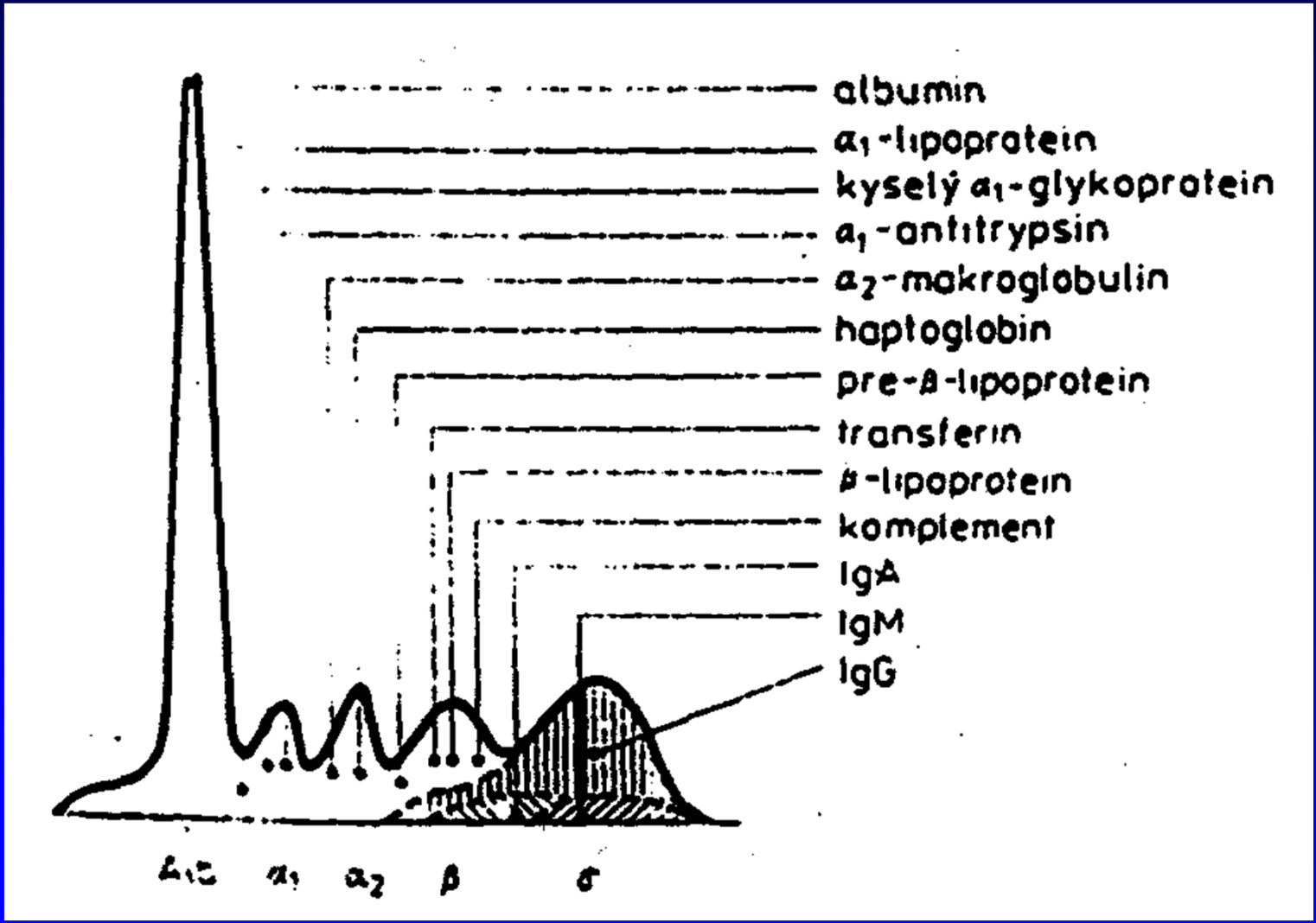
Agamma-globulinaemia



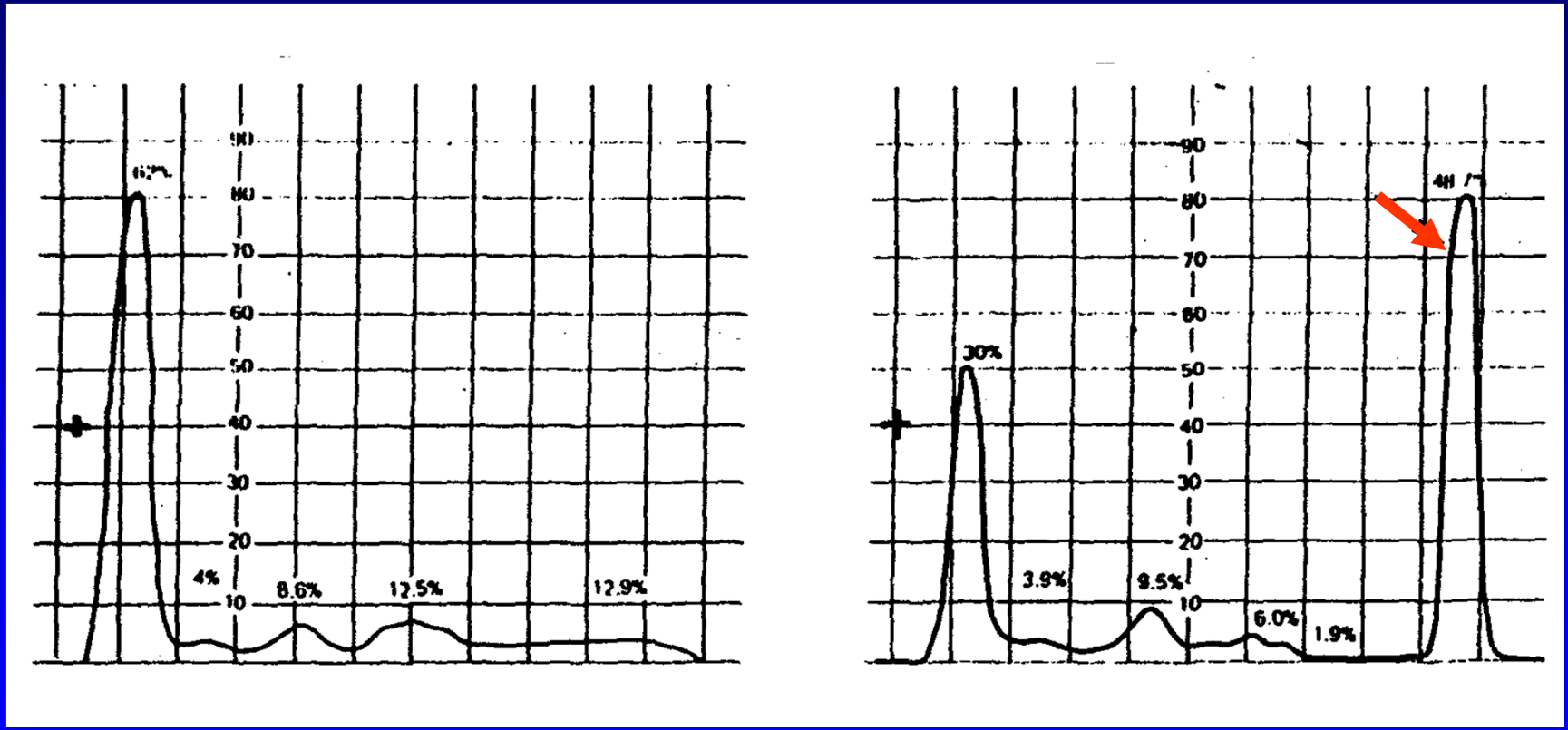
Monoclonal gammapathy



Proteins components on acetatcelulose



Reference sample Monoclonal rise

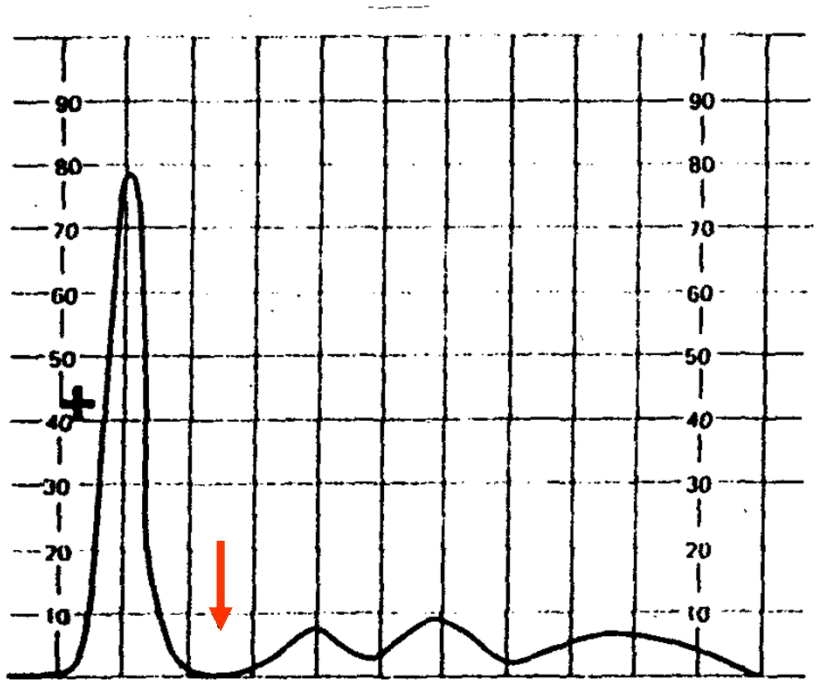


