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COORDINATORS:
OANA M. ABUREL
CLAUDIA BORZA

AUTHORS
DANINA M. MUNTEAN
MARIA D. DĂNILĂ
ANCA M. BÎNĂ
ADRIAN STURZA
THEIA STANCIU-LELCU
MIHAELA IONICĂ

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PATHOPHYSIOLOGY LAB NOTES

FOR MEDICAL STUDENTS

GHIDURI ȘI ÎNDRUMĂTOARE DE LABORATOR

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Editura „Victor Babeş”

Piaţa Eftimie Murgu nr. 2, cam. 316, 300041 Timişoara

Tel./Fax 0256 495 210

e-mail: evb@umft.ro

www.umft.ro/editura

Director general: Prof. univ. emerit dr. Dan V. Poenaru

Colecţia: GHIDURI ŞI ÎNDRUMĂTOARE DE LABORATOR

Referent ştiinţific: Prof. univ. dr. Cătălin Marian

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FOREWORD

Recent years were marked by a continuous improvement of the curriculum of medical schools, the main goal being that of forming new generations of medical doctors highly capable of scientific inference based on constantly updated information.

Hence, the study of Pathophysiology ensures the necessary knowledge for understanding the causes and mechanisms responsible for the occurrence and evolution of various pathological processes, as well as how functional imbalances arise as a consequence of disease.

The present manual is structured in a manner which enables the learning of the basic principles involved in the etiopathogenesis of disease as well as the current investigation techniques. The present collection of work is in accordance to the didactic concept and intent of our team to organize, in an updated and systematic manner, the paraclinical investigations and laboratory analyses currently applied in the clinical practice.

The learning objectives presented at the beginning of each chapter describe methods of obtaining a common understanding of the concept of disease as well as facilitating the development of an analytical approach to medical reasoning, which is an essential component of preparing students for clinical practice.

The final part of each chapter is represented by multiple choice questions and representative clinical cases which aim to assess the degree of understanding of the importance of investigations associated with the studied pathology and the interpretation of subsequent pathological alterations in order to correctly formulate the paraclinical diagnosis. Lastly, the manner in which the information is systematized in this manual is presented during the practical laboratory aims to create an interactive communication with students, in order to correlate the pathophysiological mechanisms of disease generation (presented in the lectures) with the paraclinical and laboratory changes which they induce (exemplified by case studies).

We hope that this didactic material will be regarded as essential in providing a correct and complete medical reasoning and will prove invaluable to students during university studies.

We express our gratitude to all who will use this proposed educational instrument and we are open to any suggestions which may contribute to the continuous improvement of this didactic material. We firmly believe that a scientific field becomes truly prolific when both readers and authors engage in a constructive dialogue.

The authors

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1. INVESTIGATION OF INFLAMMATION. TUMOR MARKERS

LEARNING OBJECTIVES

At the end of this chapter, students are expected to:

1. *Request the general tests of inflammation and identify their pathological changes in acute and chronic pathologies.*
2. *Interpret the general tests of inflammation.*
3. *Know the clinical value of procalcitonin in assessing the severity of a systemic bacterial infection.*
4. *Know the laboratory changes in the infection caused by SARS-COV 2 virus.*
5. *Request and interpret increased values of tumor markers in breast, ovarian, and prostate cancers.*

I. INVESTIGATION OF INFLAMMATION

- **Definition:** the dynamic process by which the *living tissues* react to various types of injuries: physical, mechanical, chemical, infectious or immune.
- **Classification:**
  a) ACUTE inflammation (< 2 weeks) is a *TRANSIENT,* ***non-specific DEFENSE reaction*** that occurs in the presence of:
    - *infections*: bacterial, viral
    - *tissue necrosis*: burns, myocardial infarction, after injuries (including bone fractures) or after surgery

  b) CHRONIC inflammation (> 2 weeks, up to months, years) is a ***PERSISTENT, PATHOLOGIC process*** associated with:
    - Chronic rheumatic diseases
    - Autoimmune diseases (LES, rheumatoid arthritis)
    - Degenerative and metabolic disorders
    - Malignancy
    - Chronic cardiovascular and pulmonary diseases *(„low grade chronic inflammation").*

The acute phase reaction is a **systemic host reaction**, irrespective of the **etiology** or **magnitude** (localized or systemic) of the triggering event, diagnosed by:

- **laboratory changes** of the **4 general tests of inflammation:** white blood cells (leukocytes) count, erythrocyte sedimentation rate, the acute phase reactants and protein electrophoresis.
- **clinical changes:** non-specific manifestations - fever, fatigue, inappetence.

A. WHITE BLOOD CELLS (WBCs) count

1. WBCs Count

- **Principle:** WBCs are counted using the flow cytometry technique and fluorescent dyes (leukocytes are stained by fluorescent probes with high affinity for nucleic acids)
- **Normal values:**
  - adults: 4, 000 – 11,000/ mm^3 or /μL (4 -11 x 10^9/L)
- **Pathological changes: leukocytosis** is **the increase in number** of **circulating leukocytes** that occurs typically in **the acute phase response.**

2. Differential WBCs count

- **Principle:** assessment of the leukocyte type distribution, by means of flow cytometry. One hundred WBCs are identified and classified according to their morphology. A relative percentage of each type of cell is subsequently reported.
- **Normal values:**
  - **Adults:**
    - Neutrophils (NE): 2 000 – 7 500/mm^3 or /μL (2 – 7,5 x 10^9/L) = 45-75%
    - Lymphocytes (LY): 1 500 – 4 000/mm^3 o /μL (1,5 – 4 x 10^9/L) = 20-55%
    - Monocytes (MO): 200 – 800/mm^3 or /μL (0,2 –0,8 x 10^9/L) = 2-8%
    - Eosinophils (EO): 40 – 400/mm^3 or /μL (0,04 – 0,4 x 10^9/L) = 1-4 %
    - Basophils (BA): 40 – 400/mm^3 or /μL (0,01 – 0,1 x 10^9/L) = < 1%
  - **Children:** values are age dependent, the ratio between lymphocytes and neutrophils is inverted

- **Pathologic changes: leukocytosis is associated with:**
  - **neutrophilia** – in **<u>acute</u> bacterial infections**
  - **eosinophilia** – in **parasitic infections and allergies**
  - **lymphocytosis** *(± monocytosis)* – in **viral infections** and **<u>chronic</u> bacterial infections (e.g., tuberculosis)**

B. Erythrocyte Sedimentation Rate (ESR)

- **Definition:** the rate at which red blood cells (in anticoagulated well-mixed venous blood) descend in a standardized tube over a 1 h period**.** ESR represents the height of the plasma column that is separated from erythrocytes in 1 h.
- **Factors that modify the ESR:**
  a. **The number of erythrocytes** - the *decrease* of RBC mass in **anemias** ⇒ ↑ ESR
  b. **Protein concentration -** increase in globulins (positively charged) in **acute** and **chronic** inflammation, promotes red blood cell sedimentation by reducing electrostatic rejection ⇒ ↑ ESR
  c. **Fibrinogen concentration -** increase of fibrinogen in chronic inflammation, promotes red blood cell sedimentation by reducing electrostatic rejection ⇒ ↑ ESR
- **Normal values**: **< 15 mm/h**, with variations dependent on age and gender (increased values in female ad elderly people):
- **Pathological changes**:
  - ESR increases in **the first 24 hours after the onset of an acute inflammation** and decreases in the next 4-6 days
  - ESR is **persistently increased** in **chronic inflammation and malignancy.**
- **Clinical value:**
  - **is a *non-specific screening* test** used to **diagnose inflammation** in **bacterial infections** and **autoimmune diseases**
  - **is a *non-specific inflammatory* test** used to **monitor the anti-inflammatory therapy in chronic rheumatic and collagen diseases** (e.g., systemic lupus erythematosus - SLE), when the **level of the C reactive protein (CRP) is *normal or mildly increased.***

C. ACUTE PHASE proteins or reactants

- **Definition:** Increased serum levels of proteins normally found in health, as well as the appearance of novel proteins (that serve as markers of a pathological event) within 8 – 12 hours after the onset of infection or trauma. **The liver** increases the synthetic rate of these proteins, its major stimulus being **interleukin-6** (**IL-6).**
- **Classification:**
  - *Coagulation factors*: fibrinogen
  - *Protease inhibitors*: alpha-1-antitryipsin, alpha-2-macroglobulin
  - *Carrier proteins*: haptoglobin, ceruloplasmin and ferritin
  - *Serum complement*: C3 fraction
  - *Other proteins*: C reactive protein, fibrinogen, serum amyloid A

Remember!
The main acute phase reactants (APR) are: **C-reactive protein (CRP), fibrinogen** and **serum amyloid (SAA):**

a. In ***acute inflammation*** the APR increase is **correlated** with the severity of inflammation
b. In ***chronic inflammation*** the APR increase is **NOT correlated** with the severity of the disease

- **CRP and fibrinogen** are **the most important APR** that are currently measured as *laboratory markers of the hepatic acute phase protein response**.***

1. C-Reactive Protein (CRP)

- **Definition:** CRP is the main APR that was firstly described in 1930 due to its ability to precipitate with the C-polysaccharide extract of Pneumococcus
- **Source:** mainly synthesized by hepatocytes in response to: infection (bacterial, fungal), tissue injury (myocardial infarction, acute pancreatitis, trauma) and chronic inflammation (autoimmune diseases, rheumatic disorders, malignancies)
- **Roles:**
  - **favorable**: acts as *opsonin* for various pathogens and *activates the complement system*
  - **unfavorable**:

  • facilitates infiltration of circulating monocytes within the vascular walls (where they become macrophages)

• stimulates the production of proinflammatory cytokines and oxygen free radicals by macrophages (with the oxidation of the LDL → LDLox)

• favors LDL/LDLox phagocytosis by macrophages → aggravation of atherosclerosis

- **Normal values: < 5 mg/L**
- **Pathological changes**:
  - **a. Acute bacterial infections**:
  - CRP increases ***markedly*,** reaching values **> 100 mg/L** and *rapidly*, 4-6 hours after the onset of disease
  - a dynamic assessment of CRP is useful to *monitor the response to the antibacterial treatment (Figure 1.1)*.
  - **b. Chronic inflammation and malignancy:**
  - CRP increases ***moderately***, between **10 - 100 mg/L**
  - a dynamic assessment of CRP is useful to *monitor the evolution of chronic inflammatory rheumatic diseases*
  - **c. Post-surgery**:
  - CRP is a *useful marker to monitor patients after surgery*
  - in *uncomplicated cases,* serum levels peak between 48-72 hours and return to normal by day 7 post-surgery
  - If the postoperative course is complicated by sepsis or chronic inflammation, CRP level remains elevated
- **Clinical value:**
  - **CRP** assay is a **more sensitive** and **useful** inflammatory marker in **clinical practice** as compared to ESR because its serum concentration increases **early** and is **NOT** affected by changes of red blood cells (shape, size or number) or by hyper-gammaglobulinemia.
  - its changes are *absent to mild-moderate* in viral infections, depending on their severity. However, a superimposed bacterial infection will trigger a marked increase in CRP, independent of the underlying viral pathology.
  - CRP is a *useful marker to monitor patients* with *chronic rheumatic diseases (e.g., rheumatoid arthritis) or other chronic pathologies (e.g., inflammatory bowel disease).*

2. Fibrinogen

- **Definition:** a large protein produced by liver as the precursor of the insoluble fibrin during the clotting process.
- **Normal values: 200 – 400 mg/dL**
- **Clinical value:**
- high values (2-3 times) can be detected within the **first 24 - 48 h** of an **acute inflammation.**
- serum levels *are persistently increased in chronic inflammation.*
- fibrinogen is a *useful marker to monitor patients with chronic rheumatic diseases*
- increased plasma level is an *independent risk factor* for coronary artery disease and cardiovascular events

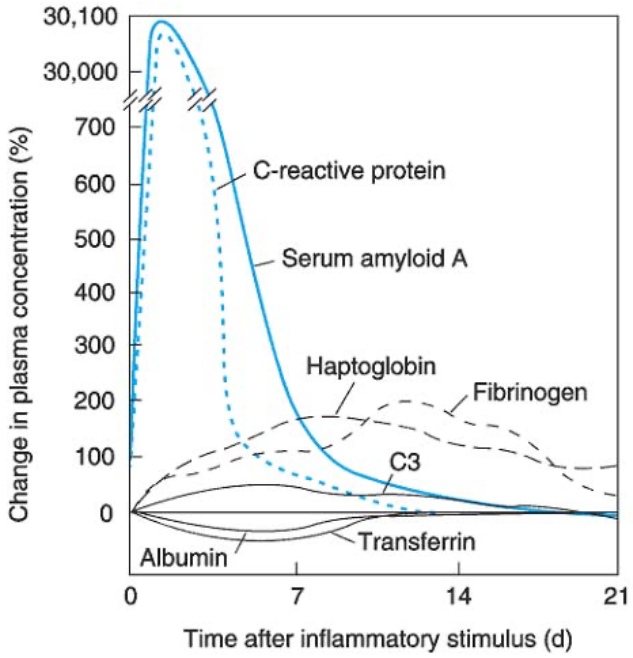


Figure 1.1. The dynamics of APR.
(*Modified after:* http://www.bhlinc.com.)

D. Serum Protein Electrophoresis

- **Principle:** in the setting of inflammation the liver synthesizes a **high amount** of **α- and γ-globulins** and subsequently, less albumins. The **decrease of the albumin/globulin ratio** is referred to as **dysproteinemia.**
- **Normal values:**
  Albumins: 50 – 60%
  Alpha-1 globulins: 3 – 6%
  Alpha-2 globulins: 7 – 10%
  Beta-globulins: 11 – 14%
  Gamma-globulins: 15 – 23%

- **Pathological variations:**

a. Dysproteinemia from acute inflammation:
- Decreased albumins
- Increased **alpha-1** and **alpha-2 globulins** due to increased liver synthesis of the acute phase proteins

b. Dysproteinemia from chronic inflammation:
- Decreased albumins
- Increased **alpha-1** and **alpha-2 globulins** due to the persistence of increased acute phase proteins
- Increased **gamma-globulins** due to increased production of immunoglobulins by immune cells

E. Procalcitonin (PCT)

- **Definition:** precursor of **calcitonin** whose serum level increases in **severe bacterial infections**.
- **Source:**
  - Under *physiological conditions* PCT is the precursor of calcitonin (CT) being produced by **endocrine cells** (C cells of the thyroid gland) and neuroendocrine cells, and released into circulation as calcitonin with a central role in calcium homeostasis.
  - In **inflammatory conditions** associated with **bacterial infections**, under the action of bacterial toxins and, respectively, of pro-inflammatory mediators (IL-6 and TNF-α), **non-neuroendocrine cells**, mainly **adipocytes**, secrete circulating PCT (without CT generation).
- **Normal value: < 0.5 μg/L(ng/mL)**
- **Clinical value:**
  - Differential diagnosis of **severe systemic inflammations** due to **bacterial infections** *vs.* **non-infectious** causes
  - Monitoring of patients at high risk of **severe infectious complications**:
    - After surgery
    - After organ transplantation
    - During immunosuppressive treatment
    - Post trauma
  - Assessment of the severity of the **systemic inflammatory response syndrome (SIRS)** and of the **risk of its progression** towards: septic state → septic shock →multiorgan dysfunction syndrome (MODS):
    - **< 0.5 μg/L** - no risk
    - **> 10 μg/L**- increased risk
  - Guidance of antibiotic therapy
  - PCT becomes detectable within **2-4 hours** after the appearance of bacterial toxins in the systemic circulation and reaches a maximum value after **12-24 hours**
  - The serum level of PCT returns to normal within **24-36 hours** after the toxins' disappearance from the circulation.

  Observation: PCT levels are usually **normal** in **viral infections, autoimmune diseases and chronic inflammatory diseases.**

Laboratory changes associated with COVID-19

COVID-19 (for Coronavirus Disease 2019) is a disease determined by the infection with the severe acute respiratory syndrome 2 virus (SARS-CoV-2), a strain that was detected in humans in China, in December 2019.

The disease takes variable forms, but the infection is constantly associated with **systemic inflammation** in the **moderate forms** and with the so-called **"hyper-inflammation"** and **"cytokine storm"** in **severe forms,** characterized by the massive release of proinflammatory cytokines IL-6, IL -1 (routinely measured).

Changes in general inflammation and blood count tests in COVID-19 consist of:

- increased CRP (but values do NOT constantly correlate with increases in IL-1 and IL-6)
- increased fibrinogen (but possibly decrease in advanced forms with lethal evolution)
- increased procalcitonin
- leukocytosis with lymphopenia
- normal /increased platelets (initially) and later a low platelet count (thrombocytopenia is a poor prognostic factor)

II. TUMOR MARKERS

- **Definition**: any macromolecule whose *presence* or *increased concentration* in the body can be related to the presence or progression of a tumor.
- **Characteristics**:
  1. Tumor markers are produced by either **tumor cells** or **non-tumor cells** of the host in response to the presence of cancer
  2. The level of a tumor marker increases in:- **serum, urine** or **in other body fluids** (*humoral markers*) and/or
     -**the tissue** in which the cancer originates (*tissue markers*)
  3. Increased concentration of certain tumor markers can be found in **non-malignant pathologies** (e.g., benign tumors, chronic inflammatory diseases); therefore, a positive result **must always be confirmed by the clinical exam and tissue biopsy**
  4. Increased concentration of a certain tumor marker can be encountered **in several types of cancers** (i.e., carcinoembryonic antigen)
  5. The serum level of a tumor marker is **influenced by numerous factors:** *the total number of cells producing the markers and their release rate, the degree of tumor expansion, the presence of a "non-secretory" tumor type (that expresses the marker but does not release it), the tumor vascularization.*
- **Classification:** according to the origin and biochemical structure, there are different classes of tumor markers (Table 1.1)

Table 1.1. Main classes of tumor markers.

Class	Tumor markers
Oncofetal antigens	AFP (alpha-feto-protein) CEA (carcinoembryonic antigen)
Tumor associated antigens (cancer antigens, CA)	CA125, CA19-9, CA15-3
Proliferation/differentiation antigens	SCCA (*squamous cell carcinoma antigen*)
Tumor tissue proteins	S-100 (protein S-100) HE4 (*human epididymis protein 4)*
Enzymes and isoenzymes	PSA (prostate specific antigen)
Hormones and ectopic precursors products	β-HCG (beta-human chorionic gonadotropin) Calcitonin Thyroglobulin

Observation! An ***ideal*** **tumor marker** should present:

- high tumor specificity (not detectable in benign disorders or in healthy subjects)
- high tumor sensitivity (detectable even in the presence of a small number of tumor cells)
- organ specificity
- a good positive predictive value (the presence of the malignant tumor in subjects with positive results) and negative predictive value (the absence of the malignant tumor in subjects with negative results)
- a good correlation with the disease stage and the tumor size
- a good prognostic value
- clinical value in therapy monitoring
- clinical value in early detection of recurrences and metastases
- assessment through a cost-effective method

Remember!

NEITHER of the available tumor markers meets the criteria of an ideal marker, therefore **combined assessment of several markers (Table 1.2) is required** in order to **define the tumor profile in clinical practice**, thus allowing:

- *early diagnosis*
- *clinical staging*
- *treatment monitoring*
- *early detection of recurrence and metastasis*
- *prognosis evaluation* (Figure 1.2)

Table 1.2. Tumor markers in different types of cancers.

Oncologic profile	**First line markers**
Malignant melanoma	Protein S 100
Testicular cancer	AFP, β – HCG
Bile ducts cancer	CA 19-9, CEA
Colorectal cancer	CEA, CA 19-9
Cervical (cervix uteri) cancer	SCC, CEA
Esophageal cancer	CEA, SCC, CA 19-9
Gastric cancer	CA 72-4, CEA, CA 19-9
Hepatic cancer	AFP, CEA
Ovarian cancer	CA 125, HE4 (ROMA score)
Thyroid cancer	Calcitonin, CEA, Thyroglobulin
Breast cancer	CA 15-3, CEA
Pancreatic cancer	CEA, CA 19-9
Prostate cancer	Total PSA, free PSA
Pulmonary cancer	SCC, CEA

Clinical value of tumor markers:

1) Screening tests

- are tests that evaluate the possibility of the existence of malignant tumors in high-risk groups of asymptomatic patients with a history of malignant disease in the family. Screening tests have no definitive diagnostic value but establish the need for further investigation of the patient in order to confirm/refute the malignant pathology.
- generally, tumor markers **are not appropriate** for screening of asymptomatic patients, **except for:**
  - **PSA** for *prostate cancer*
  - **calcitonin** for *thyroid cancer*

2) Early diagnosis

- Role: to early detect malignant tumors in *symptomatic patients with a high probability of malignancy*
- generally, tumor markers are not suitable for early diagnosis, **except for:**
  - **AFP** in patients with hepatic cirrhosis, together with abdominal ultrasound, for the detection of *hepatic cancer*
  - **AFP** and **β-HCG**in patients with a **gonadal tumor,** together with the *testicular ultrasound* for the detection of *testicular cancer*
  - **PSA** in patients > 50 years of age with **prostate adenoma**, together with digital rectal examination (DRE) and *transrectal ultrasound* for the detection of *prostate cancer*
  - **CA 125** and **HE4** (ROMA score) in patients with a pelvic tumor mass, together with the digital vaginal examination and transvaginal ultrasound, for the detection of *ovarian cancer*

3) Stadialization and prognosis

- Role: usually, increased pre-therapeutic levels (high "basal level") of tumor markers tends to correlate with advanced stages of neoplasia (metastasis), and therefore with a reserved prognosis
- a decreased concentration of tumor markers at the end of the treatment indicates tumor eradication and remission and a good prognosis

4) Monitoring the treatment response

- Role: is **the most important clinical use** of tumor markers
- a decrease towards the basal level suggests tumor *eradication/remission*
- lack of/minor decrease of the marker indicates:
  - *incomplete tumor resection/multiple tumors*
  - *resistance to chemotherapy/radiotherapy*
  - *metastasis (pre-therapy)*

5) Early detection of metastasis and tumor recurrence:

- an increase of the tumor markers level following an initial post-therapeutic normalization is strongly suggestive for **metastasis/recurrence**
- the repeated assessment of tumor markers is an important, **non-invasive post-therapeutic monitoring tool**

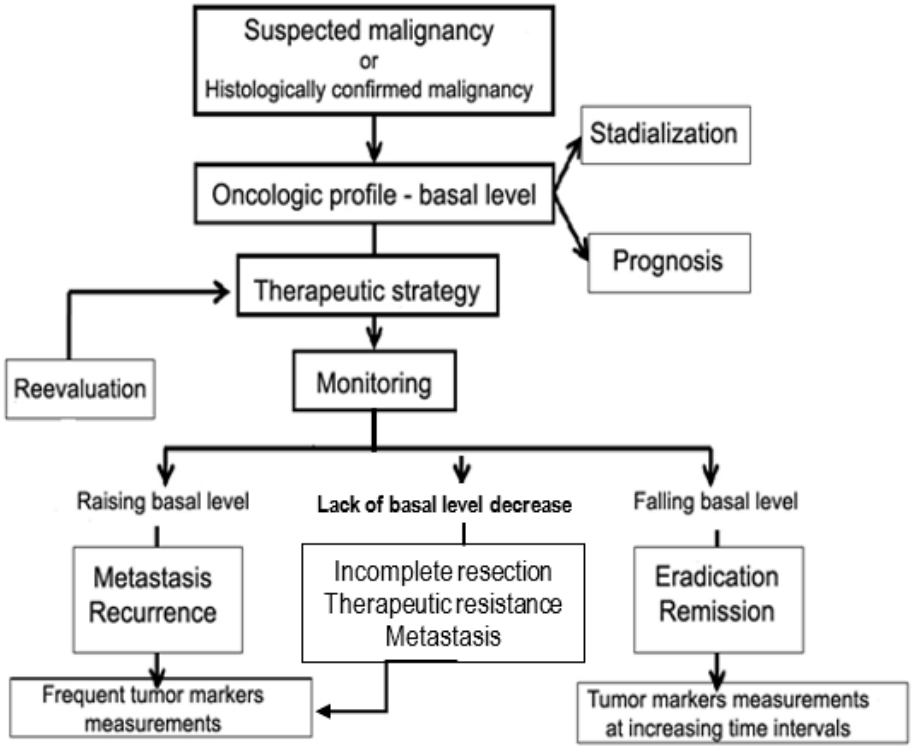


Figure 1.2. Algorithm for the clinical use of tumor markers.

Observation: Research to identify new tumor markers with diagnostic and prognostic accuracy is ongoing and one of the newest directions is represented by ***microRNAs***. These have been identified by a large number of studies as potential biomarkers for the *diagnosis, prognosis* and *treatment monitoring* of different types of neoplasms.

A. Tumor profile in BREAST CANCER

Table 1.3. Main tumor markers in breast cancer.

Marker	Screening	Early diagnosis	Treatment monitoring	Detection of metastasis	Recurrence detection	Prognosis
CA 15-3	-	-	+	+	+	-
CEA	-	-	+	-	+	+

Important!

- The use of CA 15-3 is NOT recommended for screening!
- The diagnosis is based on **mammography** (irregular stella opacity) and confirmed by **mammary biopsy.**

B. Tumor profile in OVARIAN CANCER

Table 1.4. Main tumor markers in ovarian cancer.

Marker	Screening	Early diagnosis	Treatment monitoring	Detection of metastasis	Recurrence detection	Prognosis
CA 125	-	+	+	+	+	+
HE4	-	+	-	-	-	-
CEA	-	-	+	-	+	+

Important!

- The use of CA 125 is NOT recommended for screening!
- The diagnosis is formulated based on **transvaginal ultrasound** and confirmed by **biopsy!**
- The combined measurement of **CA 125 + HE4**isused for the **ROMA score** (*Risk of Ovarian Malignancy Algorithm*) (Table 1.5) calculation which allows ovarian cancer risk stratification in women with a pelvic tumor mass

Table 1.5. Interpretation of the ROMA score in women with a pelvic mass

Period	**HIGH risk**	**LOW risk**
Premenopausal	Score≥ 11,4%	Score< 11,4%
Postmenopausal	Score≥ 29,9%	Score< 29,9%

C. Tumor profile in PROSTATE CANCER

Table 1.6. Main tumor markers in prostate cancer

Marker	**Screening**	**Early diagnosis**	**Treatment monitoring**	**Detection of metastasis**	**Recurrence detection**	**Prognosis**
Total PSA	**+**	**+**	**+**	**+**	**+**	**+**

Important!

- The diagnosis is based on **transrectal ultrasound** and is confirmed by **biopsy**
- Total PSA includes: the fraction fixed on α-antichymotrypsin (PSA-ACT) and the free fraction (free-PSA)

a) Total PSA:

- is evaluated in **men > 50 years** only combined with digital rectal examination and transrectal ultrasound
- is interpreted according to Table 1.7:

Table 1.7. Interpretation of total PSA values

Age	**Normal value**
< 40	≤ 1,4 ng/mL
40-50	≤ 2 ng/mL
50-60	≤ 3,1 ng/mL
60-70	≤ 4,1 ng/mL
> 70	≤ 4,4 ng/mL

Risk level	**Total PSA**
Low	≤ 10 ng/mL
Intermediate	11-20 mg/mL
High	> 20 mg/mL

b) Free PSA:

- Is assessed in the same serum as total PSA if **total PSA = 4-10 ng/ml** at repeated measurements and a first prostate biopsy is negative for prostate cancer
- Is interpreted as **free PSA / total PSA ratio** (Table 1.8)

Table 1.8. The free PSA/total PSA ratio interpretation

Total PSA	**Normal Ratio**
2-4 ng/ml	>10%
4-10 ng/ml	>25%

A ratio **free PSA/total PSA < 25%** indicates the suspicion of prostate cancer and dictates a second prostate biopsy!

Obs.: Approximately 5% of breast cancers and 10% of ovarian cancers have a genetic predisposition, the best known being the mutation of the ***BRCA1 / BRCA2 genes***. Among the population carrying this mutation, the risk of developing breast or ovarian cancer increases up to 70% and that of prostate cancer 5-7 times. Thus, in the presence of a positive family history, the evaluation of BRCA1 / BRCA2 mutations by venous blood sampling could be on the one hand a method of *genetic screening* and on the other hand would broaden the spectrum of *therapeutic options*, given the favorable response to a class of tumor cell DNA repair inhibitors.

CHECKPOINT!

***1. Which of the following statements about ESR is CORRECT?**

A. Increases after 4-6 hours after the onset of an acute inflammation
B. Depends on the number of white blood cells
C. Is a non-specific inflammatory test
D. It has a high sensitivity in viral infections
E. It is a test with high clinical value for monitoring antimicrobial treatment

***2. Which of the following statements about CRP is CORRECT?**

A. Is an acute phase protein produced by the liver
B. Depends on the serum albumin / globulin ratio
C. Its plasma concentration is influenced by the erythrocyte mass
D. Greatly increases in viral infections
E. Has a lower value in clinical practice than ESR

3. **Which of the following measurements are useful for monitoring the evolution of chronic inflammatory rheumatisms?**

A. ESR
B. Electrophoresis
C. Serum amyloid A
D. CRP
E. Leukocyte count

4. **Which of the following statements about fibrinogen are correct?**

A. It is a test of clinical value in acute inflammation
B. It is a test of clinical value in chronic inflammation
C. Its increase causes ESR increase
D. It is a monitoring test useful after surgery
E. It is a marker of the increased risk of systemic bacterial infection

5. **Which of the following ELFO fractions increase in acute inflammation dysproteinemia?**

A. Albumin
B. α1-globulins
C. α2-globulins
D. β-globulins
E. γ-globulins

6*. Procalcitonin:

A. It is produced by the C cells of the thyroid gland under pathological conditions
B. Elevated values indicate an increased risk of systemic infectious complication
C. It is the marker of viral infections
D. Decreases in advanced septic shock
E. Decreases in severe sock complicated with MODS

7. Laboratory investigations associated with SARS-COV2 infection reveal:

A. Lymphocytosis
B. Decreased procalcitonin
C. Thrombocytopenia in severe forms
D. Decreased PCR
E. Lymphopenia

8. **Which of the following statements are true:**

A. Leukocytosis with neutrophilia is present in viral infections
B. C-reactive protein decreases LDL uptake by macrophages
C. In case of postoperative complications, the level of protein C remains elevated
D. In severe forms of COVID-19,hyperinflammation is associated with cytokine storm
E. Serum PCR level depends on globulin concentration and erythrocyte changes

9. *** Which of the following combined tumor markers are of clinical use in the early diagnosis of ovarian cancer?**

A. CA 15-3 + CEA
B. CA 125 + HE4
C. Total PSA + free PSA
D. AFP + β - HCG
E. AFP + CEA

10. **Which of the following tumor markers are part of the tumor profile of breast cancer?**

A. CA19-9
B. AFP
C. CA15-3
D. CA125
E. CEA

CASE STUDIES

1. A 19-year-old patient diagnosed with type 1 diabetes from the age of 13 presents for a pricked wound in the right leg. The patient is feverish(38°C). Surrounding the wound, the skin is red and warm, while the leg is edematous and painful.

   - *Leukocyte count:* WBCs = 15.000/mm^3
     NE = 7,8 $x10^9$/L, EO = 0,1 $x10^9$/L, BA = 0,01 $x10^9$/L, LY = 1,5 $x10^9$/L, MO = 0,6 $x10^9$/L

Which is the most probable diagnosis?

List the investigations you would further recommend.

.....

.....

.....

2**.** A 52-year-old female presents to the physician for a right mammary node recently detected by breast self-*examination*. Clinical breast examination revealed a tough, irregular tumor attached to deep structures accompanied by skin dimpling.

What is the most probable diagnosis?

List the investigations you would further recommend.

.....

.....

.....

NOTES

2. INVESTIGATION OF THE PATHOLOGICAL IMMUNE RESPONSE

LEARNING OBJECTIVES

At the end of this chapter, students are expected to:

1.Ask for and interpret the main investigations of the anaphylactic reaction.
2.Ask for and interpret the main investigations of the cytotoxic reaction.
3.Ask for and interpret the main investigations of the immune complex-mediated reaction.
4.Ask for and interpret the main investigations of the cell-mediated reaction.
5.Ask for and interpret the main tests required for the laboratory diagnosis of the main autoimmune diseases:systemic lupus erythematosus and rheumatoid arthritis.
6. Ask for and interpret the main tests required for the laboratory diagnosis of the infection caused by SARS-COV-2 virus

- **Definition:** the pathological, over activated immune response is characterized by:
  - *excessive increase in the intensity and/or duration of the mechanisms* involved in the body's specific defense against foreign antigens in the case of **hypersensitivity reactions**
  - *targeting the self-antigens* in the **autoimmune diseases**

I. INVESTIGATION OF HYPERSENSITIVITY REACTIONS

- **Definition:** *induction by foreign ("non-self" or exogenous) antigens of an excessive increase in the intensity and/or duration of the immune response (IR),* which can be:
  - **humoral** - mediated by B lymphocytes
  - **cellular** - mediated by cytotoxic T lymphocytes
- **Classification:** according to the mechanism of tissue injury production, **4 types of hypersensitivity (HS) reactions** were described (Gell & Coombs classification,1963):
  - ✓ **Type I:** Anaphylactic reaction
  - ✓ **Type II: Cytotoxic reaction**
  - ✓ **Type III:** Immune complex-mediated reaction
  - ✓ **Type IV:** Cell-mediated reaction
- **Characteristics:**
  - *Types I, II, III* – belong to the **immediate HS reactions** that occur in *minutes-hours* from the exposure to the sensitizing antigen (Ag) and are mediated by an excessive **humoral immune response**
  - *Type IV* – **delayed HS** occurs 48-72 hours after the exposure to the sensitizing Ag and is mediated by an excessive **cellular immune response.**

A. Type I HS: ANAPHYLACTIC Reaction

- **Definition:** HS reaction mediated by **IgE antibodies** (Ab) as a response to specific Ag called **allergens** which induce *mast cell degranulation.*
  - Allergens enter the body through **4 pathways**:
    - **Inhalant:** pollen, dust mites, mold, animal products (hair, feathers, dander, saliva, urine), chemical substances (sprays, scented sticks)
    - **Digestive:** eggs, milk, peanuts, almonds, sea food, strawberries, peaches, antibiotics
    - **Parenteral:** drugs, insect bites
    - **Dermal:** cosmetics, animal products
- Occurs in **atopic** individuals who present a **hereditary predisposition for allergic manifestations.**
- **Clinical forms –** according to the site of the Ag-Ab reaction, **2 forms of type I HS** occur:

  a) Local - Ag-Ab reaction at TISSUE level:
  - *Allergic rhinitis*
  - *Extrinsic allergic asthma*
  - *Atopic dermatitis (eczema)*
  - *Allergic gastroenteritis*

  b) Systemic - Ag-Ab reaction at INTRAVASCULAR level:
  - *Angioedema - subcutaneous edema* (eyelids, lips, etc.)
  - *Urticaria*
  - *Anaphylactic shock – vascular collapse, death by laryngeal edema and asphyxiation (medical emergency!)*

- **Evaluation of the type I HS reaction:**

1. Multi-allergenic tests (Phadiatop, Phadiatop Infant)
2. Measurement of total IgEs in serum
3. Measurement of specific IgEs in serum
4. Prick tests
5. Specific provocation testing (nasal, bronchial, conjunctival)
6. Differential WBCs count: *eosinophilia* (may be missing in ≥ 1/3 of cases)

1. MULTI-ALLERGENIC tests (Phadiatop, Phadiatop Infant)

- **Definition:** assays for the measurement of serum IgEs that target a homogenous mixture of allergens (food and inhalant Ag), relevant for the age of the patient
- **Clinical value: screening test for ATOPY** (predisposition of the body to trigger the anaphylactic reaction) (Figure 2.1):
  - *A positive* result indicates the **presence of atopy** and requires total IgE measurement, followed by prick tests and measurement of specific IgEs for the *common allergens* in order to diagnose the **typical allergy.**
  - *A negative* result indicates that clinical symptoms are caused by *unusual allergens*, i.e. **atypical allergy** or are NOT due to allergy.

2. Measurement of TOTAL serum IgEs

- **Indication: NEGATIVE** multi-allergenic tests
- **Normal Values :** < 100 UI/ml
- **Clinical value:** A value of total IgE > **150 UI/mL** is suggestive for the presence of **atopy,** i.e. the ***innate predisposition for allergic disorders***. Of note, total IgE can also be increased in the absence of atopy in case of *parasitic diseases* and in *smokers*. On the other hand, total IgE is normal in 20-30% of patients with documented allergy.

