Preanalytical variables. POCT. Metabolic diseases

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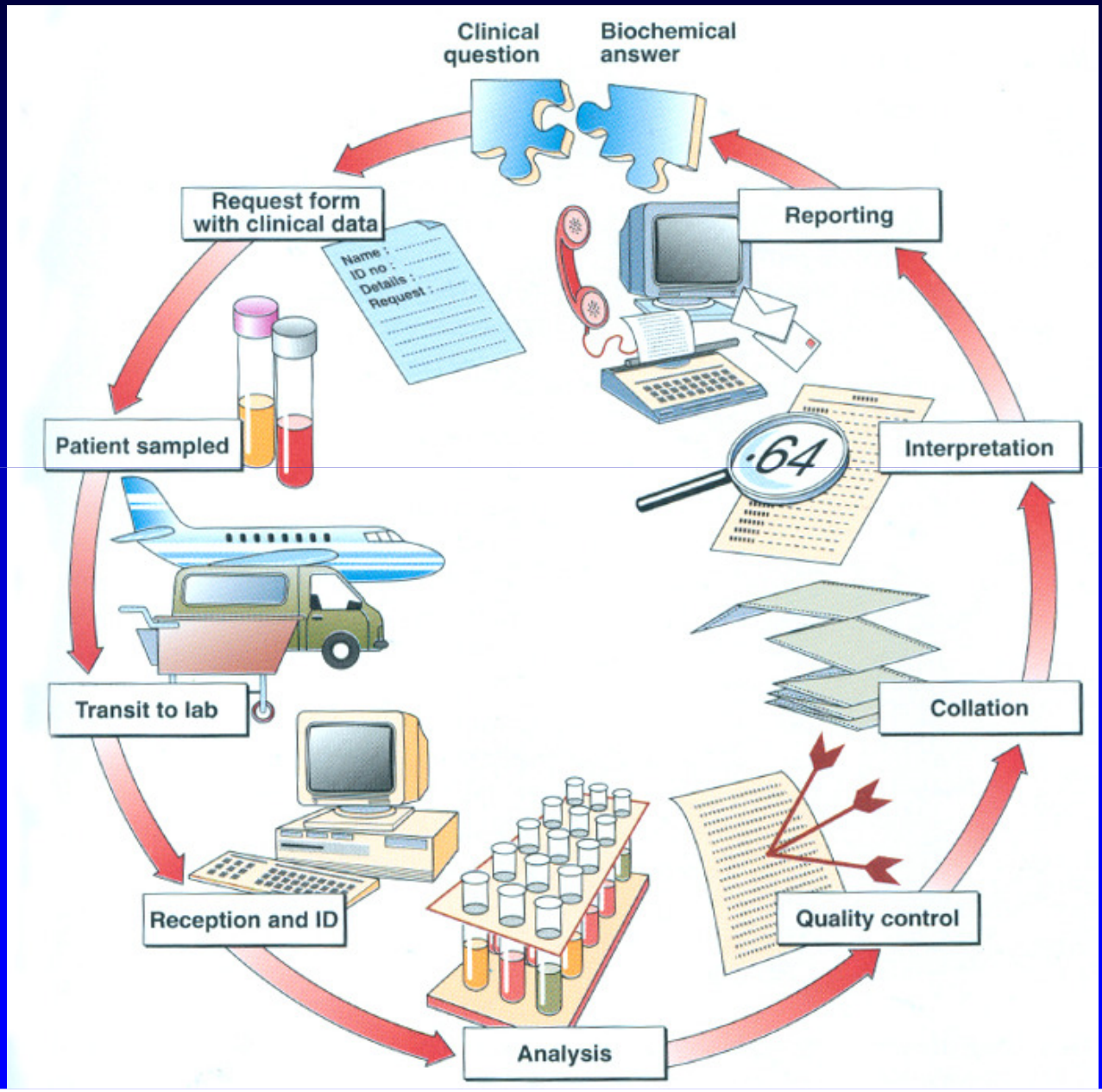
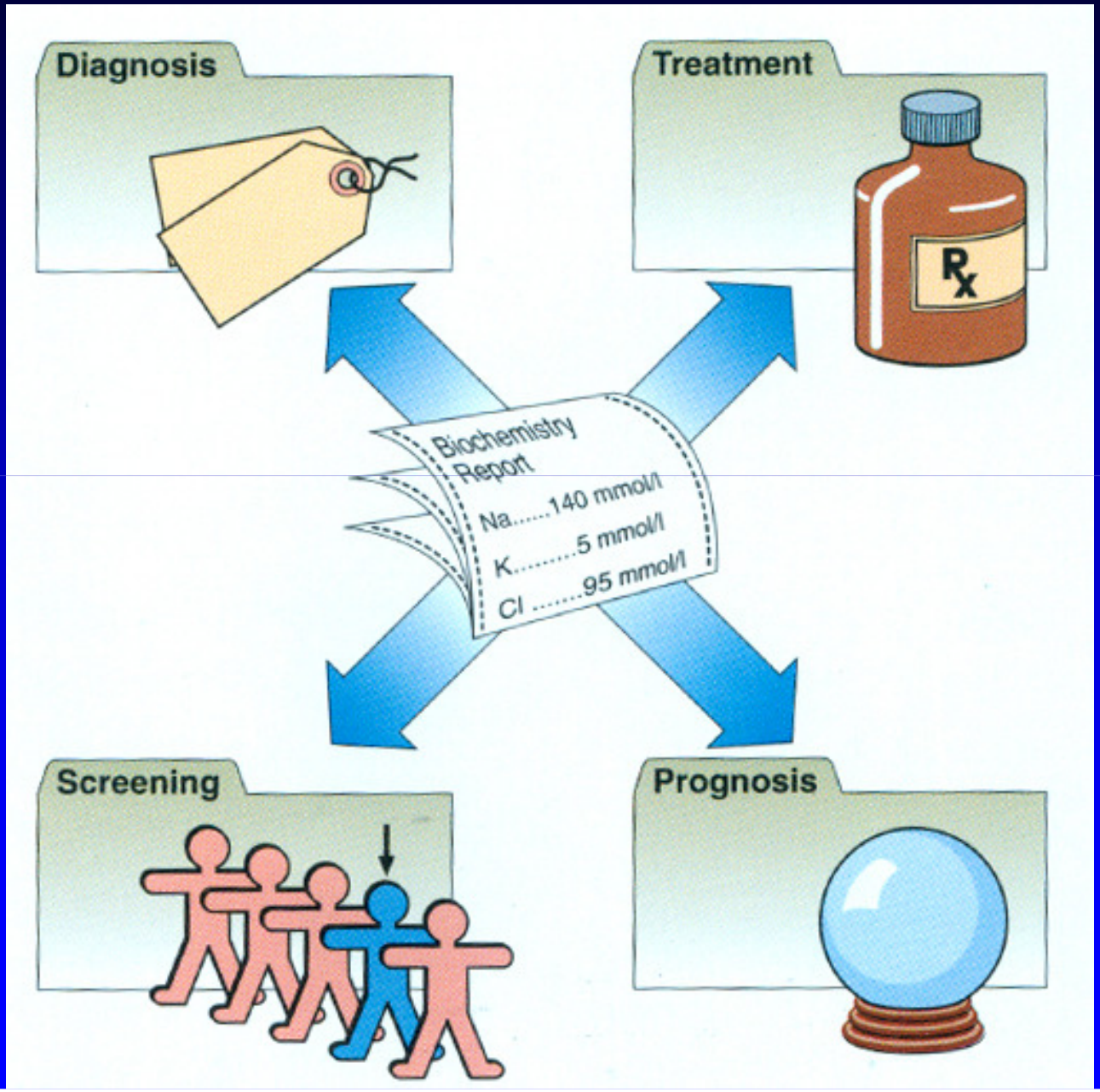
Circuit diagram of the clinical biochemistry process