β-2-microglobulin

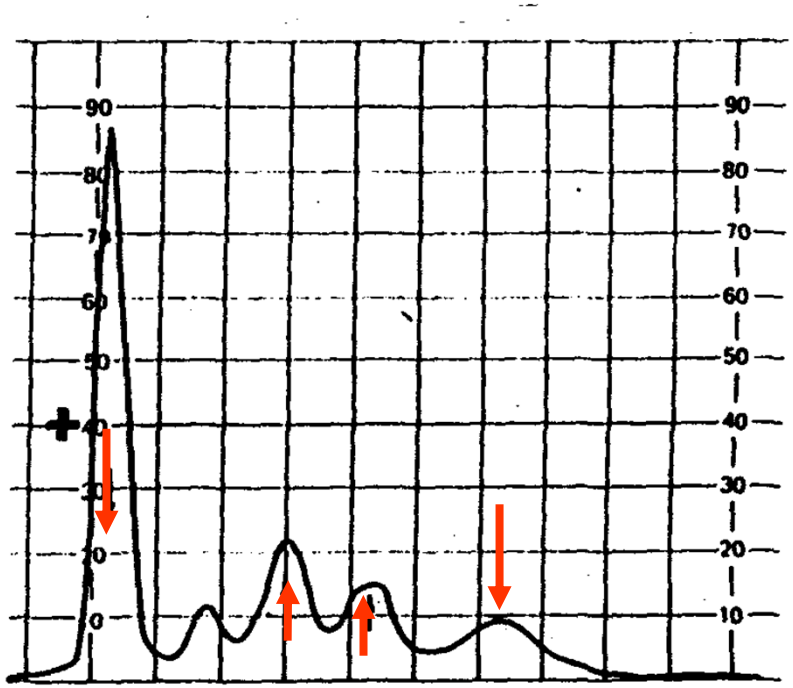
- non-glycosylated polypeptide 100 AA, Mr 11 000
  - 0.8 – 2.4 mg/l
  - part of HLA system

- TU marker – NHL, CLL, myeloma
- tubular damage

Antitrypsine def.

