Preanalytical variables. POCT.
Metabolic diseases

Ivan Šebesta

ÚLBLD 1.LF UK
Circuit diagram of the clinical biochemistry process
How biochemical tests are used
INFORMATION ABOUT PATIENT

• case history

• physical examination

• imaging studies (x-ray, EEG, etc.)

• laboratory tests
  • clinical chemistry (60 – 70%)
  • haematology
  • microbiology
  • immunology
The results of laboratory tests are useful and effective informations under following conditions:

- proper indication
- rapid availability
- accurancy
- proper interpretation
The most useful is rapid information from bedside testing near the patient (POCT). This can be done in:

1) simple test – bed side testing

2) general practitioner’s office

3) primary health care laboratory
A portable bench analyser
CLINICAL CHEMISTRY INFORMATION

• gives information about metabolic functions

• has wide range and high specificity

• is there quantification

• is relatively easy available

• is relatively harmless to the patient
Blood specimen tubes for specific biochemical tests

- Plain tube: no anticoagulant
  - Clot forms
  - General

- Plain tube: contains SST gel
  - General

- EDTA anticoagulant
  - Whole blood analysis
  - Red cell analysis
  - Lipids and lipoproteins

- Lithium heparin anticoagulant
  - General

- Fluoride oxalate
  - Glucose
  - Lactate

- Heparinized syringe
  - Arterial blood sampling
INTERPRETING RESULTS

Before considering diagnosis or treatment based on an analytical results the clinicians should ask himself three questions:

1) If it is the first time the estimation has been performed in this patients, IS IT NORMAL OR ABNORMAL?

2) If it is abnormal, IS THE ABNORMALITY OF DIAGNOSTIC VALUE or is it a no-specific finding?

3) If it is one of a series of results, HAS THERE BEEN A CHANGE, AND IF SO, IS THIS CHANGE CLINICALLY SIGNIFICANT?
Several commonly asked questions may be answered, at least in part, by laboratory testing.

1) Is the diagnosis correct?
   Proper selected laboratory tests may corroborate or refute a working diagnosis.

2) What is the etiology of the disease?

3) How severe is the disease?

4) Has the patient’s condition improved or deteriorated?

5) Is the patient at risk for disease or is there a disease not clinically apparent?
Some of the factors that can cause a test result to misrepresent the physiology of the patient

**Interpreting a Test**

- Inappropriate Reference Range
- Wrong Patient
- Wrong Container
- Sample Site
- Sample Mishandling
- Storage
- Analytical Problem
- Misreported
- Patient Preparation
  - Stress
  - Sex
  - Age
  - Drugs
  - Medical history
  - Food/Drink

LABORATORY
Schematic diagram of lipoprotein particle:

- Apolipoprotein
- Polar shell
- Non-polar core
- Phospholipids
- Esterified cholesterol
- Free cholesterol
- Triacylglycerols
Determination of lipoproteins:

- **An ultracentrifugation** (to distinguish various classes according to the hydrated density): VLDL, IDL, LDL, HDL
- **Electrophoretically**: α-lipoproteins, pre-β-lipoproteins, β-lipoproteins, chylomicrons
- **Immunochemical methods**: Apo A, Apo B, Apo C, Apo D, Apo E,
Lipoprotein electrophoresis

α-lipoproteins
Pre-β-lipoproteins
chylomicrons

Lp(a)
β-lipoproteins
Basic investigations of lipid metabolism

- **Cholesterol**: 3.8 - 5.2 mmol/l
- **TAG**: 0.9 - 1.7 mmol/l
- **HDL**: > 0.9 mmol/l
- **LDL**: < 4.5 mmol/l
Target values of Czech Society for Atherosclerosis

- **Cholesterol** 4.5 – 5.0 mmol/l  
  (at individuals with decreased risk to 6.0 mmol/l)
- **HDL** > 0.9 mmol/l
- **LDL**  
  < 2.5 mmol/l  at secondary prevention  
  < 3 – 3.5 mmol/l  at increased risk  
  < 4 – 4.5 mmol/l  at decreased risk
- **TAG**  < 2.3 mmol/l
Additional tests

- **calculation of LDL cholesterol after Friedewald formula:**
  
  \( \text{LDL} = \text{total cholesterol} - (\text{HDL} + \text{TAG} \times 0.37) \) [mmol/l]

- atherogenic index \( \text{AI} = \frac{\text{total cholesterol} - \text{HDL}}{\text{HDL}} \)

- investigation of apo A-I and apo B-100
- electrophoresis of lipoproteins
Primary hypercholesterolelemias