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

DRY CHEMISTRY

The most useful is rapid information

Examination near the patient (POCT)

1) simple test – bed side testing

2) general practitioner's office

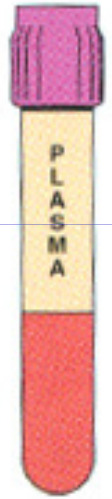
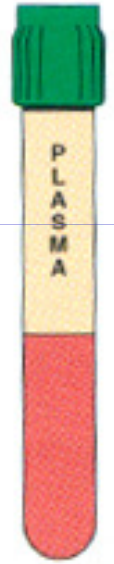
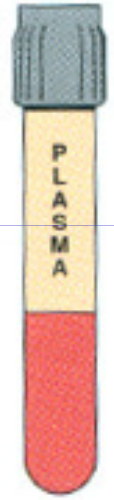
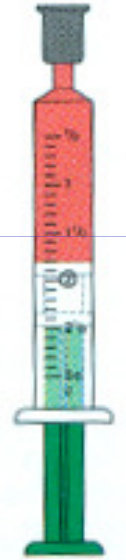
3) primary health care laboratory

A portable bench analyser

CLINICAL CHEMISTRY INFORMATION

- gives information about metabolic functions
- has wide range and high specifity
- 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	Plain tube: contains SST gel	EDTA anticoagulant	Lithium heparin anticoagulant	Fluoride oxalate	Heparinized syringe
					
• General	• General	• Whole blood analysis • Red cell analysis • Lipids and lipoproteins	• General	• Glucose • Lactate	• 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?

CHOOSING LABORATORY TESTS

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?

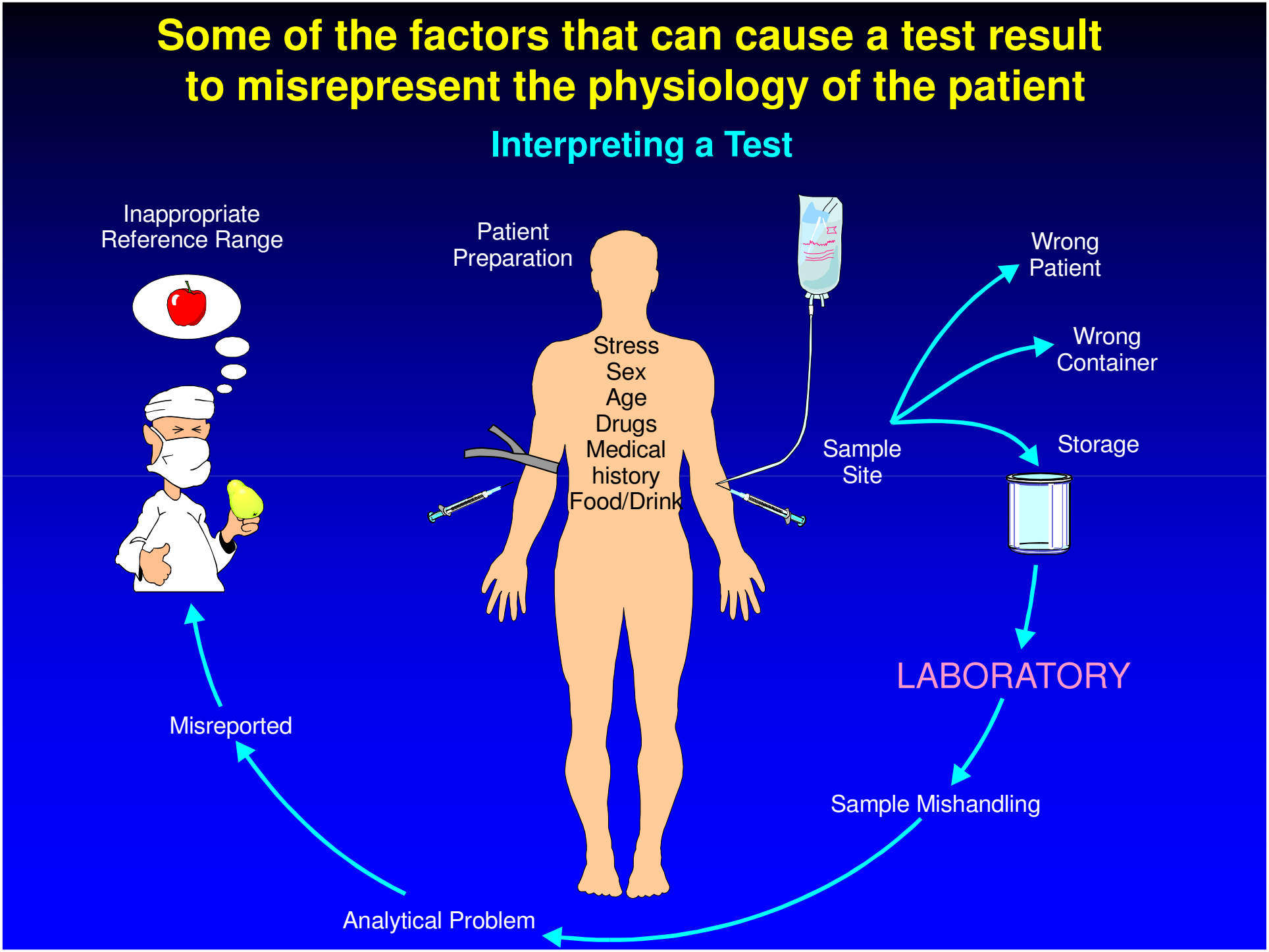
3) How severe is the disease?