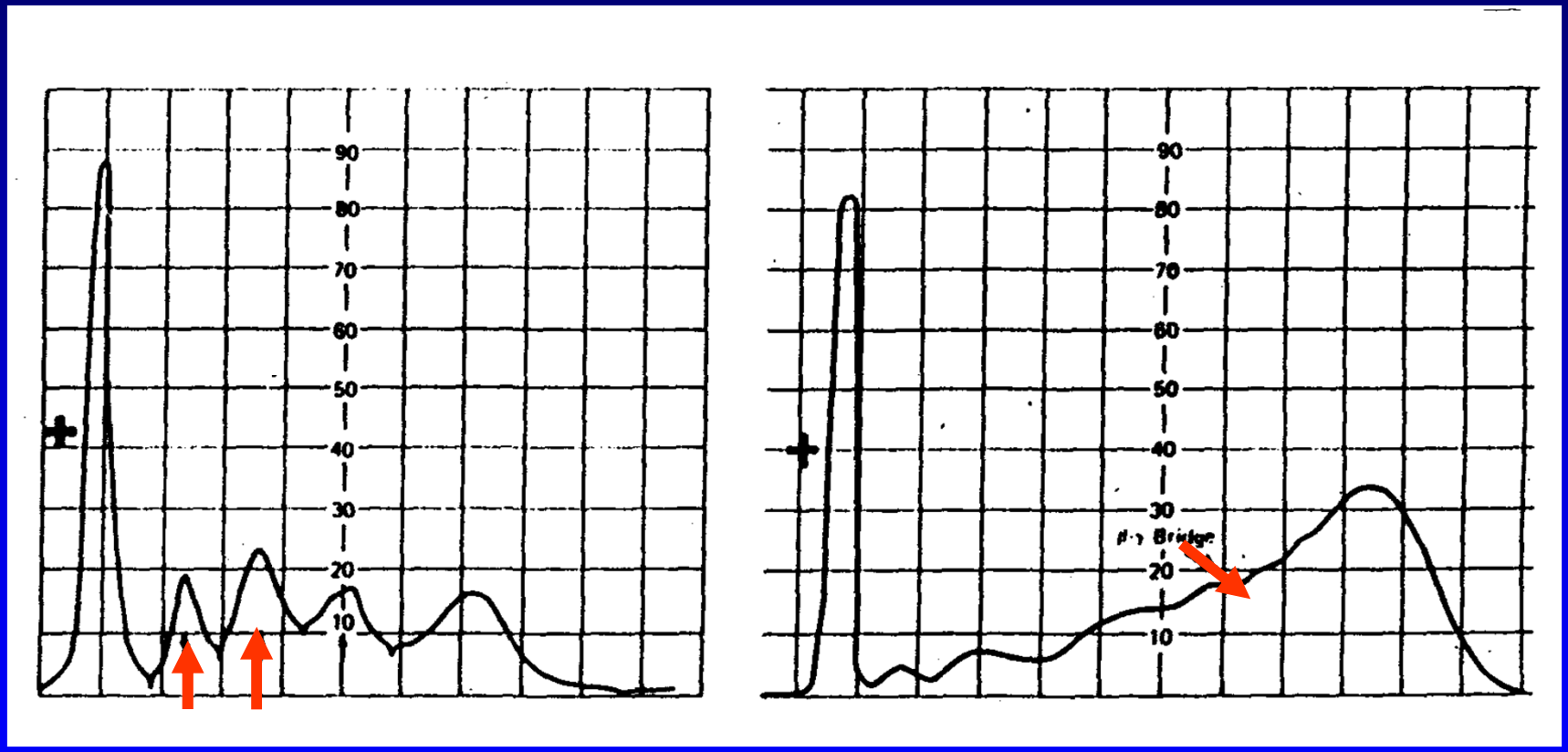


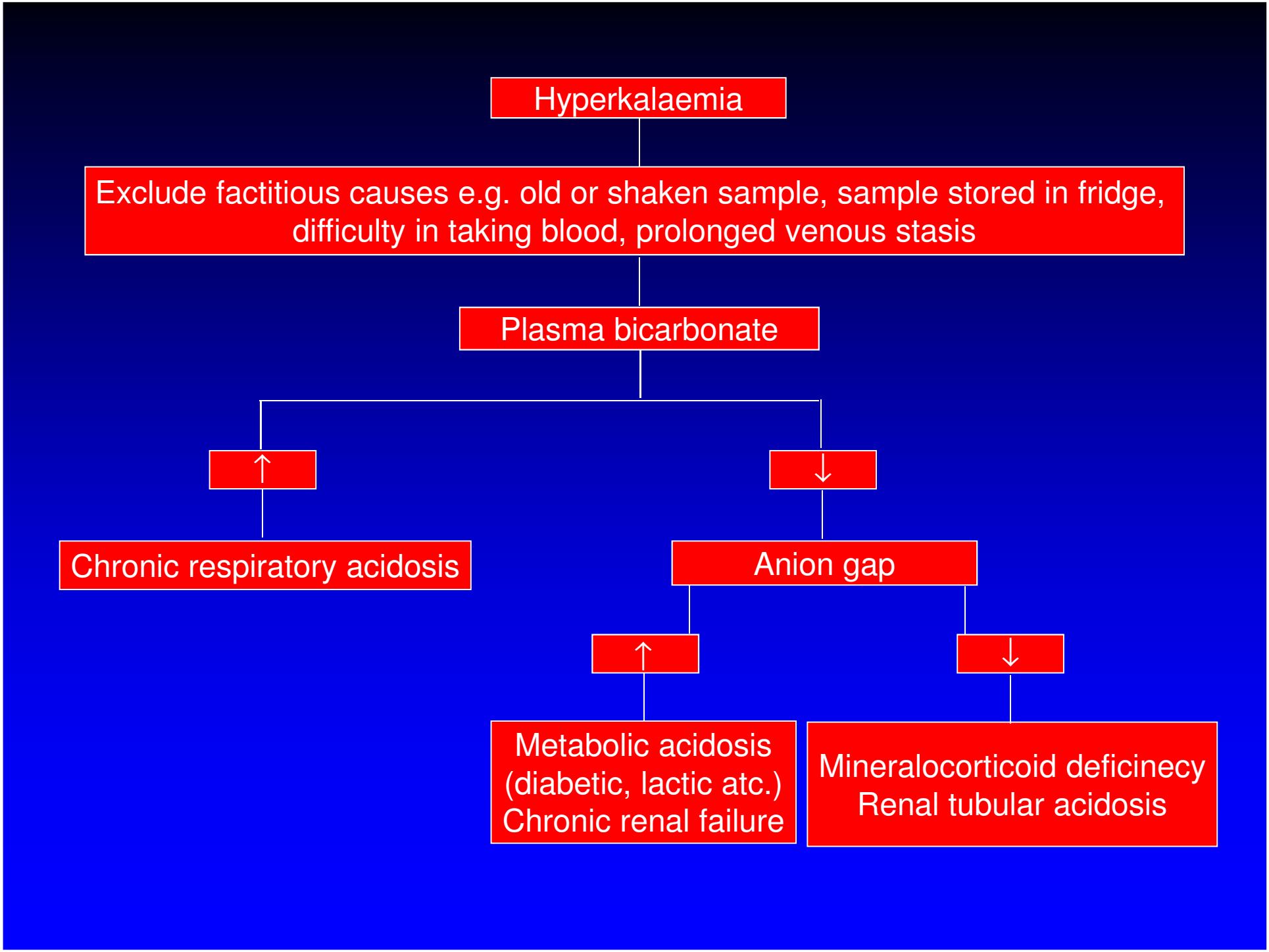
Nephrotic syndrome

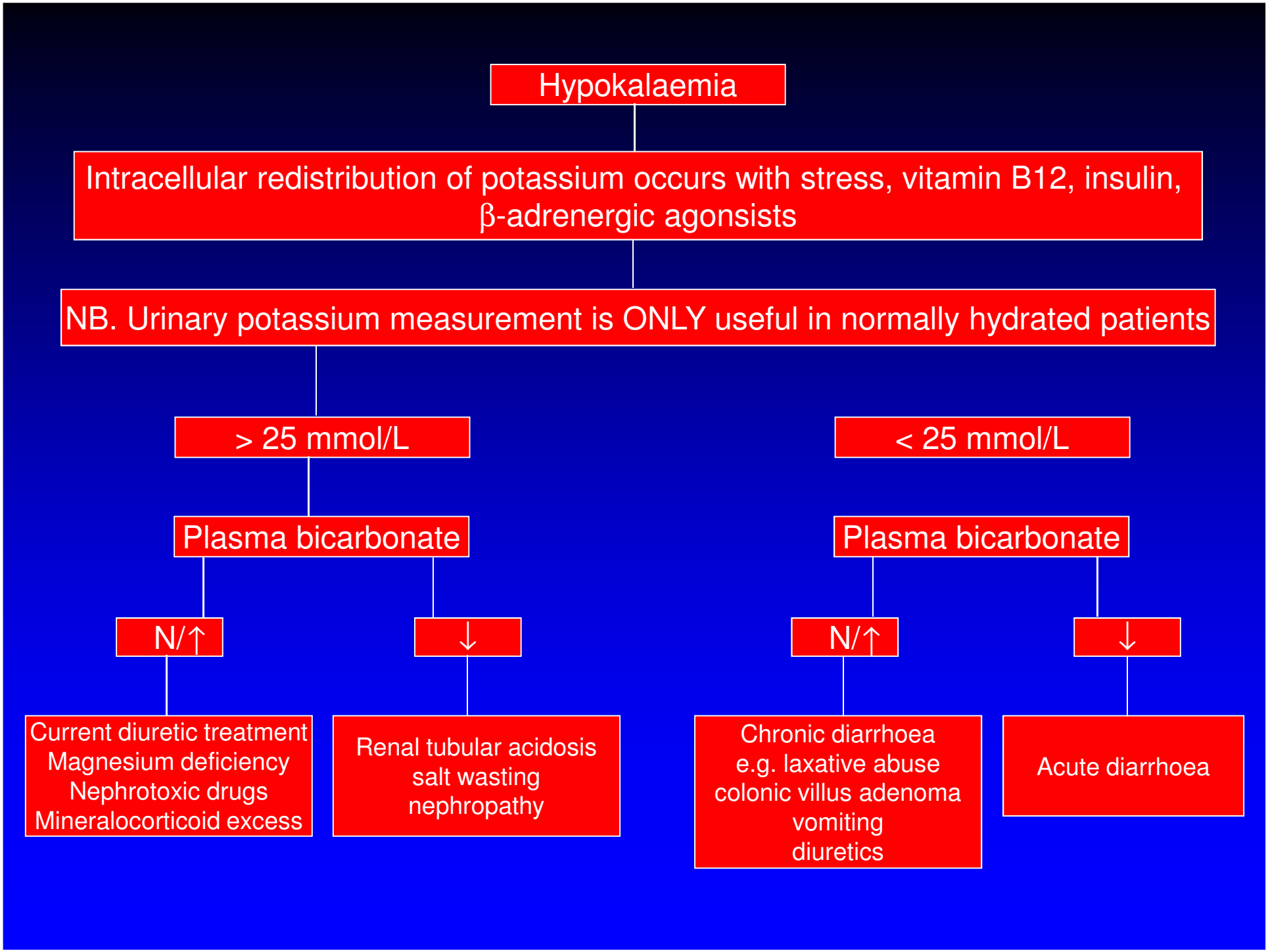


Inflammation

Cirrhosis




Hyperkalaemia
Exclude factitious causes e.g. old or shaken sample, sample stored in fridge, difficulty in taking blood, prolonged venous stasis
Plasma bicarbonate
↑
↓
Chronic respiratory acidosis
Anion gap
↑
↓
Metabolic acidosis (diabetic, lactic atc.) Chronic renal failure
Mineralocorticoid deficinecy Renal tubular acidosis