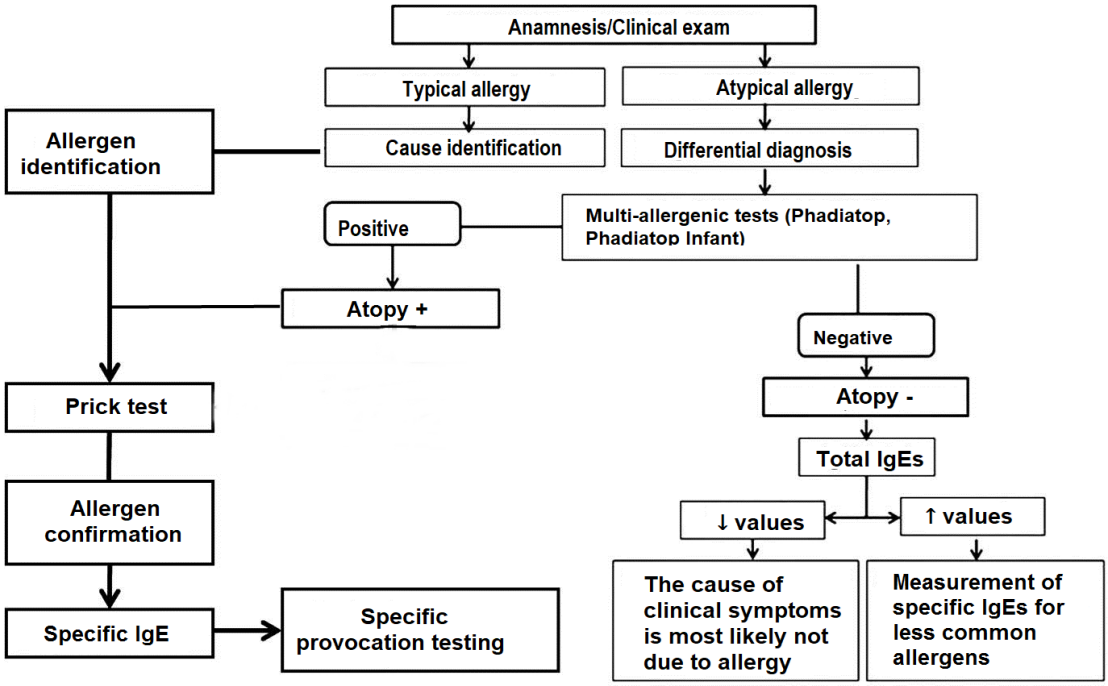


Figure 2.1. Diagnostic algorithm for type I HS reaction.

3. Measurement of SPECIFIC serum IgEs

- **Indication: POSITIVE** multi-allergen tests in:
- Children with *positive familial history* of allergic disorders
- Children and adults suspected of *allergic disorders*
- **Normal Values:** < 0.35 UI/ml
- **Clinical value:**

a) Diagnostic value - identification of the **allergen responsible** for diseases mediated by type I HS reaction: *angioedema, extrinsic asthma, atopic dermatitis*

b) Prognostic value - the presence of specific IgE for *food and contact allergens* in the first year of life is associated with an increased risk of sensitivity towards inhalant allergens and the development of allergic asthma in childhood (7-10 years).

4. Prick tests (scratch tests)

- **Definition:** tests that show the presence of specific IgEs at **skin mast cells** level by exposing them to the suspected allergen
- **Principle:** the allergen is introduced into the **epidermis**, at the level of the inner face of the forearm by pricking the skin with a very fine needle that bears a drop of the diluted allergen solution.
- **Interpretation:**
  - the test is positive if after **15 minutes** at the puncture site a papule **> 3 mm** in diameter is present.
- **Clinical value:**
  the test is positive if after **15 minutes** at the puncture site a papule **> 3 mm** in diameter is present.
  - **Confirmation of atopy** and **identification of the allergen** responsible for the anaphylactic reaction and for the symptomatology
    For the majority of allergens a good correlation between the papule diameter and the serum level of specific IgEs exists.

!Remember: Prick-testing is performed only after **discontinuation, 48 hours in advance**, of any **antiallergic treatment**

5. Specific provocation tests

- **Definition:** tests used to identify the presence of specific IgEs on **mast cells at the level of mucous membranes** *via* their exposure to a suspected allergen.
- **Types:**
- Nasal **for the diagnosis of allergic rhinitis**
- Bronchial **for the diagnosis of extrinsic (allergic) asthma**
- Conjunctival **for the diagnosis of allergic conjunctivitis**
- **Clinical value:** the tests are useful when a ***professional allergy*** is suspected.

B. Type II HS: CYTOTOXIC reaction

- **Definition**: HS reaction triggered against antigens *attached* to the surface of the "target cells" with the formation of antibodies of the **IgG** or **IgM** class
- **Clinical forms**:
  - Transfusion reactions
  - Hemolytic anemia
  - Fetal erythroblastosis
  - Autoimmune disorders: Basedow-Graves disease, pernicious anemia (Addison-Biermer), myasthenia gravis
- **Evaluation of type II HS reaction**
  1. Coombs test – evaluation of anti-erythrocytes antibodies (Ab)
  2. Measurement of auto-antibodies in autoimmune disorders

1. The Coombs test

- **Definition**: a test that demonstrates the presence of **Ab against red blood cells** by triggering an agglutination reaction in the presence of specific Ag expressed on the surface of the erythrocyte membrane ("target cell")
- **Types**:
  - DIRECT Coombs test
  - INDIRECT Coombs test

a. The Direct Coombs test (Figure 2.2a)

- **Principle:** identification of **anti-erythrocyte antibodies attached** to **erythrocytes**. The contact of the patient's red blood cells with the **antiglobulinic serum (anti-IgG)** leads to erythrocytes agglutination if there are anti-erythrocyte antibodies (IgG class) on their surface.

- **Clinical value***:* positive diagnosis of **autoimmune hemolytic anemia**

b. The indirect Coombs test (Figure. 2.2b)

- **Principle:** identification of **anti-erythrocyte antibodies free in the serum**. After the incubation of healthy erythrocytes with patient's serum, the free anti-erythrocyte antibodies become attached to the surface of the red blood cells and trigger their agglutination when exposed to the anti-globulinic serum.
- **Clinical value:** detection of **anti-Rh Ab** in Rh (-) pregnant women with an Rh (+) pregnancy that carry the risk for fetal erythroblastosis.

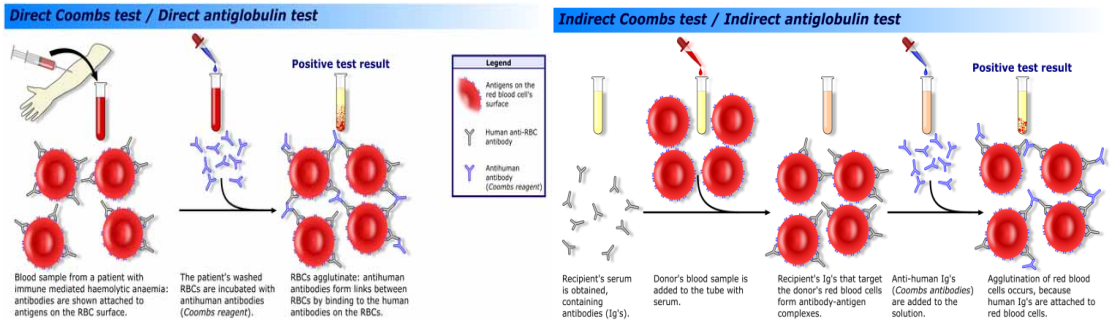


Figure 2.2. Direct (a) and indirect (b) Coombs test.

2. Auto-antibodies measurement

• **Clinical value:** useful in the diagnosis of **autoimmune diseases**

C. Type III HS: IMMUNE COMPLEX-mediated

- **Definition:** HS reaction in which tissue injury occurs as a result of the **formation and deposition of immune complexes (IC)** at the level of **basal membranes:** *vascular, glomerular, and synovial*.
- **Clinical forms:**

a) SYSTEMIC forms – deposition of IC affects one or more tissues or organs in:
  - Poststreptococcal glomerulonephritis
  - Autoimmune disorders, e.g., systemic lupus erythematosus, rheumatoid arthritis (+ type IV HS)

b) LOCAL forms – IC are deposited at the entry site of the antigens in:
  - Extrinsic allergic alveolitis: e.g., pigeon fancier's lung

- **Evaluation of type III HS reaction**
  1. Detection of circulating immune complexes (CIC) in serum
  2. Detection of tissue Ag-Ab complexes in tissue biopsy (e.g., diagnosis of glomerulonephritis by immunofluorescence)
  3. Detection of auto-antibodies in the serum for the diagnosis of *autoimmune diseases* mediated by type III HS reaction:
     - **Anti-nuclear Ab (ANA)** in **systemic lupus erythematosus (SLE)**
     - **Rheumatoid factor** (auto-Ab of the IgM class against IgGs) and **Anti-cyclic citrullinated peptides** (anti-CCP) **Ab** in **rheumatoid arthritis (RA)**

D. Type IV: CELL-MEDIATED reaction

- **Definition:** HS reaction that occurs **48-72 hours** (hence, delayed) after the contact with an Ag and is characterized by: i) the destruction of Ag-carrying „target cells" by **cytotoxic T lymphocytes** and ii) a chronic inflammation (often of granulomatous type) mediated by macrophages**.**
- **Clinical forms:**

a. SYSTEMIC forms:

– Chronic intracellular bacterial infections, e.g., tuberculosis
– Autoimmune disorders with :
  ○ multi-organ damage: SLE, RA (+ type III HS)
  ○ Organ specificity: type 1 diabetes mellitus, Hashimoto thyroiditis, pernicious anemia (+ type II HS)

b. LOCAL forms:
- Contact dermatitis induced by:
  - Tree sap (ivy, oak, etc)
  - Paraphenylendiamine (hair dye, tannery)
  - Nickel compounds (jewelry)
  - Parabens (cosmetic products)
  - Ethylenediamine (preservatives for skin care products, ophtalmic solutions)
  - Formaldehide (cloth dyes)
  - Antibiotics (bacitracine, neomicine)
- Extrinsic allergic alveolitis (+ HS type III)
- Tuberculin intradermoreaction

- **Evaluation of type IV HS reaction:**
  - **QuantiFERON-TB Gold**
  - **Mantoux test (PPD test)**
  - **Patch-tests**

1) QuantiFERON-TB Gold

- **Principle: is the *„gold standard"*** in TB diagnosis, being based on the stimulation of mycobacterium proteins, with the subsequent induction of Lymphocytes' response and the release of IFN-γ.
- **Interpretation:**

a. The test is positive both in **patients with** *M. tubercolsis* **infections**, as well as in those with TB latent infection

b. The test is inconclusive (i.e., a negative LfT response to mycobacterian proteins) in the following situations**:** reduced activity of LfT; insufficient number of LfT; LfT inability to produce IFN-γ; improper samples manipulation.

2) Mantoux test (PPD test)

- **Principle:** the intradermal injection of 5 units of PPD (*Tuberculin Purified Protein Derivative = an extract of Mycobacterium tuberculosis*) on the volar surface of the forearm elicits an erythematous, indurated papule ± vesicles at the injection site after **72 hours**
- **Interpretation**

a. The test is positive, suggesting **tuberculosis infection** if the papule's diameter is:
- ≥ **15 mm** in a *low-risk person* (person without known risk factors for TB infection)
- ≥ **10 mm** in a person at *medium risk of TB infection* (patients with chronic pathologies such as diabetes mellitus, chronic renal failure, neoplasms, microbiology laboratory staff) or in those with BCG vaccination > 15 years earlier
- ≥ **5 mm** in immunocompromised patients or in a person at *high risk of TB infection*

b. PPD test conversion consists in **a negative test being followed by a positive one** and indicates a **recent tuberculosis infection,** if it is not the consequence of a BCG vaccination.

c. A negative reaction does not necessarily rule out the tuberculosis infection, since there is also the possibility that an infected organism may be in the ante-allergic phase or in the context of an anergising disease.

3) Patch-tests

- **Principle:** assays that reproduce the type IV HS reaction at skin level towards a wide variety of contact antigens, after their application in the form of pre-impregnated patches on the intact skin of people suspected of **contact dermatitis**. Suspected allergens ("European standard allergen kit") are placed on the surface of the skin with the aid of **Finn patches**, removed after 48 hours.
- **Interpretation:**
- negative - no reaction
- equivocal/irritative - just erythema
- positive - erythema + vesicles
- extremely positive - erythema + vesicles + edema
- **Clinical value:**
- **"gold *standard*" for the identification of contact Ag**
- Allows the differential diagnosis of contact dermatitis by skin reexamination after another 48 hours:
  - **Persistence of manifestations ⇒ contact dermatitis**
  - Disappearance of manifestations ⇒ **irritative/inflammatory dermatitis**

II. INVESTIGATION OF AUTOIMMUNE DISORDERS

- **Definition:** diseases caused by a decrease in tolerance towards „self" (endogenous) antigens and the onset of an immunological self-aggression process mediated by both types of IR, humoral – Auto-Ab production and/or cellular – mediated by T lymphocytes.
- **Clasification:**

a. Systemic diseases: the immune reaction is oriented against antigens found in **several organs**:

– Systemic lupus erythematosus (SLE)
– Rheumatoid arthritis (RA)
– Scleroderma

b. Organ specific diseases: the immune reaction is oriented against antigens in a **specific organ**

– *Endocrine diseases*: Graves-Basedow disease, Hashimoto autoimmune thyroiditis, Addison's disease, type 1 diabetes.
– *Anemias*: pernicious, autoimmune haemolytic.
– *Myopathies*: miastenia gravis
– *Nephropathies*: Goodpasture syndrome
– *Liver diseases*: primitive biliary cirrhosis
– *Enteropathies*: celiac disease

1. Systemic lupus erythematosus (SLE)

- **Definition: prototype of autoimmune disease**, characterized by **multiorgan damage** and the presence of auto-Ab directed against **nuclear Ag (ANA, Antinuclear Antibodies)** - markers of a type III HS reaction
- **Characteristics:**

– it occurs especially in young women between 20-40 years of age, with chronic evolution marked by exacerbations
– is induced by *"trigger factors"*: sun exposure, drug administration (i.e., procainamide, isoniazid)
– responsable for drug-induced SLE/lupoid syndrome), certain viruses (ex. Epstein-Barr)

- **Laboratory diagnosis:**

– **Measurement of antinuclear antibodies (ANA) = screening test**
– **Complete blood count**: may highlight the consequences of the presence of auto-Ab against blood cells:

o *autoimmune haemolytic anemia* ($\Rightarrow$ positive Coombs test) or resulting from the chronic inflammatory process
o *leukopenia* $\Rightarrow$ L < 4 000/mm^3
o *thrombocytopenia* $\Rightarrow$ PLT < 100 000/mm^3

– **Serum complement fractions:** low C3 and/or C4
– **Chronic inflammatory syndrome:** increased CRP, fibrinogen and ESR
– **Low serum albumin** and **high urinary albumin/creatinine ratio** indicate the presence of lupus nephritis
– **Skin biopsy:** highlights band deposits *('lupus band')* of IgG, IgM and complement fractions at the dermis/epidermis junction
– **Identification of lupus cells** (neutrophils that have phagocytosed DNA remnants) - initially a diagnostic criterion for SLE, currently replaced by the determination of antinuclear auto-Ab (ANA). However, the identification of lupus cells remains a useful test, especially in the presence of atypical clinical or serological manifestations of SLE.

Antinuclear Ab occur in the highest titer in patients with SLE, but are found in smaller amounts in patients with rheumatoid arthritis and can also be detected in the elderly population

! Observation:

According to the **EULAR/ACR 2019** *(European League Against Rheumatism / American College of Rheumatology)* classification, the **mandatory condition for the diagnosis of SLE is the presence of antinuclear Ab (ANA) detected by immunofluorescence in the patient's serum**. If ANA are absent, the diagnosis of SLE is unlikely. If ANA are **present**, **additional criteria** (clinical and laboratory) will be used to calculate a score for the positive diagnosis - the patient presents SLE at a **score ≥ 10**.

Table 2.1. The main autoimmune diseases associated with organ-specific autoantibodies

Autoimmune disease	Auto-Ab
1. Basedow-Graves disease	Anti-TSH receptor Ab
2. DM type 1	Anti-pancreatic beta cells Ab
	Anti-insulin Ab
3. Pernicious anemia	Anti-gastric parietal cells Ab
	Anti-intrinsic factor Ab
4. Myasthenia gravis	Anti-acetylcholine receptor Ab
	Anti-Muscle Specific Kinase (anti-MuSK) Ab
5. Goodpasture Sdr.	Anti-glomerular basement membrane Ab
6. Primary biliary cirrhosis	Anti-mitochondrial Ab
7. Celiac disease	Anti-gliadin Ab

Table 2.2. The clinical value of auto-Ab determined for the diagnosis of SLE.

Auto-Ab	CLINICAL Value
Anti-double stranded DNA Ab	**Confirmation test**
Anti-Sm Ab *Smith nuclear protein (Sm)*	**Confirmation test**
Anti-phospholipid Ab	**Diagnosis of *secondary* antiphospholipid syndrome** (*primary*, in the absence of other pathologies) clinically characterized by episodes of recurrent arterial and/or venous thrombosis and repeated spontaneous miscarriages. ***Anti-phospholipid Ab are:*** - ***Anticardiolipin Ab*** = Ab (IgG, IgM) detected by binding to a phospholipid called ***cardiolipin*** - ***Anti-β2-glycoprotein 1 Ab*** = Ab detected by binding to ***β2-glycoprotein 1*** which in turn interacts with phospholipids - ***Lupus anticoagulant*** • despite its name, it is not a test for the diagnosis of SLE, but its name derives from the fact that lupus-type anticoagulants were initially identified in this pathological context. • the term "anticoagulant" refers to the interaction of lupus-type anticoagulants with phospholipid-protein complexes, which ***in vitro*** elongates phospholipid-dependent coagulation tests (ex. aPTT), without inhibiting the activity of clotting factors. ***In vivo***, however, because the balance of prothrombotic factors differs, lupus-type anticoagulants will show a ***procoagulant*** effect. To confirm the diagnosis of antiphospholipid sdr. blood tests for the detection of anti-phospholipid Ab must be positive at least **2 times** at an interval of **≥ 12 weeks**.

2. Rheumatoid arthritis (RA)

- **Definition:** clinical representation of a type III HS, characterized by inflammatory reactions with multiple locations caused by **auto-antibodies of the IgM class** called **rheumatoid factor**, directed against the Fc fragment of IgG.
- **Characteristics:**
  - more frequent in women between 30-50 years old
  - its etiology includes genetic or environmental factors (smoking, alteration of oral, respiratory and intestinal commensal flora, viruses - eg, Epstein-Barr)
  - typical symptoms include ***symmetrical and progressive damage to small joints***, with pain and stiffness mainly in the morning at this level, accompanied by periarticular muscle atrophy.
  - predominant synovial impairment: ***erosive arthritis + reactive fibrosis + ankylosis***
  - *extra-articular* clinical manifestations appear in evolution, e.g. subcutaneous nodules at the level of pressure points - elbows, Achilles tendon, as well as general clinical manifestations, e.g. fatigue, sleep disorders
- **Laboratory diagnosis:**
  - **Rheumatoid factor** (RF) – increased
  - **Anti–cyclic – citrulinated peptide antibody (anti - CCP)** - **the confirmation test**, being positive in patients with negative RF, even before the clinical onset of the disease
  - **Complete blood count** - may show hematological manifestations of a chronic inflammation:
    - anemia reactive
    - thrombocytosis
  - **Chronic inflammatory syndrome:** increased ESR, fibrinogen and CRP
  - **Joint ultrasound** and **joint MRI** are useful for early detection of synovitis and joint erosions
  - **Joint fluid examination** – used for the differential diagnosis of arthritis

! Observation:
In the case of RA as well, the early positive diagnosis of RA forms without radiological changes is made based on the calculation of a score - the patient has rheumatoid arthritis at a **score ≥ 6**.

III. DIAGNOSIS OF SARS-CoV 2 INFECTION

COVID-19 is the most severe infectious disease of our century caused by the new coronavirus of severe acute respiratory syndrome, SARS-CoV2, whose positive diagnosis requires (Figure 2.3)

1. RT-PCR (Real Time - Polymerization Chain Reaction)
- the **"gold standard"** method for diagnosing SARS-CoV-2 virus infection
- biological material is harvested from the upper airways (nasopharyngeal / oropharyngeal exudate) or from the lower airways (sputum, tracheal aspirate, tracheobronchial lavage) in patients with severe acute respiratory impairment, followed by viral RNA (ribonucleic acid) laboratory processing.

2. Rapid tests based on antigen detection
- identifies the presence of coronavirus proteins (viral antigens) in nasopharyngeal secretion samples collected from patients

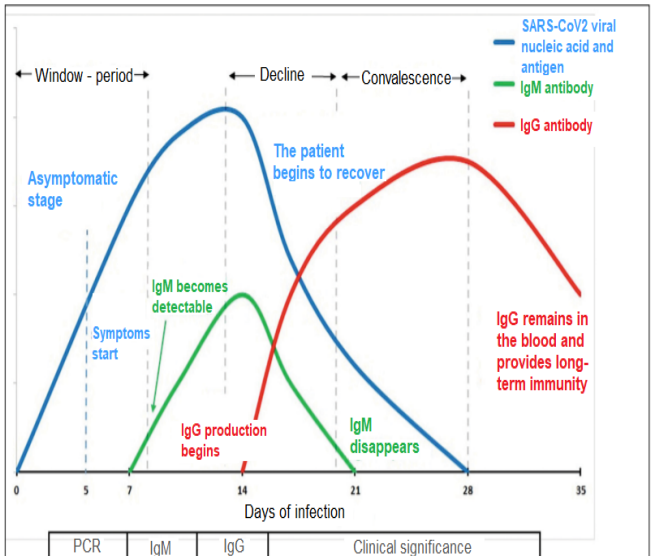
3. Rapid tests based on antibody detection (qualitative tests)
- identifies the presence of antibodies (immunoglobulins M and G) in the blood of people who had previous COVID-19 infection (or are vaccinated)

4. Serological tests (quantitative tests)
- Provide the assessment of the serum levels of IgM and IgG antibodies produced by the body when exposed to COVID-19 infection (or after vaccination). The development of these antibodies takes place over time. Thus, IgM antibodies can be detected in the blood from day 7 after infectious contact, while IgG antibodies appear after day 10.
- The appearance of these antibodies is an indicator of the development of the immune response

5. **Genome sequencing**

It is performed using Novel Generation Sequencing (NGS) technology. It provides an alternative to real-time monitoring of the dynamics of viral transmission by identifying sequences that aggregate together in clusters and, respectively, their correlation with clinical and epidemiological data

Complete genomic sequences are available in international databases (GenBank, GISAID), and the evolution of the pandemic can be monitored in real time on the website https://nextstrain.org/ncov/global, which allows phylogenetic analysis and rate analysis viral variability.



PCR Test	IgM Test	IgG Test	Clinical significance
+	-	-	immunological "window"
+	+	-	early stage of infection
+	+	+	active stage of infection
+	-	+	late/recurrent stage of infection
-	+	-	early stage; PCR can be false-negative
-	-	+	the patient went through infection / recovery
-	+	+	recovery stage; PCR can be false-negative

Figure 2.3. Dynamics of the results of investigations used in the diagnosis in SARS-CoV-2 infection – (Adapted from CliniSciences).

CHECKPOINT!

***1. Which of the following is an anaphylactic reaction assessement test?**
A. Measurement of circulating immune complexes
B. Serum IgE measurement
C. The Coombs test
D. Patch tests
E. Measurement of auto-antibodies in serum

***2. The Coombs test evaluates:**
A.Type I HS reaction
B. Type III HS reaction
C. The presence of antierythrocyte autoantibodies
D. The presence of antinuclear Ab (ANA)
E. Type IV HS reaction

***3. In which of the following situations does the Mantoux test signal a tuberculosis infection:**
A. Papule diameter ≥ 15 mm in a subject with low TB risk
B. Papule diameter ≥ 5 mm in an immunocompromised patient
C. Only erythema occurs at the site of PPD injection
D. Papule diameter is ≤ 2 mm in an immunocompromised patient
E. Only petechiae appear at the site of PPD injection

4. According to the diagnostic algorithm for type I HS reaction:
A. The multi-allergen test allows the identification of specific allergens
B. In the presence of atopy, specific IgE is determined
C. In the absence of atopy, total IgE is determined
D. Prick test is performed to confirm the atopy
E. If total IgE is elevated, clinical symptoms are most likely not due to allergy

***5.Specific provocation tests:**
A. They are useful in exploring the type IV HS reaction
B. Highlight the presence of specific IgE in the skin mast cells
C. Highlight the presence of specific IgE in the mucous membranes
D. They are screening tests to identify atopy
E. Are negative in the presence of atopy

6. Investigation of the type III HS reaction comprises:
A. Identification of contact antigens using patch tests
B. Detection of immune complexes in the serum or in tissue preparations
C. Mantoux test
D. Measurement of antinuclear autoAb in SLE
E. Direct and indirect Coombs test

7. Select the correct statements regarding the type IV HS reaction:
A. It is mediated by humoral immunity
B. It is at the basis of the Mantoux test principle
C. It is at the basis of the Phadiatop test principle
D. It has atopic dermatitis as a clinical manifestation
E. It has contact dermatitis as a clinical manifestation

8. Which of the following are organ-specific autoimmune diseases?
A. Diabetes mellitus
B. Systemic lupus erythematosus
C. Autoimmune hemolytic anemia
D. Rheumatoid arthritis
E. Primary biliary cirrhosis

***9. Which of the following is the mandatory condition for the diagnosis of SLE?**
A. Presence of antinuclear antibodies
B. Identification of lupus cells
C. Increased C-reactive protein
D. Autoimmune hemolytic anemia
E. Leukopenia

10. The diagnosis of rheumatoid arthritis requires:
A. The presence of anti-nuclear antibodies
B. Increased ESR
C. Anti–cyclic – citrulinated peptide antibody (anti - CCP)
D. Skin biopsy
E. Joint ultrasound

CASE STUDIES

1. An 18-year-old patient has been suffering for several years from rhinoconjunctivitis that becomes more severe in August-September. The patient's mother was diagnosed with asthma and the father with contact dermatitis.

Leukocytes = 5 x $10^3/\mu l$
Leukocyte count: NE = 1,7 x 10^9/L, EO = 1 x 10^9/L, BA = 0,1 x 10^9/L, LY = 3,9 x 10^9/L, MO= 0,6 x 10^9/L
ESR = 10 mm/h
CRP = 2 mg/L
Fibrinogen = 244 mg/dL

What is the suspected diagnosis?

List the further investigations you would recommend.

……

……

……

2. A 32-year-old patient, a medical assistant in the Pneumology Department, presents with prolonged fever (> 2 weeks), accompanied by persistent dry cough, fatigue, night sweats, weight loss.

Leukocytes= 14 x $10^3/\mu l$
Leukocyte count: NE = 1,5 x 10^9/L, EO = 0,04 x 10^9/L, BA = 0,05 x 10^9/L, LY = 6 x 10^9/L, MO= 0,7 x 10^9/L
ESR = 40 mm/h
CRP = 20 mg/L
Fibrinogen = 466 mg/dL

What is the suspected diagnosis?

List the further investigations you would recommend.

……

……

……

NOTES

3. INVESTIGATION OF RESPIRATORY DISEASES

LEARNING OBJECTIVES

At the end of this chapter, students are expected to:

1. *Define the principles of pulmonary function tests and the measured parameters.*
2. *Interpret the results of spirometry and plethysmography.*
3. *Interpret the results of bronchomotor tests.*
4. *Understand the role of PEFR monitoring and explain its changes in asthma.*
5. *Define DLCO and list the causes of its pathological values*

I. SPIROMETRY

- **Principle: :** the most **frequently used** pulmonary function test (PFT) that measures how an individual inhales and exhales **volumes of air** as a function of **time**. It allows the recording of the**: *time-volume curve*, *flow-volume loop, direct maximal ventilation curve*** *through* which **static** and **dynamic** lung volumes and capacities are automatically determined. Values are expressed as a percentage (%) of the predicted (ideal) values calculated according *age*, gender, height, ethnicity.
- **Necessary equipment**: spirometer
- **Technique:**
  - The subject sits upright in a chair with legs uncrossed and feet flat on the ground. Nose clips are applied to prevent air leaking through the nose. The subject is asked to exhale as forcefully as possible after taking in a full, deep breath in order to obtain **the volume-time curve** and **the flow-volume loop.**
  - At least 3 technically acceptable determination must be obtained, from which the personal best is chosen
  - There are 2 types of spirometry:
    - *Slow spirometry* – the recording of 2-3 ventilation cycles at rest followed by a slow maximal ventilation cycle, which consists of a prolonged slow maximal exhalation followed by a maximal inhalation, in order to obtain the **volume-time curve.**
    - *Forced spirometry* – recording of a maximal forced ventilation cycle which consists of a maximal inhalation followed by a prolonged forced maximal exhalation (at least 6 seconds), in order to obtain the forced **flow-volume loop**.
- **Clinical value:** spirometry is the cornerstone of PFTs and the most useful tool for the ***diagnosis*** *and* ***follow-up*** *of ventilatory defects* in chronic lung diseases.

A. ASSESSING PULMONARY STATIC VOLUMES

- **Principle:** through slow spirometry a time-volume curve (Fig.3.1) is obtained, on which the **static pulmonary volumes** are determined.

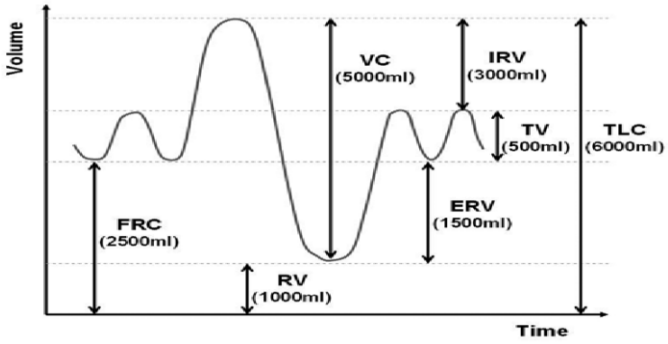


Figure 3.1. Time-volume curve in slow spirometry. Static pulmonary volumes.

- **Evaluated parameters:**
  - **Tidal Volume (TV) –** volume of air inhaled or exhaled with each breath during quiet breathing (6-8 ml/kg) 500 ml
  - **Inspiratory Reserve Volume (IRV)** – maximum volume of air inhaled from the end-inspiratory tidal position
  - **Expiratory Reserve Volume (ERV)** - maximum volume of air that can be exhaled from resting end-expiratory tidal position
  - **Slow Vital Capacity (SVC)** - The volume of air that is exhaled from the lung during a maximal forced expiration effort starting after a maximal forced inspiration.

    SVC = TV + IRV + ERV

– **Inspiratory Capacity (IC)** = the maximum volume of air that can be inhaled from the end-expiratory tidal position.

IC = SVC – ERV

B. ASSESSING PULMONARY DYNAMIC VOLUMES

- **Principle:** by using forced spirometry you obtain a *volume-time curve* (Figure 3.2) on which the **dynamic pulmonary volumes** are determined.

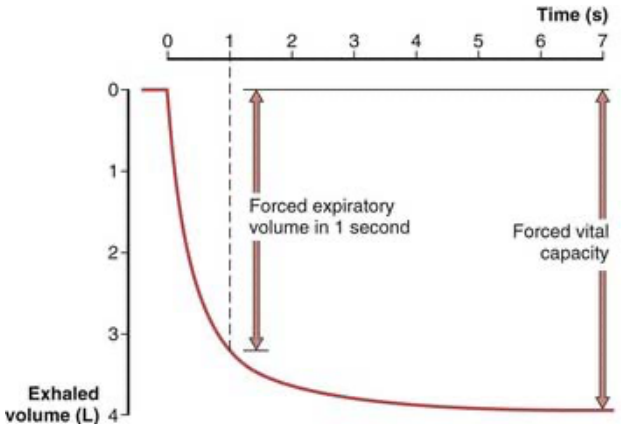


Figure 3.2. Volume-time curve in forced spirometry. Dynamic pulmonary volumes

- **Evaluated parameters:**

– ***Forced vital capacity*** **(FVC)** – the **maximum volume of air** that can be breathed out as **forcefully** and **rapidly** as possible following a maximum inspiration.

– ***Forced Expiratory Volume in the First Second*** **(FEV_1)** – **FEV1** the volume exhaled during the first second of the FVC maneuver.

– ***FEV_1/FCV ratio*** – provides an excellent measure of **airflow limitation**

- **Normal values:**
  – Current FVC ≥ 80% predicted FVC
  – Current FEV_1 ≥ 80% predicted FEV_1
  – FEV_1/FVC ≥ 70% predicted FEV_1/FVC

- **Clinical value:**

1. **FVC** – evaluates the mass of functional pulmonary tissue and the effort the subject performs during ventilation.
   Values < 80% are indicative of ***restrictive ventilatory defect***
2. **FEV_1** – evaluates the degree of permeability (airflow resistance) in the proximal airways (bronchi with a diameter > 2 mm), pulmonary elastic recoil and the effort the subject performs during ventilation.
   – Values < 80% appear in all ventilatory defects and indicate their severity*:*
   - Mild ≥ 70
   - Moderate 60-69
   - Moderate-Severe 50-59
   - Severe 35-49
   - Very severe < 35%

3. **FEV_1/FCV ratio**
   – evaluates proximal airway permeability (diametre > 2 mm), pulmonary elastic recoil and the effort the subject performs during ventilation.
   – Values <70% are indicative of ***obstructive respiratory defect***.

C. ASSESSMENT OF LUNG FLOW RATES

- **Principle:** provides a graphical analysis of inspiratory and expiratory flow from various inspired lung volumes.

1. FLOW-VOLUME LOOP

- **Principle:** by performing a ventilation cycle at rest followed by a forced maximal ventilation cycle you obtain the flow-volume curve (Figure 3.3) which has **2 phases:**
  - *inhalation* with a rounded aspect
  - *exhalation* which quickly reaches a peak at high pulmonary volume values and then decreases linearly or with a slight concave aspect at low pulmonary volumes
- **Evaluated parameters:**
- **Peak Expiratory Flow Rate (PEFR)** = Maximum flow rate during an FVC maneuver, occurs in initial 0.1 sec. After a maximal inspiration, the patient expires as forcefully and quickly as he can and the maximum flow rate of air is measured.

– ***Forced mid-expiratory flow 25-75%***= the mean value of airflow after eliminating 25% of FVC up to the elimination of 75% of FVC.

– ***Forced expiratory flow*** **(FEFx%) =** maximum air flow at the moment when 25%, 50% or 75% of FVC has been eliminated

– ***Maximum expiratory flow*** **(MEFx%)** = maximum air flow at the moment when 25%, 50% or 75% of FVC has yet to be eliminated.

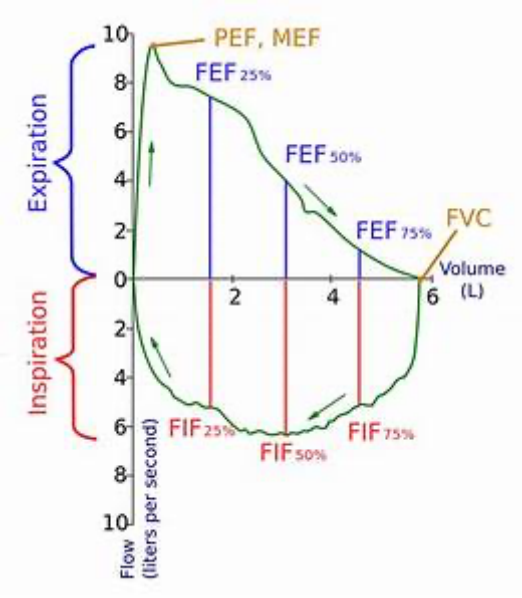


Figure 3.3. Flow-volume loop. Forced expiratory flows.

- **Normal values:**

–actual PEFR ≥ 80% of predicted PEF

–actual $FEF_{25-75\%}$ ≥ 65% of predicted $FEF_{25-75\%}$

- **Clinical value:**

– **PEFR** – has the same significance as FEV_1 and achieving PEFR before eliminating 25% of FVC is an indicator for a **good forced exhalation.** The parameter used to monitor the variability of bronchial obstruction in asthma *(ΔPEFR)*.

– **$FEF_{25-75\%}$** – assesses the degree of permeability (airflow resistance) in the distal airways (bronchi with a diameter < 2 mm), pulmonary elastic recoil and does not depend on the ventilation effort performed by the subject. Values <65% define **distal bronchial obstructive syndrome** which is associated with FEV_1 and FEV_1/FVC ratio at the lower limit of normal values.

2. THE MAXIMAL VOLUNTARY VENTILATION (MVV)

- **Principle:** The subject is asked to breathe as quickly and as deeply as possible for 12 secs and the measured volume is extrapolated to 1min. Periods longer than 15 seconds should not be allowed because prolonged hyperventilation leads to fainting due to excessive lowering of arterial pCO2 and H+.

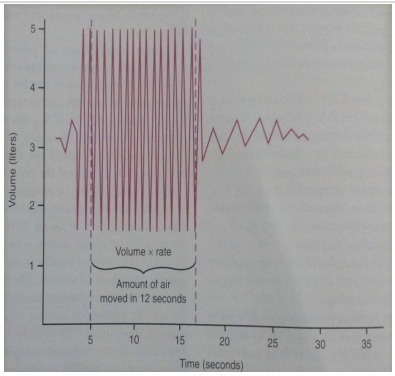


Figure 3.4. The maximal voluntary ventilation.

Remember!