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

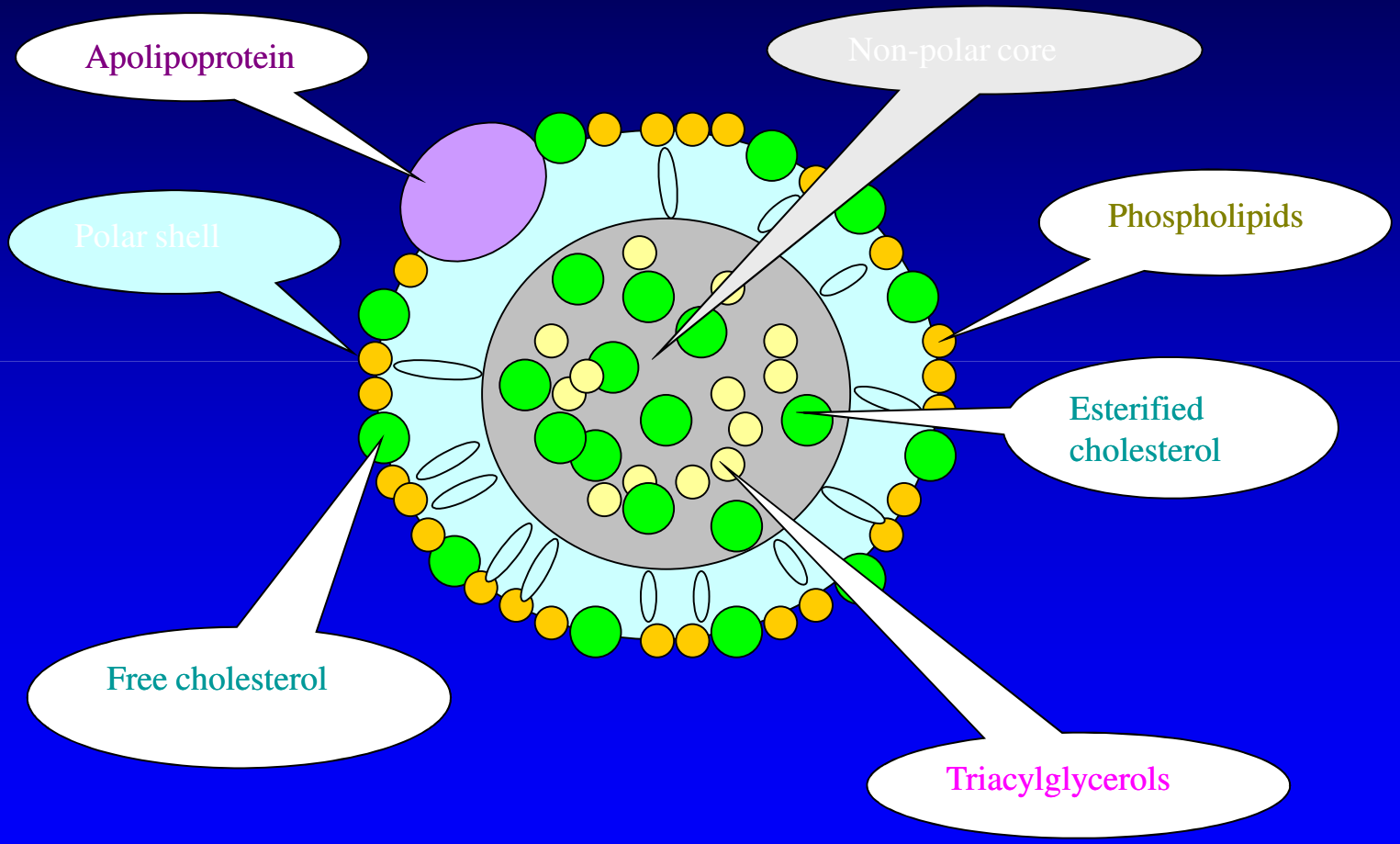
5) Is the patient at risk for disease or is there a disease not clinically apparent?

Some of the factors that can cause a test result to misrepresent the physiology of the patient

Interpreting a Test



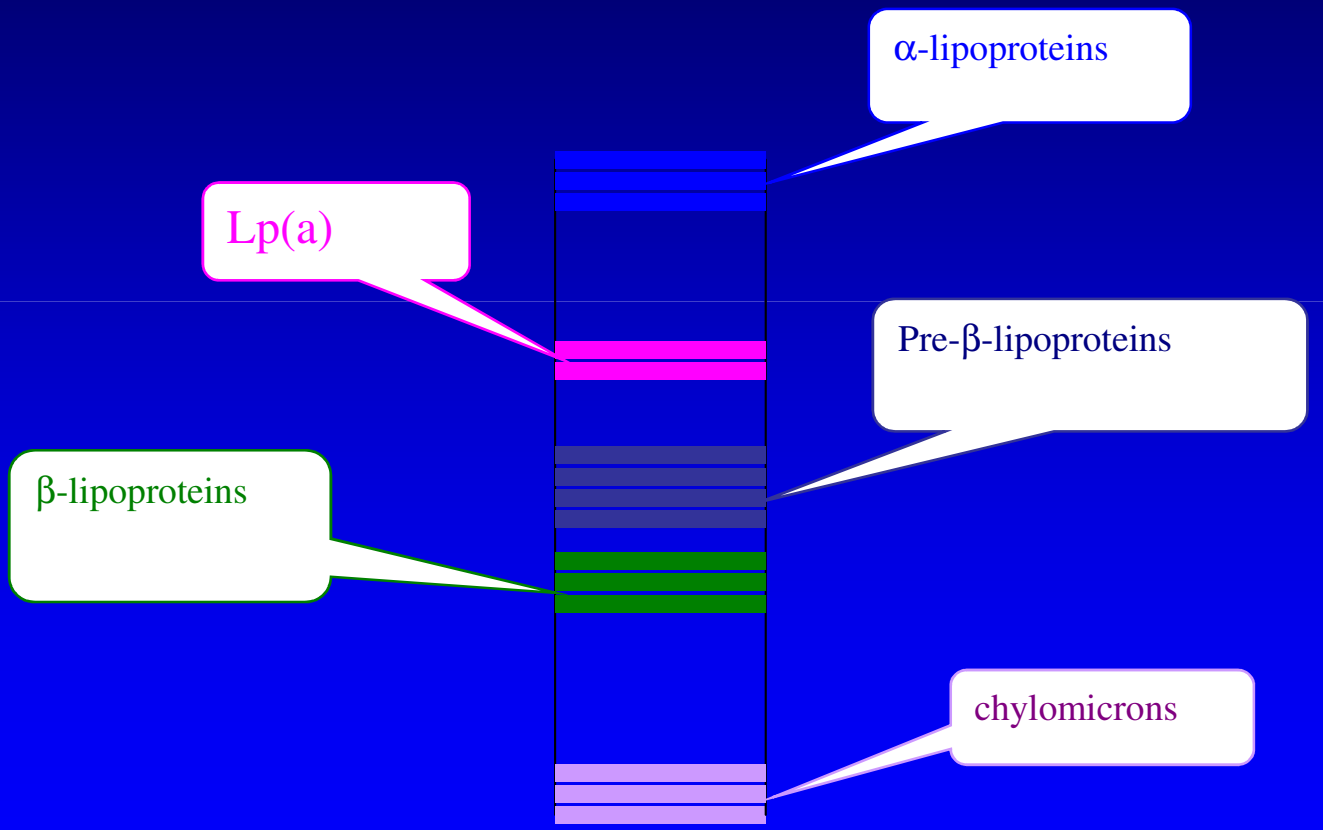
Schematic diagram of lipoprotein particle:



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



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 :***

(*the formula cannot be used if the concentration of TAG › 4,5 mmol/l*)

LDL = total cholesterol - (HDL + TAG x 0.37) [mmol/l]

atherogenic index

$$AI = \frac{\text{total cholesterol - HDL}}{\text{HDL}}$$

- **investigation of apo A-I and apo B-100**
- **electrophoresis of lipoproteins**

Primary hypercholesterolemias

- **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 dysbetalipoproteinemia**
  - a defective gene for ApoE - pathological lipoprotein β-VLDL
  - cholesterol 7.5 - 25 mmol/l
    TAG 2 - 10(20) mmol/l

Primary hypertriacylglycerolemias

- **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 descreased 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 discribed 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 hyperlipoproteinemias

❶ **Diabetes mellitus type I**

- insulin is an activator of lipoprotein lipase
- if DM is decompensated
  ⇨ ketoacidosis, hypertriglyceridemia and sometimes increased cholesterol as well

❷ **Diabetes mellitus type II**

- a more intensive synthesis of VLDL in liver, insulin resistence, HDL reduction, TAG rise
- if DM is decompensated
  ⇨ glycosylation of apo B