Hypokalaemia
Intracellular redistribution of potassium occurs with stress, vitamin B12, insulin, β-adrenergic agonsists
NB. Urinary potassium measurement is ONLY useful in normally hydrated patients
> 25 mmol/L
< 25 mmol/L
Plasma bicarbonate
Plasma bicarbonate
N/↑
↓
N/↑
↓
Current diuretic treatment
Magnesium deficiency
Nephrotoxic drugs
Mineralocorticoid excess
Renal tubular acidosis
salt wasting
nephropathy
Chronic diarrhoea
e.g. laxative abuse
colonic villus adenoma
vomiting
diuretics
Acute diarrhoea

Indication to protein investigation

- **oedema**
- **polyuria**
- **bleeding**
- **chronic renal disease**
- **chronic diarrhoea**
- **chronic liver disease**
- **chronic binder diseases**
- **bone pains**
- **lymphoproliferative diseases**
- **high prevalence of infections**
- **pathological finding - high ESR, proteinuria**

Relationship among diagnosis and biochemical tests

- depletion of proteins ..... inadequate synthesis, retention of water, salts
  - TP, albumin
  - ELPHO
- dehydratation
- acute and chronic inflammation
- monoclonal gammapathy ···· Immunofix
- anaemia of iron deficiency ..... transferrin, ferritin, soluble Trf receptor
- anaemia of chron. dis.
- m.Wilson ..... ceruloplasmin, copper
- haemochromatosis ..... transferrin, TIBC, ferritin
- malnutrition ..... Trf, alb, prealb

Total protein

- **Consists of**: cca 100 - 120 proteins
- **Half-life** : prealbumin 12-24 hrs
- transferrin 8 days
- albumin, 20 days
- IgE 2 days
- **Ref.range:** 65 - 85 g/l
- **Synthesis :** liver, lymphocytes, plasmocytes (Ig, complements components)

- **Degradation:** excretion, endogenous catabolism

- **Physical consequences:** colloid-osmotic pressure, transporters, enzymes, antibodies, hormones and receptors, haemostasis and coagulation, nutrition, buffer

Total protein

Decreasing

- malabsorption
- liver diseases
- enhanced losses
  - gut, urine, burns
- enhanced catabolism
  - inflammation,malignity
  - chronic diseases
- dilution
- catabolism
  - starvation

Increasing

+ hypergammaglobulinaemia
+ hypovolaemia

Types and markers of proteinuria

Form	*Causes*	*Marker*
Selective glomerular	Diabetes Hypertension Early stage	Albumin, transferin
Non-selective glomerular	postural proteinuria, glomerulopathies fever, exercise	Albumin, IgG
Tubular	Bact.Pyelonephritis Interstitial nephritis Toxic nephropaties	α_1 microglobulin, β2 microglobulin
Mixed	DM, hypertensive nephropathy Burns, chronic pyelonephritis	Albumin, total protein
Prerenal	Intravascular hemolysis, rhabdomyolysis, myeloma	Hemoglobin, myoglobin, Ig- light chains
Postrenal	Postrenal hematuria (stones, tumors)	IgG/albumin, α2 macroglobulin/ albumin

Proteinuria

roughly, quantitative
GBM up to 150 000 MW,
norm 120 mg/d
tubulointerstitial disorders generally < 1.0 g/d