- Due to the difficulty of obtaining the maximal direct ventilation curve in some patients, it is preferred to calculate the maximal indirect ventilation ($V'max._{indirect}$) using the principle that maximal ventilation is attained if the patient takes 30 breaths with the amplitude of FEV_1
- **Computation: MVV = FEV_1 x 30**
- **Normal value: MVV ≥ 80% of predicted MVV**
- **Clinical value:** - Measures the speed and efficiency of filling & emptying of the lungs during increased respiratory effort, i.e., It reflects *peak ventilation in physiological demands*

- Useful in sports medicine

Pathological changes: MVV is markedly decreased in patients with

- Emphysema
- Airway obstruction
- Poor respiratory muscle strength

D. INTERPRETATION OF VENTILATION ABNORMALITIES ACCORDING TO SPIROMETRY

The type of dysfunction is established by:

– determining FVC, FEV_1 and $FEF_{25-75\%}$

– calculating FEV_1/FVC

– analyzing the flow-volume loop aspect

1. RESTRICTIVE VENTILATORY DEFECT (RVD)

- **Causes**:

– Parenchymal *RVD*: diffuse interstitial pulmonary fibrosis, pulmonary tuberculosis, extrinsic allergic alveolitis, pneumoconiosis.

– Extraparenchymal RVD: extrapulmonary conditions limiting the expansion of the chest during forced breathing: obesity, ankylosing spondylitis, myasthenia gravis, muscular dystrophy, kyphoscoliosis.

- **Diagnosis:**

1. *FVC < 80%*
2. FEV_1/FVC *normal or > 70%*
3. FEV_1 *and V'max.*$_{indirect}$ *< 80%*
4. $FEF_{25-75\%}$ *normal* or > 65%
5. *Flow-volume loop* (Figure.3.5) – it is narrower due to decreased pulmonary volumes, but generally has the same shape as a normal flow-volume loop.

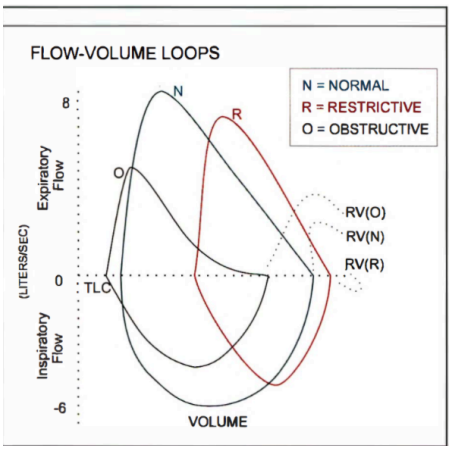


Figure 3.5. Flow- volume loop in OVD and RVD (RV - residual volume; TLC - total lung capacity).

2. OBSTRUCTIVE VENTILATORY DEFECT (OVD)

- **Causes:**

– *typical OVD:* Asthma, COPD (chronic obstructive pulmonary disease)
– *central OVD: stenosis of the superior airways (larynx, trachea) through tumors and intrabronchial foreign bodies*

- **Diagnosis:**

1. *FVC ≥ 80%* or *< 80%* (through an increase in RV in OVD with "captive air")
2. FEV_1/FVC *< 70%*
3. FEV_1 *and V'max.*$_{indirect}$ *< 80%*
4. $FEF_{25-75\%}$ *< 65%*
5. *Flow-volume loop (Figure 3.5)*:

– *Typical OVD* – expiratory flow rates are decreased and $FEF_{50\%} << FIF_{50\%}$
– *Central OVD* – expiratory and inspiratory flow rates are decreased (the peak and the base of the curve are flattened) and $FEF_{50\%} = FIF_{50\%}$.

3. DISTAL BRONCHIAL OBSTRUCTIVE SYNDROME (DBOS)

- **Causes:** chronic tobacco bronchitis, asthma (without crisis), COPD (beginning)
- **Diagnosis:**

1. *FVC normal*
2. FEV_1/FVC *lower limit of the normal range*
3. *FVC and V'max.*$_{indirect}$ lower limit of the normal range
4. $FEF_{25-75\%}$ *< 65%*
5. *Flow-volume loop* (Figure 3.6)*:* accentuation of the concave shape of the expiratory phase

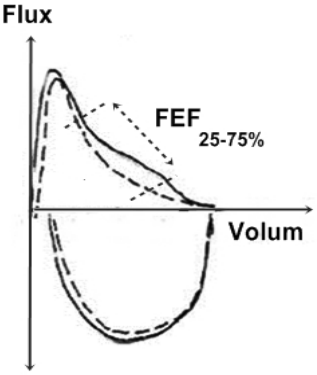


Figure 3.6. Flow-volume loop in distal bronchial obstructive syndrome.

4. MIXED VENTILATORY DEFECT (MVD)

- **Causes**:
  – Pulmonary TB associated with chronic bronchitis
  – Asthma (severe crisis)
- **Diagnosis:**
  – *FVC < 80%*
  – FEV_1/FVC *< 70%*
  – FEV_1 *and V'max.*$_{indirect}$ *< 30%*
  – $FEF_{25-75\%}$ *< 65%*
  – *Flow-volume loop: narrow, very low expiratory flows*

Remember!

The diagnosis of MVD must be confirmed by a decrease of the TLC (the restrictive component) determined through plethysmography

Table 3.1. Diagnosis of ventilatory defects through forced spirometry

Parameter	DBOS	Restrictive VD	Obstructive VD	Mixed VD
FVC	N	↓	N (possibly ↓)	↓
FEV_1	N	↓	↓	↓↓
FEV_1/FVC	N	N	↓	↓
$FEF_{25-75\%}$	↓	N possibly↑)	↓	↓

Note: N - normal, ↑ - increased, ↓ - decreased

II. BODY PLETHYSMOGRAPHY

- **Principle:** according to Boyle's law the product of pressure and volume is constant (P x V = constant), this allows the determination of the functional residual capacity and the calculation of the residual volume and the total pulmonary capacity (static pulmonary parameters).
- **Evaluated parameters:**

– **Functional residual capacity (FRC)** – the volume of air in the lungs at the end-expiratory tidal position

FRC = ERV + RV

– **Residual volume (RV)** – Volume of air remaining in lungs after maximum exhalation

VR = FRC – ERV

– **Total lung capacity (TLC)** – the volume of air in the lungs at the end of a maximal inhalation

TLC = Slow VC + RV

- **Necessary material:** body plethysmograph
- **Technique:**

– A patient is placed in a sitting position in a closed body box with a known volume. The patient pants with an open glottis against a closed shutter to produce changes in the box pressure proportionate to the volume of air in the chest.

– The subject then takes the slow spirometry test to obtain the VC ant the ERV (Figure 3.7)

– At the end of a normal exhalation the flow interrupter is turned on so that during inhalation air will NOT enter the lungs:

  - The volume that corresponds to FRC is decompressed
  - The volume of air in the cabin is compressed due to the expansion of the ribcage
  - The modifications in pressure in the lungs (measured in the mouthpiece) and those in the cabin are inversely proportional with the volumes of gas in the lungs and the cabin

– Knowing that the cabin's volume is 600 liters and measuring the 2 pressure variations (from the lungs and the cabin) it is possible to calculate the volume of air in the lungs which is equal to FRC

– Knowing FRC and ERV it is possible to calculate RV (RV=FRC-ERV)

– Knowing the slow VC and RV it is possible to calculate TLC (TLC=VC+RV)

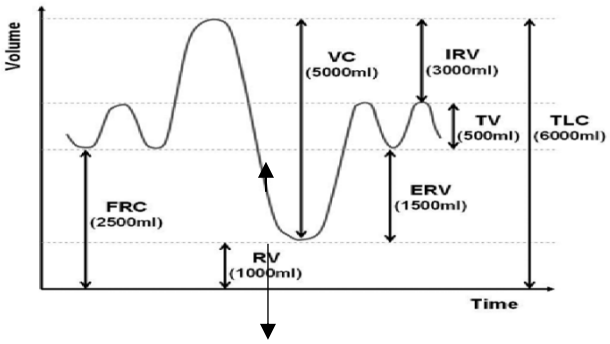


Figure 3.7. Time-volume curve. Parameters determined through slow spirometry and body plethysmography.

- **Normal values:**

– Current RV = 80-135% predicted RV

– Current TLC = 80-120% predicted TLC

RV/TLC x 100 ≤ 35%

- **Clinical value:**

– The diagnosis of the etiopathogenic forms of **Typical OVD:**

  - **OVD** with pulmonary hyperinflation characteristic for **COPD**
  - **OVD** with "air trapping" characteristic for **asthma**

– The diagnosis of the etiopathogenic forms of **Typical RVD**

  - **RVD** - parenchymal in **pulmonary fibrosis**
  - **RVD**- extraparenchymal in **pleural disease, obesity**

– Confirming the diagnosis of **MVD**

Table 3.2. Diagnosis of ventilatory defects through SLOW SPIROMETRY AND BODY PLETISMOGRAPHY

Parameter	DBOS	RVD		OVD	
		Parenchymal	Extraparenchymal	Hyperinflation	*„Air trapping"*
SVC	N	↓	↓	N	↓
RV (FRC)	N/↑	↓	N/↑	↑↑	↑↑
TLC	N	↓	↓	↑	N/slightly↑
RV/TLC	N/↑	N	↑	↑	↑↑

III. BRONCHOMOTOR TESTS

- **Principle:** the bronchomotor effect of substances administered in gaseous form are evaluated through the modifications in **FEV_1** (determined through spirometry) or flow resistance **(Raw)** (determined by body plethysmography)

A. Bronchoconstrictor tests

- **Principle:** inducing bronchial obstruction in patients with **normal FEV1 or Raw** but with clinical suspicion of **bronchial asthma**
  - *Nonspecific tests (methacholine, histamine, acetylcholine, etc.)* prove the patient has a hyperreactive airway (HRA)
  - *Specific tests with different allergens* determine a bronchoconstrictor reaction through type I hypersensitivity
- **Interpretation:** the test is positive if *FEV_1 decreases with more than 20% or Raw increases with more than 40%*
- **Clinical value:**
  - for the diagnosis of asthma
  - a negative test has diagnostic value for the absence of bronchial hyperresponsiveness from COPD

The methacholine challenge test

- **Principle:** progressively higher inhalator doses of methacholine are administered and FEV_1 is determined after each dose. The concentration of methacholine at which FEV_1 is decreased by 20% is identified *($PC_{20\%}$)*.
- **Interpretation**: a $PC_{20\%} < 16$ mg/ml is significant for the presence of HRA which can be:
  - Borderline: $PC_{20\%}$ = 4-16 mg/mL
  - Mild: $PC_{20\%}$ = 1-4 mg/mL
  - Moderate: $PC_{20\%}$ = 0,25-1 mg/mL
  - Severe: $PC_{20\%} < 0,25$ mg/mL

B. Bronchodilator tests

- **Principle:** fast acting β-adrenergic agents are used (Salbutamol) on patients who already have obstructive syndrome (FEV_1 decreased, Raw increased)
- **Interpretation:** the test is positive if *FEV_1 increases with more that 12% or Raw decreases with more than 40 %.*
- **Clinical value:**
  - *diagnostic* – proving the spastic origin and the reversibility of the bronchial obstruction to confirm asthma
  - *therapeutic* – to determine the efficacy of the bronchodilator medication

Remember!

Sometimes in severe asthma the response to the bronchodilator can be weak due to bronchial inflammation. In such cases short term corticoid therapy is indicated (7-10 days) followed by the reevaluation of the bronchomotor test. An increase of $FEV_1 \geq 15\%$ is interpreted as a positive test for bronchial hyperresponsiveness in bronchial asthma.

IV. PEFR MONITORING

- **Principle**: through repeated measurements of PEFR (*peak expiratory flow rate*), in different moments of the day, it is possible to determine the daily variability of PEFR (ΔPEFR)
- **Necessary Material:** peak flowmeter
- **Interpretation:** in healthy subjects the daily variability of PEFR is under 20%. In subjects with asthma the values are **greater than 20%**, proportional to the severity of the asthma. Modern electronic devices assure a simplified interpretation of ΔPEFR by the help of color codes: green – controlled asthma, yellow – requires therapeutic adjustment, red – requires medical reevaluation
- **Clinical value:**

-assessment of asthma severity

- adjustment of BD treatment

$$\Delta PEFR\% = \frac{PEF\ maxim - PEF\ minim}{PEF\ maxim} \times 100$$

V. MEASURING THE DIFFUSING CAPACITY FOR CARBON MONOXIDE CO (DL_{CO})

- **Principle:** the *transfer factor of the lung* (TL) measures the diffusion capacity of the lungs (DL) defined as the volume of gas (measured in milliliters) that diffuses through the alveolocapillary membrane in one minute for a gradient of partial pressure between the alveolar air and the capillary blood of 1 mmHg.
  - DL_{CO} depends on 2 categories of factors:
  - *Membrane factors: the size and the thickness of the exchange surface*
  - *Circulatory factors*: the volume of capillary blood and the concentration of Hb in the blood

Remember!

- *Although the physiological gas of the exchange is O_2, measuring its partial pressure in the capillary blood is difficult. In clinical practice the transfer factor of the lung for CO or the diffusion capacity of the lungs for CO are used because hemoglobin has a very high affinity for CO and the partial pressure of CO in the capillary blood is very small, which allows us to ignore it in the calculus of DL_{CO}.*
- *In smokers, Hb is partially saturated with CO, which lowers its affinity and modifies the result. That is why, the patient is forbidden to smoke a few hours before determining DL_{CO}!*

- **Technique:** usually determining DL_{CO} is done through the **single respiration technique**
  - The subject makes a complete inhalation from the level of RV from a gaseous mix that contains a small, known concentration of CO and holds his breath for 10 seconds and then exhales slowly and completely
  - The concentration of exhaled CO is measured (the air sample is taken from the end of the exhalation to make sure that it is from the alveoli) and the quantity of gas absorbed in the pulmonary capillaries in during the period of apnea is calculated (expressed in ml/min/mmHg)
  - The obtained DL_{CO} value is corrected according to the concentration of hemoglobin in the patient's blood
  - The corrected DL_{CO} value is divided by the volume of air in the alveoli (VA) and the transfer constant is obtained (K_{CO})

$$\mathbf{DL_{CO} = K_{CO} \times VA}$$

- **NORMAL value:**
  - DLCO corrected to Hb value
  - DLCO (corr) = **80-120%** of the predicted value
  - The severity of the decrease of DL_{CO} can be :
    - *mild*: $DL_{CO} > 60\%$ pred
    - *moderate*: DL_{CO} = 40-60% pred
    - *severe*: $DL_{CO} < 40\%$ pred
- ↓ **DLCO with more than 20%** of the predicted value reflects:

1. The reduction of the exchange surface:

- the reduction of pulmonary volumes: lung resection
- the destruction of the alveolocapillary membrane: emphysema

2. The thickening of the alveolocapillary membrane

- interstitial oedema
- pulmonary fibrosis

VI. MEASUREMENT OF THE FRACTION OF EXHALED NITRIC OXIDE (FE_{NO})

- **Principle:** measurement of **FE_{NO}** represents a simple and non-invasive method to appreciate the eosinophilic inflammation within the airways. The analyzed parameter:
  - Responds more rapidly than spirometry to inflammatory changes induced by exposure to allergens = a more sensitive disease *marker*;
  - There is a minimal correlation between the level of **FE_{NO}** and the pulmonary functional tests.

- **NORMAL values: FE_{NO} = 5 - 25 ppm**
  - CLINICAL value: **useful in *diagnosing the eosinophilic bronchial inflammation, monitoring the response to corticotherapy*** *and certain selected cases of refractory* severe asthma
- **Interpretation:**
  - **FE_{NO} < 5 ppm**: primary cilliary dyskinesia, cystic fibrosis, bronchopulmonary dysplasia;
  - **FE_{NO} = 25 - 50 ppm**: possible eosinophilic inflammation;
  - **FE_{NO} > 50 ppm**: permanent eosinophilic inflammation.

CHECKPOINT!

***1. Which of the parameters decreases in distal bronchial obstructive syndrome?**

A. FVC
B. FEV_1
C. FEV_1/FVC
D. PEF
E. $FEF_{25-75\%}$

***2. Which of the folowing parameters is considered a good indicator of expiratory force?**

A. PEFR
B. RV
C. SVC
D. $V'max._{indirect}$
E. FEV_1/FVC

***3. Hyper reactive airway (HRA):**

A. Is defined by decreased FEV_1 with more than 20% at doses of methacholine > 16 mg/ml
B. Is defined by decreased FVC with more than 20 % at doses of methacholine < 16 mg/ml
C. Is characteristic of asthma
D. Is characteristic of COPD
E. Is always reversible (NEGATIVE bronchodilator test) in asthma

***4. Which of the following represents the characteristic modification of the flow-volume loop in the central OVD?**

A. Very narrow flow-volume loop
B. A greater than normal rate of forced exhalation
C. The peak and the base of the curve greatly flattened
D. An accentuation of the concave shape of the flow-volume loop
E. The aspect of "miniloop"

***5. Which of the following values define OVD in asthma?**

A. SVC increased, RV increased, TLC normal, RV/TLC increased
B. SVC decreased, RV increased, TLC normal, RV/TLC ratio increased
C. Increased Raw ≥ 20% at the methacholine test
D. ∆PEFR < 15%
E. FEV_1 increases with more than 20% at the methacholine test

6. Which of the following define the OVD in COPD?

A. FEV1 < 80%
B. SVC < 80%
C. RV > 135%
D. TLC = 80 – 120%
E. RV/TLC ratio > 35%

7. Which of the following define the RVD in pulmonary fibrosis?

A. FVC ≥ 80%
B. SVC < 80%
C. TLC < 80%
D. RV/TLC > 35%
E. RV < 80%

8. Which of the following define the RVD in myasthenia gravis?

A. SVC < 80%
B. TLC < 80%
C. RV/TLC < 35%
D. RV < 80%
E. FEV_1 > 80%

9. Which of the following is the significance of a positive bronchodilator test?

A. The presence of a pulmonary emphysema
B. The presence of a chronic bronchial obstruction caused by COPD
C. The presence of a chronic bronchial obstruction caused by asthma
D. Presence of bronchial hyperresponsiveness
E. The presence of a reversible bronchial obstruction

10. Monitoring PEFR:

A. Is done with the peakflowmeter
B. It is used in the diagnosis of COPD
C. It is useful in the adjustment of the bronchodilator treatment in COPD
D. Shows the great variability of the bronchial obstruction in COPD
E. Is useful to establish the severity of asthma

CASE STUDIES

1. 40-year-old female patient who is a bird breeder, has difficulty breathing, persistent dry cough, inappetence and weight loss. The patient does not smoke and is not allergic. Through forced spirometry the following parameters have been determined: FVC = 55%, FEV_1 = 67%, FEV_1/FVC = 80%, $FEF_{25-75\%}$ = 83%. Through plethysmography the following parameters have been determined: VC = 55%, RV = 50%, FRC = 47%, TLC = 64%, RV/TLC = 22%.

What type of ventilatory defect does the patient have and what is its most probable cause?
Provide reasons for your answer.

……

……

……

2. 39-year-old male patient accuses difficulty breathing, productive cough during the night. The patient is an occasional smoker and has allergic rhinitis. Due to the history of allergies, there is a suspicion of allergic asthma. Through forced spirometry the following parameters have been determined: FVC = 58%, FEV_1 = 39%, FEV_1/FVC = 43%, $FEF_{25-75\%}$ = 23%. Through plethysmography the following parameters have been determined: SVC = 60%, RV = 175%, FRC = 150%, TLC = 117%, RV/TLC = 47%.

What type of ventilatory defect does the patient have and what is its most probable cause?
Provide reasons for your answer.

……

……

……

NOTES

4. INVESTIGATION OF HYPERTENSION AND PERIPHERAL ARTERY DISEASE

LEARNING OBJECTIVES

At the end of this chapter, students are expected to:

1. *Define and enumerate the grades of arterial hypertension*
2. *Interpret the investigations currently used in the diagnosis and management of hypertension*
3. *Interpret the investigations currently used in the diagnosis of peripheral artery disease*

I. INVESTIGATION OF HYPERTENSION

- **Definition:** Arterial hypertension (HT) is defined as the **persistent increase of systolic blood pressure (SBP) values ≥140 mmHg and/or diastolic blood pressure (DBP) values ≥ 90 mmHg** associated with a high risk for ***hypertension-mediated organ damage (HMOD)*** which refers to the occurrence of *cardiovascular, cerebrovascular, ocular* and *renal* complications.

Classification:

- According to the etiology:
  - **primary/essential HT** - 95% of the cases
  - **secondary** HT - 5% of the cases
- According to SBP and DBP values, several categories of HT have been described (Table 4.1).

BP category is defined according to the office BP measurements, with the patient seated, and the higher values of systolic and/or diastolic BP are used as for diagnostic.

Observation! A particular form of hypertension is "**white-coat" HT**, in which *BP values measured in the office are elevated*, but *normal when measured by ambulatory monitoring or at home* (in both treated or untreated patients). It mainly reflects the pressor response to the alerting reaction elicited by office BP measurements by the doctor/nurse and can be associated with all grades of hypertension; however, its prevalence is greatest in grade 1 HT.

Table 4.1. Classification of HT according to *European Society of Cardiology (ESC) Guidelines 2018*

Category	SBP (mmHg)		DBP (mmHg)
Optimal	< 120	and	< 80
Normal	120 – 129	and/or	80 – 84
High normal	130 – 139	and/or	85 – 89
1st grade HT	140 – 159	and/or	90 – 99
2nd grade HT	160 – 179	and/or	100 – 109
3rd grade HT	≥ 180	and/or	≥ 110
Isolated systolic HT	≥ 140	and	< 90

- **Diagnosis of HT requires:**
  - Identification of the increased BP values at repeated office BP measurements, during ambulatory/24 h monitoring and/or at home monitoring
  - Identification of possible causes of secondary HT - imaging and laboratory investigations
  - Assessment of hypertension-mediated organ damage (HMOD)
  - Assessment of cardiovascular risk

A. BLOOD PRESSURE Measurement

- **Principle:** HT diagnosis is based on repeated office BP measurements (classically, at least 3), unless the values are markedly increased (e.g. grade 3 hypertension) or there is evidence of HMOD, e.g., left ventricular hypertrophy, hypertensive retinopathy, or chronic kidney disease (CKD). The 2018 ESC Guidelines also support the use of out-of-office BP measurements, i.e. ambulatory and/or home monitoring as an alternative strategy to

repeated office BP measurements to confirm the diagnosis of hypertension when these measurements are logistically and economically feasible.

Out-of-office BP measurement are indicated in specific circumstances, e.g., when there is considerable variability in the office BP, in the presence of white-coat HT, etc. Ambulatory BP monitoring is particularly indicated for the assessment of BP values at night when nocturnal hypertension is suspected, such as in sleep apnea, CKD, diabetes.

- **Definitions of HT** according to the consulting room, ambulatory, and home blood pressure (BP) values are presented in Table 4.2.

B. LABORATORY and IMAGING Investigations

- **Clinical value:** are mandatory for the assessment of the HT :
  - *etiology* (primary/secondary)
  - *prognosis* (identifying the associated cardiovascular risk factors and organ damage)
  - *treatmen*t (lifestyle particularities, the response to antihypertensive treatment, the degree of compliance to the treatment).

Table 4.2. Definitions of HT according to consulting room, ambulatory, and home BP values *(ESC Guidelines 2018)*.

Category	SBP (mmHg)		DBP (mmHg)
BP measured in consulting room	**≥ 140**	**and/or**	**≥ 90**
Ambulatory blood pressure monitoring (ABPM) with portable devices :			
Diurnal mean value	**≥ 135**	**and/or**	**≥ 85**
Nocturnal mean value	**≥ 120**	**and/or**	**≥ 70**
24 hours mean value	**≥ 130**	**and/or**	**≥ 80**
Mean values of BP measured by the patient at home (auto-measurement)	**≥ 135**	**and/or**	**≥ 85**

1. ROUTINE Laboratory Tests

- **Definition:** laboratory investigations that are considered routine screening tests for the evaluation of hypertensive patients are:

1. Identifying cardiovascular risk factors / comorbidities (DM, hyper/dyslipidemia, metabolic syndrome):
- Fasting blood glucose (+ Hb A1c, in hyperglycemic conditions)
- Serum lipid profile:
  - Total Cholesterol
  - LDL-cholesterol, HDL-cholesterol
  - Triglycerides

2. Heart evaluation:
- 12-leads ECG

3. Kidney evaluation:
- Serum creatinine
- Serum uric acid
- eGFR with MDRD (Modification of Diet in Renal Disease Study)
- Urine exam: qualitative proteinuria (dipstick)

4. Identification of the secondary effects of diuretics (if it's the case):
- Serum potassium and sodium

2. Investigations for the Assessment of HYPERTENSION-MEDIATED ORGAN DAMAGE (HMOD)

- **Definition:** - 2 types of investigations are indicated by the ESC Guideline (Table 4.3):

a. ***Basic screening tests for HMOD:***
- 12-lead ECG
- Urinary albumin: creatinine ratio
- Blood creatinine and eGFR
- Fundoscopy

b. ***More detailed screening tests for HMOD:***
- Echocardiography
- Carotid Doppler ultrasound
- Abdominal Doppler ultrasound and Doppler studies (to evaluate renal size and structure, the adrenal glands and the renal arteries plus the abdominal aorta, respectively)
- Pulse wave velocity (PWV) - a novel index of aortic stiffness and underlying arteriosclerosis
- Ankle-brachial index (ABI) - for the screening of peripheral artery disease (lower extremity artery disease)
- Brain imaging (Table 4.3)

Table 4.3. Assessment of hypertension-mediated organ damage (*ESC Guidelines*, 2018).

Target Organ	Investigation	Indication and interpretation
Heart	*ECG*	Screening for: – left ventricular hypertrophy (LVH) • Sokolow-Lyon index > 35 mm • R wave in aVL ≥11 mm – ischemia – arrhythmias - frequently, atrial fibrillation
	Echocardiography	Assessment of cardiac structure and function, when this information will influence treatment decision: – left ventricular hypertrophy: LV mass > 115 g/m^2 in males and > 95 g/m^2 in females – assessment of left ventricular systolic and diastolic function
Arteries	*Carotid ultrasound*	Detection of the presence of carotid plaque or stenosis: – thickening of the arterial wall (IMT > 0,9 mm) – atherosclerotic carotid lesions (IMT > 1,5 mm)
	Doppler ultrasound	Screening for lower extremity artery disease (LEAD): – Ankle Brachial Index (ABI) < 0,9
Kidneys	*Blood creatinine and eGFR*	– decreased glomerular filtration rate – indicated in all hypertensive patients
	Albuminuria *Urine albumin: creatinine ratio*	– reflects the damage of the glomerular membrane with increased permeability – indicated in all hypertensive patients
Eyes	*Fundoscopy*	Detection of hypertensive retinopathy in patients with grade 2 or 3 hypertension and in all hypertensive patients with diabetes
Brain	*CT scan* *MRI/angio MRI*	To evaluate the presence of ischemic or hemorrhagic brain injury, especially in patients with a history of cerebrovascular disease or cognitive decline

IMT (intimal medial thickness)

3. COMPLEMENTARY Investigations

- **Definition**: are specific tests which are indicated when secondary hypertension is suspected (Table 4.4) or in particular conditions, as follows:
  - Younger patients (< 40 years) with grade 2 hypertension or onset of any grade of hypertension in childhood
  - Clinical or biochemical features suggestive of endocrine causes of hypertension or CKD
  - Clinical features suggestive of obstructive sleep apnea
  - Symptoms suggestive of phaeochromocytoma or family history of phaeochromocytoma
  - Acute worsening hypertension in patients with previously documented chronically stable normotension
  - Resistant hypertension
  - Severe (grade 3) hypertension or a hypertension emergency
  - Presence of extensive HMOD

Table 4.4. Complementary investigations for secondary hypertension.

Type of secondary HT	Screening investigations and their modifications
Obstructive sleep apnea	– Epworth score and ambulatory polysomnography
Renal parenchymal HT	– eGFR < 60 ml/min/1.73 m^2 – increased serum creatinine – urine dipstick for protein/blood, urinary albumin: creatinine ratio – hematuria/leukocyturia – abnormal renal ultrasound
Renal vascular HT	– increased plasma aldosterone (secondary hyperaldosteronism) – increased plasma renin – hypokalemia – proteinuria, usually moderated – increased serum creatinine
Primary hyperaldosteronism	– increased plasma aldosterone – decreased plasma renin – hypokalemia
Cushing syndrome	– increased free cortisol excretion in 24 h urine – negative dexamethasone suppression test (diagnosis confirmation)
Pheochromocytoma	– increased plasma metanephrines – increased of metanephrines and catecholamines in 24 h urine
Thyroid disease (HYPER- or hypothyroidism)	– thyroid hormones (T3,T4) – TSH – Holter monitoring and thyroid function tests
Hyperparathyroidism	– increased PTH – hypercalcemia – hypophosphatemia
Coarctation of aorta	– usually detected in children or adolescence – different BP (≥20/10 mmHg) between upper–lower extremities – rib notching on chest X-ray – echocardiography – decreased ABI

4. BLOOD PRESSURE TREATMENT TARGETS

The aim of hypertension therapy is to lower the BP values under **140/90 mmHg** for all patients and if the treatment is well tolerated, the treatment target BP values should be 130/80 mmHg.

- For all patients under **65 years**, the target systolic BP should be **< 130 mmHg but NOT < 120, and for the patients over 65 years** the target SBP = **130-139 mmHg**. For patients over 80 years, the same SBP target is recommended if they can tolerate the treatment.
- Treatment target for diastolic BP: It is recommended to maintain the DBP values **70-79 mmHg** but **NOT < 70** regardless of the associated comorbidities and cardiovascular risk.

II. INVESTIGATION OF PERIPHERAL ARTERY DISEASE

- **Indications:** According to the 2017 European Society of Cardiology *Guidelines for the diagnosis and treatment of peripheral artery diseases* (PAD), investigations of peripheral arteries are indicated as follows:
  - subjects with a family history of cardiovascular disease
  - symptoms such as angina, dyspnea
  - transient or permanent neurological symptoms
  - any clinical manifestation during walking (fatigue, muscle pain/cramps) located in the gluteal region, thighs, ankles or feet, which disappears at rest
  - any pain at rest localized in the gluteal region, thighs, ankle or feet in orthostatism or clinostatism
  - any foot injuries with reduced healing capacity
  - any upper limb pain induced by exercise, associated with dizziness or vertigo (sign of vertebro-basilar insufficiency)
  - abdominal pain and postprandial diarrhea, which can be recapitulated by alimentation, and are associated with weight loss
  - personal history of hypertension, diabetes, dyslipidemia, renal failure or smoking
  - erectile dysfunction
- **Clinical value:** the screening investigations in PAD allow *the diagnostic* of disease, identify the *anatomical location of lesions*, assess the *severity of disease* progression, and the *treatment response.*

A. NON-INVASIVE Investigations

- **Definition:** a series of tests that can provide information about the location and severity of arterial lesions and the efficiency of therapeutic interventions - post-revascularization.

1. Ultrasonography/ Doppler Ultrasound

- **Principle:** the analysis of the Doppler signal obtained as a result of changes of the signal frequency emitted by the Doppler transducer, caused by the movement in red blood cells (the Doppler effect) - provides information about the:
  - **presence of arterial blood flow**
  - **direction of arterial blood flow** - the blood flow that is approaching the device is recorded as a positive phase and the one that is distancing is recorded as a negative phase
  - **speed of the blood flow** - according to the amplitude of the signal (the amplitude varies inversely with the vessel diameter)
  - **type of the blood flow (laminar or turbulent)**
- **Techniques:** the analysis of the Doppler signal correlated with data provided by the two-dimensional echography are referred as:
  - **duplex ultrasonography** - combination of *bidimensional echography* and *pulsed Doppler* ultrasound
  - **triplex ultrasonography** - adds color Doppler (that reveal the direction of blood flow) or power Doppler (more sensitive than color Doppler for the detection and demonstration of blood flow) to duplex ultrasonography

In these combined techniques:

  - the ***bidimensional echography*** increases the diagnosis accuracy - by providing information about the arterial tract, structure of the vascular wall and perivascular structures
  - the ***pulsed Doppler*** (the same ultrasound transducer sends and receives signals alternately) – allows the generation of the Doppler curve.
- **Clinical value:**
  - Doppler ultrasound is considered the **method of choice** for: i) *diagnosing the presence and the magnitude of arterial lesions and* ii) *the assessment of patients with revascularization after angioplasty or bypass.*
  - It is also used for the evaluation of the degree of collateral circulation as well as for the study

of hemodynamic impact of arterial stenosis on the distal vascular bed
- used for both diagnostic and preoperative evaluation (informations regarding the patency and quality of the distal vascular bed are useful in choosing a revascularization strategy)
- used for patient follow-up after revascularization intervention (bypass or angioplasty)

Ankle/brachial pressure index (ABI)

- **Definition:** is the ratio of the systolic blood pressure at the ankle and the systolic blood pressure in the upper arm, using Doppler ultrasound.

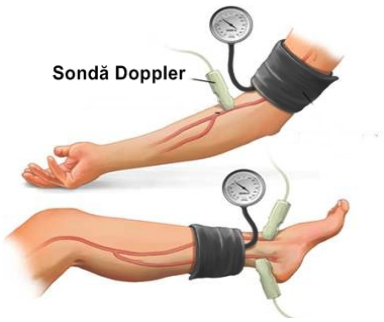


Figure 4.1. Ankle/brachial pressure index *(Modified after http://www.riversideonline.com)*

- **Technique:**
  - patient in dorsal decubitus
  - the pressure cuff is placed with the bottom of the cuff approximately 2-3 cm above the cubital fossa on the arms and the malleolus at the ankle
  - transmission gel is applied, then the tip of the Doppler transducer is placed at a 45° angle pointed towards the patient's head and patient's knee until an audible pulse signal is obtained
  - the pressure cuff is inflated 20-30 mmHg above the point where the pulse is no longer audible
  - the pressure cuff is deflated at a rate of 2-3 mmHg per second, noting the manometer reading at which the first pulse signal is heard and the systolic pressure is recorded
  - the procedure is repeated to measure the pressure on the other arm and leg
  - the highest of the right and left arm and leg's systolic pressures will be used to calculate the ABI
- ***Interpretation:***
  - ***0.9-1.4: NORMAL***. In the presence of a clinical suspicion of peripheral ischemia (intermittent claudication), an effort test with pre- and post-exercise ABI is performed.
  - ***< 0.9*** - suggests lower limb arterial stenosis:
    - mild stenosis: ABI = 0.8-0.9
    - moderate stenosis: ABI = 0.6-0.8
    - severe stenosis: ABI = 0.4-0.6
    - *critical ischemia: ABI < 0,4*
  - ***> 1,4*** - indicates arterial calcification frequent in patients with diabetes and chronic kidney disease (CKD).

B. INVASIVE Investigations

- **Definition:** They represent imaging tests used to determine arterial lesions with indication for endovascular or surgical revascularization

1. Standard angiography with contrast media

- **Definition:** an imaging procedure that uses X-rays to highlight the arterial lumen; it is performed after the injection of iodinated contrast agents using an arterial approach (puncture of the femoral, brachial or radial artery).
- **Clinical value:**
  - *Was considered* ***„golden standard"*** **for PAD diagnosis** & **evaluation of causes, localization**, and severity of lesions (the degree of collateral circulation development) in patients with PAD **before the revascularization interventions.**
  - today, due to invasive approach & risk of complications, less invasive techniques are preferred, with the exception of *vascular damage under the knee*
  - ☞ still an important advantage: it offers the possibility of interventional cure of diagnosed lesions during the same intervention (if these lesions can be resolved with percutaneous revascularization)

2. Computed tomography & angiography (Angio-CT)

- **Clinical value:** is useful in the diagnosis and treatment of peripheral arterial disease
  - limitations of this method are related to the use of iodinated contrast agents (which should be limited in patients with CKD and should be used with caution in case of allergies), radiation

exposure and interpretation difficulties due to the presence of calcium deposits and stents that are a source of artifacts
  - the advantages include: fast and non-invasive acquisition of the image, high availability, high resolution and 3D reconstruction
- it displays a vascularization „map", essential for choosing the interventional strategies (location, severity of arterial lesions)

3. Magnetic resonance angiography (MR angiography)

- **Clinical value:** is a method with a better reliability when compared to other imaging techniques; it provides higher quality images and allows the examination of a three-dimensional image. The contrast media used (gadolinium) has minimal side effects; thus is a highly valuable method for patients with mild to moderate chronic kidney disease.
- **Limitations**: are related to the use of the magnetic field which does not allow the examination of patients with defibrillators, cochlear implants, metal prostheses, etc.

4. Intravascular Ultrasonography (IVUS)

- **Definition:** is a modern methodology using a specially designed catheter with a miniaturized ultrasound probe attached to the distal end of the catheter while its proximal end is attached to the ultrasound machine.
  The major advantage is the visualization not only of the *vascular lumen* but also the of *inner wall* of blood vessels.
- **Clinical value:**
  - the most valuable use of IVUS is to visualize the *structure of the atheroma plaques*, which cannot be seen by angiography (especially useful in situations in which angiographic imaging is considered unreliable)
  - thus, IVUS allows to determine both *plaque volume* within the wall of the artery and the *degree of stenosis* of the arterial lumen

CHECKPOINT!

