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, 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
<table>
<thead>
<tr>
<th>Test</th>
<th>Unit</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>Prerenal uraemia</strong></td>
</tr>
<tr>
<td>U-Na⁺</td>
<td>mmol/l</td>
<td>5-35</td>
</tr>
<tr>
<td>FE_{Na⁺}</td>
<td>%</td>
<td>0.2-1.0</td>
</tr>
<tr>
<td>U-Cl⁻</td>
<td>mmol/l</td>
<td>5-25</td>
</tr>
<tr>
<td>FE_{Cl⁻}</td>
<td>%</td>
<td>0.10-1.0</td>
</tr>
<tr>
<td>U/S osmolality</td>
<td></td>
<td>1.0-3.0</td>
</tr>
<tr>
<td>U/S urea</td>
<td></td>
<td>0.5-90 (depends on protein intake, eventually on breakdown of tissue proteins)</td>
</tr>
<tr>
<td>U/S creatinine</td>
<td></td>
<td>20-130</td>
</tr>
<tr>
<td>S-urea/creatinine</td>
<td></td>
<td>30-140</td>
</tr>
</tbody>
</table>
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 $\rightarrow$

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

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)
Electrophoresis with high resolution

<table>
<thead>
<tr>
<th>Zones</th>
<th>Serum Proteins Found in Zones</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. PREALBUMIN ZONE</td>
<td>- Prealbumin</td>
</tr>
<tr>
<td>2. ALBUMIN ZONE</td>
<td>- Albumin</td>
</tr>
<tr>
<td>3. ALBUMIN-ALPHA-1 INTERZONE</td>
<td>- Alpha-lipoprotein (Alpha-1-antitrypsin)</td>
</tr>
<tr>
<td>4. ALPHA-1 ZONE</td>
<td>- Alpha-1-acid glycoprotein</td>
</tr>
<tr>
<td>5. ALPHA-1-ALPHA-2 INTERZONE</td>
<td>- C3</td>
</tr>
<tr>
<td>6. ALPHA-2 ZONE</td>
<td>- IgA, (Fibrinogen), IgM (Monoclonal Ig’s, light chains)</td>
</tr>
<tr>
<td>7. ALPHA-2-BETA-1 INTERZONE</td>
<td>- IgG, (C-reactive protein) (Monoclonal Ig’s, light chains)</td>
</tr>
<tr>
<td>8. BETA-1 ZONE</td>
<td></td>
</tr>
<tr>
<td>9. BETA-1-BETA-2 INTERZONE</td>
<td></td>
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<tr>
<td>10. BETA-2 ZONE</td>
<td></td>
</tr>
<tr>
<td>11. GAMMA-1 ZONE</td>
<td></td>
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<tr>
<td>12. GAMMA-2 ZONE</td>
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<tr>
<td>CUST.</td>
<td>P-COM.</td>
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</tbody>
</table>
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. Inflamm, DM:** ↑ β-fraction
- **Age:** ↓ Albumin, ↑ α-2 fraction, ↑ β-fraction
Results of capillary electrophoresis

Electrophoresis of serum proteins

<table>
<thead>
<tr>
<th>Fractions</th>
<th>%</th>
<th>Range [%]</th>
<th>Range g/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>60.7</td>
<td>55.1 - 65.7</td>
<td>40.1 - 47.8</td>
</tr>
<tr>
<td>Alpha 1</td>
<td>4.3</td>
<td>3.1 - 5.6</td>
<td>2.2 - 4.1</td>
</tr>
<tr>
<td>Alpha 2</td>
<td>10.2</td>
<td>8.0 - 12.7</td>
<td>5.8 - 9.2</td>
</tr>
<tr>
<td>Beta 1</td>
<td>6.0</td>
<td>4.9 - 7.2</td>
<td>3.6 - 5.2</td>
</tr>
<tr>
<td>Beta 2</td>
<td>4.8</td>
<td>3.1 - 6.1</td>
<td>2.2 - 4.5</td>
</tr>
<tr>
<td>Gamma</td>
<td>14.0</td>
<td>10.3 - 18.2</td>
<td>7.5 - 13.2</td>
</tr>
</tbody>
</table>

Electrophoresis of normal serum

<table>
<thead>
<tr>
<th>Fractions</th>
<th>%</th>
<th>Range [%]</th>
<th>Range g/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>56.4</td>
<td>55.1 - 65.7</td>
<td>40.1 - 47.8</td>
</tr>
<tr>
<td>Alpha 1</td>
<td>3.1</td>
<td>3.1 - 5.6</td>
<td>2.2 - 4.1</td>
</tr>
<tr>
<td>Alpha 2</td>
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<tr>
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</tr>
<tr>
<td>Gamma</td>
<td>20.3</td>
<td>10.3 - 18.2</td>
<td>7.5 - 13.2</td>
</tr>
</tbody>
</table>