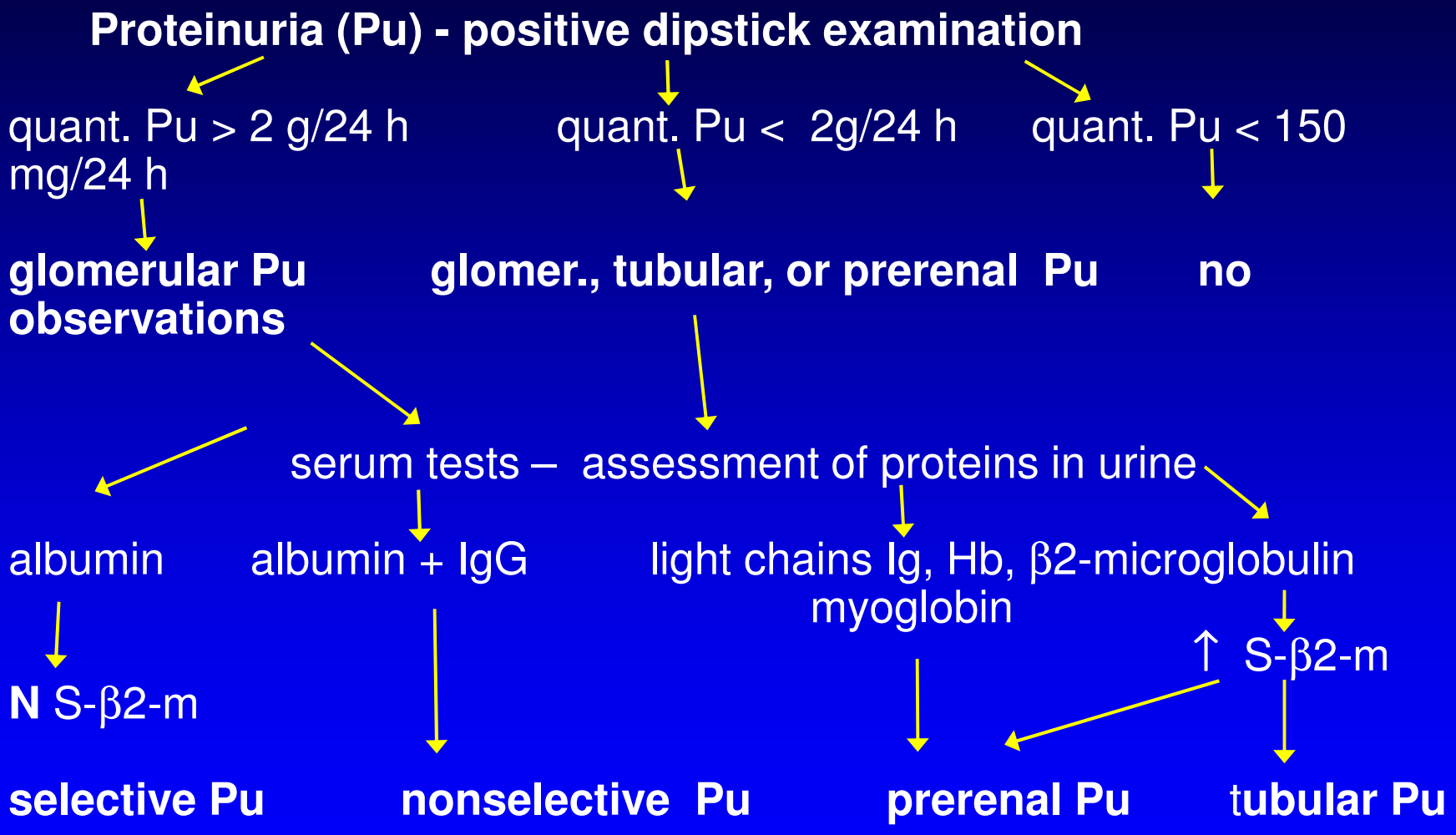
tubular proteinuria - β-2-microglobulin, α-1-microglobulin
selective - albumin & transferrin without Ig; MW < 100 000
nonselective – if immunoglobulins MW > 100 000

glomerular proteinuria - selective and non-selective

Type of proteinuria – index of selectivity : $\frac{\frac{IgG\ m}{IgG\ s}}{\frac{Trf\ m}{Trf\ s}}$

< 0,1 selective, > 0.2 non-selective
0,1 – 0,2 moderately selective

Diagnostic algorithm in patients with proteinuria



Classification of albuminuria and proteinuria

Categories of albumin-uria	**Albumin-uria** mg/24 hod	**ACR** (albumin/ creatinine ratio) mg/mmol
A1	< 30	< 3
A2	30-300	3-30
A3	>300	> 30

Categories of protein-uria	**Protein-uria** mg/24 hod	**PCR** (protein/ creatinine ratio) mg/mmol
Normal to mildly incr.	<150	<15
Moderately increased	150-500	15-50
Severaly increased	>500	>50

Albumin

Reduction

- malnutrition
- malabsoption
- increase consumption during pregnancy
- impaired synthesis- cirhosis
- increase breakdown
- losses
- dilution
- inflammation

Increase

+ dehydratation
+ i. v. application of albumin

Diagnostics of kidney diseases

- Dipstick tests for urine
  - Haematuria, glucose, ketone bodies, bilirubin, nitrites, leukocytes
- Microscopic examination of urine
  - Erythrocytes, leukocytes, tubular cells, casts
  - Hamburger's sediment
- Renal functional tests
  - Creatinine, urea, other routine parameters
  - GFR – collection, formulas, cystatin C
  - Tubular function – wastes, β-2-microglobulin
- Examination of albuminuria or proteinuria
- Immunological assays

Glomerular filtration (GFR)

Indicator of kidney function – filtration in all nephrons:
GFR = N x SNGF

SNGF = Ki x (P-π)- amount of filtration in one glomerulus

K_i – filtration coefficient

$K_i = k \times S$ (k – effective hydraulic permeability, S – filtration surface area)

P – mean transcapillary hydraulic gradient

π – mean difference of oncotic pressure

N- number of functional nephrons

Decrease of GFR in chronic renal diseases may not correspond to number of destroyed glomeruli – hypertrophic changes of residual nephrons

Factors influencing GF

Changes in renal blood flow

Changes in hydrostatic pressure in the glomerular capillaries

Changes in systemic blood pressure

Changes in hydrostatic pressure in the Bowman's capsule

Obstruction of the ureter

Renal edema in the hard case

Changes in the concentration of plasma proteins : dehydration, hypoproteinemia, etc. (less significant factors)

Kf changes

Glomerular capillary permeability changes

Currently, the most frequently used to determine the GFR are direct methods based on the principles set by Rehberger or calculation methodology based on variable values of serum creatinine or cystatin C.

- Methods for measuring GFR with the collection of urine
- Methods for measuring GFR without the collection of urine

Methods of calculation

- **CKD-EPI for creatinine and cystatine**
- **MDRD formula**
- **Prediction Ckr according to Cockcroft and Gault - obsolentní**
- **Schwarz formula for children**
- **…**

CKD-EPI for creatinine – basic recommended equation

CKD-EPI for creatinine (Levey et al. 2009)	**eGFR (ml/s/1,73 m^2)**
Women, S-creatinine ≤ 62 µmol/l	$2{,}4 \cdot (Skr/61{,}9)^{-0{,}329} \cdot 0{,}993^{age}$ ($\cdot$ 1,159 – black population)
Women, S-creatinine > 62 µmol/l	$2{,}4 \cdot (Skr/61{,}9)^{-1{,}209} \cdot 0{,}993^{age}$ ($\cdot$ 1,159 – black population)
Men, S-creatinine ≤ 80 µmol/l	$2{,}35 \cdot (Skr/79{,}6)^{-0{,}411} \cdot 0{,}993^{age}$ ($\cdot$ 1,159 – black population)
Men, S-creatinine > 80 µmol/l	$2{,}35 \cdot (Skr/79{,}6)^{-1{,}209} \cdot 0{,}993^{age}$ ($\cdot$ 1,159 – black population)