*__1 Which of the following values of blood pressure corresponds to 1st degree hypertension?__
A. SBP = 135 mmHg, DBP = 85 mmHg
B. SBP = 140 mmHg, DBP = 95 mmHg
C. SBP = 165 mmHg, DBP = 105 mmHg
D. SBP = 170 mmHg, DBP = 95 mmHg
E. SBP = 185 mmHg, DBP = 110 mmHg

*__2. Which of the following investigations isn't part of the routine tests in the case of a hypertensive patient?__
A. Fasting plasma glucose
B. Serum creatinine
C. Echocardiography
D. Serum potassium
E. ECG

*__3. Which of the following laboratory tests is NOT characteristic for hypertension in primary hyperaldosteronism?__
A. Hypokalemia
B. Hypernatremia
C. Hyper-reninemia
D. Hypo-reninemia
E. Increased plasma aldosterone level

__4. Therapeutic target for HT in patients over 65 years of age is represented by:__
A. SBP = 130-139 mmHg
B. SBP = 140-149 mmHg
C. SBP $<$ 130 mmHg
D. DBP = 70-79 mmHg
E. DBP $<$ 70 mmHg

*__5. Which of the following is the best method for postoperative evaluation of patients with revascularization after angioplasty or bypass?__
A. Ultrasound / Doppler
B. Segmental plethysmography
C. Determination of transcutaneous PO_2
D. Standard angiography
E. Magnetic resonance angiography

__6. Which of these investigations show a hypertension-mediated organ damage in a hypertensive patient?__
A. Echocardiography
B. Hemoglobin and hematocrit
C. Serum uric acid
D. Urine albumin-creatinine ratio
E. Fundoscopy

__7. Secondary hypertension is suggested by:__
A. Very high values of BP, especially in the elderly
B. Acute worsening hypertension in patients with previously documented chronically stable normotension
C. Younger patients (<40 years) with grade 2 hypertension
D. Resistant hypertension
E. Slow deterioration of the renal function in a hypertensive patient

__8. A patient with renovascular hypertension presents:__
A. Hyperaldosteronism
B. Decreased renin level
C. Increased renin level
D. Hyperkalaemia
E. Increased excretion of cortisol

*__9. Critical ischemia of the lower limbs is defined by:__
A. Ankle-brachial index < 0.9
B. Ankle-brachial index < 0.4
C. Ankle-brachial index >1.4
D. Ankle-brachial index: 0.6-0.8
E. Ankle-brachial index: 0.4-0.6

__*10. A patient with secondary HT due to Cushing syndrome presents:__
A. Increased free plasma metanephrines
B. Increased urinary excretion of cortisol
C. Decreased serum creatinine
D. Increased parathyroid hormone
E. Abnormal Epworth score

CASE STUDIES

1. A 62-y old patient, diagnosed with hypertension for 6 years, is referred to the hospital for effort dyspnea, palpitations, headache and dizziness. Clinical examination revealed a BP = 165/100 mmHg, HR = 115 b/min and an irregular pulse.

Which investigations would you request for this patient? Which are the treatment targets in this case?

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2. A 58-y old diabetic patient, with history of heavy smoking, presents to the GP for pain (muscle cramp) in the right calf that occurs during walking and mild physical activity. The right foot is pale and cold and has low pulse amplitude at posterior tibial and dorsalis pedis arteries.

Which is the most likely diagnostic?
Which investigations are required to establish the diagnostic & therapy?

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NOTES

5. NORMAL ECG – BRIEF REVISION. ECG IN CARDIAC HYPERTROPHY

LEARNING OBJECTIVES

At the end of this chapter, students are expected to:

1. Recap the normal ECG components.
2. Assess heart rate and QRS axis on ECG.
3. Describe and recognize on ECG the criteria for right and left atrial hypertrophy (enlargement).
4. Describe and recognize on ECG the criteria for right and left ventricular hypertrophy (enlargement).

I. NORMAL ECG – BRIEF REVISION

- **Definition:** A plot of the voltages as a function of time is termed the surface **electrocardiogram (ECG)**.

- **Principles:**
  In order to record an ECG, 10 electrodes are distributed across the body. Electrodes (measuring wires) are placed on the skin, and the wiring for the individual tracing and the tracings themselves are termed "leads".
  ECG electrodes are distributed across the body in a pattern designed to represent two separate planes: a ***frontal plane*** and a ***horizontal plane***:
  - **the frontal plane** – the limb or peripheral leads (bipolar or unipolar leads) obtained by placing **3 recording electrodes** as follows:
    - **R** (the electrode placed on the right arm)
    - **L** (the electrode placed on the left arm)
    - **F** (the electrode placed on the left leg)
  - **the horizontal plane** – the chest or precordial leads (unipolar) obtained by placing **6 recording electrodes**: V1-V6

A. ECG LEADS

1. The Bipolar Limb Leads (standard leads: I, II, III)

- generate an equilateral triangle called Einthoven's triangle (Figure 5.1) with the heart in the middle that detects the electric potential difference between an exploring **(positive)** and a reference **(negative)** electrode, as follows:
  - **Lead I -** between the right arm (RA) and left arm (LA), the left arm being positive
  - **Lead II -** between the right arm (RA) and left leg (LL), the left leg being positive
  - **Lead III -** between the left arm (LA) and left leg (LL), the left leg being positive**.**

2. The Unipolar Limb Leads (aVR, aVL and aVF)

- Are the bisectors of Einthoven's triangle (Figure 5.1).
- The same three leads that form the standard leads also form the three unipolar leads known as the augmented voltage leads (aV). These three leads are referred to as aVR (R for arm), aVL (L for arm) and aVF (F for foot) and also record a change in electric potential in the frontal plane. The exploring (positive) electrode is compared with a reference (negative) which is based on an average between of other two limbs electrodes:
  - aVR - R (+) composed by L and F electrodes averaging
  - aVL - L (+) composed by R and F electrodes averaging
  - aVF - F (+) composed by R and L electrodes averaging

On a triaxial frame system, aVR, aVL and aVF can be projected by connecting the apices of Einthoven's triangle with the center of the equilateral triangle. By producing the leads on negative halves, all six limb leads can be obtained on a **hexaxial reference system** (Figure 5.3). The information from the limb and chest electrodes is combined:

- leads II, III, and aVF view the inferior surface of the heart;
- leads V1 and V2 view the anterior surface, the septum (anteroseptal) and right ventricle;
- leads V3 and V4 view the anterior surface and the apex of the heart (anteroapical);
- leads I, aVL, V5, and V6 view the left ventricle and lateral surface (anterolateral);
- leads V1 and aVR look through the right atrium directly into the cavity of the left ventricle.

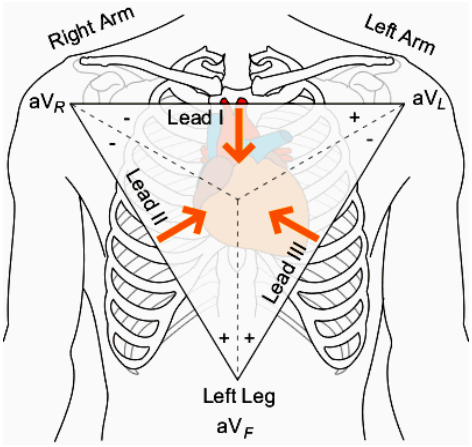


Figure 5.1. ECG limb leads.
Einthoven triangle.
(*Modified after http://www.nottingham.ac.uk)*

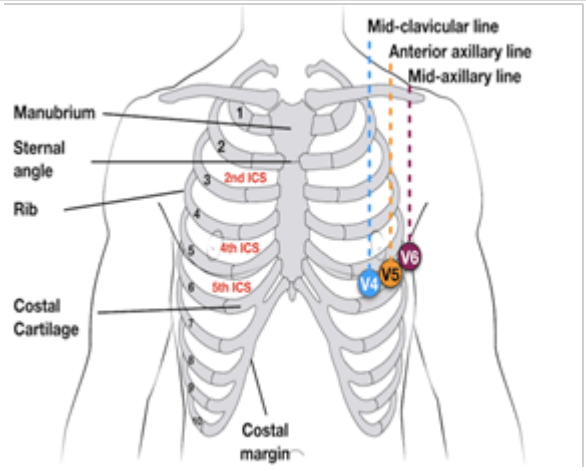


Figure 5.2. The chest electrodes exploring the heart in the horizontal plane.
(Modified after http://www.nottingham.ac.uk*)*

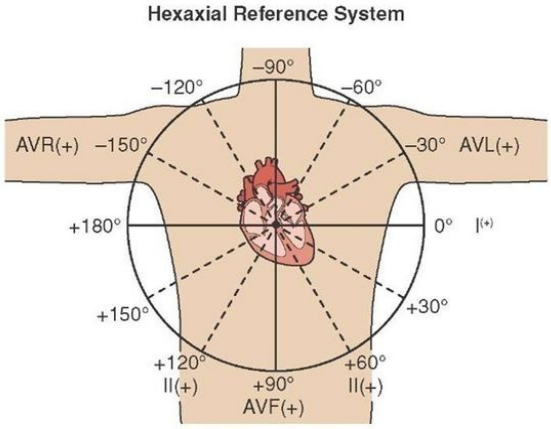


Figure 5.3. Hexaxial reference system
(*After* https://ro.pinterest.com/pin/723391377489012097*)*

3. Precordial leads (chest leads V1 – V6)

– These are six unipolar leads, each in a different position on the chest, designated V1 to V6 - are the exploring leads (the reference electrode is obtained by connecting the left arm, right arm, and left leg electrodes together). The precordial leads are recording the electric potential changes along the horizontal plane (perpendicular to the frontal plane).

They are distributed on body surface as follows:

- **V1**: Positioned in the 4th intercostal space just to the right of the sternum.
- **V2**: Positioned in the 4th intercostal space just to the left of the sternum.
- **V3**: Positioned halfway between V2 and V4.
- **V4**: Positioned at the 5th intercostal space in the mid-clavicular line.
- **V5**: Positioned in the anterior axillary line at the same level as V4.
- **V6:** Positioned in the mid axillary line at the same level as V4 and V5.

Remember!

– *V1 and V2 explore the right ventricle and are called **right precordial leads.***
– *V3 and V4 explore the interventricular septum and are called **septal or transient leads** .*
– *V5 and V6 explore the left ventricle and are called **left precordial leads**.*

B. Normal ECG Elements

An ECG trace records the electrical activity of the heart as **waves, segments and intervals.** ECG is recorded on millimeter ruled paper as follows: the large squares are measuring 5 mm wide and each large square is further divided in 5 small squares of 1 mm width. In order to simplify interpretation, a standard ruling is used. (Figure 5.4):

- the calibration for signal **amplitud**e (voltage) is 1 mV = 10 mm (1 cm). Thus, one large square is equivalent to 0.5 mV and each small square to 0.1 mV. At the beginning of each tracing, a 1 millivolt calibration signal should optimally be visible.
- the calibration for the **time** depends upon the paper speed, usually 25 mm/sec. In this case, the large square is equivalent to 0.2 sec. (5/25) and the small one corresponds to 0.04 sec (1/25).

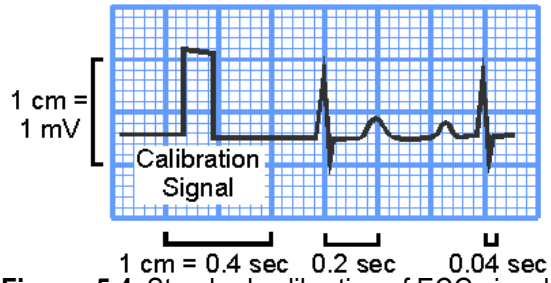


Figure 5.4. Standard calibration of ECG signal.

1. **P wave** (Figure 5.5) - represents the **atrial depolarization** and defines the sinus rhythm
   Characteristics:
   - round, symmetrical, possibly notched / bifid (DII) or byphasic (+/) in V1
   - always positive in leads II, III, and aVF
   - always negative in aVR
   - duration = 0.08 - 0.10 sec
   - amplitude = maxim (2.5 mm) in lead II

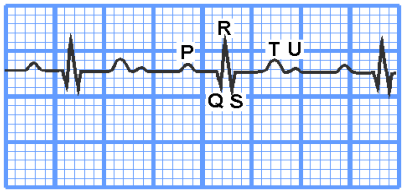


Figure 5.5. ECG waves.

2. **PQ (PR) Interval** (Figure 5.6):
   - represents the atrioventricular conduction (AV node, His bundle and bundle branches depolarization)
   - is measured between the onset of the P wave (atrial depolarization) and the onset of the QRS complex (ventricular depolarization)

   Characteristics:
   - duration = 0.12 - 0.20 sec (inversely related to HR), constant

     !!! PQ interval variation (PR) may occur in *respiratory sinus arrhythmia*

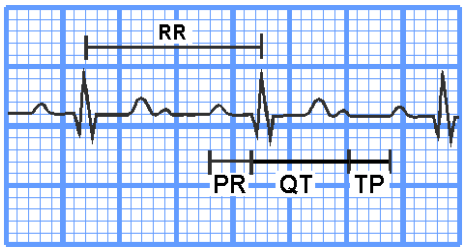


Figure 5.6. ECG intervals.

3. **QRS Complex** (Figure 5.5) – represents the **ventricular depolarization** and includes:
   - *Q wave (the first negative wave)* - depolarization of the *interventricular septum*
   - *R wave (the first positive wave)* - simultaneous depolarization of the right ventricle and of the central and apical region of the left ventricle
   - *S wave (the second negative wave)* - depolarization of the postero-basal region of the left ventricle

   Characteristics:
   - Morphology: depends on the position of the exploring electrode and the electrical position of the heart; positive in most leads, negative in aVR, V1, and V2
   - Duration: 0.08-0.10 sec

The Q wave:

is the first downward deflection (designated normally q)

- represents depolarization of the interventricular septum - normally, always from left to right
- normal: duration < 0.04 s
- amplitude < 1/4 of the following R wave (less than 2 mm)
- visible in leads I, III, aVL, V5, and V6

Remember !

Isolated large or deep Q wave may be seen in **leads III and aVR** as a normal variant. The ***'normal' Q wave diminishes or disappears on deep inspiration*** because of an alteration in the position of the heart, whilst the 'pathological' Q wave of infarction persists

The R wave:

- is the first upward deflection (designated normally R)
- represents depolarization of ventricles (both are activated simultaneously)

- normal: maximal amplitude in lead II (frontal plan) and variable in horizontal plan, increases progressively across the precordial leads to maximum in V5 and V6
- any second upward deflection (pathological) is designated R' or r' prime

The S wave:

- is the second downward deflection (designated normally S)
- represents depolarization of the postero-basal region of the left ventricle
- normal: is the deepest in the right chest leads V1 and V2, decreases in amplitude across the precordium and is often absent in leads V5 and V6.

Reminder!

In the precordial leads, the QRS complex morphology gradually changes from predominant *negative* in leads V1 and V2 to predominant positive in leads V5 and V6. Normally lead **V_1** will show an **rS** type of complex. Then, the R wave will steadily increases in amplitude from lead V1 to V6, with a corresponding decrease in S wave depth, culminating in a predominantly positive complex in **V5** and **V6** (the classical **qRS** complex). This increase in height of the R wave, which usually reaches a maximum in lead V_5, is called **normal R wave progression**.At some point, generally around the V_3 or V_4 position, the R/S ratio becomes 1. This point, where the amplitude of the R wave equals that of the S wave, is called the **transitional zone.** .

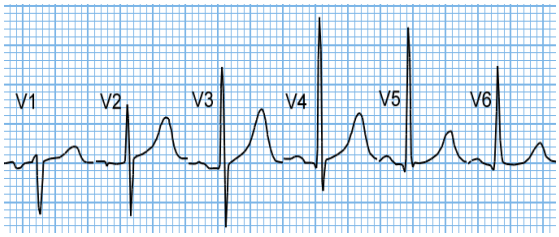


Figure 5.7. Typical changes in QRS morphology across the chest leads

4. **ST segment** – represents the initial part of the ventricular repolarization

Characteristics:

- represents the isoelectric segment recorded between the end of QRS complex and the beginning of T wave
- the junction between the end of the QRS complex and the beginning of the ST segment is referred to as the **J point**

5. **T wave** (Figure 5.6) – represents the final part of the ventricular repolarization

– **Characteristics:**
– asymmetrical shape (rises slowly and returns abruptly to the baseline)
– amplitude is 1/3 from that of the preceding QRS complex
– is always positive in leads I, II, V2, V3, V4, V5, and V6 and negative in aVR

6. **QT interval** (Figure 5.6) – defines ventricular depolarization and repolarization.

Characteristics:

– comprises the **QRS complex,** the **ST segment** and the **T wave**
– QT duration varies with ***heart rate*** (the faster the heart rate, the faster the repolarization, and therefore the shorter the Q-T interval. With slow heart rates, the Q-T interval is longer)
– rate-corrected QT intervals are used in practice and expressed as **QT corrected (QTc)** intervals calculated from the Bazett equation: $QTc = QT/\sqrt{RR}$ (RR = 60/HR)
– normal duration of QTc is up to 0.45 sec
– **abnormal** QT may be:
= prolonged - congenital long QT syndrome, antiarrhythmic drugs (quinidine, procainamide), hypocalcemia, myocardial ischemia and infarction or
= shortened - digitalis, hypercalcemia
– **QT prolongation** may predispose patients to potentially **lethal ventricular arrhythmias** e.g., torsade de pointes.

7. **U wave** (Figure 5.5) – is generated by ventricular post-depolarization, repolarization of Purkinje fibers or papillary muscles.

Characteristics:

– is a small positive deflection that seldom follows the T wave in young people and with bradycardic rhythms (in most ECGs, U wave is not seen at all)
– has the same polarity as the T wave
– the maximum amplitude is in V2, V3 and is less than 1/3 of the amplitude of the T wave

C. Assessment of heart rate (HR)

Methods:

1. If the rhythm is regular - *use the Dale Dubin rule (Figure 5.8):* simply count the number of large squares

between R waves with the following rates: 300 - 150 - 100 - 75 – 60 – 50 -43

Practically:

– start with finding on the ECG tracing an R wave which superimposes on a thick line: if the next R wave falls on the:
  - first thick line (5 mm) ⇒ HR = 300 beats/min (1500/5 = 300)
  - second thick line (10 mm) ⇒ HR = 150 beats/min (1500/10 = 150)

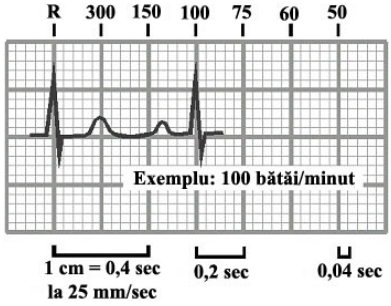


Figure 5.8. Dale Dubin rule

2. If the rhythm is irregular and the ECG does have 30 large squares recorded in one lead

– In such cases a time-averaged rate should be determined by counting the number of QRS complexes along **30 large boxes** (which each represent 0.2 seconds, i.e., 30 x 0.2 **= 6 s**) and multiply by 10 to obtain the heart rate in beats/minute.

3. If the rhythm is irregular and the ECG does not have 30 large squares recorded-use the formula:

– In such cases the **arithmetic mean of 3 RR intervals (mm)** - usually in lead II - is computed and the value is introduced in the HR formula:

HR = 1500 / RR interval (mm).

4. Extrasystoles are included in the HR calculation

5. If the atrial rate is different from the ventricular rate> they are both separately calculated according to the PP intervals for atria and RR intervals for ventricles, respectively

D. Electrical axis determination

Definition:

The **cardiac axis** or the **mean QRS electrical axis** refers to ***the mean direction of the wave of ventricular depolarization in the frontal plan*** and its major diagnostic use is **to identify left and right axis deviations** in this plan.

The mean QRS axis is determined by two major factors:

– the heart anatomy, i.e. position and size

– the direction of ventricular depolarization

Method: The rapid determination of the electrical axis is based on the principle that the morphology of the QRS complex depends on the projection of its resulting vector on the ECG leads.

- AQRS can be determined by analyzing the morphology of the QRS complex in I and III leads (or I and aVF):

– RI - RIII pattern = **normal QRS**

– RI - SIII pattern = **left axis deviation** - **„DIVERGENT**" appearance

– SI - RIII pattern = right axis deviation - "**CONVERGENT**" appearance

Reminder!

- In case of RI SIII pattern **lead II** is also examined to ensure that the deviation is pathological:
- if the QRS complex is predominantly positive in II
  ➔ horizontal normal axis
- if the QRS complex is predominantly negative in II
  ➔ left axis deviation

4. Interpretation (Figure 5.9):

- **Normal QRS axis:** between –30 and +90 (some authors accept 110) degrees
- **left axis deviation** : between –30 and –90 degrees
- **right axis deviation :** between +110 and +180 degrees
- **extreme right axis deviation:** between– 180 and – 90 degrees

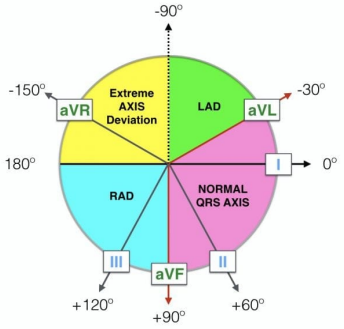


Figure 5.9. QRS Axis.

Observation!

The changes of the heart axis in the horizontal plane are called **rotations** and occur along the longitudinal axis of the heart:

- **clockwise rotation** moves the transitional zone towards the left precordial leads **V5-V6** and is associated with **right ventricular hypertrophy**
- **counterclockwise rotation** moves the transitional zone towards the right precordial leads **V1 V2** and is associated with **left ventricular hypertrophy**.

II. ECG IN CARDIAC HYPERTROPHY

A. ATRIAL HYPERTROPHY

1. Right atrial hypertrophy (RAH)

- **Causes**
  - Pulmonary hypertension
  - Tricuspid regurgitation or stenosis
  - Atrial septal defect
- **ECG criteria** (Figure 5.10):
  - Tall, peaked P wave > 2.5 mm in lead II, also called **„P pulmonale"**

or

  - Biphasic P wave in lead V1 with a predominant positive deflection

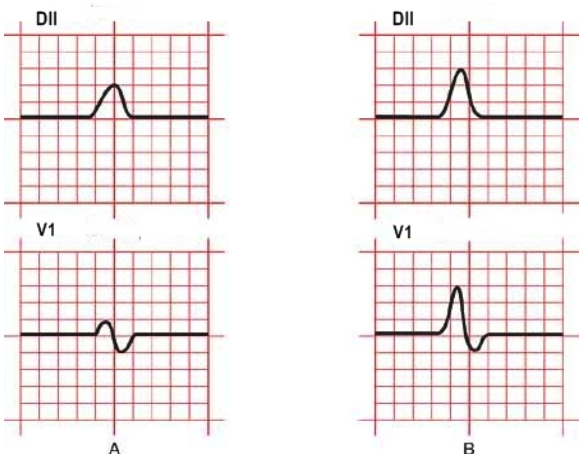


Figure 5.10. ECG in right atrial hypertrophy (A – normal ECG, B – RAH).

2. Left Atrial Hypertrophy (LAH)

- **Causes:**
  - Mitral regurgitation or stenosis
  - Aortic regurgitation or stenosis
  - Left cardiac failure
- **ECG criteria** (Figure 5.11):
  - Broad, notched P wave > 0.12 sec in lead II, also called **„P mitrale"**
  - Biphasic P wave in V1 with a predominant wide negative deflection

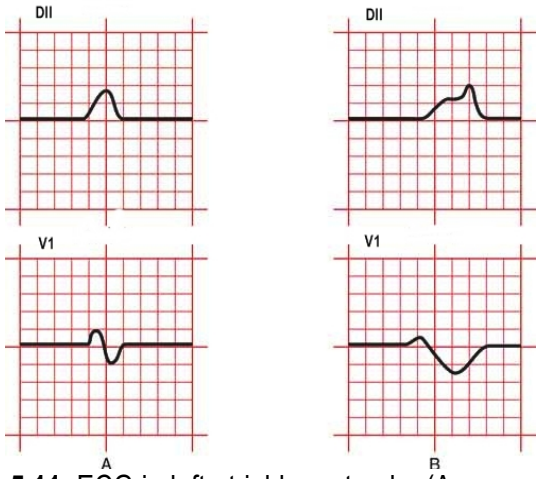


Figure 5.11. ECG in left atrial hypertrophy (A – normal ECG, B – LAH).

B. VENTRICULAR HYPERTROPHY

- **Principle:** myocardial hypertrophy induces changes in both cardiac depolarization and repolarization.
  - **a. Abnormal ventricular depolarization:**
    - Amplitude criteria:

    - increased amplitude of the R waves in the leads that explore the hypertrophied ventricle
    - Duration criteria:

    - QRS duration = 0.08-0.12 sec
    - Electrical axis criteria:

    - axis deviation towards the hypertrophied ventricle

  - **b. Abnormal ventricular repolarization – secondary ST-T changes –** ST segment and T wave are usually opposing to the QRS complex; Inverted (asymmetric) T-wave
    - in direct leads: *down sloping ST segment; negative and inverted T wave*
    - in indirect leads: *ST segment elevation; positive and asymmetric T wave*

1. Right ventricular hypertrophy

- **Causes:**
  - Pulmonary hypertension
  - Pulmonary embolism
  - Mitral stenosis
- **ECG criteria** (Figure 5.12):
  - *Amplitude:*
    - R wave ≥ 5 mm in **V1, V2** lead *or* R/S ≥ 1 in V1
    - Deep S wave in V5, V6 lead
  - *Heart axis:*
    - *Right axis deviation*
  - *Duration:*
    - QRS = 0.10-0.12 sec
  - *ST-T changes:*
    - ST segment depression and inverted T wave in V1, V2

± *Right atrial hypertrophy*

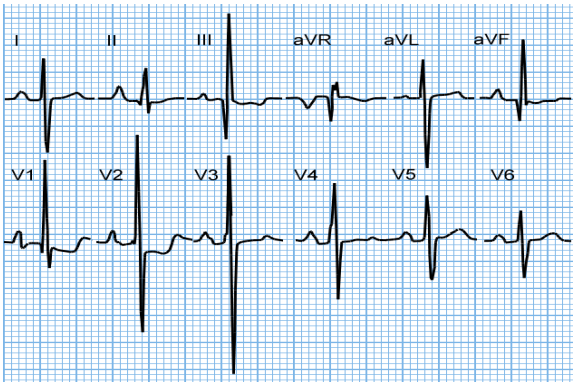


Figure 5.12. Right ventricular hypertrophy.

2. Left ventricular hypertrophy

- **Causes:**
  - Arterial hypertension
  - Aortic stenosis or regurgitation
  - Mitral regurgitation
- **ECG criteria** (Figure 5.13):
  - *Amplitude:*
    - R wave ≥ 11 mm in **aVL**
    - S form **V1** + R from **V5 or V6 > 35 mm** = **Sokolov-Lyon index**
    - Deep S wave in V1, V2
  - *Heart axis:*
    - *Left axis deviation*
  - *Duration :*
    - QRS = 0.10-0.12 sec
  - *ST-T changes:*
    - ST segment depression and inverted T waves in V5, V6
  - ± *Left atrial hypertrophy*

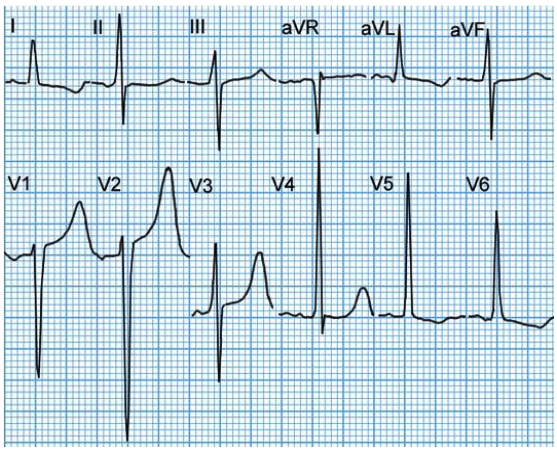


Figure 5.13. Left ventricular hypertrophy.

Reminder!

The assessment of hypertrophy on ECG is just a diagnosis guidance (a Sokolov-Lyon index> 35 mm may also be present in young people as an expression of a hyper voltage trace), thereby echocardiography or novel imaging techniques (CT/ cardiac MRI) are usually required for diagnosis confirmation.

CHECKPOINT!

***1.Which of the following is a characteristic of the normal P wave?**
A. Round and asymmetrical
B. Positive in aVR
C. Duration = 0,08-0,10 sec
D. Amplitude $<$ ¼ of the R wave amplitude
E. Amplitude $>$ 3 mm in lead II

***2. Wich of the following elements has a duration that is inversely related to the heart rate?**
A. P wave
B. ST Segment
C.QRS complex
D. QT interval
E. T wave

***3. Which of the following is a characteristic of the normal Q wave?**
A. Presence in the aVR lead
B. Presence in V1-V3 leads
C. High amplitude in lead III with disappearance in the deep inspiration
D. Duration of 0,04 sec
E. Amplitude $<$ 1/3 of the R wave amplitude

***4. What is the heart rate if the second R wave is on the fourth thick line?**
A. 100 b/min
B. 75 b/min
C. 60 b/min
D. 50 b/min
E. 43 b/min

***5. Which of the following ECG changes indicates right atrial hypertrophy?**
A. P wave with $>$ 2,5 mm amplitude in V1
B. P wave with $>$ 0,12 sec duration
C. P pulmonale in lead II
D. P mitrale in lead II
E. Modified P wave in the presence of mitral stenosis

6. What is the heart axis if the QRS aspect is RI-SIII, i.e., the „divergent" pattern?
A. Normal QRS axis
B. Pathological left axis deviation
C. Pathological right axis deviation
D. Between –30 and –90 degrees
E. Between +110 and 180 degrees

7. Which of the following ECG changes defines left atrial hypertrophy?
A. Broad, notched P wave $<$ 0.12 sec in lead II
B. Broad, notched P wave $>$ 0.12 sec in lead II
C. P wave $>$ 2.5 mm in V1
D. Biphasic P wave in V1 with predominant negative deflection
E. Modified P wave in the presence of tricuspid regurgitation

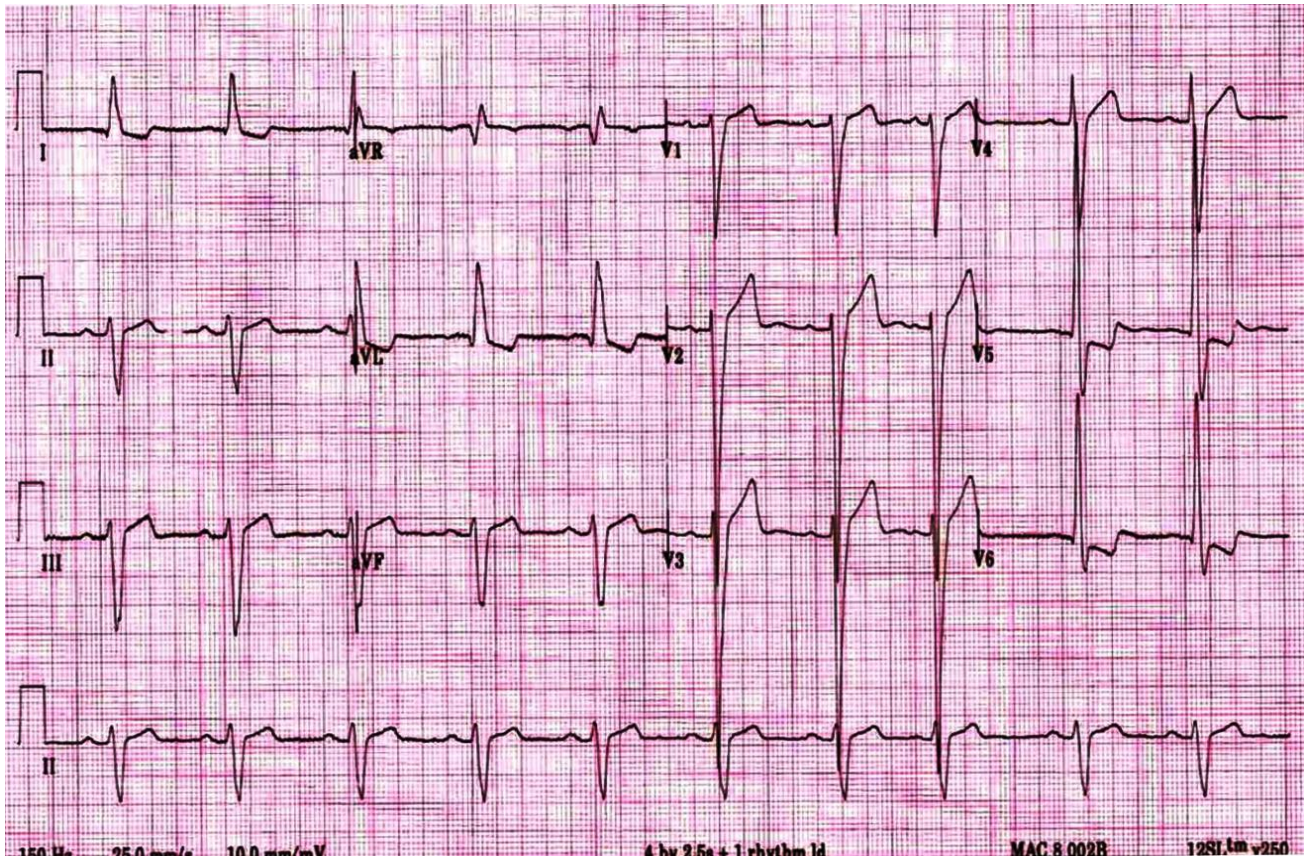
8. Which of following ECG changes defines right ventricular hypertrophy?
A. R wave $\geq$ 25 mm amplitude in V5,V6
B. Deep S wave in V1, V2
C. SI RIII aspect
D. RI SIII aspect
E. ST segment depression and inverted T wave in V1,V2

9. Which of following ECG changes defines left ventricular hypertrophy?
A. R wave $>$ 5 mm in V5, V6
B. Deep S wave in V1,V2
C. Sokolov-Lyon index $>$ 35 mm
D. ST-T changes is V1, V2
E. ST-T changes in V5, V6

10.Which of the following ECG changes are present in both left and right ventricular hypertrophy ?
A. Sokolov-Lyon index $>$ 35 mm
B. QRS = 0,10-0,12 sec duration
C. ST segment depression in all precordial leads
D. Inverted T wave in all limb leads
E. Inverted T wave in precordial leads which explore hypertrophic ventricle.

CASE STUDIES

1. Analyze the following ECG of a hypertensive 64 years old patient who is undergoing a routine check-up. Set the rhythm, heart rate and the electrical axis of the heart and describe the pathological elements identified..



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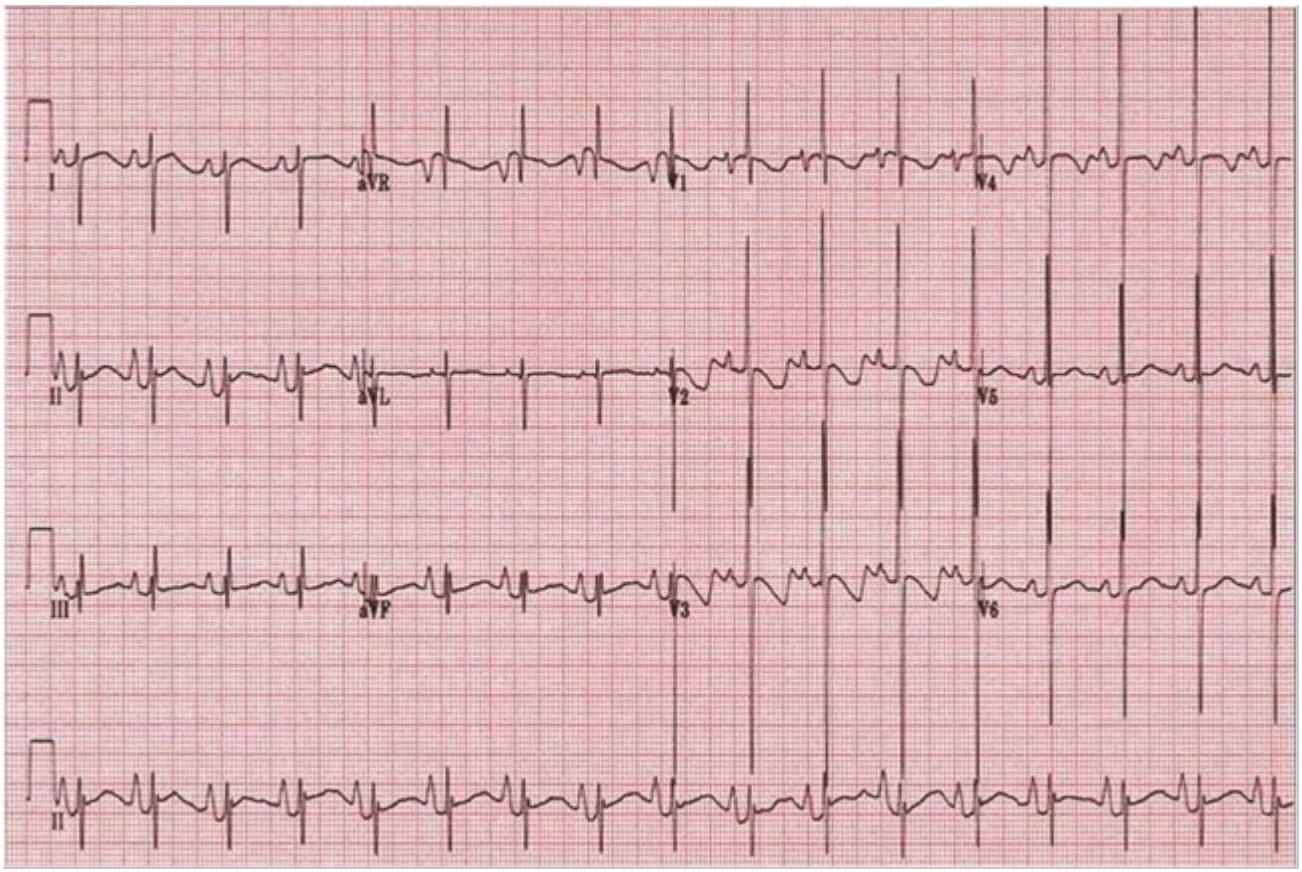
2. Analyze the following ECG of a 65 years old patient diagnosed with pulmonary chronic cord who is undergoing a routine check-up. Check the rhythm, heart rate and the electrical axis of the heart and describe the pathological elements.