- **Familial hypercholesterolemia**
  - a disorder of LDL receptors
  - cholesterol:
    - heterozygotes 7-15 mmol/l (ICD 30-50 years)
    - homozygotes 15-30 mmol/l (MI to 20 years)
  - increased concentration of LDL cholesterol and Apo B
Primary hypercholesterolemias

- Familial defective Apo B100
  - a point mutation and a replacement of one amino acid in the position 3500 on the huge Apo B100 molecule
  - cholesterol: 7-10 mmol/l

- Polygenic hypercholesterolemia
  - a combination of adverse genetic and external factors
  - cholesterol: 8 mmol/l approximately
Combined hyperlipidemias

- **Familial combined hyperlipidemia**
  - an intensive Apo B synthesis in liver with a concomitant increased production of VLDL and LDL (high atherogenic particles)
  - a frequent cause of ICD and MI to 60 years
  - cholesterol 10 - 15 mmol/l
  - TAG 2.3 - 5.7 mmol/l

- **Familial dysbetalipoproteine mia**
  - a defective gene for ApoE - pathological lipoprotein β-VLDL
  - cholesterol 7.5 - 25 mmol/l
  - TAG 2 - 10(20) mmol/l
Primary hypertriacylglycerolelemias

- **Familial hyperlipoproteinemia type V**
  - rather uncommon disorder
  - more frequently in adults, obese, with DM and with hyperuricemia
  - an inductive factor: alcohol, drugs containing estrogens, renal insufficiency
  - increased in ELPHO: pre-β-lipoproteins and chylomicrons
  - cholesterol 7 - 13 mmol/l
  - TAG 10 - 20 mmol/l

- **Familial hyperchylomicronemia**
  - a deficit of lipoprotein lipase or Apo CII
  - TAG 20 - 120 mmol/l
  - Treatment: fats containing FA with medium chains
Primary hyperlipoproteinemias

Familial hypertriacylglycerolemia
- autosomal dominant transfer of disorder
- increased concentration of VLDL
- decreased concentration of HDL
- non-insulin-dependent diabetes mellitus adds in seniors
- cholesterol normal
- TAG to 6 mmol/l
Hypolipoproteinemias

- **Familial hypo-β-lipoproteinemia**
  - a longevity
  - low values of LDL cholesterol
  - a normal catabolism of LDL
  - a reduced production of apo B

- **A-β-lipoproteinemia**
  - a rare autosomal recessive disorder
  - heterozygotes have decreased LDL cholesterol
  - other lipids are in norm
  - homozygotes have a total deficit of lipoprotein particles containing apo B (malabsorption of fat, steatorrhea, retard grow, progressive degeneration of CNS, reduced visual sharpness, hemeralopia)
Hypolipoproteinemias

- **Hypo-α-lipoproteinemia**
  - lower HDL levels
  - a defective apo A-I (according to the location of the described case – Apo-A-I-Milano)
  - HDL cannot be produced without apo A-I
  - Apo C-II cannot be transported back into liver – relative deficiency of apo C-II
  - an increased level of VLDL

- **An-α-lipoproteinemia (Tangier disease)**
  - absence of HDL in plasma
  - extremely low levels of apo A-I and apo A-II
  - abnormally fast catabolism of HDL and apo A-I
Secondary hyperlipoproteinemia

1. **Diabetes mellitus type I**
   - insulin is an activator of lipoprotein lipase
   - if DM is decompensated
     - ketoacidosis, hypertriglyceridemia and sometimes increased cholesterol as well

2. **Diabetes mellitus type II**
   - a more intensive synthesis of VLDL in liver, insulin resistance, HDL reduction, TAG rise
   - if DM is decompensated
     - glycosylation of apo B
Secondary hyperlipoproteinemias

3 Hypothyreoidism
• thyroxine increases the biosynthesis of LDL receptors in liver and an activity of lipoprotein lipase in adipocytes (by action of cAMP) as well

4 Nephrotic syndrome
• hypoalbuminemia
• a stimulation of lipoprotein synthesis.
• increased cholesterol and TAG
Secondary hyperlipoproteinemias

5 Chronic renal failure
   • an inhibition of lipoprotein lipase in the plasma of uremic patients
   • elevated TAG

6 Primary biliary cirrhosis
   • hypercholesterolemia

7 Obesity - TAG

8 Alcoholism - TAG

9 Treatment with hormones and diuretic drugs

10 Mental anorexia
To How to recognize a patient with risk of coronary disease?

Cholesterol > 5.2 mmol/l

Yes

Binary decision:
- Male?
- Smoker?
- Diabetic?
- Hypertension?
- Familial history of early MI?
- Angina pectoris?
- Oral contraception?