MDRD equation

- $MDRD_7$ (Levey) :

  $170. S_{cr}^{-0,999} . age^{-0,176}. N_{urea}^{-0,170} . albumin^{0,318}.0,762(♀) . 1,18$ (black population)
- The updated equation for SI units
- $eGF = 547,1535 . (S_{cr})^{-1,154} . age^{-0,203} . 0,742$ (females) . 1,21 (black population) $[ml.s^{-1}.1,73m^{-2}]$

- When using standardized methods for the determination of creatinine modified equation has the form:

  $eGF = 515,3832 . (stand\ S_{kr})^{-1,154} . age^{-0,203} . 0,742$ (females) . 1,21 (black population) $[ml.s^{-1}.1,73m^{-2}]$
-

Evaluation of MDRD eGFR

- In the calculated values higher than 1,5 $ml.s^{-1}.1,73m^{-2}$ the value recommended is ≥ 1,5 $ml.s^{-1}.1,73m^{-2}$ in view of equation inaccuracies in this area
- Values 1,0 až 1,5 $ml.s^{-1}.1,73m^{-2}$ must be individually evaluated in relation to the clinical picture
- .
- The value of EGF by the MDRD less than 1.0 is considered pathological value.
- Estimation of GFR using the MDRD formula is not recommended for use in children and pregnant women

Factors affecting examination of GF

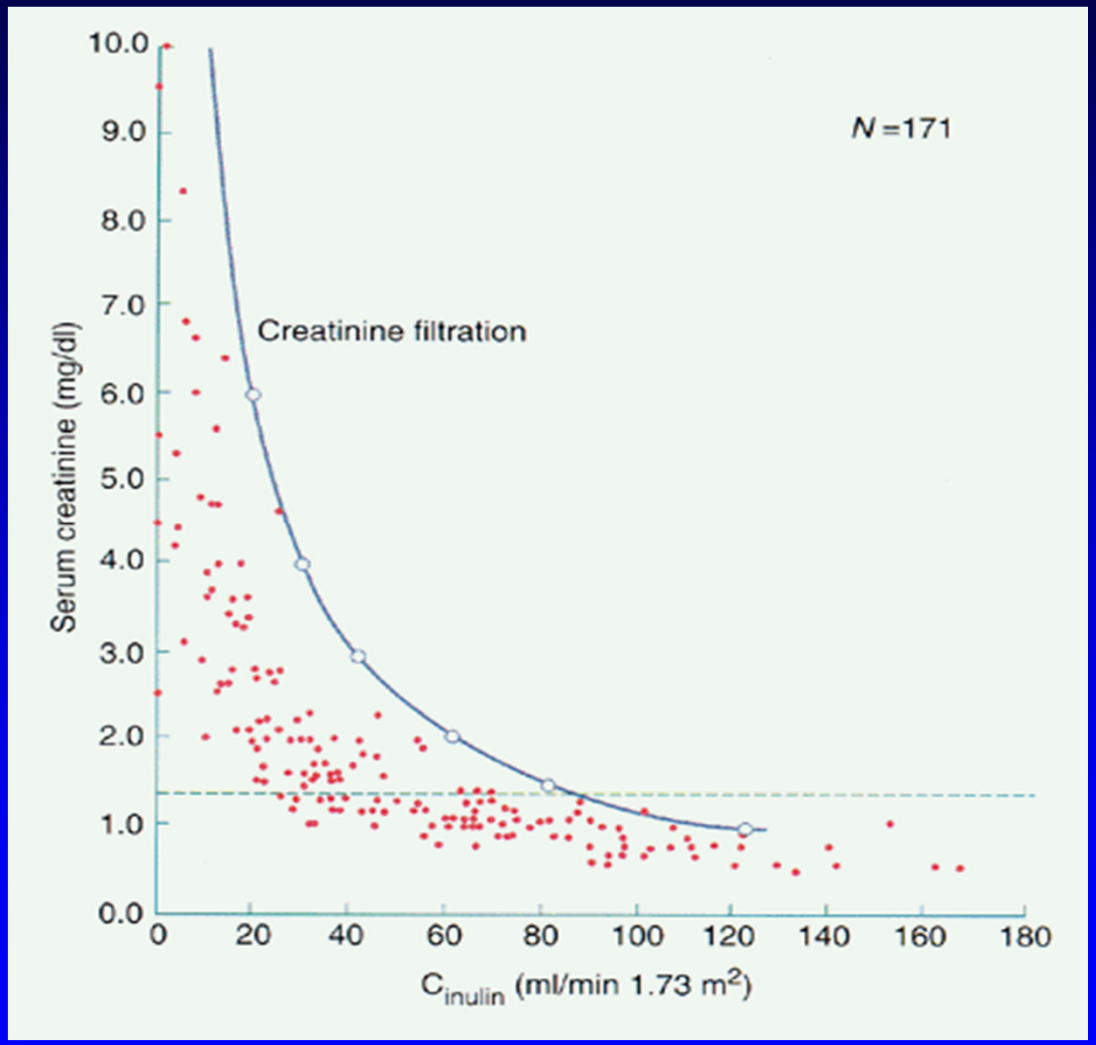
the biggest problem is the accurate collection of urine
and determination of creatine

Creatinine

- Non-enzymatic formation from creatinephosphate in muscle
- Creation reflects the size of muscle mass and is at physical rest and meatfree diet stable
- **Excretion by glomerular filtration**, secretion from tubules in increased concentration
- **Creatinine in urine** - secretion is relatively constant, reflecting the size and activity of the muscle mass, an indicator of the accuracy of 24 hour urine collection

Influence of concentration of creatinine

- **Effect of muscle mass (such as atrophy)**
- **Intake of proteins and aminoacids**
- **Age – childred and elderly**
- **Obesity, malnutrition**
- **Drugs - cefalosporins, trimetroprim, cimetidine**
- **Extrarenal creatinine excretion (especially by gut in individuals with advanced stages of CKD)**
- **Changes in creatinine distribution space (particularly in individuals with large swelling)**
- **Liver function – creatine (creatinine substrate) is formed in the liver**



Relation between serum creatinine concentration and glomerular filtration rate, measured as clearance of inulin, in patients with glomerular disease.