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NOTES

6. INVESTIGATION OF CORONARY ARTERY DISEASE

LEARNING OBJECTIVES:

1. *Request and interpret investigations used in the diagnosis of chronic coronary syndromes.*
2. *Request and interpret the investigations used in the diagnosis of acute coronary syndromes.*
3. *Recognize, establish the evolution stage and location of myocardial infarction*

Cardiovascular diseases are currently the leading cause of death in both industrialized and developing countries. Of these, the first place is held by coronary artery disease (CAD), which is characterized by the presence of atheromatous plaques in the coronary arteries, responsible for the occurrence of ***myocardial ischemia***. The severity of this chronic disease is due to its *progressive* nature and the risk of exacerbations caused by complicated atheromatous plaques (thrombosis, erosion, rupture). The clinical presentation of CAD includes: silent ischemia, stable and unstable angina, myocardial infarction, heart failure and sudden death.

The current classification of CAD includes 2 types of coronary syndromes (CS): **chronic** (CCS) - clinical correspondent, angina pectoris and **acute** (ACS) - clinical correspondent, myocardial infarction (European Society of Cardiology – ESC – Guideline 2019).

A. Investigations in CHRONIC CORONARY SYNDROMES - ANGINA PECTORIS

1. *Basic (First-line)* testing

a) Laboratory biochemical testing

- **Definition**: tests used to identify the causes of ischemia, cardiovascular risk factors and associated comorbidities and to establish the prognosis of the disease:

– **Complete blood count**: may reveal *anemia* (decreased oxygen supply to the myocardium) or *infection* (increased oxygen demand)
– **Serum lipid profile**: comprising total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides: may reveal *hyper/dyslipidemia* (important risk factor for coronary artery disease)
– **Fasting plasma glucose** and **glycated Hb (HbA1c)**: may reveal *diabetes* (important risk factor for coronary artery disease)
– **Serum creatinine** and **estimated glomerular filtration rate (eGFR)** - in case of suspected *chronic kidney disease*
– **Thyroid hormones** - in case of clinical suspicion of *thyroid dysfunction* (hyperthyroidism - increased oxygen demand, hypothyroidism - dyslipidemia)
– **Markers of myocardial necrosis** - troponin (preferably highly sensitive), when the clinical examination shows signs of instability.

b) Resting electrocardiogram (ECG)

- **Principle: Angina pectoris** elicits **PRIMARY changes** of **ventricular REPOLARIZATION, i.e**:
  – **ST segment -** signals the ***electrical INJURY***
  – **T wave -** signals the ***electrical ISCHEMIA***

that should be present in **two or more contiguous leads.**

Observation!
*ECG changes are **not** mandatory in angina pectoris, many patients may have normal ECGs if they do not present angina pain at the moment of examination.*

I. ST segment changes

- **Subendocardial ECG injury** (Figure 6.1)**:**

– is due to **chronic ischemia** in the case of stable/unstable angina pectoris, silent ischemia or ischemia during exercise testing
– elicits ***ST segment depression ≥ 1 mm*** that can be **horizontal <u>or</u> down- sloping** with a *duration > 0.08 sec. after the J point.*

Remember!
***Rapidly upsloping ST segment** is considered **normal**, without diagnostic value for angina pectoris.* It may occur physiologically during exercise or in sinus tachycardia regardless its etiology.

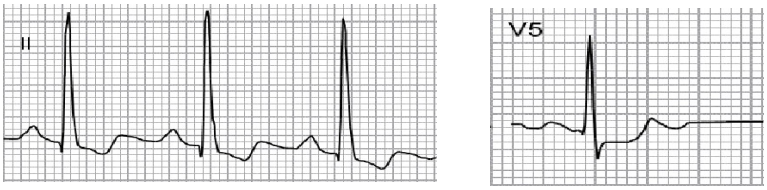


Figure 6.1. Types of ST segment depression in angina pectoris (down-sloping - lead II, horizontal - V5).

- **Transmural ECG injury** (Figure 6.2)
  - is due to a **severe acute ischemia**, such as that during unstable angina, including a particular type called **Prinzmetal angina** (or vasospastic, variant angina) but can also be triggered by exercise testing; it is suggestive for severe coronary stenosis or coronary spasm.
  - elicits **ST segment elevation ≥ 1 mm** that is **transient** - disappears in minutes/hours after the cessation of pain/exercise.

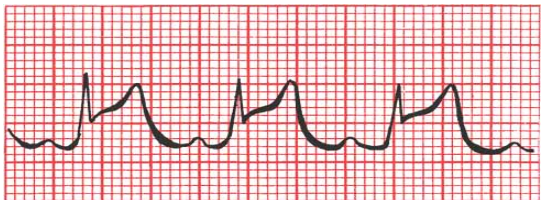


Figure 6.2. ECG in transmural injury.

Remember!
ST segment elevation ≥ **1 mm** that is ***persistent*** for several days is the hallmark of the **acute** stage of ST elevation myocardial infarction (STEMI).

II. T wave changes

Signal the electrical **ISCHEMIA** and comprise several patterns:

- **flattened**, **isoelectric** T waves ***or***
- **inverted, negative** T waves ***or***
- **peaked, symmetrical** T waves (the normal T wave is rounded, asymmetrical)

Remember!
Isolated T wave changes *without* ST segment changes *are suggestive* for myocardial ischemia but ***do not confirm the diagnosis*** *of myocardial acute/chronic ischemia*. Positive diagnosis requires the presence of coronary obstruction through coronary angiography or changes in parietal kinetics by means of imaging investigations.

When analyzing the ST segment changes in the V2-V3 leads, it should be noted that an elevation up to 2 mm in men and up to 1.5 mm in women is considered NORMAL.

Sometimes persistent ST elevations (that may include the T wave as well) may exist on the ECG in an old myocardial infarction ("frozen image") - suggestive aspect for the existence of a ventricular aneurysm as a complication of MI.

c) Resting echocardiography

CLINICAL value: Although it does not directly reveal myocardial ischemia, by evaluating the structure and function of the heart it brings important information for the diagnosis and prognosis of coronary patients by assessing the systolic and diastolic function of the left ventricle and revealing: segmental abnormalities of parietal kinetics, left ventricular hypertrophy suggestive for unsatisfactorily controlled hypertension (and at the same time for an increased oxygen demand), some associated valvopathies

d) Chest X-ray

- **CLINICAL value**: May be useful in the differential diagnosis of chest pain or for the evaluation of patients with heart failure or associated pulmonary pathology.

2. Investigations used to *reveal myocardial ischemia*

- **CLINICAL value**: These are useful investigations to confirm the diagnosis of chronic coronary syndrome, but especially *to identify patients who require invasive angio-coronary investigations and myocardial revascularization*.

a) Exercise electrocardiogram

- **CLINICAL value**:

The test uses a physiological way of cardiac stress induction and is a good option for patients with normal resting ECG, who can be subjected to effort.

Exercise ECG is recommended to assess: the degree of exercise tolerance, symptomatology, the occurrence of cardiac arrhythmias during exercise, the increase in blood pressure and the risk of coronary events in selected patients.

The test can NOT be used to detect ischemia in patients with pre-existing ECG changes (conduction

disorders, pre-excitation syndromes, stimulated rhythm or pre-existing ST-segment depression).

Observation! The ESC 2019 Guideline recommends using the exercise test as a non-invasive test for inducible ischemia ONLY when non-invasive imaging tests are not available.

b) Stress imaging tests

- **CLINICAL value**: Induction of ischemia is done by physical exertion or by using positive inotropic pharmacological agents (dobutamine) or vasodilators of the coronary microcirculation (adenosine, dipyridamole):

– ***stress echocardiography*** - reveals new changes in parietal kinetics and ischemia-induced myocardial thickening deficit (signaling contraction deficit)
– ***stress scintigraphy*** - reveals the decrease in blood flow in the ischemic myocardium (via the decrease in the uptake of radioactive tracer in that territory); can also evaluate the viability of the myocardium, the radioactive tracer permeating only viable myocardial cells, the areas with myocardial fibrosis being hypocaptating. There are 2 technical variants:
  - Single Photon Emission Computer Tomography (**SPECT**) technique
  - Positron Emission Tomography (**PET**) technique
– ***stress magnetic resonance*** - highlights the cardiac perfusion with the help of the gadolinium tracer; can also assess myocardial viability.

3. Investigations used to reveal *coronary anatomy*

a) Computed tomography coronary angiography (angioCT)

- **CLINICAL value**: is a ***non-invasive*** imaging technique that uses iodine-based contrast agents and allows:

– Visualization of ***both the lumen as well as the vascular wall***, thus highlighting coronary atherosclerosis, both hemodynamically significant and insignificant
– Evaluation of coronary calcifications, respectively the presence and extent of coronary artery disease by ***calculating the calcium score (Agatston score)***. Numerous clinical studies have shown that the degree of coronary calcification is proportional to coronary atherosclerosis (although it does not take into account uncalcified plaques) and is of prognostic importance even in hemodynamically insignificant lesions, regardless of age, gender or race.

Observation! The ESC 2019 Guideline recommends the use of angioCT as a preferred test for investigating patients with a low probability of having coronary artery disease, previously undiagnosed with coronary artery disease and with a high likelihood of good image quality. This is because coronary angioCT is a strictly diagnostic investigation, which does not offer the possibility to treat the lesions during the same session, a subsequent invasive coronary angiography being necessary which implies the administration of a higher total amount of contrast substance. In patients with an increased probability of coronary artery disease, invasive coronary angiography is recommended because it offers the possibility of resolution of the lesions within the same intervention.

b) Coronary angiography

- **CLINICAL value**:

It is an ***invasive*** technique that uses iodine-based contrast agents, performed both to confirm the diagnosis of coronary artery disease and to establish the need for revascularization, which can be performed on the spot, following the diagnostic procedure.

The technique does NOT give information about the vascular wall, but only about the lumen (lumenography), neither does it give indications about the hemodynamic significance of the stenosis. Therefore, complementary techniques have been developed that can be performed during coronary angiography: **Intravascular Ultrasound** (IVUS), **Optical Coherence Tomography** (OCT) and the **measurement of the coronary flow reserve** ("Fractional Flow Reserve", FFR). They allow the evaluation of the vascular wall (IVUS and OCT) and the hemodynamic significance of stenoses (FFR).

B. Investigations in ACUTE CORONARY SYNDROMES – MYOCARDIAL INFARCTION (MI)

1. RESTING ELECTROCARDIOGRAM

- **Principle:** Myocardial infarction (MI) elicits **PRIMARY changes** of ventricular **DEPOLARIZATION** and **REPOLARIZATION,** i.e. Q wave, ST segment and T wave abnormalities, respectively. ECG changes occur in leads facing the infarcted area – called **direct leads** and also in those who explore infarction through a normal myocardial wall (opposite to the infarction area, causing the occurrence of „mirror images") – called **indirect leads**.

- **Classification**: 2 types of MI are encountered:

✓ **STEMI - ST Elevation Myocardial Infarction** (formerly known as the Q wave-MI), which is associated with **transmural necrosis**.

✓ **Non-STEMI – Non ST Elevation Myocardial Infarction** (formerly known as the non-Q MI) which is associated with **subendocardial necrosis**.

Remember!

The category of acute coronary syndromes without ST-segment elevation also includes unstable angina. The difference between this and non-STEMI myocardial infarction consists in the duration and intensity of painful symptoms (subjective aspect) and, most importantly, in the serum values of enzymatic markers (in unstable angina the values are normal !).

a) Stages of STEMI as seen on the ECG

DIRECT SIGNS:

I. ACUTE STEMI stage:

- **ST elevation:**
  - is the **FIRST** ECG sign in the AMI evolution
  - occurs in minimum **2 contiguous leads** and is ≥ **1 mm** in limb leads and ≥ **2 mm** in chest leads
  - ECG patterns:
    - ***"monophasic wave"*** pattern (Figure 6.3 left)
    - ***"tombstone"*** appearance - convex ST segment elevation that includes the T wave (Figure 6.3 right)

Remember!

Persistent ST segment elevation signals electrical INJURY.

Sometimes, in the first 1 to 3 hours after the onset of pain (the "superacute" phase of AMI) a very wide, tall, sharp T wave called **"hyperacute T"** appears - a change rarely seen on the ECG.

- **Pathological Q wave:**
  - is the **SECOND** ECG sign in the AMI evolution
  - appears at 8 - 10 hours after the onset of pain
  - **pathological Q** is defined by **duration ≥ 0.04 sec** and **amplitude** ≥ 25% (1/4) of the ensuing R wave
  - **QS complex** (a single large negative deflection) when present in 2 contiguous leads is the equivalent of the pathological Q wave.

Remember!

The pathological Q wave or the QS complex signals electrical NECROSIS

Observation!

Isolated Q waves in lead III and aVL or **isolated QS complex in V1, V2** DO NOT indicate NECROSIS.

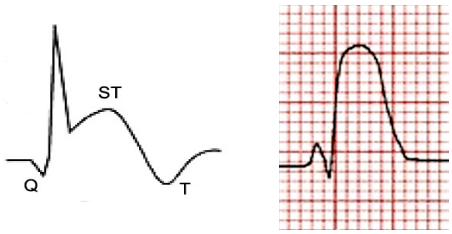


Figure 6.3. The acute STEMI stage direct signs: "monophasic wave" (left) and "tombstone" pattern (right).

- **Pathological T wave:**
  - is the **THIRD** ECG sign in the AMI evolution (Figure 6.3 left)
  - it represents a deep, symmetrical T wave inversion

Remember!

The negative T wave signals the electrical ISCHEMIA!

ECG changes in the acute STEMI stage are shown in Figure 6.4 (A-D).

II. SUBACUTE (RECENT) STEMI stage

- is associated with (Figure 6.4 E):
  - ST segment returns progressively to the isoelectric line - ***disappearance of INJURY***
  - persistent negative, inverted or isoelectric or positive T waves - ***persistence or disappearance of ISCHEMIA***
  - pathological Q wave or QS complex - ***persistence of NECROSIS***.

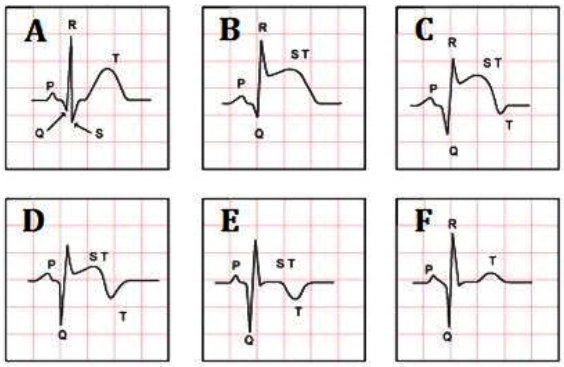


Figure 6.4. ECG findings in the evolution of STEMI: **A.** "*Hyperacute*" T wave (rare); **B. Acute stage** with ST elevation; **C. Acute stage** with ST elevation and pathological Q wave; **D. Acute stage** with ST elevation, pathological Q wave and negative, sharp, symmetrical T wave **E. Subacute stage** with isoelectric ST segment, negative T wave, pathological Q wave. **F. Chronic stage** with pathological Q wave that persist for the rest of the patient's life. *(Modified after https://www.dreamstime.com/stock-illustration-ecg-evolution-step-step-stemi-st-elevation-myocardial-infarction-acute-coronary-syndrome-angina-pectoris)*

III. CHRONIC STEMI stage

- is associated with (Figure 6.4 F):
  - isoelectric ST segment – disappearance of injury
  - isoelectric or positive T wave – disappearance of ischemia
  - pathological Q wave or QS complex as permanent ECG changes - PERSISTENCE of NECROSIS for the entire LIFETIME.

Remember!

The presence of complete left bundle branch block (LBBB) on the ECG renders the diagnosis of acute or recent STEMI very difficult. In order to be able to confirm the presence of myocardial infarction in patients with complete LBBB, the Sgarbossa criteria of ST segment elevation are used (See Cardiology).

The appearance on the ECG of a new LBBB is always pathological and can be a sign of AMI.

b) Myocardial Infarction LOCALIZATION

I. Acute STEMI localization

- **Principle:** STEMI location is based on the identification of ECG leads that show the ***direct signs*** of infarction (Table 6.1):
  - ST segment elevation
  - Pathological Q waves
  - Negative, symmetrical T waves

Table 6.1. Myocardial infarction location **-** direct ECG signs.

AMI location	ECG changes
Anterior	V1-V4
Antero-septal	V1-V2
Antero-apical	V3-V4
Extended anterior	V1-V6
Lateral	V5-V6, lead I, aVL
Inferior	Leads II, III, aVF

II. POSTERIOR AMI localization

- **Principle**: posterior infarction does not elicit direct signs in standard ECG leads, only in V8, V9 and esophageal leads.

  The ECG identification is therefore based on the **indirect signs** that appear in **V1** and **V2 leads** (possible V3 as well) and consist of:
  - ***R/S ratio > 1*** *= tall R waves in V1 and V2 (the mirror images of Q waves that may be recorded directly using the esophageal leads)*
  - ***ST depression in V1 and V2*** *in the acute stage* (Figure 6.5)

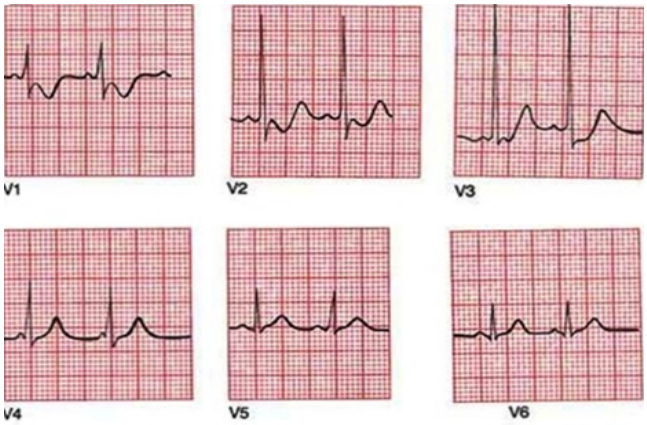


Figure 6.5. Indirect ECG signs of acute posterior MI in the V1 and V2 leads.

III. Acute NON-STEMI localization

- **Principle:** in acute non STEMI the direct signs of infarction are missing because between the standard ECG leads and the infarcted area the normal subepicardial layer is interposed.

Indirect ECG signs appear in the **precordial leads** (Figure 6.6):

- **Lack of pathological Q waves**
- **Diffuse ST segment depression**
- **Negative, inverted, symmetrical T waves**

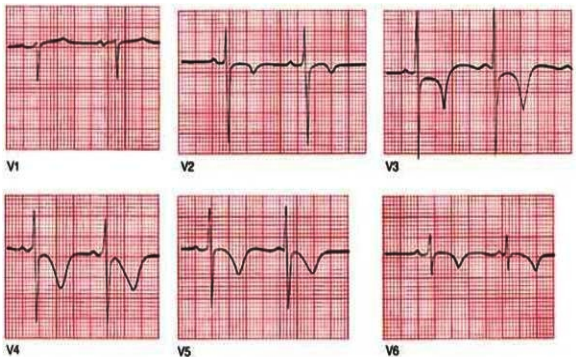


Figure 6.6. Acute non-STEMI with ST segment depression and negative T wave in the chest leads.

2. MYOCARDIAL NECROSIS SERUM BIOMARKERS

Cardiomyocyte necrosis is followed by release into the bloodstream of two types of serum biomarkers:

- ***proteins:*** cardiac troponins T and I, and myoglobin
- ***enzymes*****:** creatinkinase – isoenzyme CK-MB

The European Society of Cardiology emphasized the importance of **serial, dynamic determination** of these biomarkers up to 6-9 hours after the onset of pain, to confirm AMI. A change of ≥ 20% in the serum level between 2 samples collected 3-6 hours apart is considered significant**.** (Figure 6.7)

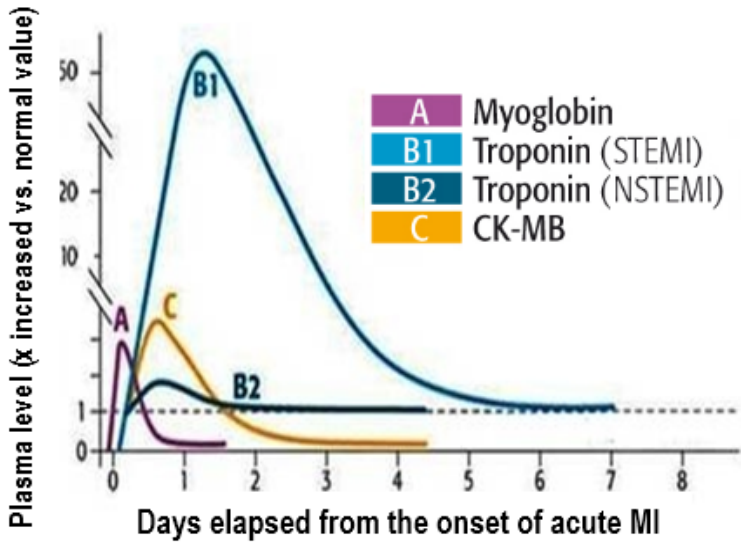


Figure 6.7. MYOCARDIAL NECROSIS serum marker dynamics
(*Modified after http://www.biomerieux-diagnostics.com/vidas-acute-coronary-syndrome-acs-panel*)

The positive diagnosis of AMI involves the presence of **at least 2 of the 3 classic criteria:** *clinical, paraclinical and laboratory - markers of necrosis.*

The positive diagnosis of AMI requires the detection of an **increase and/or decrease (in dynamics)** of myocardial necrosis biomarkers' (especially troponin) concentration with at least one unit above the 99th percentile for the reference superior limit (obtained in normal individuals) **PLUS** at least one of the following criteria:

- symptoms of ischemia: typical angina pectoris or atypical pain
- ECG changes suggestive of new ischemia:
  - changes of ST segment or newly installed left bundle branch block
  - pathological Q waves on the ECG
- echocardiographic proof of a recent loss of viable myocardium or a recent parietal kinetic alteration
- intracoronary thrombi identification during angiography or autopsy

a) Cardiac troponins I and T (cTn)

- **Definition:** troponins (cTn) are regulatory proteins involved in cardiomyocyte contraction, with two clinically relevant forms, TnT and TnI.
- **Dynamics**:

- in the context of **STEMI,** cTn begin to increase at **3-12 hours** after the onset of chest pain, reach a maximum level at **24 hours**, the increase being significant (up to more than 20 times the reference limit) and regain their normal level after **7-14 days** (Figure 6.7 B1)
- in the context of **NON-STEMI** cTn begin to increase at 3-12 hours after the onset of chest pain, but the increase is ***modest*** *and* ***transient*** (Figure 6.7 B2).

Remember!

Differential diagnosis between **acute non-STEMI** and unstable angina is based upon the ***absence of increased cTn*** determined in dynamics in unstable angina, due to the lack of necrosis/cardiomyocyte destruction.

- **CLINICAL value**:
  - currently considered the **SPECIFIC markers for myocardial necrosis**
  - are **cardiosensitive,** the higher the serum level, the higher the probability of MI
  - cTnI and cTnT serum levels can be increased up to 14 days (in extensive STEMIs) and are useful for **subacute MI diagnosis**
  - increased levels, WITHOUT A DYNAMIC MODIFICATION may also appear in the context of **non-coronary myocardial lesions.**

Also, there are several pathological states that can lead to moderate increases in serum levels of cTn (Table 6.2).

Table 6.2. Non-coronary causes for increased TROPONINES

CARDIAC causes	NON-CARDIAC causes
Tachyarrhythmias or bradyarrhiytmias	Ischemic or hemorrhagic stroke
Severe decompensated heart failure (acute or chronic), cardiogenic shock	Critical patients (hypovolemic or septic shock, severe burns)
Pulmonary emboly / Severe pulmonary HT	Acute or chronic renal failure
Hypertensive crisis	Rhabdomyolysis / Extreme physical activity
Myocarditis	Hypo- / Hyperthyroidism
Cardiomyopathy: hypertrophic and Tako-Tsubo	Severe respiratory failure
Aortic dissection	
Aortic stenosis	
Cardiotoxic medication (doxorubicin, 5 – fluorouracil)	
Cardiac maneuvers (percutaneous angioplasty, coronary bypass, cardioversion, ablation, endomyocardial biopsy)	
Infiltrative disorders (sarcoidosis, amyloidosis, sclerodemy, hemochromatosis)	

Remember!
In case of acute STEMI, if chest pain and ST segment elevation are present, one must not wait for serum cTn analysis in order to initiate reperfusion therapy (thrombolysis or angioplasty).

b) High-sensitivity cardiac troponins (hs-cTn I, hs-cTn T)

- **Definition:** determination of serum levels of cTnI and cTnT by use of methods that allow early detection, in the first **1-3 hours** after the onset of the symptoms, of cTn increases in the range of **ng/mL**.
- **Clinical value:**
  - a positive result will have a ***positive predictive*** value for AMI and improves the sensitivity of cTn determination in the first 3 hours after the onset of necrosis („*troponin blind period*").

c) Creatin kinase – isoenzyme MB (CK-MB)

- **Definition:** creatin kinase (CK) is an enzyme found in the heart, muscle and brain, presenting 3 isoenzymes:
  - isoenzyme CK-MM – specific for skeletal muscle
  - isoenzyme CK-BB – specific for brain
  - isoenzyme CK-MB – specific for myocardium
- **Dynamics:** CK-MB values start to elevate **3-6 hours** after pain onset, reaching a maximum at **24 hours** and returning to normal after **2-4 days**(Figure 6.7 C).

- **CLINICAL value**:
  - A **specific marker** for the diagnosis of AMI, **without being superior to troponins**
  - **Serial determinations** upon admission and 6-9 hours after the onset of pain **are mandatory** in order to demonstrate an increase and/or decrease beyond the 99th percentile of the reference population (may be repeated between 12-24 hours, if the initial values were not increased)
  - Values are **correlated with the severity of the necrosis** (infarct area **extension**)
  - The return to normal values followed by a new rise indicates **reinfarction**
  - Elevated values *in association* with an increase of **cTn** have a **negative prognosis** (increased risk of postinfarction mortality)
  - **Non-coronary** CK-MB elevations can be seen in: myocarditis, pulmonary embolism, shock, skeletal muscle trauma, intramuscular injections, convulsions, major physical effort

d) Myoglobin

- **Definition:** hemoprotein with a role in binding O_2, located in the skeletal and cardiac muscle
- **Dynamics:** myoglobin starts to increase **2-4 hours** after the onset of the AMI, reaches a maximum between **6-9 hours** and returns to normal after **24 hours**.
- **CLINICAL value:**
  - Is the **first enzyme** (early marker) that increases in the blood in the case of AMI, but this rise is **NOT specific to myocardial injury**, elevated values being associated with rhabdomyolysis in general.
  - The normal value of myoglobin helps in **excluding the diagnosis of AMI**.

3. IMAGING TECHNIQUES

- **CLINICAL value**: they have an important role in demonstrating the new loss of viable myocardium or regional disorders of parietal kinetics, the imaging criterion being part of the paraclinical criteria that establish the diagnosis of AMI (where these techniques are available).

a) Echocardiography

It is performed in the emergency department for all patients with cardiorespiratory arrest, cardiogenic shock, hemodynamic instability for:

- the differential diagnosis of chest pain with highlighting other possible causes: aortic dissection, pericarditis, myocarditis, valvular diseases, pulmonary thromboembolism, cardiomyopathies
- evaluation of parietal kinetics disorders
- diagnosis of mechanical complications in patients with myocardial infarction.

b) Coronary angiography

It is indicated for diagnostic and therapeutic purposes ***to all patients with STEMI***, as soon as possible in the course of infarction, and to patients with non-STEMI according to the information provided by noninvasive clinical and paraclinical data that allow risk stratification (hemodynamic or electrical instability, the existence of a large area at risk).

c) Cardiac magnetic resonance

It has a limited use, for diagnostic purposes, in the acute phase of MI, being more useful in the ***subacute and chronic stages of MI*** by assessing myocardial perfusion, identifying disorders of segmental kinetics, myocardial area at risk, edema and myocardial fibrosis.

d) Radionuclide imaging techniques (SPECT, PET)

It provides information about myocardial perfusion and cardiac function, but is used sparingly in the acute phase of MI due to the delay in image acquisition (up to 90 min).

CHECKPOINT!

***1. Which of the following ECG criteria occurring in the direct leads defines the *subendocardial* injury in angina pectoris?**
A. Down-sloping ST segment depression < 1 mm
B. Horizontal ST segment depression > 1 mm
C. ST segment elevation > 1 mm
D. Negative, sharp and symmetrical T wave
E. Unchanged ST segment

***2. Which of the following ECG changes is present in leads V1 / V2 in the acute posterior MI?**
A. Significant elevation of the ST segment
B. ST segment depression > 1 mm
C. Pathological Q waves
D. R/S ratio < 1
E. Positive, symmetrical T wave

3. Which of the following statements are true about *transmural* injury?
A. May occur in unstable angina
B. It is common in chronic ischemia
C. Is transient in Prinzmetal angina
D. Is persistent in acute STEMI
E. Elicits ST depression in direct leads

4. The acute stage of non-STEMI is characterized by:
A. Lack of Q wave
B. Pathological Q-waves or QS complex in direct leads
C. Pathological Q-waves or QS complex in indirect leads
D. Diffuse ST segment depression
E. Atrioventricular dissociation

5. Which of the following changes when present in direct leads define STEMI in the subacute stage?
A. ST segment elevation
B. Isoelectric ST segment
C. Normal Q wave
D. ST segment depression
E. Isoelectric or positive T wave

6. Select the correct statements:
A. Isolated changes of the T wave not accompanied by changes of the ST segment have diagnosis value for myocardial ischemia
B. The direct ECG signs appear in the inferior infarction in the V2-V4 leads
C. The 'tombstone' pattern is characteristic for subendocardial infarction
D. The pathological Q wave signals myocardial necrosis
E. The QS complex is equivalent to the pathological Q wave

7. Select the correct statements:
A. AngioCT is an invasive technique
B. Coronary angioCT visualizes both the lumen and the vascular wall
C. Stress scintigraphy allows to highlight areas of myocardial fibrosis
D. Coronary angiography allows revascularization therapy
E. Coronary angiography allows the quantification of the degree of coronary calcification

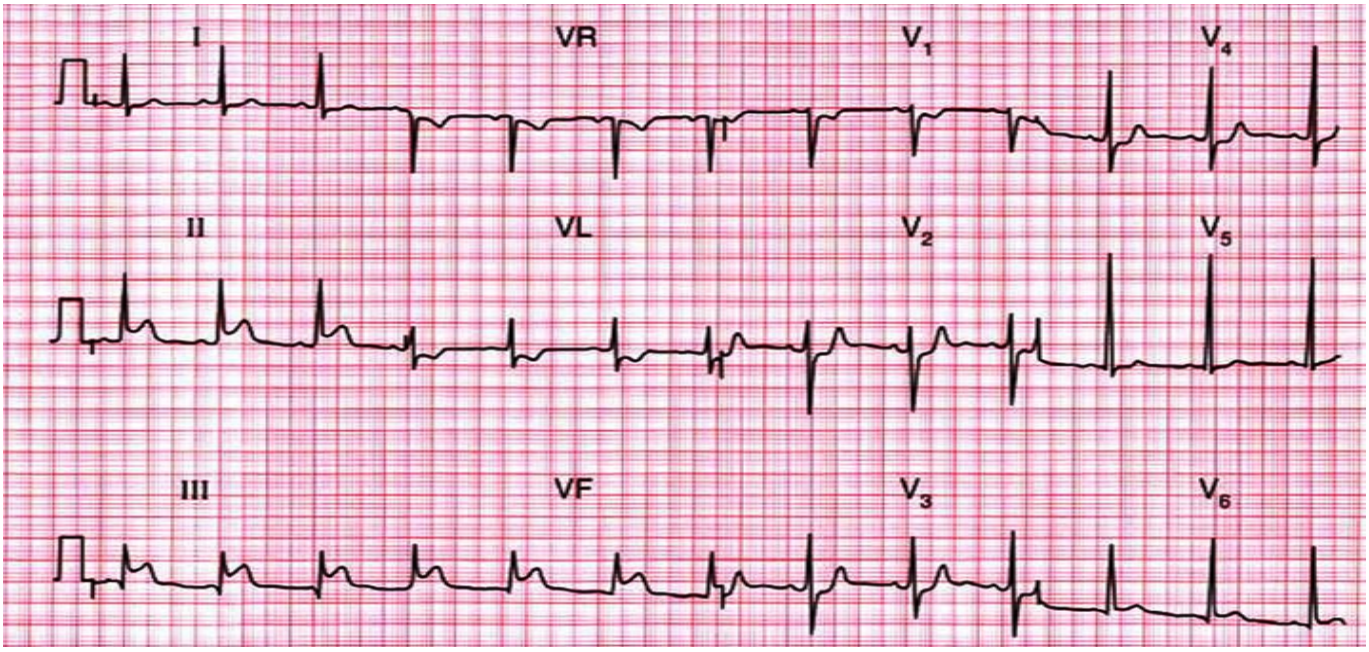
***8. The following statements are true about cardiac troponins, EXCEPT for:**
A. They are useful in the late diagnosis of myocardial infarction
B. They are released later than myoglobin
C. They are the most specific serum markers of myocardial necrosis
D. Troponins reach their maximum concentration 5 hours after a MI
E. They are increased in both STEMI as well as non-STEMI infarction

***9. The following statements about CK-MB are true, EXCEPT for:**
A. It is an isoenzyme specific to the heart muscle
B. Its increase after having returned to normal values indicates reinfarction
C. Reaches a maximum at 24 hours
D. Returns to normal after 6-8 days
E. It increases in other heart conditions as well

10. Which of the following tests are helpful in acute STEMI?
A. Resting ECG
B. Cardiac troponins
C. Stress echocardiography
D. Coronary angiography
E. Radionuclide imaging techniques (SPECT, PET)

CASE STUDIES

1. A 50-year-old man is brought to the emergency room, accusing severe chest pain that began 4 hours ago. What is your ECG diagnostic? What supplementary investigations would you recommend?



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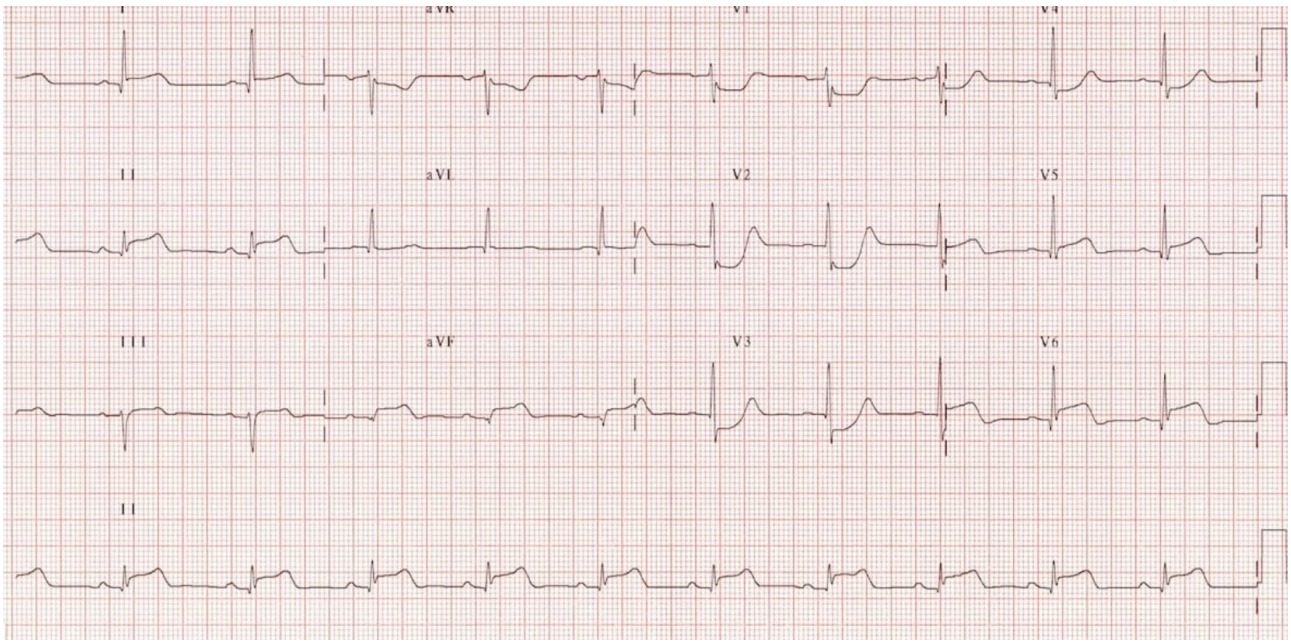
2. A 50-year-old patient, is brought to the emergency room accusing chest pain with a constrictive character, with irradiation at epigastric level, accompanied by nausea and vomiting, symptoms that have started 5 hours previously. What is your ECG diagnostic? What supplementary investigations would you recommend?