Secondary hyperlipoproteinemias

❸ **Hypothyreoidism**

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

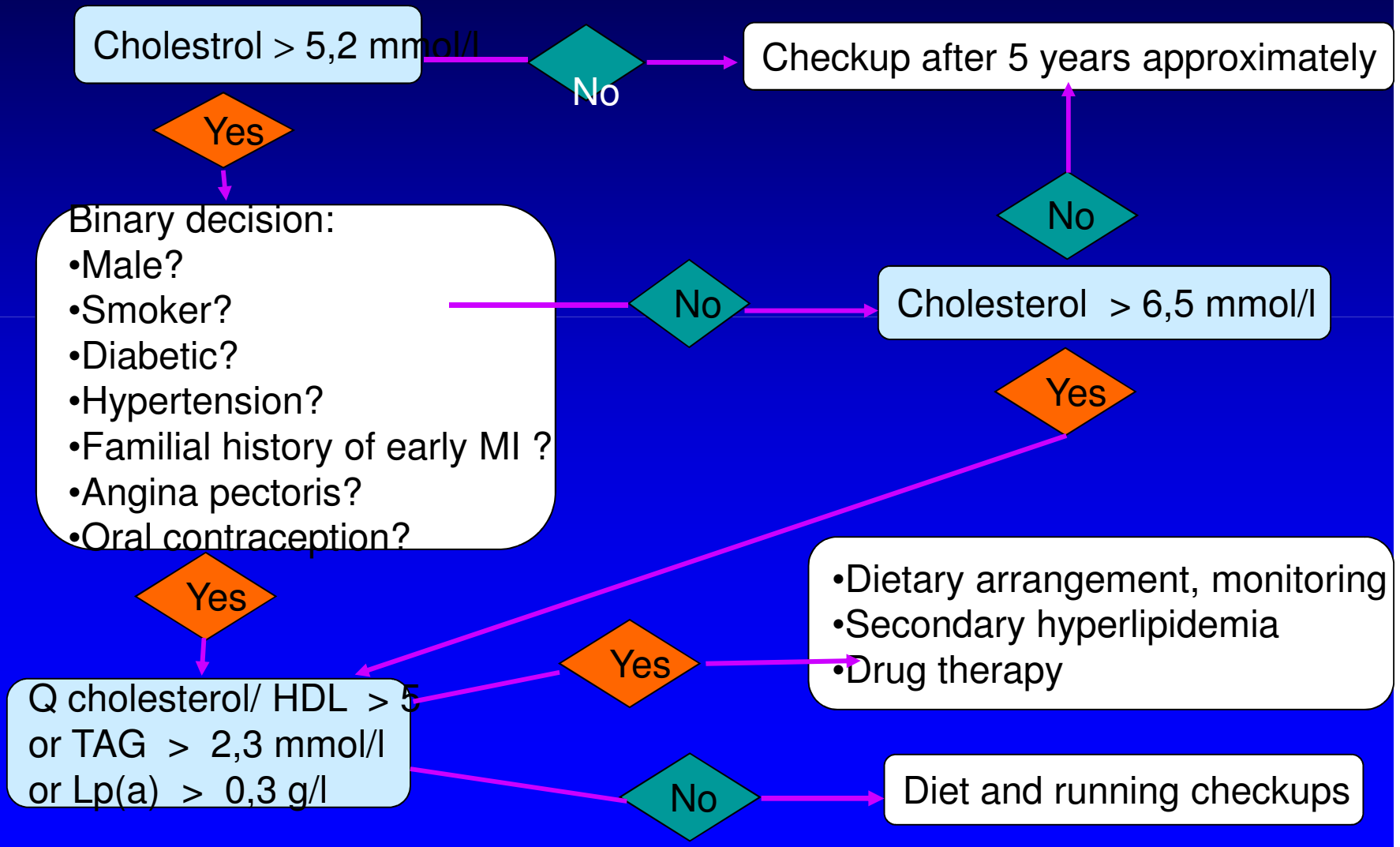
❹ **Nephrotic syndrome**

- 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 ?



URIC ACID

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
- urolithisasis
- hypertonia
- renal diseases
- early severe atherosclerosis
- fatty liver infiltration
- family predisposition
- hemoblastoses
- cytostatic therapy and x-ray radiation
- pre-eclampsia