From 40 years of age gradual *decline* of GF occurs, which at age of 80 to 90 years is approximately half the value compared to individuals between 20 to 30 years This decline is probably the result of *decline in the flow of blood and plasma through the kidney*.

Slow decline in GFR - aproximately 0.17 $ml.s^{-1}.1.73m^{-2}$ per decade of life.

Cystatin C is a polypeptide belonging to the group of inhibitors of cysteine proteases. It is freely filtered and subsequently absorbed and metabolized in the tubules.

CKD-EPI formula for cystatine (2012) – used in special cases - confirmation of CKD, pregnancy, children…

Combined CKD-EPI formula for cystatine and creatinine.

Acute inflammation has 2 phases:

- early :
- increase - haptoglobin, orosomukoid, ceruloplasmin, α-1-antitrypsin, fibrinogen, CRP, C3

- decrease - negative acute phase reactants *albumin, prealbumin, transferrin*

- late:
- increase - γ-globuliny

Prealbumin (Transthyretin)

- **Mr 21 000 , combination with TBG, half-life 1-2 days**
- **0.1-0.4 g/l**

- **nutrition, proteosynthesis, acute phase reactant**

↑ **steroids**

↓ **liver diseases, necrosis, acute phase reactant**

CRP

- **β-fraction, MW 110 000 - 140 000**
- ***Binds C polysaccharide from pneumococci***
- **Sensitive but non-specific**
- **physiological values under 10 mg/l (8.5)**

  determination – supersensitive CRP
- ⇧ **inflammation, FW,**

  checking of immunosuppressive therapy

α_1-acid-glycoprotein = orosomucoid

- 45 % saccharides (sialic acid)
- Function unclear
- 0.5 – 1.2 g/l, MW 44 000
- Acute phase reactant, later coming than CRP (monitoring of process – relation CRP and orosomucoid)

↑ inflammation, perinatal infection, chronic inflammation, malignities, SLE

Haptoglobin

- 0.3 – 2.0 g/l, allotypes – paternity investigation formerly
  - haptoglobin binds released haemoglobin 1:1
  - at strong haemolysis zero values

⬇ liver diseases, intravascular haemolysis, haemolytic anaemia, cirrhosis,
⬆ inflammation, operation and trauma

CAVE - inflammation + haemolysis - normal value !!

Fibrinogen

- Dimer of three chains, MW 340 000
- 2-4 g/l, half-life 2-4 days

- acute-phase reactant of coagulation,

↑ inflammation, after surgery, malignancies, RA, AMI, nephrotic syndrome, obstruction of biliary tract

↓ DIC, hyperfibrinolysis, severe liver damage, fibrinolytic therapy

Amyloid

- fibrillae of amyloid

1) AL - L chains
2) secondary chains of AA
3) Transthyretin (prealbumin) – with change of 1 amino acid

- protein precursor SAA – source of amyloid deposits in organs
- ranges 50-300 mg/l

a-1-antitrypsin

- inhibitor of serine proteases
- 1.0 – 2.0 g/l, MW 53 000
- Genetic variation e.g. ZZ (10% of activity)
- inflammation, tumors, hepatopathies
- Primary pulmonary emphysema, neonatal hepatitis, cystic fibrosis, exudative enteropathy

New markers of inflammation

- Elastase from granulocytes (60-110 ug/l)
  - Reaction to bacterial inflammation
- Procalcitonin (do 0.5 ug/l)
  - 116 AK, Mr 13 000
  - stimulation by bacterial and mycotic infection
- Neopterin (2-7 nmol/l)
  - production by macrophages after stimulation by INF-γ
  - excreted by T lymphocytes
  - marker of cellular immunity

Ceruloplasmin

- **MW 140 000, six copper atoms, α-2 fraction**
  - **90 % of copper in serum**
  - **higher values at women,**
  - **0.2 – 0.6 g/l**

⇩ m. Wilson, nephrotic syndrome

⇧ intrahepatal cholestasis, inflammation, AMI

Reactive forms of oxygen and nitrogen

Radicals

- Superoxide anion (superoxide)
- Hydroxyl radical
- Peroxyl radical
- Alcoxyl radicals
- Hydroperoxyl radical
- Nitric oxide

Reactive forms

- Peroxid hydrogen
- Singlet oxygen
- Hypochlorous acid
- Ozone
- Nitrous acid
- Peroxynitrit
- Nitronium
- Nitrosyl
- Nitroxid
- Alkylperoxynitril

Metods of detection -free radical damage

direct measurement

- **electron spin resonance (ESR)**
- **radical trapping**
- **pulse radiolysis**
- **chemiluminiscence**

Metods of detection -free radical damage

Indirect measurement

- **antioxidant systems**
  - determination of individual substances (enzymes, substrates, trace elements)
  - total antioxidant capacity
- **determination of autoantibodies**
- **Determination of substances generated during radical reaction**
  - malondialdehyde
  - conjugated diens
  - hydroperoxides of lipids
  - aldehydes (4-HNE)
  - penthane, ethane
  - damaged DNA bases
  - modificated aminoacids
  - oxLDL
  - AGEs and AOPP