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NOTES

7. ECG IN CARDIAC ARRHYTHMIAS

LEARNING OBJECTIVES:

At the end of this chapter, students are expected to:

1. *Enumerate the ECG criteria of the sinus rhythm.*
2. *Describe and recognize the ECG criteria for sinus, supraventricular, ventricular arrhythmias.*

I. SINUS RHYTHM is the normal cardiac rhythm generated by the dominant heart pacemaker - the sinoatrial (SA) node.

ECG criteria of **sinus rhythm** are as follows:

1. P wave generated by the SA node - *sinus P wave*:
   - positive in leads II, III and aVF
   - negative in aVR
2. P wave precedes every QRS complex
3. PR interval constant with a duration = 0.12 - 0.20 sec
4. PP (atrial rate) and RR (ventricular rate) intervals are regular and equal
5. Heart rate: 60 - 100 beats/min.

II. ECG IN CARDIAC ARRHYTHMIAS

Cardiac arrhythmias are pathological states in which the heart is NOT in sinus rhythm due to abnormalities in either generation or conduction of myocardial excitation.

Classification of arrhythmias:

1. **According to the SITE OF ORIGIN:**
   - *sinus*
   - *supraventricular*
   - *ventricular*
2. **According to HEART RATE:**
   - *tachycardia*: >100/min
   - *bradycardia:* < 60/min
3. **According to the DURATION:**
   - *paroxysmal* (sudden onset and end)
   - *acute* (days or weeks) *chronic* (months or years)
4. **According to the ONSET**
   - paroxysmal (sudden)
   - non-paroxysmal (progressive)
5. **According to SYMPTOMATOLOGY:**
   - *asymptomatic*
   - *symptomatic, with:*
     - *mild symtoms* – palpitations;
     - *moderate symptoms* – angina, shortness of breath, dizziness;
     - *severe symptoms* – acute pulmonary edema, cardiogenic shock, faintness, syncope.

A. SINUS ARRHYTHMIAS

1. SINUS Tachycardia

- **Definition:** increase in SA node automatism, which generates impulses with a **rate > 100 beats/minute** (Figure 7.1), unrelated to or disproportionate to the level of physical or emotional stress
- **Causes:**

- Acute: physical exertion, emotions, pain, fever, infections, acute heart failure, acute pulmonary embolism, hypovolemia
- Chronic: pregnancy, anemia, hyperthyroidism

- **ECG changes:**
  - **Heart rate: 100-160 b/min** (up to 180 b/min)
  - **Rhythm:** sinus, REGULAR
  - **P waves:** normal

Observation!

Heart rate can by *gradually* decreased by vagal maneuvers: eyeball compression, carotid sinus massage, Valsalva manoever.

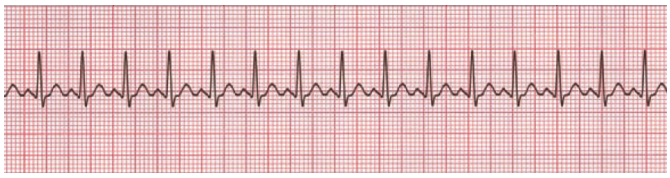


Figure 7.1. Sinus tachycardia.

2. SINUS Bradycardia

- **Definition:** decrease in SA node automatism, which generates impulses with a **rate < 60 beats/minute** (Figure 7.2), (or < 50 beats/minute, during the night)
- **Causes:**

- Extrinsic: hypothermia, hypothyroidism, treatment with beta-blockers, digitalis, antiarrhythmic drugs
- Intrinsic: acute ischemia and sinus node infarction (complication of AMI), chronic degenerative changes, e.g., sinus node fibrosis (sick sinus syndrome)

- **ECG changes:**
  - **Heart rate < 60 b/min**
  - **Rhythm:** sinus, REGULAR
  - **P waves:** normal

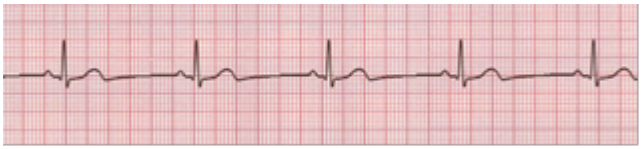


Figure 7.2. Sinus bradycardia.

3. SINUS Respiratory Arrhythmia

- **Definition:** variation of heart rate (PP/RR intervals) triggered by breathing *(heart rate increases during inspiration and decreases during expiration, typically in young, fit individuals)* due to the variations in vagal tone (efferent supply to the heart) - Figure 7.3.
- **ECG changes:**
  - **Heart rate (HR):**
    - RR intervals shorten during inspiration
    - RR intervals lengthen during expiration
  - **Rhythm:** sinus, IRREGULAR
  - **P waves:** normal

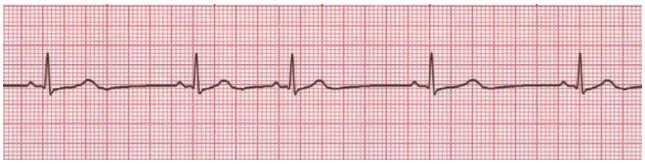


Figure 7.3. Sinus Respiratory Arrhythmia.

4. Sick SINUS syndrome (Sinus Node Disease)

- **Definition:** inability of the SA node to generate a normal rhythm, appropriate for body's needs.
- Causes: ischemia or fibrosis of the SA node; typically, it occurs in people older than 50; causes an increased propensity to supraventricular arrhythmias (atrial tachycardia, flutter or fibrillation)
- **ECG changes:** persistent sinus bradycardia, sinus arrest, sinoatrial block, bradycardia-tachycardia syndrome, that occur successively in the same patient

a. Sinus arrest or pause

- **Definition:** transient alteration in discharge by the SA node (sec. to min.) that result in a pause (no P waves) that has **no arithmetical relationship to the basic sinus rate** – i.e., the cycle length of the pause is not a multiple of the basic sinus cycle length (as happens with 2:1 or 3:1 SA nodal block).
- **ECG changes:**
  - **HR:** severe bradycardia
  - **Rhythm:** IRREGULAR
  - **P waves:** normal (when they reappear)

b. Sinoatrial block

- **Definition:** transient failure of intraatrial impulse conduction from the sinus node to the atria
- ***ECG changes:***
  - **HR**: severe bradycardia
  - **Rhythm:** IRREGULAR
  - **P waves:** normal, when present
  - The PP interval that includes the SA block **IS a multiple** of the basic sinus PP interval length

c. Bradycardia-tachycardia syndrome: slow heart rhythms that alternate with fast heart rhythms. It is typical finding in ischemic heart disease or valvulopathies. The mainstay treatment option for patients with symptomatic sinus bradyarrhythmia/SSS is permanent pacemaker implantation (Figure 7.4).

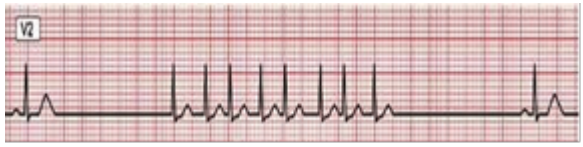


Figure 7.4. "Brady-tachy" syndrome.

B. SUPRAVENTRICULAR ARRHYTHMIAS

1. Premature ATRIAL CONTRACTIONS (PAC)

- **Definition**: also known as *atrial extrasystoles*, they are premature depolarizations due to an action potential that is generated by an ectopic atrial focus (before the next scheduled SA node action potential). PACs are present in 60% of healthy adults.
- **Causes**:
  - **physiological conditions**: excessive coffee, tobacco, alcohol, exercise
  - **pathological conditions**: coronary heart disease, valve heart diseases, cardiomyopathies, heart failure, high fever.
- **ECG changes** (Figure 7.5)**:**
  - **Rhythm:** IRREREGULAR
  - **Premature ectopic P' wave** with a morphology different from the normal P wave and that:
    - is followed by a QRS complex when the ectopic impulse finds the A-V node and the His bundle in the *excitable period* = *conducted PAC*
    - is not followed by a QRS complex when the ectopic impulse finds the A-V node or the

His bundle in the *refractory period* = *blocked PAC*

- **QRS morphology** is variable, depending on the atrio-ventricular (AV) conduction of the ectopic beat:
  - **normal (narrow) QRS**, if the PAC is physiologically conducted to the ventricles, simultaneously through both branches of the His bundle
  - **abnormal (wide) QRS**, in asynchronous (aberrant) ventricular conduction, when the extrasystolic impulse propagates through the A-V node, but finds a branch of the His bundle in the refractory period
  - **lack of QRS,** when the PAC is blocked (no AV conduction, since the AV node in refractory period).

- **The post-extrasystolic pause is (usually) less-than-fully-compensatory:** the distance between the pre-extrasystolic P wave and post-extrasystolic P wave is less than 2 normal P-P intervals (P-P' + P'-P < 2 P-P).

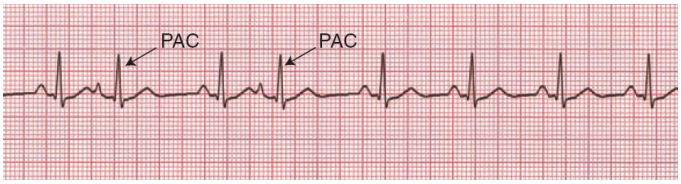


Figure 7.5. Premature Atrial Contraction.

2. Premature JUNCTIONAL CONTRACTIONS (PJC)

- **Definition:** also known as *atrio-ventricular (AV) or junctional extrasystoles (ES)*, they are premature depolarizations arising from the **AV node** or the '**junction**' of the heart. The nodal (junctional) ectopic focus can be located:
  - upper nodal → *supranodal ES*
  - middle nodal → *centronodal ES*
  - lower nodal → *infranodal ES*
- **ECG changes:**
  - **Rhythm:** IRREGULAR
  - **Premature, ectopic NEGATIVE P' wave in almost all leads** (retrograde atrial activation) that:
    - *precedes* the QRS complex with the P'R interval of the premature contracture < 0.12 sec for the supranodal ES
    - *coincides with QRS,* being masked by the QRS complex, for the centronodal ES (lack of P' wave)
    - *follows* the QRS complex for the infranodal ES
  - **Narrow QRS complexes**
  - **No compensatory pause**

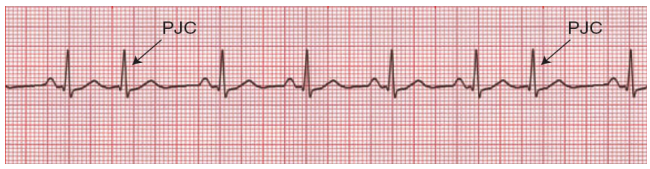


Figure 7.6. Premature Junctional Contraction.

3. SUPRAVENTRICULAR Tachycardia (SVT)

- **Definition:** regular sequence of **at least 6 PAC** or **PJC** with either **sudden onset/end** (paroxysmal SVT) or **gradual onset/end** (non-paroxysmal SVT)
- **ECG changes:**
  - **HR: 150-250 b/min** (max. 300 b/min)
  - **Rhythm:** REGULAR
  - **P' waves:** absent at high HR**;** sums up with the T wave from the previous complex = **T+P wave**
  - **QRS complex:** normal (thin, ventricular activation is synchronous)

Observation!

The term **supraventricular** is used because the origin of tachycardia, atrial or junctional, cannot be specified.

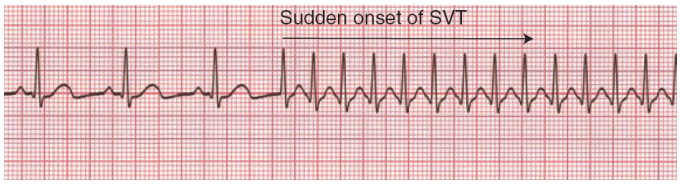


Figure 7.7 Supraventricular tachycardia.

! Observation:

- **Paroxysmal SVT** can be:
  - *atrial* (atrial tachycardia by reentry)
  - *junctional* (reentrany nodal tachycardia and reentry atrio-ventricular tachycardia from the WPW syndrome)
- **Non-paroxysmal SVT** can be:
  - *atrial* (multifocal atrial tachycardia)
  - *junctional* (junctional tachycardia)

3. Atrial FLUTTER

- **Definition: atrial arrhythmia** with **REGULAR atrial rate** between **250 - 350 b/minute.**
- **Causes:**
  - Cardiac: coronary heart disease, cardio-myopathies, mitral valvopathies
  - Extracardiac: COPD, hyperthyroidism
- **ECG changes** (Figure 7.8)**:**
  - Characteristic **"F" waves** with a '**saw-tooth' appearance** that replace the normal sinus P waves (**baseline is absent**)

- The **F waves** are visualized best in leads **II, III and aVF** and **V1**
- A **functional, *systematized* AV block** occurs, usually with **2/1, 3/1 or 4/1 AV conduction ratio** and a **REGULAR** heart **(ventricular) rate** (in most cases)
- There is a characteristic response to parasympathetic/sympathetic stimulation:
  - **vagal maneuvers** (parasympathetic stimulation) induce an **increase of the AV block degree** and *an abrupt decrease of the ventricular rate*
  - **exercise** (sympathetic stimulation) elicits a **decrease of the AV block degree** and an *abrupt increase of the ventricular rate*.

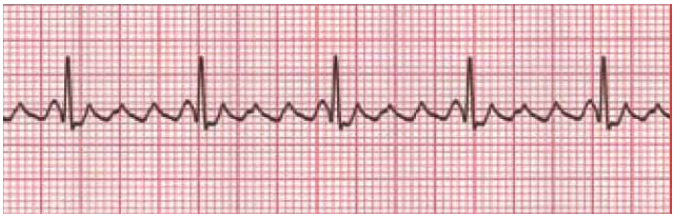


Figure 7.8. Atrial flutter.

4. Atrial FIBRILLATION (AFib)

- **Definition**: the **most FREQUENT atrial arrhythmia** with **IRREGULAR atrial rate** between **400 - 600 b/minute.**
- **Causes:**
  - Cardiac: hypertension, heart failure (congestive), mitral valve disease, cardiomyopathy, coronary heart disease, post-cardiac surgery
  - Extracardiac: hyperthyroidism (may be the only manifestation of the disease, thyroid function testing is mandatory), obesity, diabetes, COPD
-
- **ECG changes** (Figure 7.9)**:**
  - Characteristic **"f" waves** that appear as an **irregular undulation of the isoelectric line** (**baseline is absent**)
  - The **„f" waves** are visualized best in leads **V1** and **V2**
  - A **functional, *intermittent* AV block** that determines:

An **IRREGULAR ventricular rate**, with HR = 120-180 b/min in *AFib with rapid ventricular response*. HR decreases in the presence of treatment when *AF with slow ventricular response* occurs.

- The „**pulse deficit**", which is a clinical sign defined as the difference in count (mismatch) between the central (apex of the heart) and peripheral (radial) pulse with the latter being smaller. This typically occurs in *AFib with rapid ventricular rate* due to the inappropriate ventricular filling that intermittently results in hemodynamically inefficient contractions (ie, that cannot be detected in the periphery as a pulse wave).

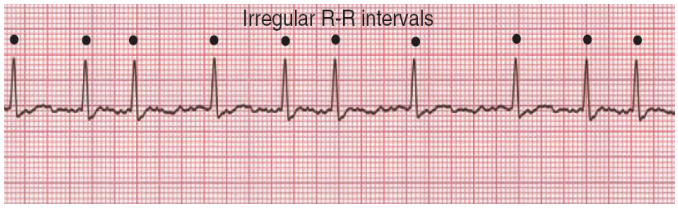


Figure 7.9. Atrial fibrillation.

C. VENTRICULAR ARRHYTHMIAS

1. Premature VENTRICULAR contractions (PVC)

- **Definition:** premature ventricular depolarizations arising from one or more ectopic foci located in the ventricles that generate an action potential before the next scheduled SA nodal action potential.
- **ECG changes:**
  - **Rhythm:** IRREGULAR
    - **Premature, wide QRS complex > 0.12 sec.** with the following characteristics:
    - abnormal morphology
    - **not** preceded by the P wave
    - **discordant** ST segment and T wave changes
    - Axis deviation in the frontal plan: the electrical axis of the PVC is opposite to the ectopic focus (e.g., a focus in the left ventricle elicits right axis deviation)
    - **The post-extrasystolic pause is (usually) compensatory -** the extra action potential causes the SA node to become refractory to generating its next scheduled beat, and thus it must "skip a beat" and will resume exactly two P-P intervals after the last normal sinus beat

(P-P' + P'-P + 2 P-P).

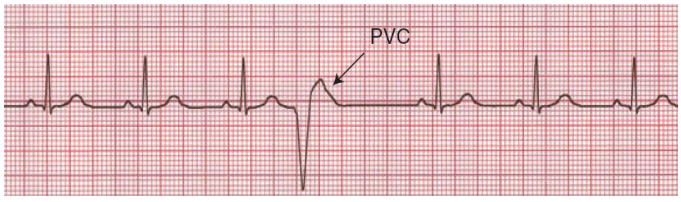


Figure 7.10. Premature Ventricular Contraction.

- **Classification:**
  - According to the *relationship with previous beats:*
    - **isolated PVC**
    - **systematized PVCs:** ***bigeminy*** (repetitive alternation between a normal QRS and a PVC), ***trigeminy*** (repetitive alternation of 2 normal QRS and a PVC), ***couplets*** (2 PVCs in a row), ***triplets*** (3 PVCs in a row)
  - According to *the morphology:*
    - **monomorphic (identical morpho-logy) - unifocal PVCs**
    - **polymorphic (various morpho-logies) - multifocal PVCs**
  - According to *their origin:*
    - **left PVCs** (originating in the LEFT ventricle) with RIGHT axis deviation (convergent, S′I - R′III pattern)
    - **right PVCs** (originating in the RIGHT ventricle) with LEFT axis deviation (divergent, R′I - S′III pattern)
  - According to *their significance:*
    - ***benign PVCs*** – isolated, may occur in the normal heart
    - ***potentially malignant PVCs*** – frequent (>10/hour), with early occurrence and *R-on-T phenomenon*, polymorphic, systematized
    - ***malignant PVCs*** – trigger ventricular tachycardia or ventricular fibrillation ⇒ risk of sudden cardiac death (especially in patients with heart failure).

2. Ventricular TACHYCARDIA (VT)

- **Definition:** a run of **more than 3 successive PVC** with **HR = 120-220 b/min.**
- **Causes:**
  - diseased heart (ischemia, fibrosis, hypertrophy, dysplasia) - eg, coronary heart disease, cardiomyopathy, mitral valve prolapse, arrhythmogenic dysplasia of the right ventricle
  - iatrogenic conditions: postoperative (after aorto-coronary bypass)
  - electrolyte imbalances: hypoK^+, hypoMg^{2+}, hypoCa^{2+}.
- **ECG changes:**
  - **Very broad QRS complexes > 0.14 sec**, with the same polarity in V1-V6
  - **AV dissociation**: P and QRS complexes at different rates (in about 1/2 of cases)
  - **Capture beats:** occur when the sinoatrial node transiently 'captures' the ventricles, in the midst of AV dissociation, to produce a QRS complex of normal duration
  - **Fusion beats**: occur when a sinus and ventricular beat coincides to produce a hybrid complex.
- **Classification:**
  - According to the *onset:*
    - *paroxysmal (sudden onset, HR >100b/min)*
    - *non-paroxysmal* (Accelerated idioventricular rhythm HR 60-100 b/min)
  - According to the *duration:*
    - *sustained* (minimum 30 sec)
    - *non-sustained (<30 sec)*
  - According to the *morphology:*
    - *monomorphic*
    - *polymorphic*

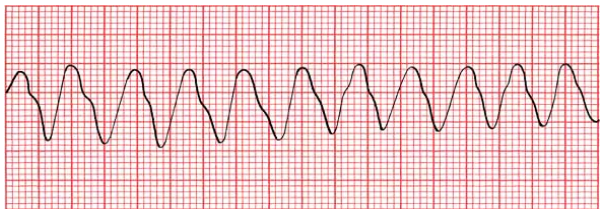


Figure 7.11a. Ventricular tachycardia

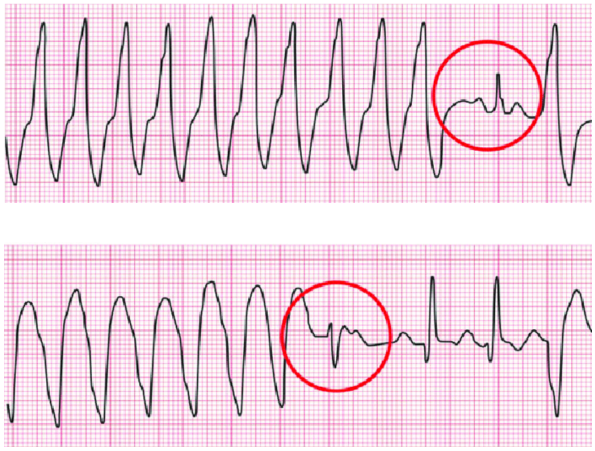


Figure 7.11b. Ventricular tachycardia with the presence of capture (upper panel) and fusion (lower panel) beats.

"Torsade de pointes"

- **Definition: severe** form of **polymorphic VT**, frequently associated with a **prolonged QT interval.**
- **ECG changes:** a French term suggesting a "twisting of the points" of the QRS complexes which swings from positive to negative and the other way

around (QRS complexes appear to "twist" around the baseline).

- This arrhythmia may spontaneously return to sinus rhythm or rapidly degenerate into ventricular fibrillation (VF).

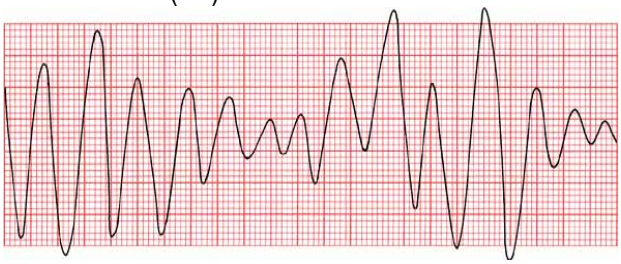


Figure 7.12. Torsade de pointes

3. Ventricular FLUTTER and FIBRILLATION

- **Definition: MALIGNANT tachyarrhythmias** with a ventricular rate of 130-300 b/min = **the main cause of sudden cardiac death**.
- **ECG changes:** QRS complexes are replaced with:
  - **a continuous sine wave** with no identifiable P waves, QRS complexes and T waves → **ventricular flutter**
  - **irregular deflections of varying amplitude and contour** due to the chaotic asynchronous fractionated activity of the heart → **ventricular fibrillation**
  - together with VT, they are known as **malignant tachyarrhythmias** because they induce cardiorespiratory arrest due to the lack/inefficiency of ventricular contractions (inability to eject a systolic blood volume compatible with the maintenance of vital functions).

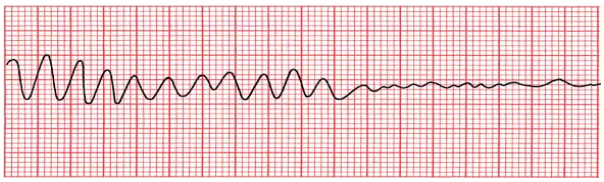


Figure 7.13. Ventricular fibrillation

CHECKPOINT!

1. Which of these arrhythmias are responsible for an *irregular* heart rhythm?

A. Sinus tachycardia
B. Sinoatrial block
C. Supraventricular tachycardia
D. Atrial flutter
E. Atrial fibrillation

***2. A regular heart rate, equal to 220 b/min and narrow QRS complexes defines:**

A. Sinus tachycardia
B. Supraventricular tachycardia
C. Atrial flutter
D. Atrial fibrillation
E. Ventricular tachycardia

3. Which of the following are characteristics of the supra nodal junctional premature contractions?

A. Elicits abnormal heart depolarization
B. The ectopic P´ wave is positive in most leads
C. The ectopic P´ wave coincides with the QRS complex
D. The ectopic P´ wave is negative and is seen after the QRS complex
E. A run of 6 PJCs can cause supraventricular tachycardia

***4. Which of the following is a characteristic of atrial flutter?**

A. P waves are replaced with "F" waves with a rate of 400-600/min
B. P waves are replaced with "f" waves with a rate of 250-350/min
C. Regular atrial rate
D. Coupling of minimum 6 consecutive PAC
E. Intermittent conduction of impulses from the SA node to the atria

5. Which of the following characteristics define the *potentially malignant* property of PVCs?

A. Isolated, on normal heart
B. Less than 10/hour
C. Monomorphous
D. Systematized
E. Early, with *R on T* phenomenon

***6. Which of the following is a characteristic of atrial fibrillation?**

A. Most frequent regular atrial arrhythmia
B. Systematized AV block
C. Regular ventricular tachyarrhythmia
D. Peripheral pulse deficit
E. Control by vagal maneuvers

7. Which of these arrhythmias are defined by changes in P waves?

A. Sinus tachycardia
B. Sinus bradycardia
C. Supraventricular tachycardia
D. Atrial flutter
E. Atrial fibrillation

8. Which of the following are characteristics of "torsade de pointes":

A. Particular form of supraventricular arrythmia
B. Is a polymorphic ventricular tachycardia
C. Is associated with short QT interval
D. Is associated with prolonged QT interval
E. Abnormal wide QRS, with slow rate, in asynchronous (aberrant) ventricular conduction

9. The following statements about bradycardia-tachycardia syndrome are true:

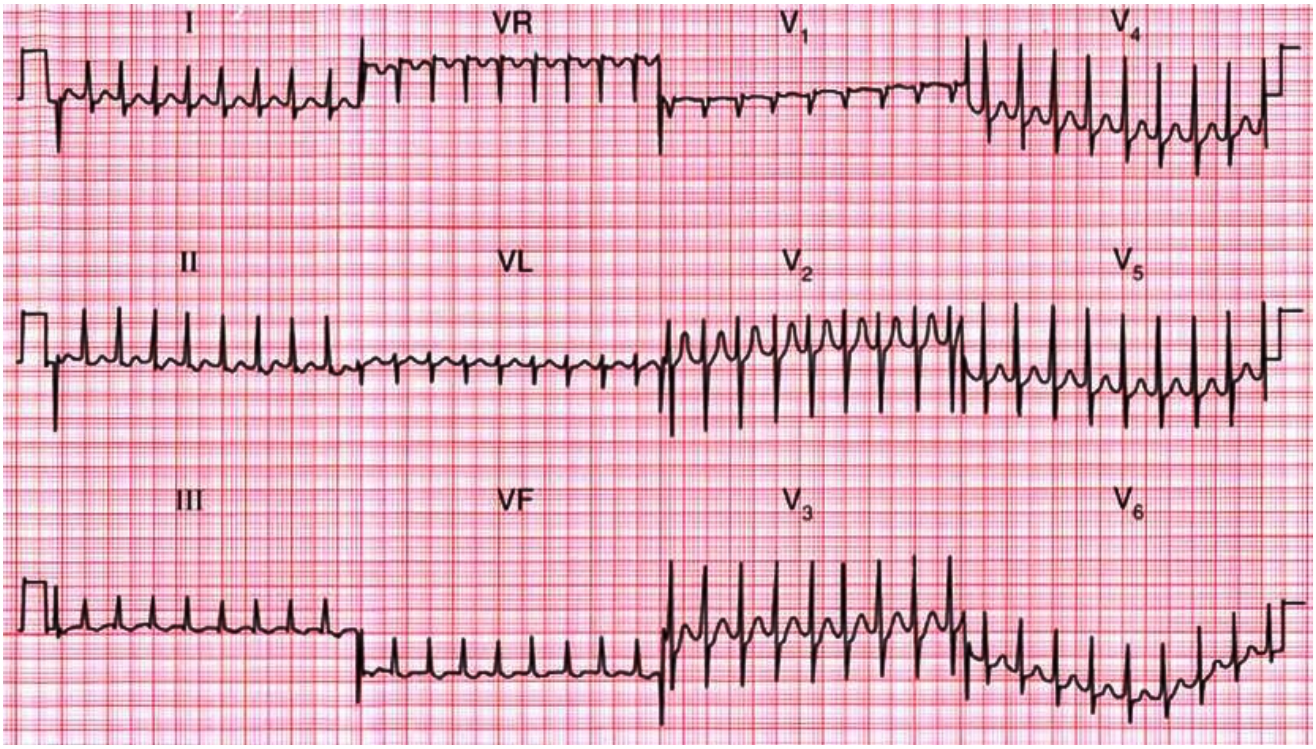
A. Is a physiological arrhythmia
B. Severe sinus bradycardia following a short access of supraventricular tachycardia
C. Is a particular form of polymorphic ventricular tachycardia
D. May degenerate into ventricular fibrillation
E. Occurs in patients with sick sinus syndrome

10. Ventricular tachycardia is characterized by:

A. Is a form of malignant tachyarrhythmia
B. Presence of capture and fusion beats
C. Systematized atrio-ventricular block
D. Ventricular rate 400-600 b/min
E. Atrioventricular dissociation

CASE STUDIES

1. A 26-year-old woman presented to the hospital accusing palpitations. She states that she has had such episodes in the past as well. Which is the ECG diagnosis?



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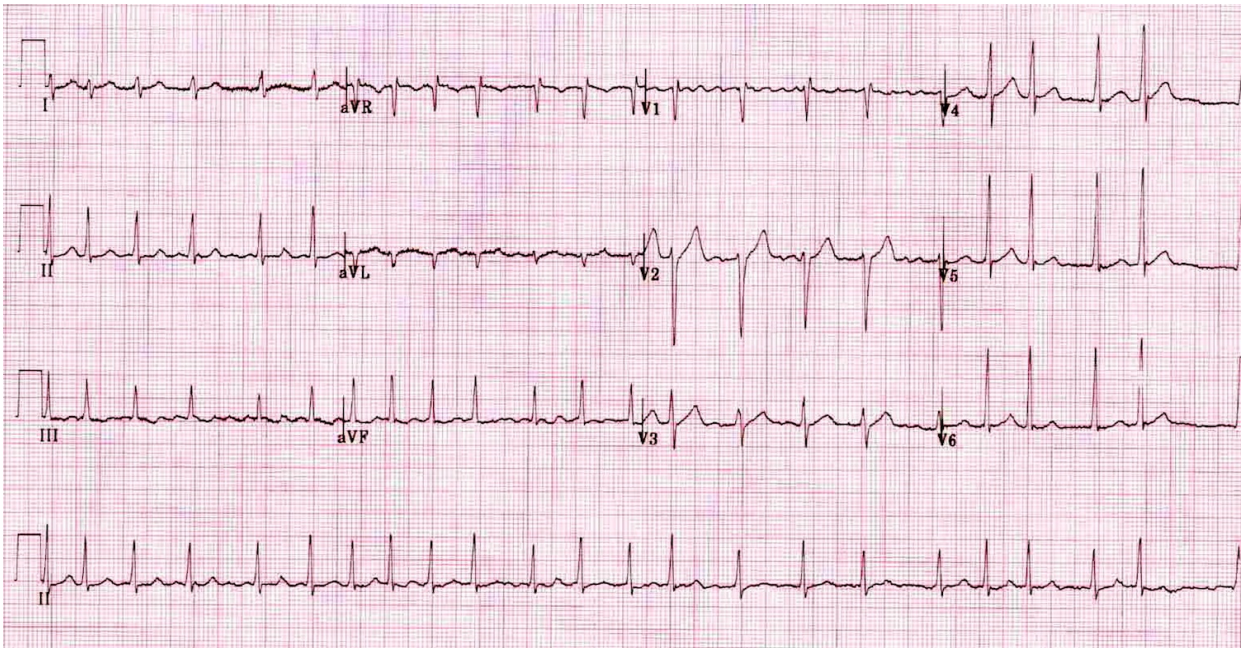
2.A 67-year-old man known with hypertension has palpitations, dyspnea at rest. Which is the ECG diagnosis?



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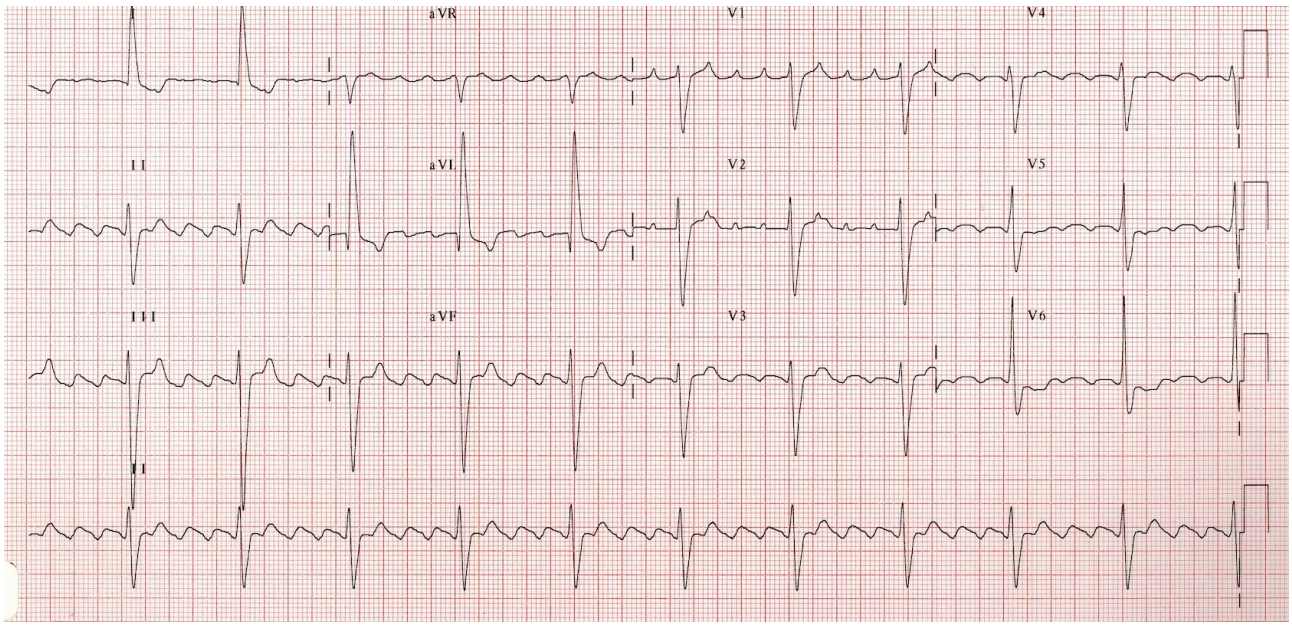
3. A 55-year-old patient presented for a cardiological check, accusing palpitations and fatigue. Which is the ECG diagnosis?



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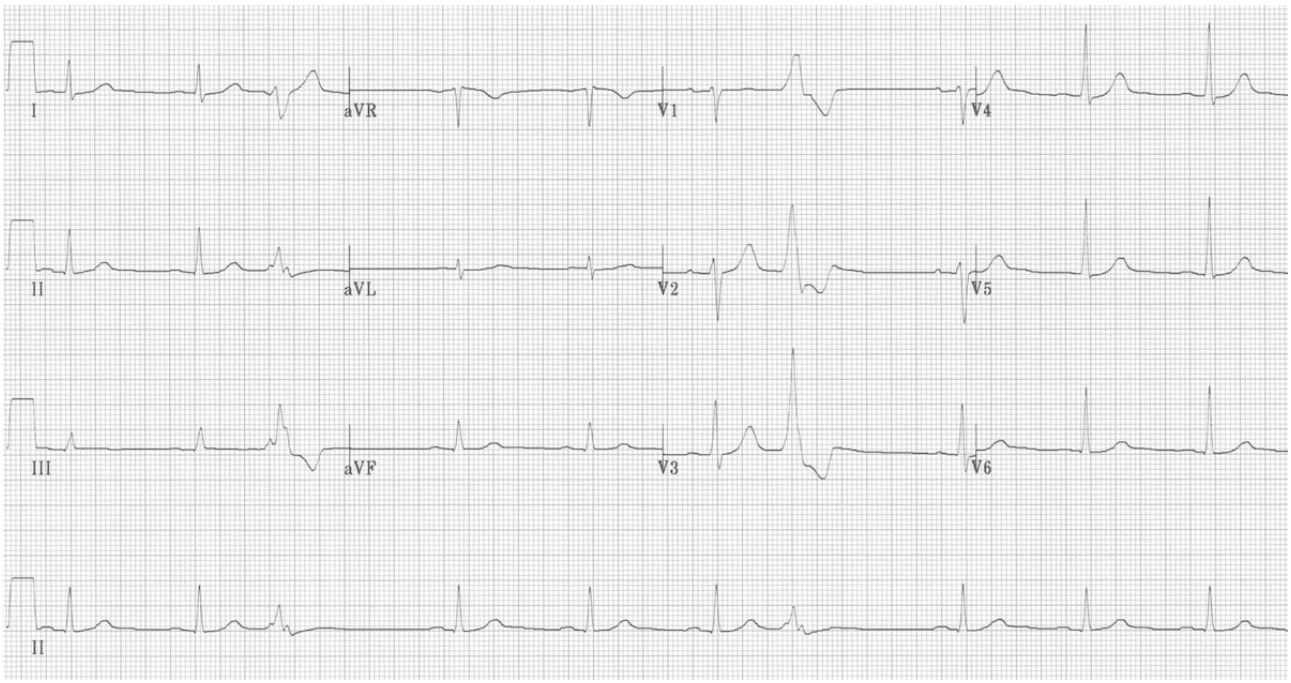
4. A 70-year-old patient, known with type 2 diabetes and coronary heart disease, has palpitations, fatigue, dyspnea and an episode of syncope. Which is the ECG diagnosis?