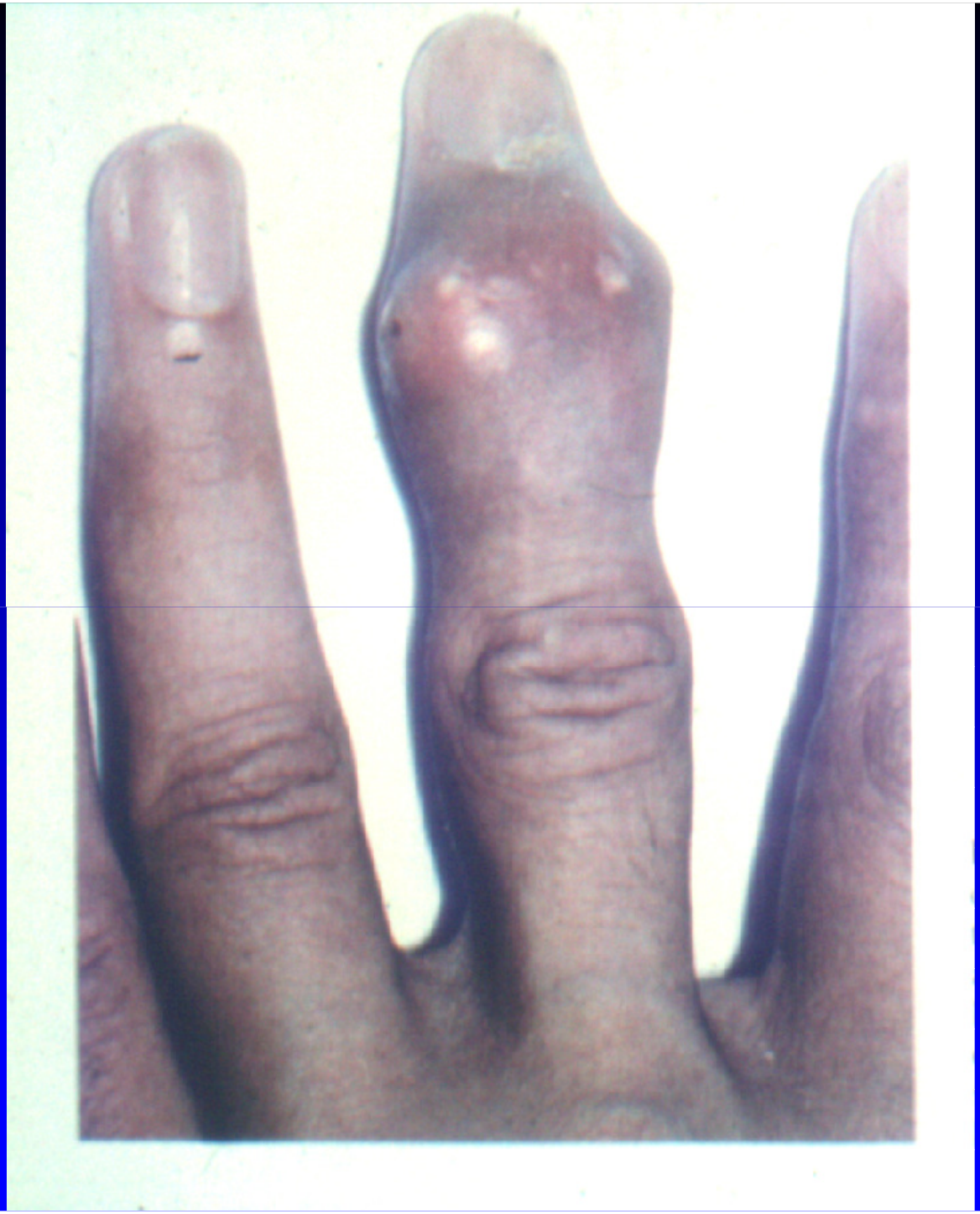
URIC ACID

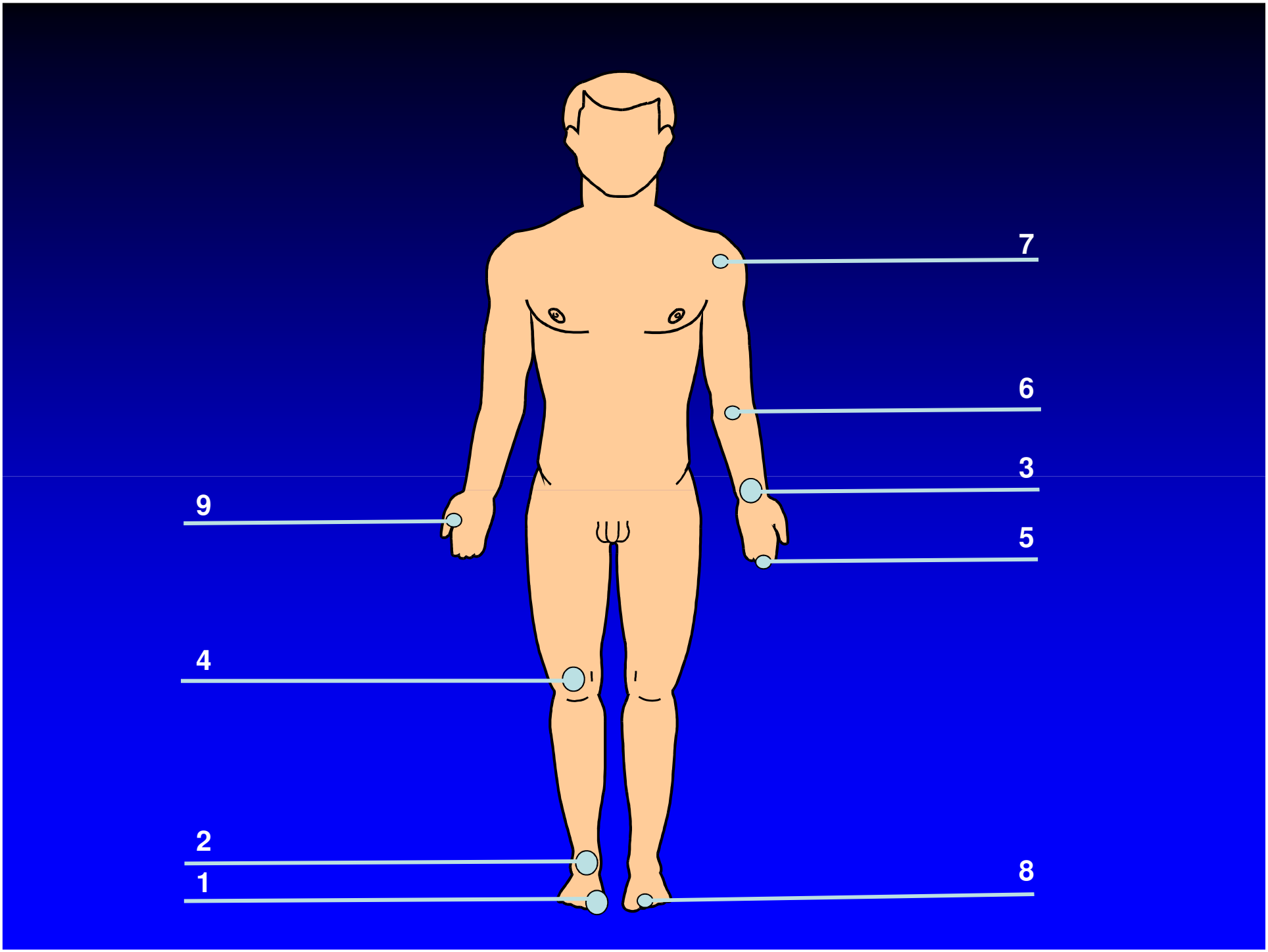
A determination of the uric acid concentration is also to be undretaken with:

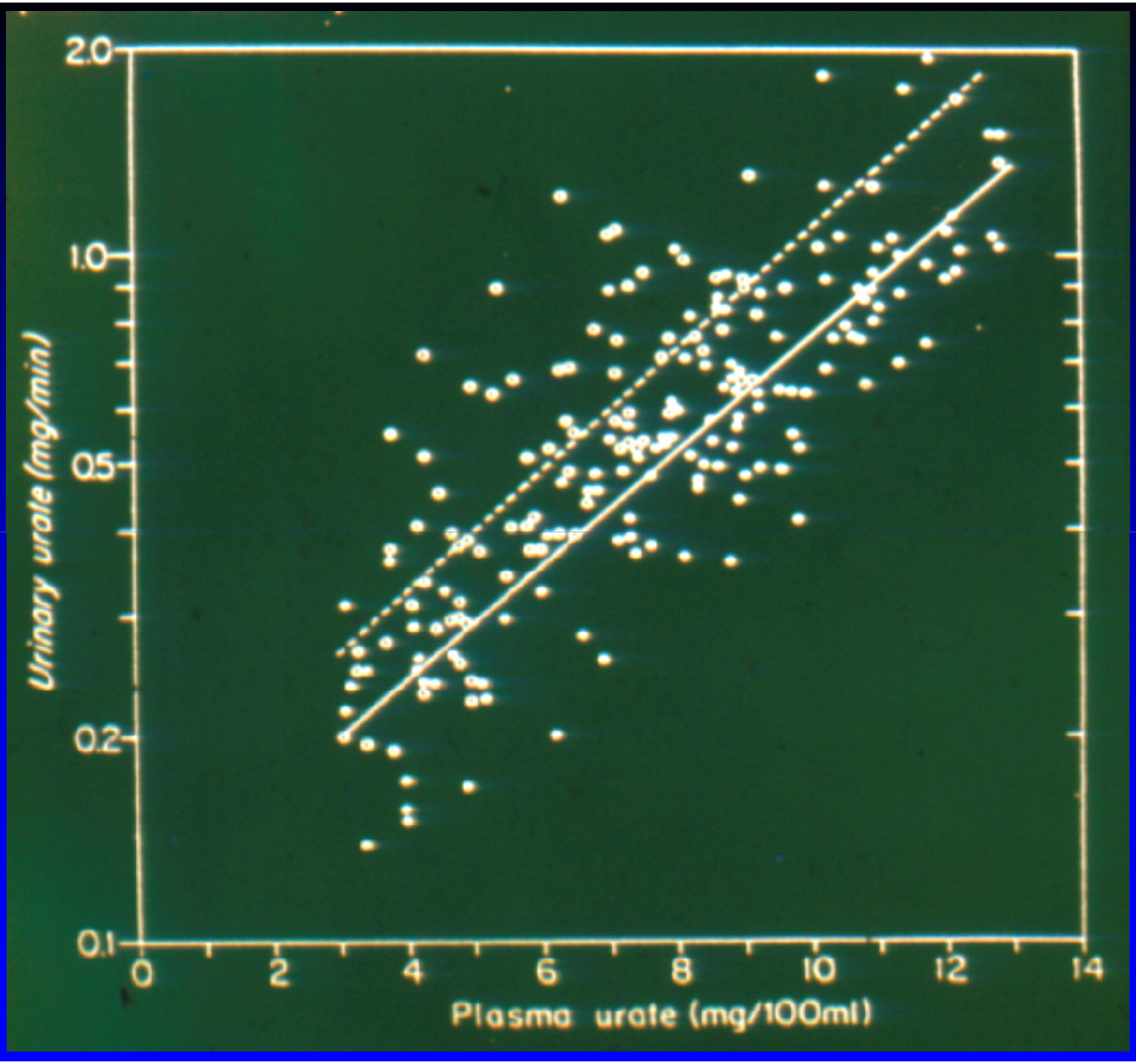
- clinical complaints, pointing to gout and with gout therapy