No

Checkup after 5 years approximately

No

Cholesterol > 6.5 mmol/l

Yes

Q cholesterol/HDL > 5 or TAG > 2.3 mmol/l or Lp(a) > 0.3 g/l

Yes

- Dietary arrangement, monitoring
- Secondary hyperlipidemia
- Drug therapy

No

Diet and running checkups

No

Yes
Symptoms and findings referring to the presence of a hyperuricemia and indicating a serum uric acid determination:

- overweight
- disturbance of carbohydrate tolerance
- disturbances in lipid metabolism
- urolithiasis
- hypertonia
- renal diseases
- early severe atherosclerosis
- fatty liver infiltration
- family predisposition
- hemoblastoses
- cytostatic therapy and x-ray radiation
- pre-eclampsia
A determination of the uric acid concentration is also to be undertaken with:

- clinical complaints, pointing to gout and with gout therapy

With regard to early gout recognition it is important to know, that ill-defined joint complaints and the presence of other metabolic disturbances may point to a developing gout.
GOUT: THE PROTOTYPICAL CRYSTAL DEPOSITION ARTHROPATHY

Monosodium Urate Crystal
Plasma Uric Acid (µmol/l)

Gout
Low purine
High purine
Normal

Diet

Plasma Uric Acid (µmol/l)
Overview of the pathogenesis of gout

LOW PURINE DIET

Are allowed:

- 2000 mg of uric acid/week
- Once a day a normal portion (cca 150 g.) of meat, milk and diary products
- One glass of alcoholic drinks, coffee, tea

Is prohibited:

- Organ meats, some fish species (lobsters, shrimps), pulses,
- Larger quantities of alcohol
TYPES OF GOUT

primary gout:

renal hypoexcretion of uric acid
excessive intake of purines in the diet

hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency

complete – Lesch-Nyhan syndrome
partial – Kelley Seegmiler syndrome

increased activity of phosphoribosylpyrophosphate synthetase (PRPPs)

familial juvenile hyperuricemic nephropathy

unknown pathogenesis
Familial juvenile hyperuricemic nephropathy

associated with:
- early onset
- men and women equally affected (autosomal dominant)

hyperuricemia
low excretion fraction of uric acid

finding of unexplained hyperuricemia with low excretion fraction of uric acid is a risk factor for severe renal damage!
Flow chart for differential diagnosis of gout

clinical - rheumatological examination
(an important information - family history)

↓

determination of serum and urinary uric acid

↓

detailed examination of purine metabolism
HYPERURICAEMIA

Exclude Secondary causes

24h urinary urate (after 3 days purine-free diet)

> 5 mmol/d

Secretors

Idiopathic gout (~20%)
G-6-P deficiency
HGPRT deficiency

< 5 mmol/d

Non-secretors

Idiopathic gout (~80%)
Detailed examination of purine metabolism.

1. Determination of serum and urinary uric acid
   * repeatedly
   * after diet (low purine)

2. Determination of purine metabolites using HPLC in
   * urine
   * plasma
   * CSF

3. Enzyme assays in ery, lymph.
   * hypoxanthin-phosphoribosyltransferase (HGPRT)
   * phosphoribosylpyrophosphat synthetase (PRPPs)
   * adenin-phosphoribosyltransferase (APRT)
   * adenosine deaminase (ADA)
   * purine nucleosidphosphorilase (PNP)

4. DNA analýza
   URAT1 (SLC22A12), GLUT9
4-component model of urate handling

glomerular filtration 100%
reabsorption 99%
post-secretory reabsorption 40%
urine excretion

**Urate transporter**

**URAT 1-gene SLC22A12**

• OMIM 607096, GeneID 116085
• 11q13, 2 transcript variants (3206 and 2940 bp) 553 amino acids
• expressed in fetal and adult kidney
Urate transport mechanisms in human renal proximal tubule
Hereditary renal hypouricemia

mutation - gene SLC22A12
W258X- prevalent mutation


Hereditary renal hypouricemia, OMIM #220150

- new transport defect of purine metabolism

- biochemical markers
  - hypouricemia ($S_{KM} < 120 \, \mu\text{mol/l}$)
  - increased excretion fraction of uric acid ($EF_{KM} > 10\%$)

- clinical features
  - urolithiasis
  - acute renal failure (exercise-induced)
INVESTIGATION OF THE PATIENT WITH RENAL CALCULI

1) If the stone is available → send it to the lab.

2) Exclude hypercalcemia and hyperuricaemia.

3) If the plasma calcium is normal collect a 24-hour specimen of urine for urinary calcium estimation.

4) If all these tests are negative and especially if there is a family history of calculi → screen urine for cystine. If the qualitative test is positive the 24-hour excretion of cystine should be estimated.