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NOTES

8. ECG CHANGES IN CONDUCTION DISORDERS AND PREEXCITATION SYNDROMES. ECG CHANGES DUE TO ELECTROLYTE IMBALANCE. ECG CHANGES INDUCED BY DIGITALIS.

LEARNING OBJECTIVES

At the end of this chapter, **students are expected to:**

1. *Recognize the ECG changes in atrioventricular and intraventricular blocks.*
2. *Recognize the ECG changes in preexcitation syndromes.*
3. *Recognize ECG changes due to electrolyte imbalance: hyper- and hypokalemia, hyper- and hypocalcemia.*
4. *Recognize ECG changes induced by digitalis in therapeutic and toxic doses, respectively.*

I. ECG CHANGES IN CONDUCTION DISORDERS

1. Atrioventricular (AV) blocks

Definition: a delay or interruption in conduction in the AV node or the His-Purkinje system (infranodal).

Etiology:

- **Acute blocks**: acute myocardial infarction (inferior - BAV with nodal site, anterior- BAV with infranodal site), postoperative (valve prosthesis), infectious diseases (bacterial endocarditis), drugs (beta blockers, digitalis), increase in the vagal tone
- **Chronic blocks:** idiopathic (degenerative lesions, the most common cause), valvopathy (aortic calcification) hypertrophic obstructive cardiomyopathy, increased vagal tone (trained athletes).

1.1 First degree AV block: (Figure 8.1)

- ***constant*** **prolongation of the PR interval > 0.2 seconds** in any lead
- **all** P waves are followed by QRS complexes, (i.e., all the atrial impulses are conducted to the ventricles which indicates a prolonged AV conduction), except for the premature contractions (extrasystoles) when present

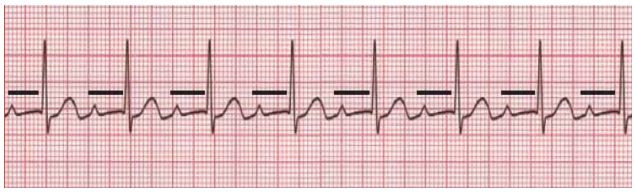


Figure 8.1. First degree AV block

1.2 Second degree AV block (Figure 8.2, 8.3)

1.2.1 Type I second degree AV block (Mobitz I or Wenckebach type) (Figure 8.2)

- ***progressive*** **prolongation of the PR interval** until a P wave is blocked with the repetition of the cycle
- the interval between 2 blocked P waves is called "the Wenckebach period"
- the PR interval preceding the blocked P wave is longer compared to the PR interval following the blocked P wave

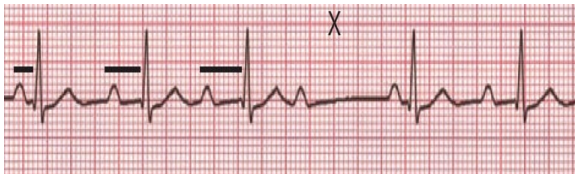


Figure 8.2. Type I second degree AV block

1.2.2 Type II second degree AV block (Mobitz II) (Figure 8.3)

- sudden interruption of the P wave conduction to the ventricles
- the PR interval of the conducted P waves is usually normal (but can also be elongated)
- **QRS complexes: normal or wide (**if the ventricular conduction is aberrant)
- **2:1 or 3:1 conduction** (Figure 8.4.):
  - occurs when every **second (2:1 block)** or **third (3:1 block**) P wave conducts to the ventricles

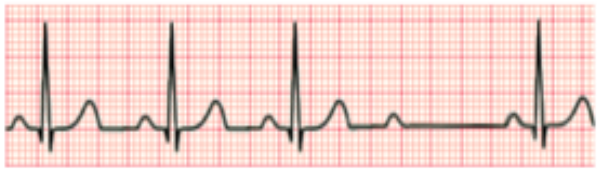


Figure 8.3. Type II second degree AV block (Mobitz II)

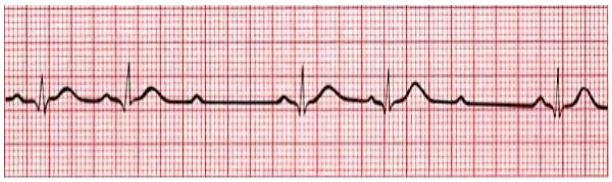


Figure 8.4. Type II second degree AV block (2:1)

***! Observation*:**

- Mobitz I AV block (Wenckebach) is caused by a conduction disorder localized at the AV node, while Mobitz II AB block is localized at the His bundle, immediately infranodal
- Second degree AV block with 2: 1 or 3: 1 conduction represents a partial interruption of conduction from the atria to the ventricles through both the AV node and the His bundle (infranodal)
- Patients with Mobitz I second degree AV block require a permanent pacemaker implant (cardio stimulation) when bradycardia becomes symptomatic (HR < 40 BPM), in the absence of a reversible / correctable cause
- Patients with Mobitz II second degree AV block, those with AV block with 2: 1 or 3: 1 conduction, as well as those with grade III BAV (complete) require a permanent pacemaker implant, regardless of the presence of symptomatic bradycardia, in the absence of a reversible cause / correction

1.3 Third degree (complete) AV block (Figure 8.5)

- total interruption of the conduction from the atria to the ventricles through both the AV node and the His bundle (infranodal)
- ECG records two independent electrical activities, a condition called **atrio-ventricular dissociation**, in which:
  - the atrial rhythm is the *sinus rhythm* or any type of *atrial arrhythmia* (e.g., fibrillation)
  - a *junctional (***AV node - > 40-60 BPM**) or *idioventricular* (**His-Purkinje system -> 20-40 BPM**) escape rhythm occurs => narrow or wide QRS
    - the ventricular rate is always lower than the atrial rate (number of QRS complexes is less than the one of P waves)
- from the moment the block is installed until the escape rhythm appears, a temporary loss of consciousness may occur - **cardiac syncope** or **Adams-Stokes seizure** due the asystole
- patients with total AV block require permanent pacemaker implantation.

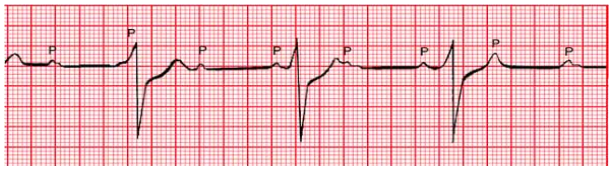


Figure 8.5. Third-degree AV block

2. Intraventricular blocks

- **Definition**: intraventricular conduction delay responsible for prolonged duration of the QRS complex:
  - if the duration is 0.10-0.12 sec, the bundle branch block is *incomplete*
  - if the duration is < 0.12 sec, the bundle branch block is *complete*

2.1 Right bundle branch block (RBBB)

Causes: right ventricular hypertrophy, *cor pulmonale*, pulmonary embolism, myocarditis, cardiomyopathies, congenital anomalies (e.g., atrial septal defect)
ECG criteria: (Figure 8.6)

- Wide **QRS complex > 0,12 sec.**
- **Right precordial** leads (**V1, V2)**
  - **rSR´** or **RsR (M-shaped' QRS complex)-** The delayed right ventricular activation produces a secondary R wave
- secondary repolarization abnormalities, with **ST depression and T wave inversion** in the **right precordial leads**
- **left precordial** leads (**V5, V6) and I, aVL:**
  - **wide S wave**

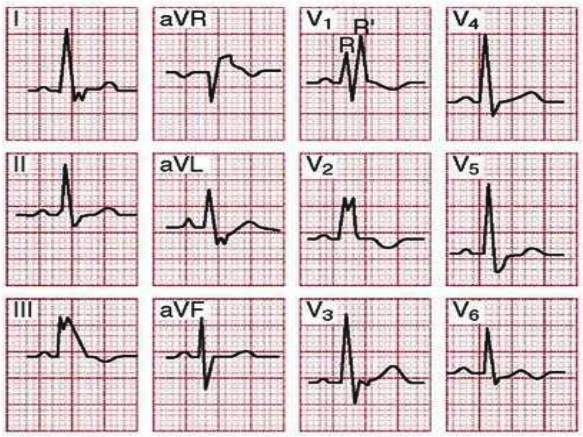


Figure 8.6. Right bundle branch block.

2.2 Left bundle branch block (LBBB)

Causes: acute myocardial infarction, dilated cardiomyopathy, hypertension, hyperkalemia

ECG criteria: (Figure 8.7)

- Wide **QRS complex > 0,12 sec.**
- **Left precordial** leads (**V5, V6, I, aVL)**
  - **rSR´** or **RsR ('M-shaped' QRS complex) -** (the delayed right ventricular activation produces a secondary R wave)
- secondary repolarization abnormalities, with **ST depression and T wave inversion** in the **left precordial leads**
- **right precordial** leads **V1, V2:**
  - **wide S wave: QS** or **rS image**

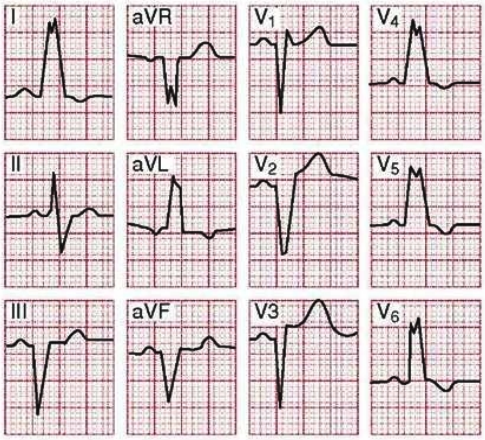


Figure 8.7. Left bundle branch block.

! Observation:

- The ECG diagnosis of acute myocardial infarction (AMI) in patients with left bundle branch block (LBBB) is often challenging and specific criteria are availble (Scarbossa criteria - see Cardiology).

2.3 Left anterior fascicular block (left anterior hemiblock, LAFB, LAHB):

ECG criteria: (Figure 8.8)

- **slightly enlarged QRS complexes**, up to **0.12 sec.**
- **left axis deviation - RI-SII-RII aspect**
  - **Small Q** waves with **tall R waves** (qR complex) in I and aVL
  - **Small R waves** with the presence of the **S waves** (rS complex) in II, III, aVF

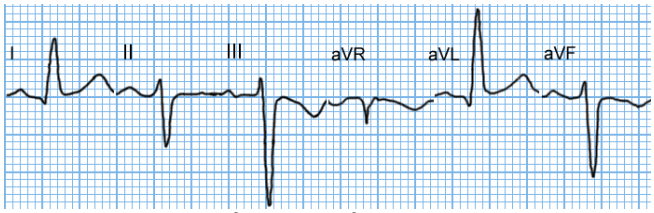


Figure 8.8. Left anterior fascicular block.

2.4 Left posterior fascicular block (left posterior hemiblock, LPFB, LPHB):

- **slightly enlarged QRS complexes**, up to **0.12 sec.**
- **right axis deviation** – SI-RII aspect
  - **Small Q** waves with **tall R waves** (qR complex) in II, III, aVF
  - **Small R waves** with the presence of **S waves** (rS complex) in I, aVL

2.5 Bifascicular Blocks

The coexistence of **RBBB plus either left anterior hemiblock (common**) or left posterior hemiblock (uncommon). The ECG will show the features of RBBB plus the axis deviation specific to each hemiblock.

II. ECG IN PREEXCITATION SYNDROMES

1. Wolf-Parkinson-White Syndrome (WPW)

Accelerated AV conduction due to the presence of an aberrant accessory pathway, called the *Kent pathway* with high conduction velocity, responsible for early ventricular activation (preexcitation before physiological depolarization)

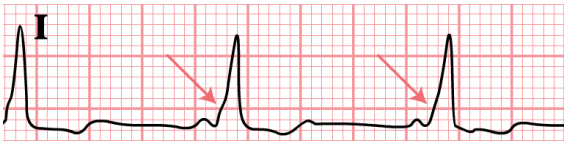


Figure 8.9. WPW Syndrome: short PR interval and the DELTA wave.

ECG criteria: (Figure 8.9)

- short **PR interval <0.12s**
- presence of the ***delta wave*** (representing early ventricular activation via the accessory pathway)
- prolonged QRS duration > 0.10s
- secondary ST-T changes

2. Lown-Ganong-Levine SYNDROME (LGL)

The LGL syndrome diagnosis requires the presence of an accessory pathway - preexcitation occurs through an aberrant atrio-nodal pathway called the *James bundle* that connects the atrium with the distal part of the AV node.

- **ECG criteria:** (Figure 8.10)
  - **PR interval < 0.12 sec**
  - No delta wave
  - Usually, no ST-T changes

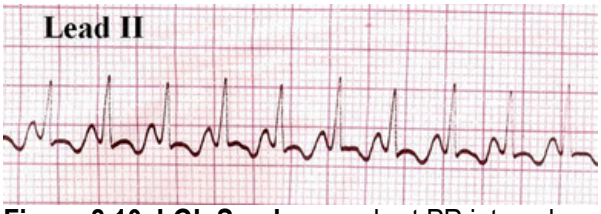


Figure 8.10. LGL Syndrome: short PR interval and NO DELTA wave.

III. ECG CHANGES DUE TO ELECTROLYTE IMBALANCE

1. Hypokalemia

Definition: serum K^+ < **3.5 mEq/L**
ECG criteria: (Figure 8.11)

- **decreased T wave amplitude** (flat or inverted)
- **ST depression**
- the appearance of **U wave**
- **long QTc** interval → high risk for VT due to R on T phenomenon
- **various atrial and ventricular arrhythmias**

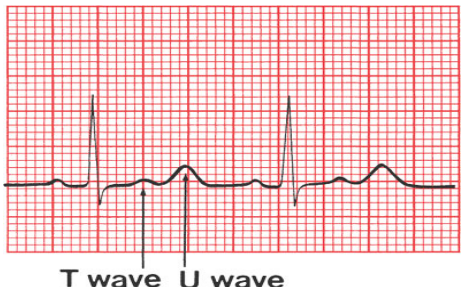


Figure 8.11. Hypokalemia.

2. HYPERkalemia

Definition: serum K^+ > **5 mEq/L**
ECG criteria: (Figure 8.12)

- **increased T wave** amplitude (tall, peaked, "tent-shaped") best seen in precordial leads
- **wide QRS** complex
- Prolongation of **PR** interval, possible AV blocks
- **loss of P** wave
- **sine wave** pattern at values of **8-9 mEq/L**
- **asystole** beyond these values.

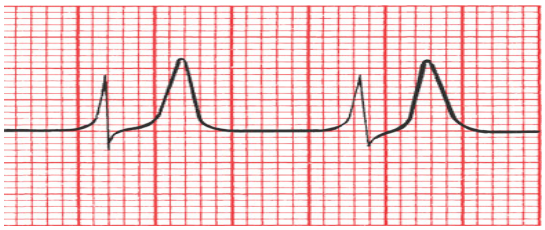


Figure 8.12. Hyperkalemia.

3. Hypocalcemia

- **Definiton:** serum Ca^{2+} < **8.5 mEq/L**
- **ECG changes:** long QT interval

4. Hypercalcemia

- **Definition:** serum Ca^{2+} > **10.5 mEq/L**
- **ECG changes:** short QT interval

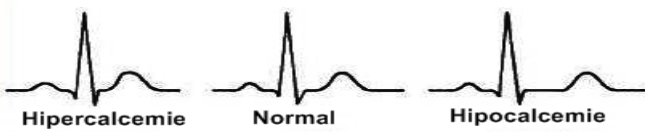


Figure 8.13. Hyper/Hypocalcemia.

IV. ECG CHANGES INDUCED BY DIGITALIS

ECG changes induced by DIGITALIS:

- **in therapeutic doses** → SA node suppression (↓ HR) and AV node (AV block) *(VAGAL effect)*
- **in toxic doses** → increasing the automaticity of ectopic pacemakers → strong suppression of SA and AV nodes

- **ECG changes in therapeutic dose – digitalis EFFECT:** (Figure 8.14)
  - decrease HR in atrial fibrillation
  - **ST depression** in all leads, "sink aspect"
  - flat T wave
  - short QT interval

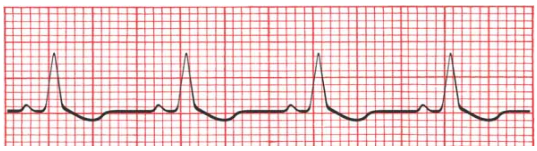


Figure 8.14. Digitalis therapeutic effect.

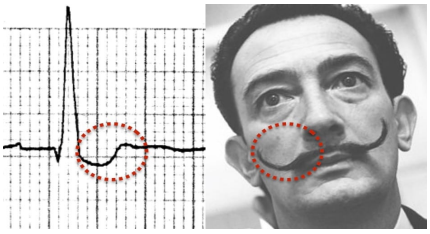


Figure 8.15. ST depression, "sink pattern".

- **ECG changes in overdose – digitalis TOXICITY:**
  - Digitalis may cause **ANY ARRHYTHMIA** or **CONDUCTION DISORDER**
  - **Via an increased automaticity of ectopic pacemakers:**
    - Atrial tachycardia
    - Junctional accelerated rhythm
    - Polymorphous PVC, bigeminy
  - **Via vagal stimulation:**
    - Sinus bradycardia
    - Ist, IInd or IIIrd degree AV block

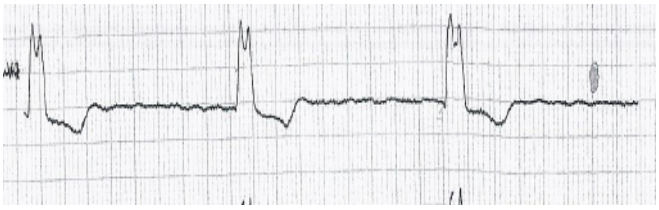
CHECKPOINT!

***1. Select the correct statement about third degree AV block:**
A. Is characterized by the presence of Wenckebach periods
B. It is characterized by PR interval prolongation > 0.20 sec
C. Hemodynamically means cardiac arrest
D. Progressively prolongation of PR interval until a P wave is blocked
E. Is the complete AV block

***2. Which of the following represent ECG diagnostic criteria of right bundle branch block?**
A. Sokolov Index Lyon > 35 mm
B. QRS duration 0.08-0.10 sec
C. rSR` image in I and aVL
D. Wide and deep S wave in I and aVL
E. Down-sloping ST depression with negative and symmetric T-wave

***3. The fascicular blocks (hemiblocks):**
A. Can be 3 degrees: Ist degree, IInd and IIIrd degree
B. Are abnormalities of impulse conduction in the right branch of the His bundle
C. Are the consequence of abnormal conduction in the left branch of the His bundle
D. Are characterized by wide QRS complex
E. Are characterized by ST segment and T wave changes

4. Which are the ECG abnormalities in the V1 lead below:



A. Atrial fibrillation
B. Right bundle branch block
C. Type 1 second degree AV block
D. Third degree AV bloc
E. Left bundle branch block

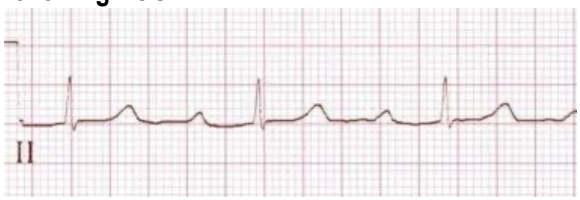
5. Which are the ECG criteria of left bundle branch block?
A. Wide QRS complex
B. rSR' image in right precordial leads
C. Wide S wave in left precordial leads
D. QS or rS image in right precordial leads
E. QS or rS image in left precordial leads

***6. Which of these ECG changes are induced by hypocalcemia?**
A. Increase in T wave amplitude
B. Wide QRS complex
C. Long QT interval
D. Short QT interval
E. Atrial and ventricular arhythmias

7. Which ECG change is determined by digitalis in therapeutic doses?
A. ST depression in all leads, "sink aspect"
B. Hypervoltated T wave
C. Sinus bradycardia
D. Ist, IInd or IIIrd degree AV block
E. Decreased HR in patients with atrial fibrillation

8. Which of these ECG changes are induced by hyperkalemia?
A. Long QTc interval
B. Increased T wave amplitude
C. Short PR interval
D. Wide QRS complex
E. Loss of P wave

***9. Which disorder is present in the lead II of the following ECG:**

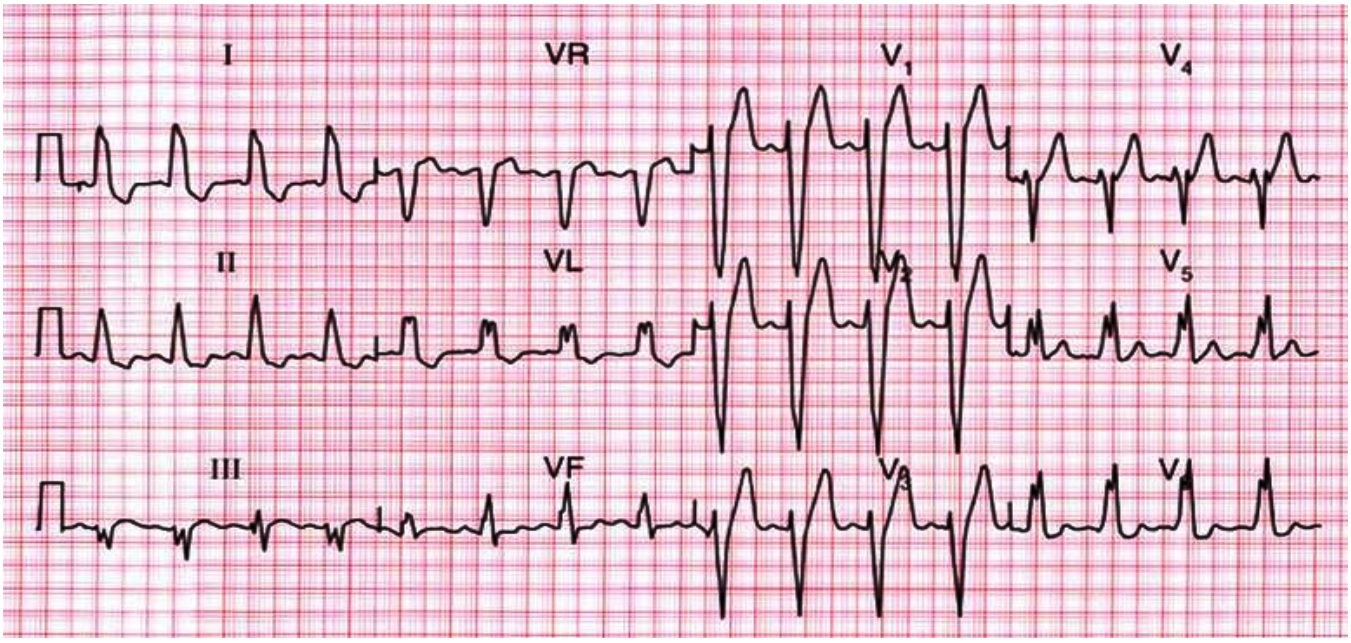


A. Right bundle branch block
B. Left bundle branch block
C. First degree AV block
D. Second degree AV block type 1
E. Atrial fibrillation

10. Which of the following statements regarding the atrio-ventricular blocks are correct:
A. First degree AV block is diagnosed in the presence of a constant PR interval with duration > 0.20 sec
B. Second degree AV block is called atrio-ventricular dissociation
C. Third degree AV block can be of type I and II
D. Second degree AV block type 1 is seen on ECG as the progressive prolongation of the PR interval until a P wave is blocked
E. In all AV blocks, the P waves are not followed by QRS complexes

CASE STUDIES

1. A 75 years old woman presented chest discomfort and dizziness when climbing up to the 2nd floor of the building where she lives. Which is your ECG diagnostic?



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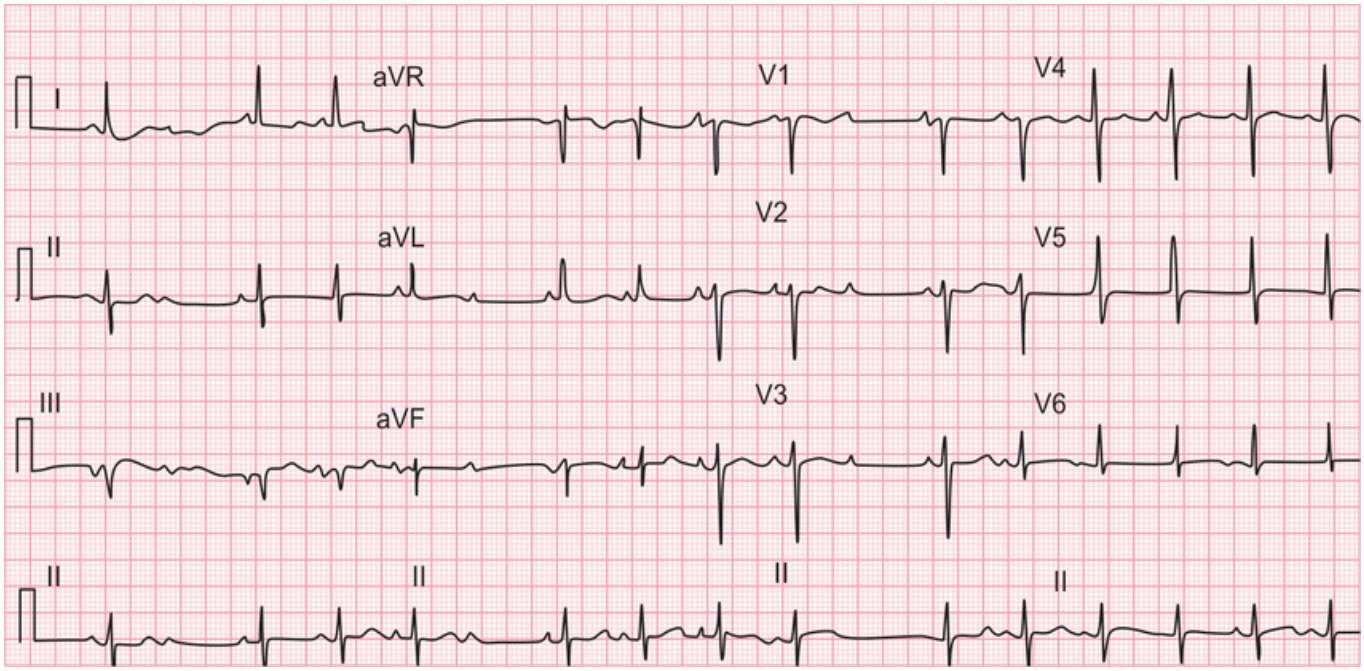
2. . Patient aged 65 years brought to the emergency department following a syncope. Which is your ECG diagnostic?



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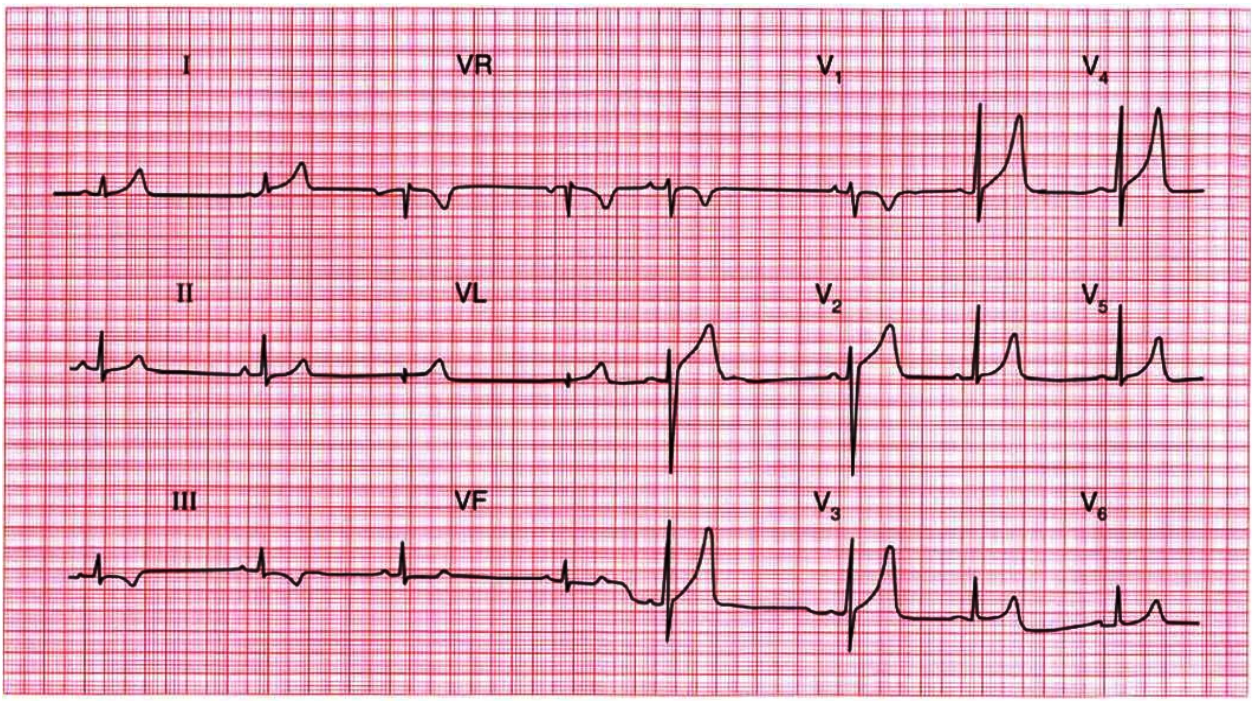
3. A 37 year-old man is hospitalized for orthopedic surgery. Preoperatively the following ECG was recorded. Which is your ECG diagnosis?



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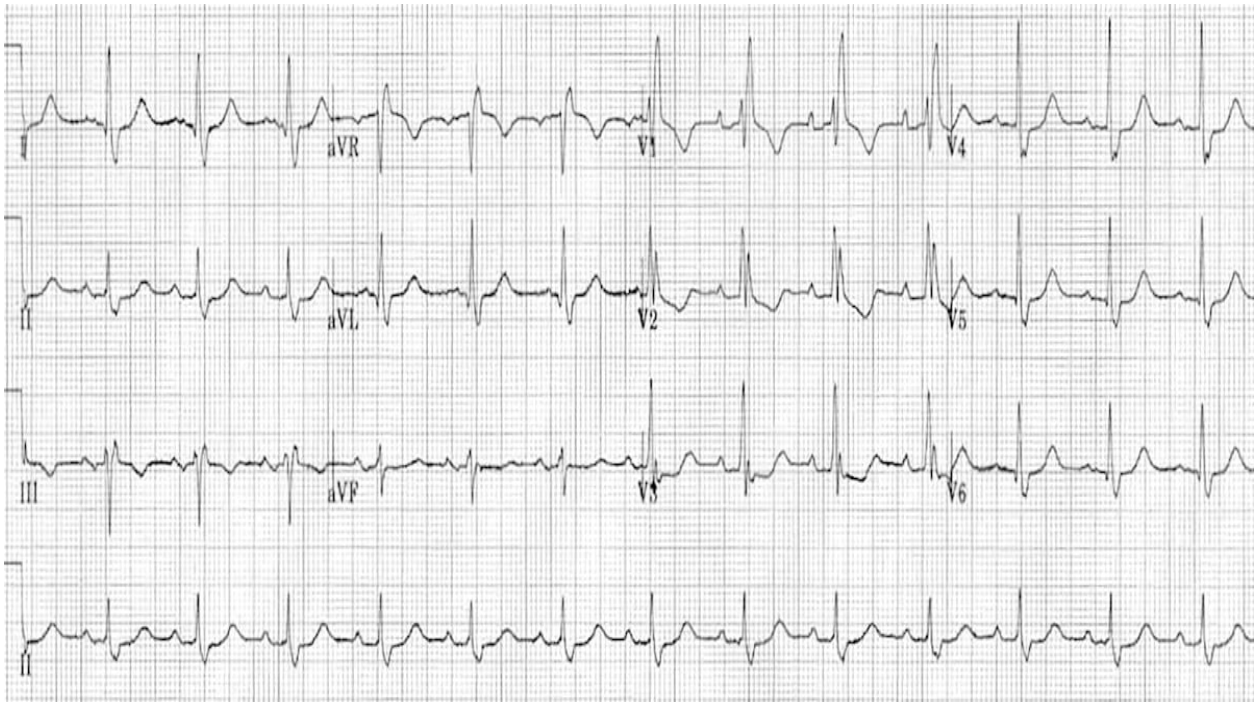
4. Interpret the following ECG:



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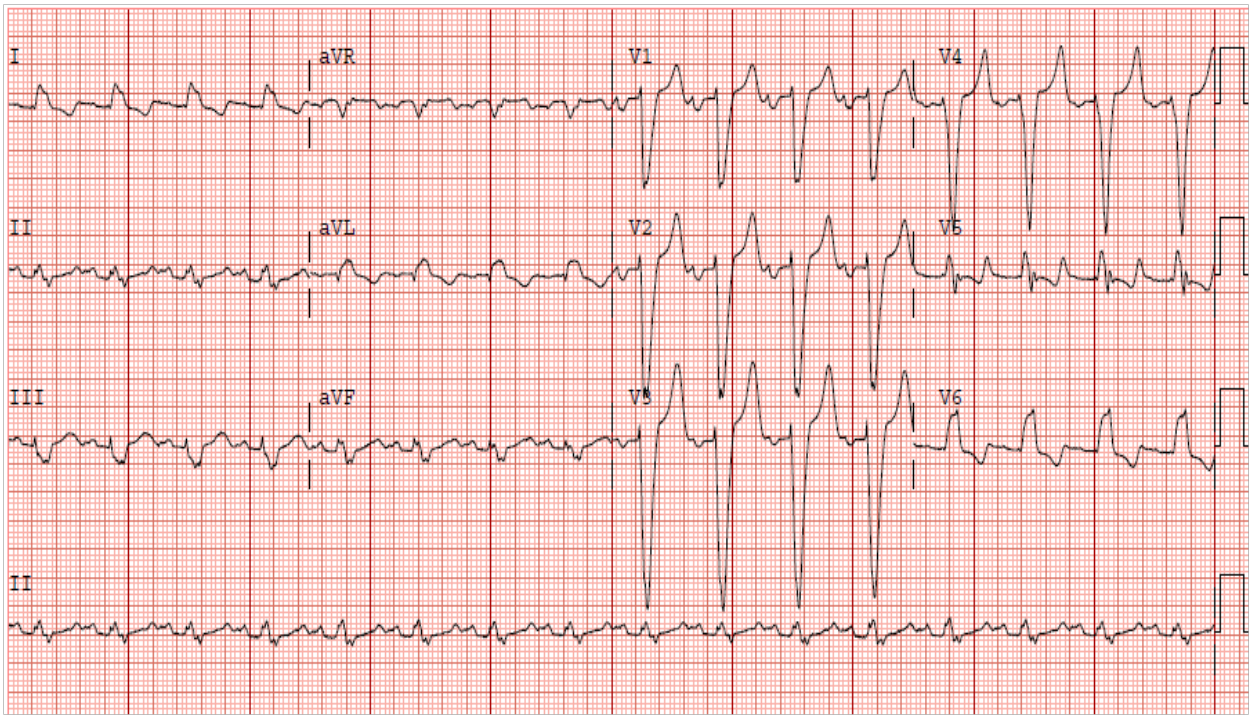
5. Interpret the following ECG:



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NOTES

9. SERUM BIOMARKERS IN CARDIOVASCULAR DISEASES. DIAGNOSTIC INVESTIGATION OF HEART FAILURE

LEARNING OBJECTIVES:

At the end of this chapter students must be able to:

1. *List the main types of serum biomarkers in cardiovascular diseases.*
2. *List and interpret the investigations used for the diagnosis of heart failure.*
3. *List the investigations used for the diagnosis of new onset acute heart failure*
4. *List and interpret the changes in high-sensitive C-reactive protein*

Currently, there is an increasing interest for the identification of novel biomarkers that can be assessed in either diagnosis or follow-up (assessment of therapeutic response and prognostic) in cardiovascular diseases.

The current opinion regarding the role of biomarkers in cardiovascular diseases is that their changes must be interpreted taking into account the clinical context, the evolutionary stage of the disease and, above all, its role in the pathophysiology of the disease.

Multimarker strategies have been mainly investigated in daily practice, for the diagnosis of **coronary artery disease (and atherosclerosis) and heart failure** – especially for risk assessment. For example, combining two or more serum biomarkers that reflect different pathophysiological mechanisms of heart failure (e.g., serum troponins as biomarkers of myocardial injury/necrosis and natriuretic peptides as biomarkers of parietal distension) will increase the predictive value of the score. Simultaneous analysis of serum biomarkers and imaging techniques (e.g., atrial natriuretic peptide and radionuclide ventriculography) could also provide a better risk stratification. However, in order to be routinely used in the clinical practice, multimarker scores must be assessed for reproducibility at population level, taking into account several geographical areas, ethnicities etc.

Importantly, any change in their serum level should be analyzed in the *clinical context*, according to the *stage of disease* and the biomarker's role in the pathophysiology of disease.

An increasing number of biomarkers have been described in the past decades, but only a few have been validated for the clinical practice (most of them being currently used for research purposes).