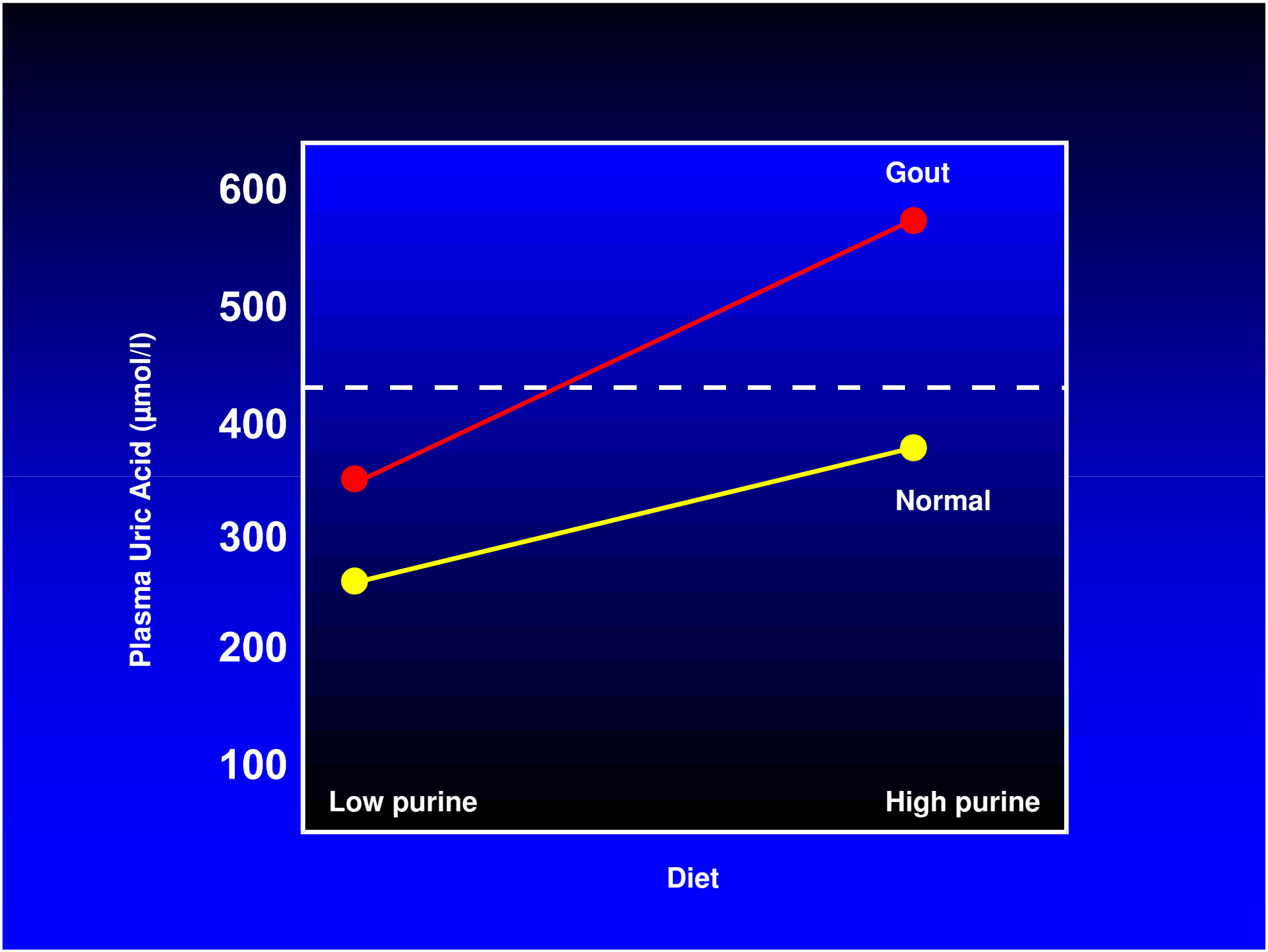
With regard to early gout recognition it is important to know, that ill-defined joint complaints and the presence of other metabolic disturbances may point to a developing gout.