Electrophoresis of biclonal PP

<table>
<thead>
<tr>
<th>Fractions</th>
<th>%</th>
<th>Range [%]</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>56.4</td>
<td>55.1 - 65.7</td>
<td>40.1 - 47.8</td>
</tr>
<tr>
<td>Alpha 1</td>
<td>3.1</td>
<td>3.1 - 5.6</td>
<td>2.2 - 4.1</td>
</tr>
<tr>
<td>Alpha 2</td>
<td>10.7</td>
<td>8.0 - 12.7</td>
<td>5.8 - 9.2</td>
</tr>
<tr>
<td>Beta 1</td>
<td>6.2</td>
<td>4.9 - 7.2</td>
<td>3.6 - 5.2</td>
</tr>
<tr>
<td>Beta 2</td>
<td>3.3</td>
<td>3.1 - 6.1</td>
<td>2.2 - 4.5</td>
</tr>
<tr>
<td>Gamma</td>
<td>20.3</td>
<td>10.3 - 18.2</td>
<td>7.5 - 13.2</td>
</tr>
</tbody>
</table>

TP : g/l

PP component M
The most important gammapathies

Normal finding

Polyclonal gammapathy

Agammaglobulinaemia

Monoclonal gammapathy
Proteins components on acetatcelulose

- albumin
- $\alpha_1$-lipoprotein
- kyselý $\alpha_1$-glykoprotein
- $\alpha_1$-antitrypsin
- $\alpha_2$-makroglobulin
- haptoglobin
- pre-$\beta$-lipoprotein
- transferin
- $\beta$-lipoprotein
- komplement
- IgA
- IgM
- IgG
Reference sample  Monoclonal rise
β-2-microglobulin

- non-glycosylated polypeptide 100 AA, Mr 11000
  - 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 deficiency
Renal tubular acidosis
Hypokalaemia

Intracellular redistribution of potassium occurs with stress, vitamin B12, insulin, β-adrenergic agonists

NB. Urinary potassium measurement is ONLY useful in normally hydrated patients

> 25 mmol/L

Plasma bicarbonate

N/↑

- Current diuretic treatment
- Magnesium deficiency
- Nephrotoxic drugs
- Mineralocorticoid excess

↓

- Renal tubular acidosis
- salt wasting nephropathy

< 25 mmol/L

Plasma bicarbonate

N/↑

- 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
- dehydration
- acute and chronic inflammation
- monoclonal gammapathy
- anaemia of iron deficiency
- anaemia of chron. dis.
- m.Wilson
- haemochromatosis
- malnutrition

- TP, albumin
- ELPHO
- Immunofix
- transferrin, ferritin, soluble Trf receptor
- ceruloplasmin, copper
- transferrin, TIBC, ferritin
- 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 (lg, 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

<table>
<thead>
<tr>
<th>Form</th>
<th>Causes</th>
<th>Marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selective glomerular</td>
<td>Diabetes, Hypertension, Early stage</td>
<td>Albumin, transferin</td>
</tr>
<tr>
<td>Non-selective glomerular</td>
<td>postural proteinuria, glomerulopathies, fever, exercise</td>
<td>Albumin, IgG</td>
</tr>
<tr>
<td>Tubular</td>
<td>Bact. Pyelonephritis, Interstitial nephritis, Toxic nephropaties</td>
<td>α₁ microglobulin, β₂ microglobulin</td>
</tr>
<tr>
<td>Mixed</td>
<td>DM, hypertensive nephropathy, Burns, chronic pyelonephritis</td>
<td>Albumin, total protein</td>
</tr>
<tr>
<td>Prerenal</td>
<td>Intravascular hemolysis, rhabdomyolysis, myeloma</td>
<td>Hemoglobin, myoglobin, Ig- light chains</td>
</tr>
<tr>
<td>Postrenal</td>
<td>Postrenal hematuria (stones, tumors)</td>
<td>IgG/albumin, α₂ macroglobulin/ albumin</td>
</tr>
</tbody>
</table>
Proteinuria

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

tubular proteinuria - \( \beta \)-2-microglobulin, \( \alpha \)-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:
\[
\frac{\text{IgG } m}{\text{IgG } s} \quad \frac{\text{Trf } m}{\text{Trf } s}
\]

< 0.1 selective, > 0.2 non-selective
0.1 – 0.2 moderately selective
Diagnosis of Proteinuria