GOUT: THE PROTOTYPICAL CRYSTAL DEPOSITION ARTHROPATHY

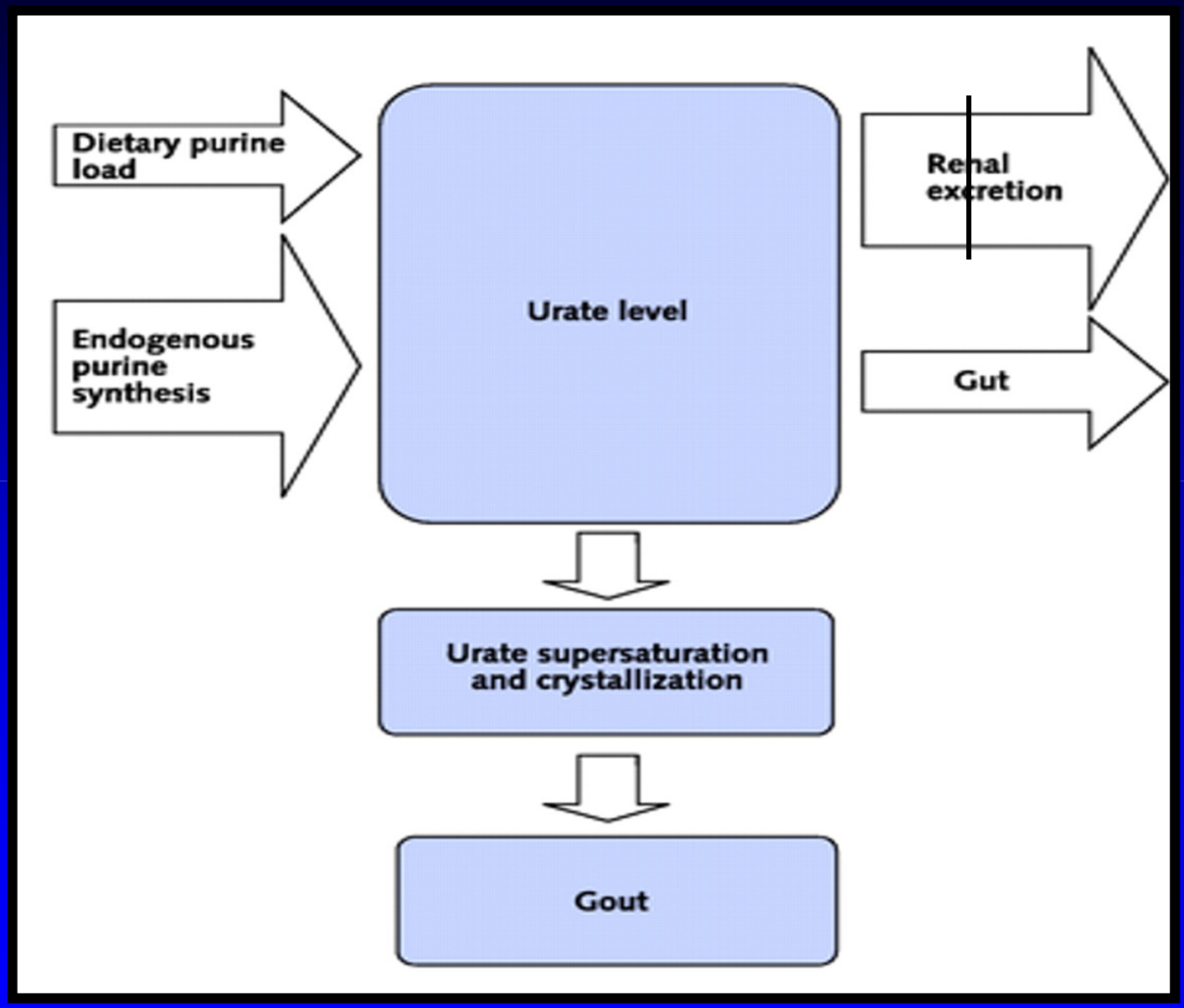



7
6
3
9
5
4
2
1
8


2.0
1.0
0.5
0.2
0.1
Urinary urate (mg/min)
0
2
4
6
8
10
12
14
Plasma urate (mg/100ml)


600
500
400
300
200
100
Plasma Uric Acid (µmol/l)
Gout
Normal
Low purine
High purine
Diet

Overview of the pathogenesis of gout



Choi, H. K. et. al. Ann Intern Med 2005;143:499-516

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

genetic defect

increased activity of phophoribosylpyrophosphate synthetase (PRPPs)

genetic defect

familial juvenile hyperuricemic nephropathy

unknown pathogenesis

Familial juvenile hyperuricemic nephropathy

nephropathy
gout

T
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 diferential diagnosis of gout

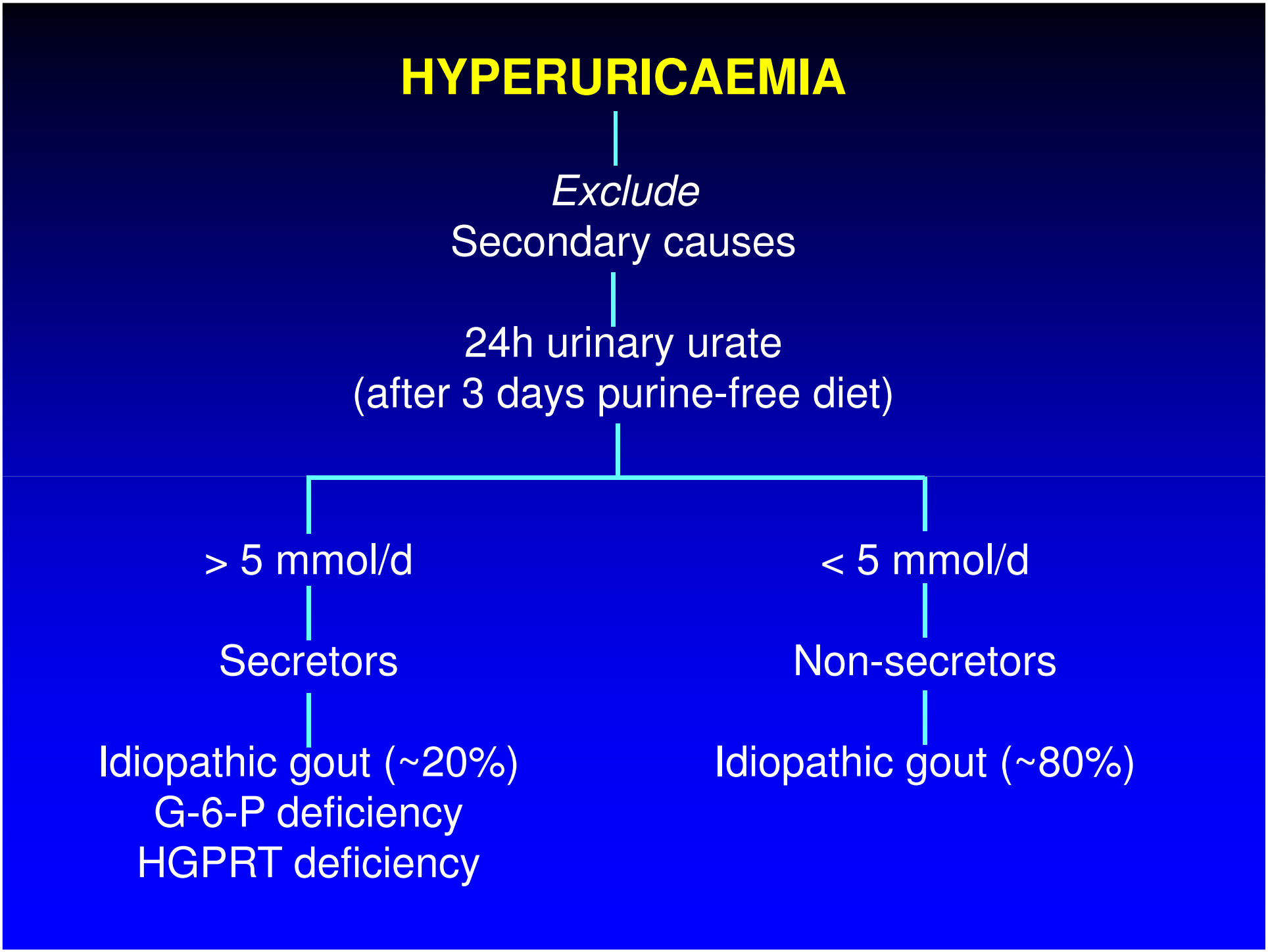
clinical - rheumatological examination
(an important information - family history

↓

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
< 5 mmol/d
Secretors
Non-secretors
Idiopathic gout (~20%)
G-6-P deficiency
HGPRT deficiency
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

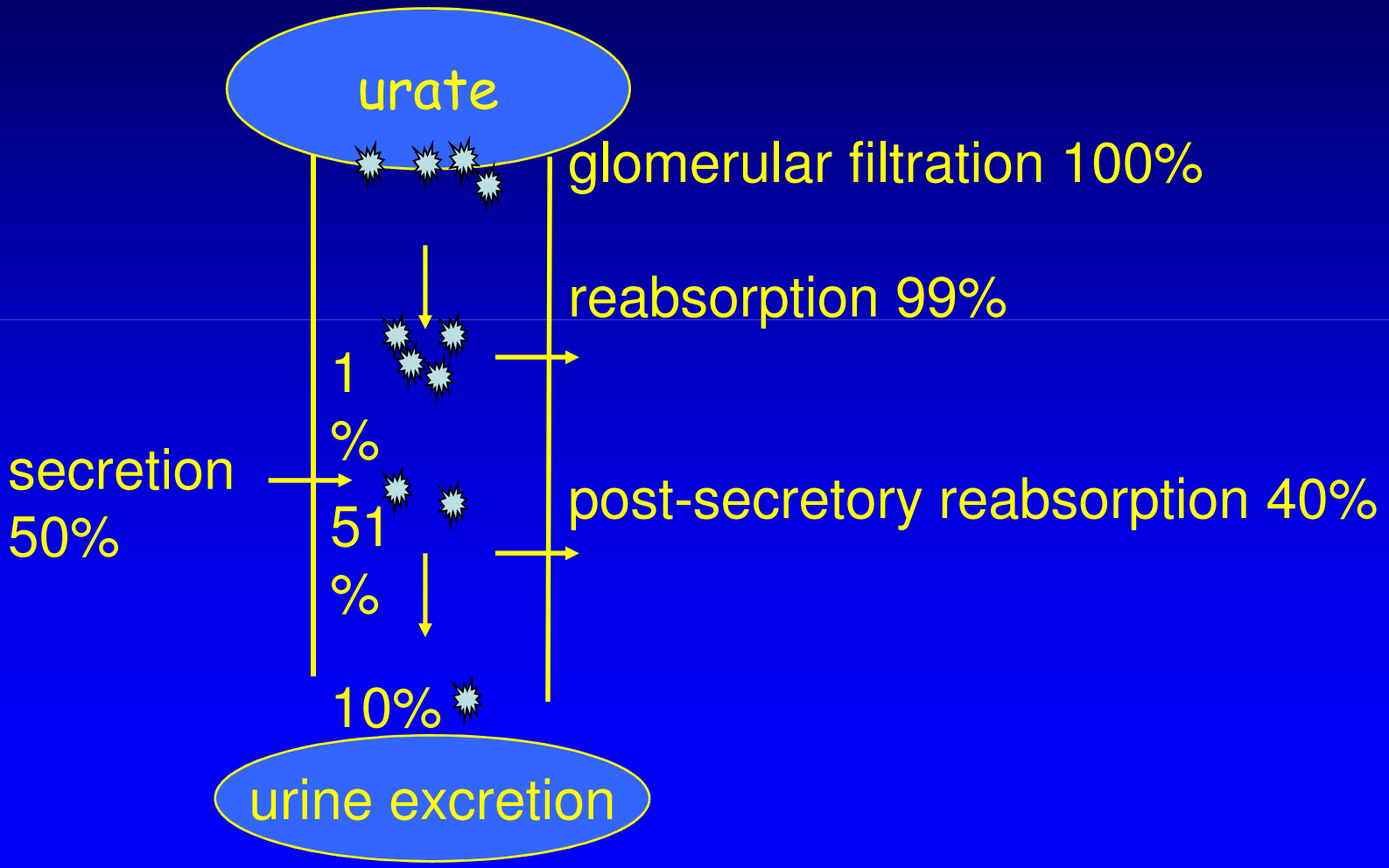
↓

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

↓

4. DNA analýza ← URAT1 (SLC22A12), GLUT9

4-component model of urate handling



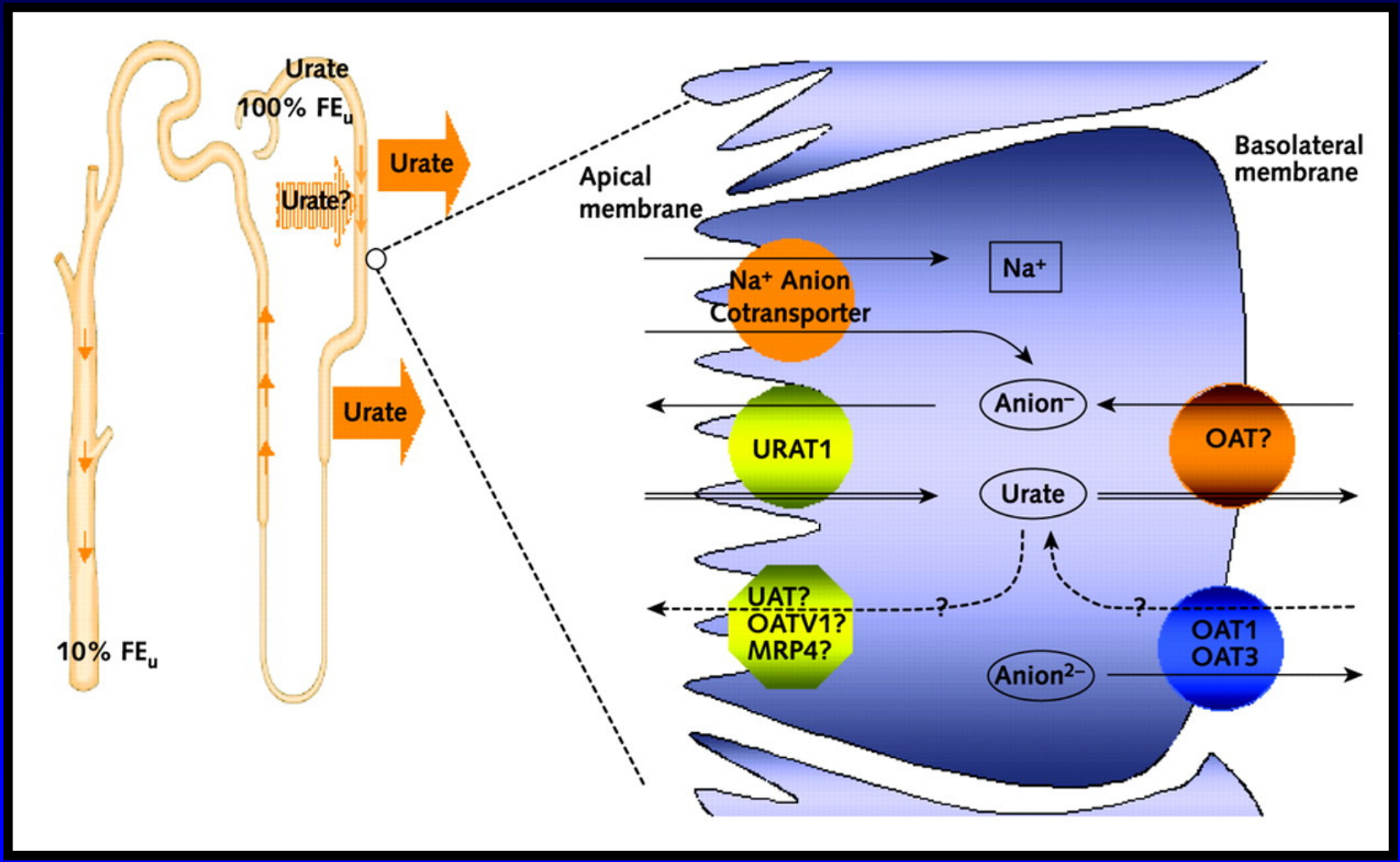
- *Enomoto, A., et al., Molecular identification of a renal urate anion exchanger that regulates blood urate levels. Nature, 2002. 417(6887): p. 447-52.*

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

Enomoto, A., et al., Nature, 2002. 417(6887): p. 447-52.

Ichida, K., et al., J Am Soc Nephrol, 2004.15:p.164-73.

***Iwai, N.,** et al., Kidney Int, 2004.66:935-44.*

***Wakida, N.,** et al., J Clin Endocrinol Metab, 2005. 90:2169-74.*

Hereditary renal hypouricemia, OMIM #220150

- new transport defect of purine metabolism
- biochemical markers
  - hypouricemia (S_{KM}<120 µmol/l)
  - inceased 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 speciemn 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.