**Proteinuria (Pu) - positive dipstick examination**

- **Quant. Pu > 2 g/24 h**
  - Glomerular Pu
  - Observations
  - Serum tests – assessment of proteins in urine
    - Albumin
    - Albumin + IgG
    - Light chains Ig, Hb, β2-microglobulin
    - Myoglobin
    - S-β2-m
- **Quant. Pu < 2 g/24 h**
  - Glomerular, tubular, or prerenal Pu
  - No observations
  - Serum tests – assessment of proteins in urine
    - Albumin
    - Albumin + IgG
    - Light chains Ig, Hb, β2-microglobulin
    - Myoglobin
    - S-β2-m
- **Quant. Pu < 150 mg/24 h**
  - Glomerular Pu
  - Observations
  - Serum tests – assessment of proteins in urine
    - Albumin
    - Albumin + IgG
    - Light chains Ig, Hb, β2-microglobulin
    - Myoglobin
    - S-β2-m

**Selective Pu**

- Nonselective Pu

**Prerenal Pu**

- Tubular Pu
# Classification of albuminuria and proteinuria

<table>
<thead>
<tr>
<th>Categories of albuminuria</th>
<th>Albuminuria mg/24 hod</th>
<th>ACR (albumin/creatinine ratio) mg/mmol</th>
<th>Categories of proteinuria</th>
<th>Proteinuria mg/24 hod</th>
<th>PCR (protein/creatinine ratio) mg/mmol</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>&lt; 30</td>
<td>&lt; 3</td>
<td>Normal to mildly incr.</td>
<td>&lt;150</td>
<td>&lt;15</td>
</tr>
<tr>
<td>A2</td>
<td>30-300</td>
<td>3-30</td>
<td>Moderately increased</td>
<td>150-500</td>
<td>15-50</td>
</tr>
<tr>
<td>A3</td>
<td>&gt;300</td>
<td>&gt; 30</td>
<td>Severely increased</td>
<td>&gt;500</td>
<td>&gt;50</td>
</tr>
</tbody>
</table>
Reduction
- malnutrition
- malabsorption
- increase consumption during pregnancy
- impaired synthesis-cirrhosis
- 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:
\[ \text{GFR} = N \times \text{SNGF} \]

\[ \text{SNGF} = K_i \times (P-\pi) \] - 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
- \( \pi \) – 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

<table>
<thead>
<tr>
<th>CKD-EPI for creatinine (Levey et al. 2009)</th>
<th>eGFR (ml/s/1.73 m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women, S-creatinine ≤ 62 µmol/l</td>
<td>2.4 . (Skr/61,9)^-0.329 . 0.993^{age} (. 1.159 – black population)</td>
</tr>
<tr>
<td>Women, S-creatinine &gt; 62 µmol/l</td>
<td>2.4 . (Skr/61,9)^-1.209 . 0.993^{age} (. 1.159 – black population)</td>
</tr>
<tr>
<td>Men, S-creatinine ≤ 80 µmol/l</td>
<td>2.35 . (Skr/79,6)^-0.411 . 0.993^{age} (. 1.159 – black population)</td>
</tr>
<tr>
<td>Men, S-creatinine &gt; 80 µmol/l</td>
<td>2.35 . (Skr/79,6)^-1.209 . 0.993^{age} (. 1.159 – black population)</td>
</tr>
</tbody>
</table>
**MDRD equation**

- **MDRD$_7$ (Levey):**
  
  \[
  170 \cdot S_{cr}^{-0.999} \cdot \text{age}^{-0.176} \cdot N_{\text{urea}}^{-0.170} \cdot \text{albumin}^{0.318} \cdot 0.762(♀) \cdot 1.18 \text{ (black population)}
  \]

- **The updated equation for SI units**

- **eGF = 547,1535 \cdot (S_{cr})^{-1.154} \cdot \text{age}^{-0.203} \cdot 0.742 \text{ (females)} \cdot 1.21 \text{ (black population)} \text{ [ml.s}^{-1}. \text{1.73m}^{-2}]**

- **When using standardized methods for the determination of creatinine modified equation has the form:**
  
  **eGF = 515,3832 \cdot (\text{stand } S_{kr})^{-1.154} \cdot \text{age}^{-0.203} \cdot 0.742 \text{ (females)} \cdot 1.21 \text{ (black population)} \text{ [ml.s}^{-1}. \text{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 \(\geq 1,5\) 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 – children 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 - approximately $0.17 \text{ ml.s}^{-1}.1.73\text{m}^{-2}$ 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
$\alpha_1$-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

\[ \text{a-1-antitrypsin} \]

- 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
- Peroxy radical
- Alcoxy 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
Methods of detection - free radical damage

**Indirect measurement**

- **Antioxidant systems**
  - determination of individual substances (enzymes, substrates, trace elements)
  - total antioxidant capacity

- **Determination of substances generated during radical reaction**
  - malondialdehyde
  - conjugated dienes
  - hydroperoxides of lipids
  - aldehydes (4-HNE)
  - penthane, ethane
  - damaged DNA bases
  - modified aminoacids
  - oxLDL
  - AGEs and AOPP

- **Determination of autoantibodies**