Their classification, according to the pathomechanism and their clinical value, respectively, is presented below:

I. Myocardial ischemia/necrosis biomarkers:

- Cardiac troponins I and T (cTn I, cTn T)
- High-sensitivity troponins, hs-cTn I, hs-cTn T
- Creatinkinase (CK) – isoform MB (CK-MB)
- Myoglobin
- Copeptin (Provasopressin, CT-proAVP)

II. Heart failure biomarkers:

- Natriuretic peptides:
  - Brain Natriuretic peptide (BNP)
  - N-terminal proBNP (NT-proBNP)
  - Mid-Regional pro A-type Natriuretic Peptide (MR-proANP)

III. Inflammation biomarkers:

- High-sensitivity C Reactive Protein (hs-CRP)
- Tumor Necrosis Factor alfa (TNF-alfa)
- Interleukins 1, 6, and 18

I. BIOMARKERS OF MYOCARDIAL NECROSIS

The increased mortality associated with acute myocardial infarction at a global level justifies the interest of the scientific community in the early diagnosis of this pathology. Biomarkers of **myocardial necrosis** can be subdivided into ***markers with clear value in practice*** (discussed in Lab 06) and ***markers with potential clinical value***, such as copeptin.

Copeptin (Pro-vasopressin, CT-proAVP)

- **Definition:** copeptin represents the C-terminal part of the precursor pre-pro-vasopressin (pre-proAVP) released from the neurohypophysis in equimolar concentrations with ADH
- **CLINICAL value:**
  - Indicator of the *acute endogenous stress* induced by acute cardiovascular events, such as:
    - acute myocardial infarction (AMI)
    - cardiac failure
    - septic and hemorrhagic shock
    - cerebral hemorrhage
    - ischemic stroke
  - Increased values of copeptin have been found in patients with AMI, immediately after ST elevation occurrence and even before the increase of classical markers (cTn, CK-MB) but due to a lack in specificity, currently it is used as a biomarker with *additive* role **in excluding the diagnosis of AMI** if hs-Tn level is normal

Observation!

In recent decades many biomarkers have been evaluated for **risk stratification** in acute coronary syndromes (ACS):

- biomarkers whose increase is associated with an *increased risk of mortality*: IL-6, cardiac fatty acid binding protein (H-FABP), etc.
- biomarkers whose increase predicts the *development of heart failure*:
  - osteoprotegerin
  - proadrenomedullin
  - neopterin

None of these have been introduced so far into clinical practice in the risk assessment strategies for patients with acute coronary syndromes.

II. DIAGNOSTIC INVESTIGATION OF HEART FAILURE (HF)

- **Definition:** Heart failure (HF) is a **clinical syndrome** characterized, at the present moment or in the antecedents, by ***symptoms*** and/or ***signs*** caused by a ***structural or functional abnormality*** associated with at least one of the following two criteria:
  1. **Increased serum natriuretic peptides**
  2. **Imaging** or **hemodynamic evidence of pulmonary or systemic congestion**, of cardiac origin, at rest or during exercise
- Typical **symptoms** of **HF** are:
  - for *left HF* – dyspnea on exertion or at rest, paroxysmal nocturnal dyspnea, orthopnea, cough, asthenia/fatigue, reduced exercise tolerance
  - for *right HF* - right hypochondrium pain/hepatalgia, bloating, anorexia
- Typical **signs** of **HF** are:
  - for *left HF* – tachycardia, pallor, sweating, symmetrical subcrepitant pulmonary rales, lateral displacement of apex shock
  - for *right HF* – jugular turgor, stasis hepatomegaly, hepatojugular reflux, peripheral edema, pleural/ascitic fluid effusions
- **Classification**: according to the mode of onset
  1. **Chronic HF (CHF)** – corresponds to the above definition and is the most common form in practice
  2. **Acute HF (HF)** – can be "**de novo"**, with an acute onset (in the case of severe acute heart disease) or, most frequently, it can appear due to the **decompensation of a chronic HF**, determined by *precipitating factors*, and which can be complicated with *acute pulmonary edema* or *cardiogenic shock*

Observation!

- HF should never be the only diagnosis of a patient - being a syndrome diagnosis, it should be

accompanied by the diagnosis of the underlying disease that led to the onset of HF

- Current guidelines recommend a series of laboratory and imaging investigations that support the diagnostic approach in HF. They include a series of initial, usual and, at the same time, essential tests for the diagnosis of HF, supplemented by additional or specific investigations where necessary.

A. Exploring CHRONIC HEART FAILURE (CHF)

Patients with suspected CHF usually have a history of myocardial infarction, hypertension, coronary artery disease, diabetes, alcohol abuse, chronic kidney disease, cardiotoxic chemotherapy, family history of alcohol-induced cardiomyopathy, or sudden death.

The **initial and essential** diagnostic tests RECOMMENDED for evaluation of patients with suspected CHF are: *natriuretic peptides*, e*lectrocardiogram*, *transthoracic echocardiography*, *chest x-ray*, and *routine blood tests for comorbidities*. Added to these are **specialized diagnostic tests** to confirm and detect reversible/treatable causes of CHF.

1. Cardiac NATRIURETIC PEPTIDES

- **Definition:** ***biomarkers with CLEAR clinical value*** belonging to the family of natriuretic peptides that includes: Atrial Natriuretic Peptide (ANP), Brain Natriuretic Peptide (BNP) and C-type Natriuretic Peptide (CNP). The main role of these peptides is to counteract the effects of RAA system activation. Current guidelines recognize only the BNP peptide and the mid- regional proANP (MR-proANP) as validated biomarkers for the diagnosis of HF.
  - **a) Brain natriuretic peptide (BNP)** – is released in high amounts by ***ventricular myocytes*** in response to ventricular distension and an increase of wall stress. It is stored as a *polypeptide precursor* (pro-BNP), being separated into **2 fragments**:
    - ①**BNP** – active metabolite, with a plasmatic half-life of approximately 20 minutes, and effects, such as:
      - **antihypertensive** (similar to ANP), through ***direct*** action – *diuretic, natriuretic, vasodilator effect* and .***indirect*** action – *inhibition of RAA system*
      - ***protective against fibrosis*** and ***cardiac remodeling*** – specific for BNP, in case of progressive heart failure
    - ②**N-terminal fragment** (**NT-proBNP)** – biologically inactive, with a plasmatic half-life of approximately 60-120 minutes (the plasmatic value of NT-proBNP is 3-5 times higher than that of BNP)
  - **b) The middle region of atrial natriuretic propeptide (Mid–Regional proANP, MR-proANP)** – is released, in equimolar concentration with ANP, by **atrial cardiomyocytes**, mainly in response to *acute atrial parietal distension*. It has a longer half-life than ANP.

- **CLINICAL value:**
  1. NP are **RECOMMENDED** as **INITIAL diagnostic tests** in all patients with symptoms suggestive of CHF to **exclude this diagnosis**. In other words, normal NP values have a ***high negative predictive*** value for the diagnosis of CHF (Figure 9.1)
     The upper value that is considered to be normal is:
     - for **BNP < 35 pg/mL**
     - for **NT-proBNP < 125 pg/mL**
     - for **MR-proANP < 40 pg/mL**
  2. **Elevated NP values support the diagnosis of CHF** when echocardiography is not immediately available
  3. **Elevated NP values** are useful for **establishing the prognosis** and may **guide** further cardiac investigations
     In the case of treated CHF:
     - the decrease in NP values suggests improved prognosis
     - the lack of decrease in values means a reserved prognosis and requires the intensification of treatment
  4. NP are **independent predictors of mortality in CHF**:
     - the increase of BNP and NT-proBNP or their maintenance at elevated levels, in the presence of treatment, signifies the progression of the disease or resistance to treatment

- MR-proANP has, apparently, a superior sensitivity in assessing the risk of mortality in CHF

5. **Increased levels of NP** are also associated with a wide variety of **cardiac and non-cardiac causes** (Table 9.1.) which compels their interpretation in a **clinical context**. As a result, the guidelines recommend further investigations to diagnose the comorbidities responsible for these increases, especially in patients diagnosed with CHF

Table 9.1. Causes of increased serum NATRIURETIC PEPTIDES.

CARDIAC causes	NON-CARDIAC causes
Heart failure	***Old age***
Acute coronary syndrome	Ischemic stroke
Pulmonary embolism	Subarachnoid hemorrhage
Myocarditis	***Renal dysfunction***
Left ventricular hypertrophy	Liver damage (cirrhosis of the liver with ascites)
Hypertrophic / restrictive cardiomyopathy	Paraneoplastic syndrome
Cardiac valvular pathology	Chronic obstructive pulmonary disease
Congenital cardiovascular diseases	Severe infections (including pneumonia) and sepsis
Atrial and ventricular tachyarrhythmias	Severe burns
Heart contusions	Severe infections (including pneumonia) and septicemia
Cardiovascular surgery	Anemia
Cardioconversion by implantable cardiac defibrillator	Metabolic and hormonal imbalances (eg diabetic ketosis, thyrotoxicosis)
Pulmonary HT	

2. 12-lead ECG

- **CLINICAL value:** it is recommended for all patients with HF
  - to detect abnormalities that increase the likelihood of a CHF diagnosis, such as: *atrial fibrillation, necrotic Q waves, LVH, widened QRS complexes*
  - the informations obtained are useful both to assess the etiology of HF (e.g., post-myocardial infarction) as well as to establish and monitor the treatment.

Observation!
ECG has low specificity for the diagnosis of HF, its major utility being to exclude this pathology (HF is unlikely in patients whose ECG is normal).

3. Transthoracic ECHOCARDIOGRAPHY

- **CLINICAL value:** represents **the most useful test** for establishing the diagnosis of HF
  - allows the establishment of the CHF phenotype, based on the measurement of the left ventricular ejection fraction (LVEF):
    - HF with reduced ejection fraction ≤ 40% (HFrEF)
    - HF with mildly reduced ejection fraction, between 41-49% (HFmrEF)
    - HF with preserved ejection fraction ≥ 50% (HFpEF)
  - provides immediate information on the size of the cardiac cavities, the presence of LVH, abnormalities of the regional wall that may suggest myocardial ischemia, right ventricular function, the presence of PHT, valvular function
  - provides only indirect information about the *structure of the myocardium* (myocardial fibrosis, pathological deposits); for the assessment of this aspect the *MRI gadolinium examination* is indicated

4. CHEST x-ray

- **CLINICAL value:**
  - useful for investigating other potential causes of **dyspnea** (e.g., lung conditions)
  - may provide additional evidence of the presence of CHF, such as the presence of **retrograde pulmonary stasis/congestion** or **cardiomegaly**

5. ROUTINE BLOOD tests for COMORBIDITIES

- **CLINICAL value:** tests RECOMMENDED in the initial evaluation of a newly diagnosed patient with HF:
  - ***Complete blood count and iron studies*** (ferritin, transferrin) – for the diagnosis of iron deficiency anemia
  - ***Electrolyte serum levels*** (sodium, potassium, chlorine) - for the diagnosis of electrolyte imbalances
  - ***Serum creatinine*** – for the diagnosis of renal dysfunction
  - ***Serum uric acid*** - for the diagnosis of hyperuricemia and gout
  - ***Liver tests*** (bilirubin, AST, ALT, GGT) - for the diagnosis of liver dysfunction
  - **Thyroid stimulating hormone (TSH) and thyroid hormones levels** – for the diagnosis of thyrotoxicosis
  - ***Glycemic balance parameters*** (fasting blood glucose, glycated Hb (HbA1c), ketonemia/ketonuria) – for the diagnosis of diabetic ketoacidosis
  - ***Lipid profile***: total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides – for the diagnosis of hyperlipidemias

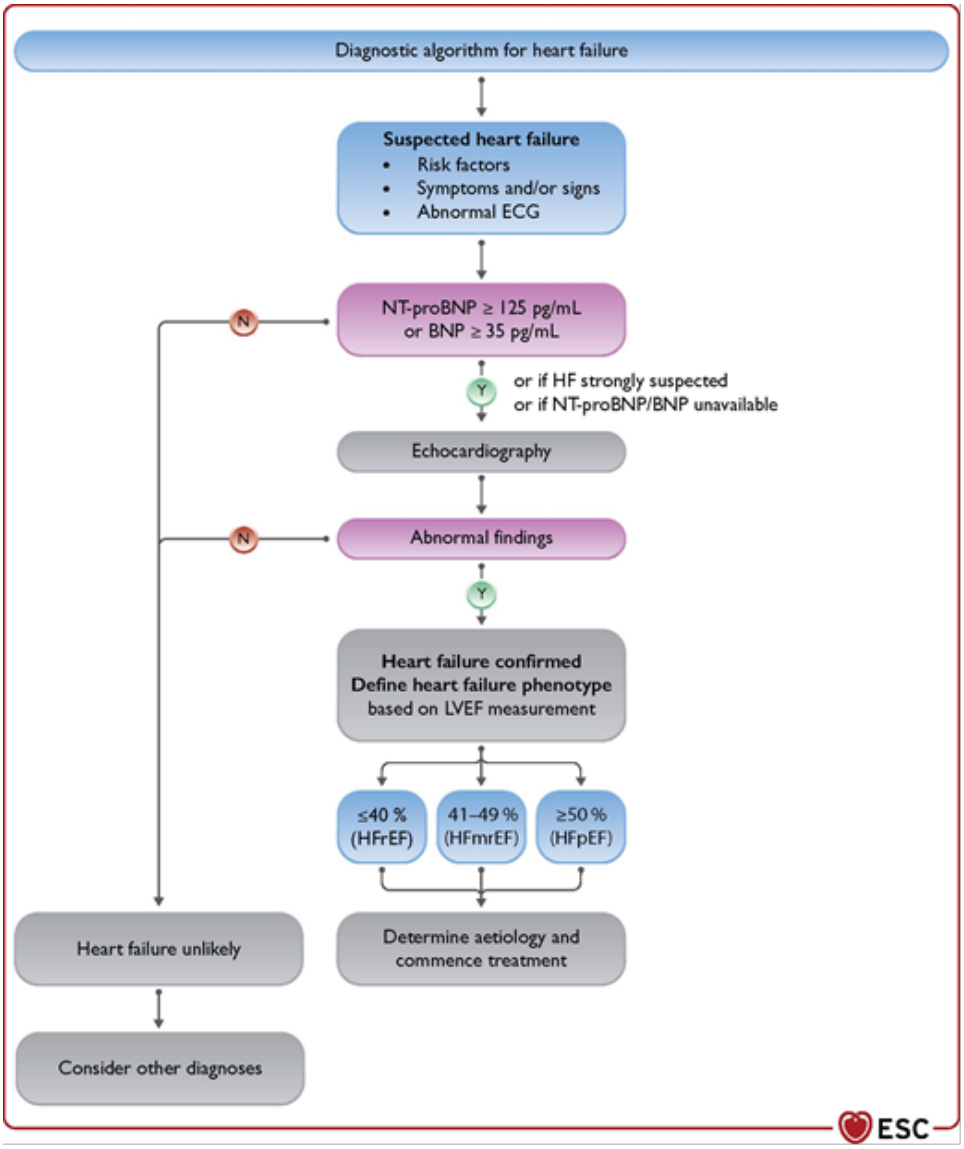

Figure 9.1. The diagnostic algorithm for heart failure. NT-proBNP = pro-B N-terminal natriuretic peptide, BNP = brain-derived natriuretic peptide, HFmrEF = heart failure with mildly reduced ejection fraction, HFpEF = heart failure with preserved ejection

fraction, HFrEF = heart failure with reduced ejection fraction. LVEF = left ventricular ejection fraction (*From ESC Guidelines for the Diagnosis and Treatment of Acute and Chronic Heart Failure,* ***2021***)

Observations!

- ***Hyperuricemia and gout*** are common in the population with HF, may be caused or aggravated by diuretic treatment and are associated with a relatively poor prognosis, especially in patients with low cardiac ejection fraction.
- ***Iron deficiency*** defined as: serum ferritin < 100 µg/L or ferritin between 100 and 299 µg/L and transferrin saturation < 20% is common in HF, being responsible for anemia and/or skeletal muscle dysfunction associated with reduced exercise capacity and quality of life.
  - Risk factors for iron deficiency include: female gender, a high class of HF, and high levels of C-reactive protein.
  - In the population with HF, iron deficiency indicates a *worse prognosis* and requires prevention measures (screening for potentially reversible/treatable causes, such as gastrointestinal bleeding) and/or treatment depending on the degree of severity.
  - Oral iron supplements have limited efficacy in patients with HF, the most widely used treatment nowadays, which has been shown to improve the prognosis and quality of life of these patients, being intravenous administration of iron preparations.

6. SPECIALIZED diagnostic tests

- **CLINICAL value** - are tests used for:
  - confirmation of the diagnosis of CHF, when the diagnostic tests recommended for the initial exploration of CHF are inconclusive
  - detection of reversible/treatable causes of CHF (basic etiology)

a. TRANSESOPHAGEAL ultrasound

- this investigation is necessary in isolated cases (e.g., the patient's symptoms are discrepant with the severity of mitral/aortic valvulopathy detected by transthoracic echocardiography).

b. PHARMACOLOGICAL and EXERCISE STRESS echocardiography

- allows the detection of *diastolic ventricular dysfunction induced by exercise* in patients with exertional dyspnea in whom resting transthoracic echocardiography revealed inconclusive diastolic parameters or preserved left ventricular ejection fraction
- is useful for assessing inducible ischemia in patients who are considered suitable for coronary revascularization

c. CARDIAC MAGNETIC RESONANCE imaging

- is the *"gold standard"* for the evaluation of myocardial structure and function in patients with inconclusive transthoracic echocardiography results
- allows the measuring of the volume, mass and ejection fraction of both ventricles
- however, the limitations of the method must be taken into account (contraindicated in patients with metal implants or eGFR < 30ml/min/ 1.73m^2 - if gadolinium is used; high costs compared to echocardiography; possibly erroneous values in the presence of tachyarrhythmias etc.).

d. Computed tomography CORONARY angiography (CTCA)

- useful for evaluating the coronary anatomy and detecting a possible stenosis at this level
- can be considered in patients with inconclusive non-invasive stress tests, to exclude the diagnosis of coronary artery disease

e. SINGLE-PHOTON EMISSION computed tomography (SPECT)

- useful in assessing ischemia and myocardial viability, myocardial inflammation or infiltration, but exposes the patient to ionizing radiation and has a high cost

f. CORONARY angiography

- useful for identifying *coronary artery disease* as the etiology of HF and establishing its severity
- performed in patients who are considered eligible for a potential coronary revascularization

B. Exploring new onset ACUTE HEART FAILURE (AHF)

Acute heart failure (AHF) refers to rapid or gradual onset of symptoms and/or signs of HF, severe enough for the patient to seek urgent medical attention, leading to an unplanned hospital admission or an emergency department visit.

AHF may be the first manifestation of HF (new onset) or, more frequently, be due to an acute decompensation of chronic HF. The precipitating factors for heart failure decompensation must be identified from the first medical contact (acute presentation) in the emergency department.

In addition to the **medical history** and **signs and/or symptoms** suggestive of a new onset acute heart failure, **to outline the diagnosis are RECOMMENDED** (Figure 9.2): *ECG, transthoracic ultrasound, initial laboratory investigations, chest radiography and pulmonary ultrasonography, other specific evaluations, and natriuretic peptide testing.* The clinical value of these investigations is presented below.

1. 12-lead ECG
- useful to rule out acute coronary syndrome or arrhythmias

2. TRANSTHORACIC echocardiography
- has a **major diagnostic value**, confirming AHF by detecting congestion, cardiac dysfunction and mechanical causes

3. Initial LAB investigations

a. Tests with DIAGNOSTIC value
- **Cardiac troponins I and T –** to rule out acute coronary syndrome. High values of cardiac troponins are present in the vast majority of patients with HF suggesting a continuous process of myocardial injury and necrosis, associated with causes of HF decompensation such as acute coronary syndromes or pulmonary thromboembolism. The elevated level of these biomarkers, in the context of AHF, has prognostic significance and is useful for risk stratification and treatment planning
- **D-dimers** – to rule out pulmonary embolism
- **Serum procalcitonin** – when an infectious etiology is suspected, such as pneumonia. Antibiotic therapy is indicated when serum procalcitonin levels are < 0.2 μg/L
- **Arterial blood gas testing and pulse oximetry** – to assess respiratory function, when respiratory insufficiency is suspected
- **Lactate** – for evaluating tissue perfusion status and diagnosing lactic acidosis
- **TSH** – when the presence of thyroid dysfunctions is suspected

b. Tests for PROGNOSIS assessment and TREATMENT determination
- **Serum creatinine** – to assess renal dysfunction
- **Serum ionogram** (sodium, potassium, chlorine) – to evaluate electrolyte imbalances
- **Iron status** (ferritin, transferrin) – to assess iron deficiency

4. CHEST x-ray and LUNG ultrasound
- can be used to confirm AHF, especially when NP testing is not available, by highlighting pulmonary stasis/congestion

5. Other SPECIFIC evaluations – include:
- **Coronary angiography** – in case of suspected acute coronary syndrome
- **Computed tomography** – in case of suspected pulmonary embolism

6. NATRIURETIC peptide testing
- NP values should be measured **if the diagnosis of AHF is uncertain**
- **normal NP concentrations make the diagnosis of AHF unlikely**
- the upper limit considered normal in the case of an **acute** presentation at **new onset** is:
  - for **BNP is < 100 pg/mL**
  - for **NT-proBNP is < 300 pg/mL**
  - for **MR-proANP is < 120 pg/mL**

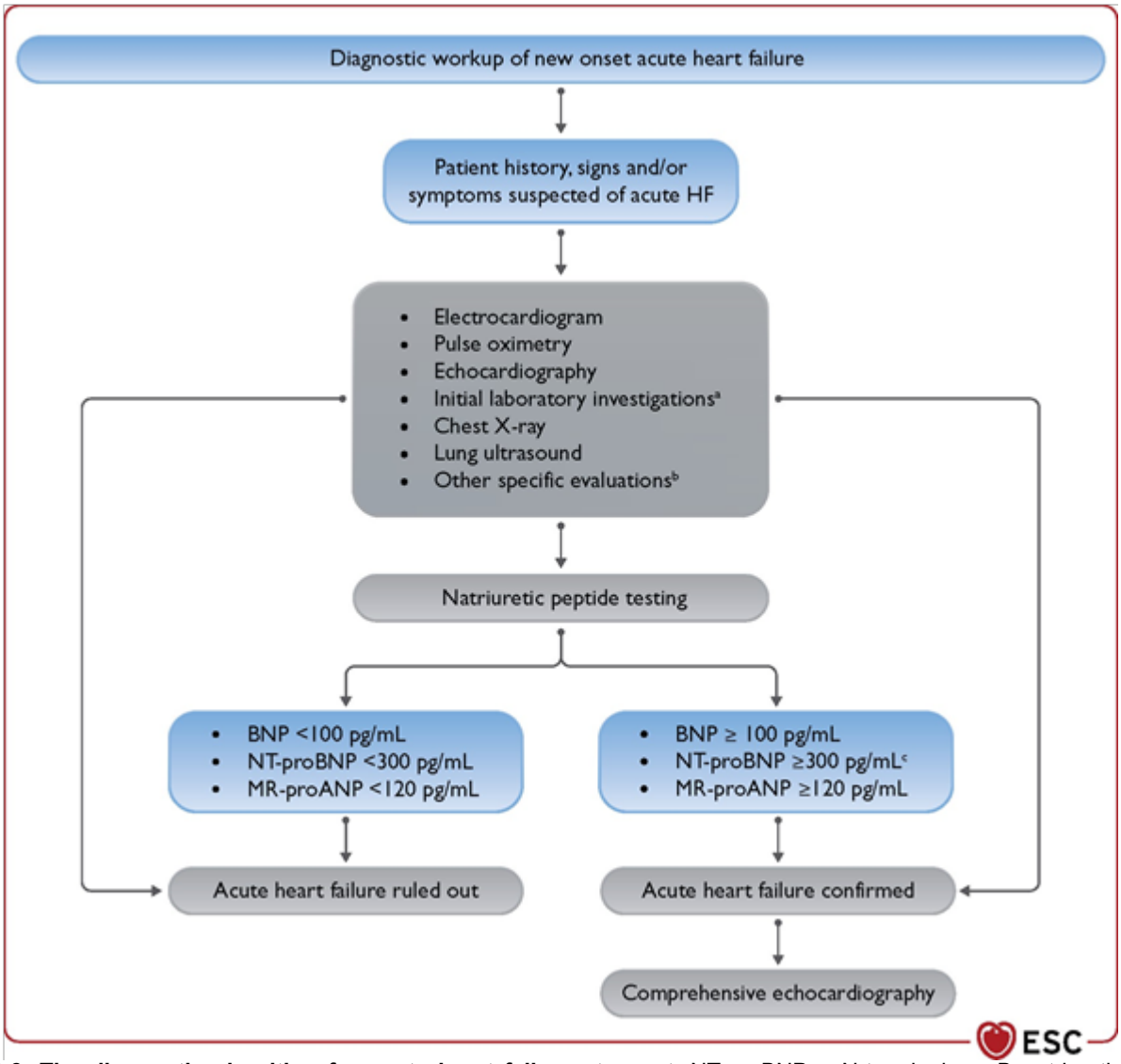

Figure 9.2. The diagnostic algorithm for acute heart failure at onset. NT-proBNP = N-terminal pro-B natriuretic peptide, BNP = brain-derived natriuretic peptide, MR-proANP = middle region of atrial natriuretic xpropeptide (*From ESC Guidelines for the Diagnosis and Treatment of Acute and Chronic Heart Failure, **2021***)

III. BIOMARKERS OF INFLAMMATION

High Sensitivity C Reactive Protein (hs-CRP)

- **Definition:** acute phase protein synthesized by the liver that is neat **biomarker of inflammation** in *coronary artery disease and heart failure*, being measured through techniques that allow the identification of concentrations lower than 3 mg/L.
- **CLINICAL value:**

① **Marker of cardiovascular events in the following 10 years** - by assigning subjects to risk groups:

- **low risk**: hs-CRP < 1.0 mg/L
- **moderate risk**: hs-CRP = 1.0-3.0 mg/L
- **major risk**: hs-CRP > 3.0 mg/L

! Observation: Two hs-CRP measurements at **2-3 weeks** distance are required to assign a patient to a risk group!

② **Marker of increased risk of ACUTE cardiovascular events** - increased hs-CRP reflects the presence of *inflammation* in the setting of:

- ATS with THROMBOSIS and AMI risk
- severe forms of HF (high mortality risk)

CHECKPOINT!

*__1. Which of the following is a neuro-humoral biomarker of cardiac failure?__
A. hs-CRP
B. Myeloperoxidase
C. Natriuretic peptide type B
D. Soluble CD40 ligand
E. Myoglobin

***2. Which of the following is a biomarker of the coronary inflammatory syndrome?**
A. Matrix metalloproteinases
B. Myoglobin
C. hs-CRP
D. Creatin kinase
E. B natriuretic peptide

***3. The upper limit of normal for BNP, in case of a non-acute presentation, is:**
A. 300 pg/ml
B. 125 pg/ml
C. 120 pg/ml
D. 100 pg/ml
E. 35 pg/ml

***4. Which of the following statements is NOT true about BNP?**
A. Has a higher sensitivity than MR-proANP in assessing the risk of mortality in HF
B. It has a protective effect against fibrosis and cardiac remodeling
C. It has a diuretic and natriuretic effect
D. It is released by the ventricular myocytes
E. It is increased in acute cardio-renal syndrome

***5. Which of the following investigations has a major diagnostic value for new onset AHF:**
A. Chest x-ray
B. Serum creatinine
C. Cardiac troponins
D. Transthoracic echocardiography
E. Natriuretic peptides

6. Which of the following are essential initial investigations for diagnosing heart failure?
A. Coronary angiography
B. Cardiac natriuretic peptides
C. ECG
D. Transesophageal ultrasound
E. Transthoracic ultrasound

7. According to the diagnostic algorithm of HF in a non-acute context, which of the following situations raises the suspicion of HF?
A. BNP ≥ 125 pg/mL
B. Normal transthoracic echocardiography
C. Typical symptoms of HF, such as: dyspnea and fatigue
D. Changes present on the ECG
E. NT-proBNP ≥ 35 pg/ml

8. Recommended laboratory investigations for the initial assessment of a newly diagnosed patient with HF include:
A. C-reactive protein
B. Complete blood count
C. Liver tests
D. CK-MB
E. Fasting blood glucose

9. Which of the following are initial LAB tests with diagnostic value for new onset AHF?
A. Cardiac troponins
B. Procalcitonine
C. Serum creatinine
D. Iron status (ferritin, transferrin)
E. D-dimers

10. Which of the following statements are true about high sensitivity C-reactive protein?
A. It is a marker of cardiovascular events for the following 10 years
B. It is a specific marker for cardiac remodeling
C. Its normal value may help to rule out the diagnosis of myocardial infarction
D. It is a marker of platelet activation
E. It is a marker of inflammation and the risk of acute cardiovascular events

CASE STUDIES

1. A 65-year-old patient, diagnosed 10 years ago with grade II hypertension, partially compliant to the recommended specialized treatment, presents to the cardiologist for dyspnea and extreme fatigue. Transthoracic echocardiography reveals thickening of the left ventricular wall and a low ventricular ejection fraction. Consecutively, the patient receives the recommendation to evaluate serum BNP and the blood count. The results reveal: BNP = 86 pg/ml and NT-proBNP = 250 pg/mL.

What diagnosis do you suspect?
What ECG changes might be present in the given case?
What additional investigations are needed to fully evaluate the patient and establish treatment?

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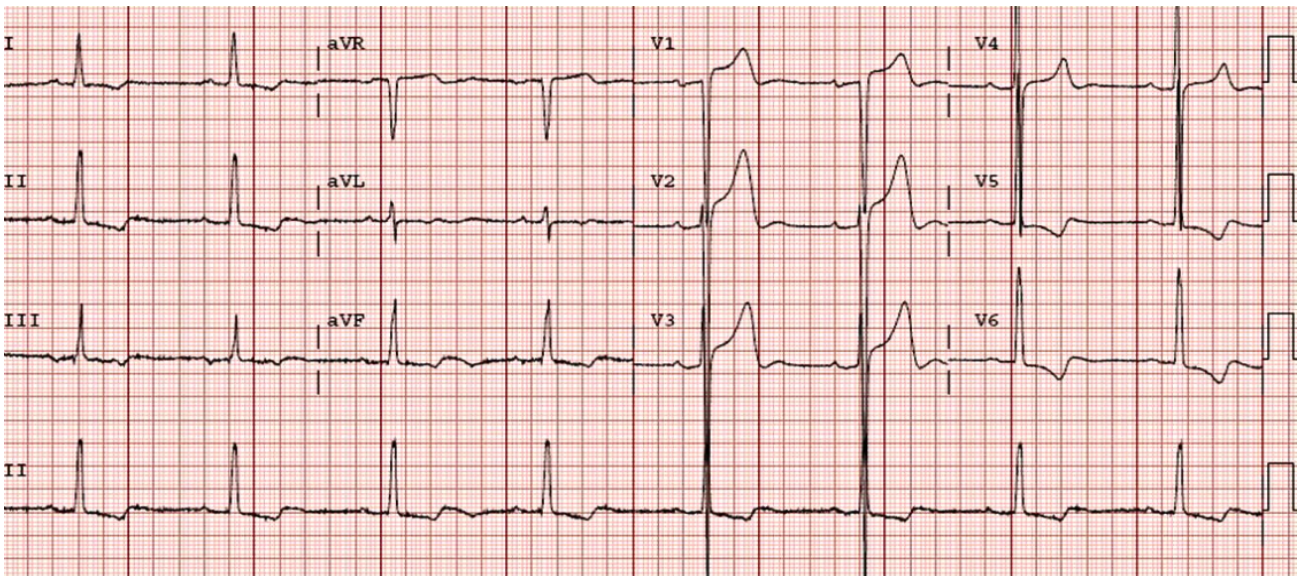
2. A 65 years old man presents to his cardiologist for the progressive worsening of fatigue. The patient was previously diagnosed with moderate aortic stenosis at the age of 45. ECG is listed below.

Analyze the ECG.
What other investigations are necessary?
Establish a diagnosis.



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NOTES

10. CARDIOPULMONARY EXERCISE TESTING (ERGOSPIROMETRY)

LEARNING OBJECTIVES:
At the end of this chapter, students are expected to:
1. *List the indications for cardiopulmonary exercise testing.*
2. *Interpret the changes in ergospirometric parameters that assess the cardio-pulmonary functional reserve.*
3. *Interpret the changes in metabolic parameters that assess the gas exchanges.*
4. *Recognize on the ECG both the physiological as well as the pathological changes elicited by exercise.*

The body's ability to adapt to exercise is the expression of the *efficient coupling* of O_2 and CO_2 exchanges at *cellular* level with those at *pulmonary* level, as a result of the interdependent functioning of four major systems: *cardiovascular, pulmonary, muscular* and *haematological*. Cardiopulmonary exercise testing or ergospirometric testing is *the most widely used method of exploring the cardiopulmonary exercise functional reserve* and is considered the *"gold standard"* for determining the *maximal aerobic capacity* and the *anaerobic threshold*. The main diagnostic and prognostic indications for cardiopulmonary exercise testing are listed in Table 10.1.

Table 10.1. Cardiopulmonary exercise testing indications according to the *Clinical Recommendations Guide for Cardiopulmonary Exercise Testing* (Guazzi et.al., 2012 updated in 2016).

• **Etiological diagnosis of unexplained <u>exertional dyspnea</u>:** – identification of cardiac, pulmonary or other causes that are responsible for exertional dyspnea
• **Evaluation of patients with <u>cardiac</u> diseases:** – functional evaluation and prognostic assessment in patients with: ▪ ***heart failure*** (with reduced or preserved ejection fraction) ▪ ***hypertrophic cardiomyopathy*** (suspected or confirmed disease) ▪ ***suspected myocardial ischemia*** ▪ ***valvular disease/dysfunction*** – selection of patients with indication for ***heart transplantation***
• **Assessment of patients with <u>respiratory</u> diseases:** – ***obstructive***: COPD, exercise-induced asthma – ***restrictive***: interstitial lung diseases – ***vascular***: primary and secondary pulmonary HT
• **Assessment of <u>exercise tolerance</u> in patients with cardio-pulmonary diseases:** – establishing the degree of effort that can be made by patients in order to design rehabilitation programs
• **Assessment of <u>perisurgical or postsurgical risk and long-term prognosis</u>** in patients who undergo **<u>major surgery</u>**: liver and lung resection/transplant, colorectal or bariatric surgery, abdominal aorta aneurysm surgery
• **Assessment of the aerobic capacity in apparently healthy individuals**
• **Assessment of patients with suspected <u>mitochondrial myopathy</u>**

A. CARDIOPULMONARY EXERCISE TESTING - TECHNIQUE

- **Principle:** cardiopulmonary exercise testing consists in a **maximal incremental effort** to be performed on the *cycle ergometer* or *treadmill*.

- **Steps:**

1. **Preliminary procedures:**
   – cardio-pulmonary exercise testing can be performed throughout the day, with ceasing of smoking and food, alcohol or coffee

ingestion at least 3 hours beforehand
- before the test:
  - *BP measurement in supine* position and on the *cycle ergometer*
  - *respirometry evaluation through spirometry* for the assessment of FEV1 and of the maximal indirect ventilation V′max.$_{indirect}$ (V′max.$_{indirect}$ = 30 x FEV1)
  - *ECG at rest* (mandatory for at least 12 leads)

2. **Warm up** – the patient pedals with no working load (0 W) for 3 min.
3. **Exercise:**
   - the patient pedals with a pre-established work rate (WR) of 5-25 W/min, 60 rotations/min, for 6-12 minutes, under continuous ECG monitoring and automatic BP measurement at 1 minute intervals
   - the exercise test is *finalized* when the patient reaches the **target maximal heart rate,** calculated according to the following formula:

tMHR = 220 – age

   - the exercise test *must be stopped* despite not having reached the target MHR in the following situations, that represent **ABSOLUTE indications for exercise test cessation:**
     - *occurrence of myocardial ischemia* (moderate/severe chest pain, ECG ischemic modifications, premature ventricular contractions)
     - *systolic BP (SBP) decrease by > 10 mmHg, in the presence of clinical and ECG signs of ischemia*
     - *systolic BP increase* > 240 mmHg or *diastolic BP (DBP) increase* > 120 mmHg
     - *severe decrease in oxygen saturation* (SpO_2 ≤ 80%) accompanied by signs/ symptoms of severe hypoxemia
     - *signs of cerebral hypoperfusion:* sudden pallor, loss of coordination, dizziness, fatigue, confusion
     - *patient's request*
     - *reaching a level of 17/18 on the Borg scale* (Table 10.2).

Table 10.2. Borg scale for the subjective assessment of exercise difficulty.

BORG scale	Difficulty degree
Very, very light	7 8
Very light	9 10 11 12
Slightly hard	13 14
Hard	15 16
Very hard	17
Very, very hard	18 19 20

4. Recovery
- the patient continues to pedal for 3 minutes, without work load (0 W) to avoid a sudden drop of BP values
- the patient will be placed in supine position with continuous ECG and BP (every 2 minutes) monitoring, for *10 minutes*
- the patient will not be released until the ECG is identical to the one before the test and will be monitored for another *1-2 hours.* For patients with a history of myocardial infarction, monitoring is required for a minimum of *24 hours.*

5. Complications
- arterial hypotension
- arrhythmias and/or AV blocks
- cardiac arrest
- acute myocardial infarction (AMI)
- stroke
- physical trauma (ex. falling)

Remember!
The exercise test is always performed with the **cardiovascular resuscitation means** at hand.

B. NON-INVASIVE PARAMETERS OF THE EXERCISE TEST

- **Types of parameters used to assess:**
  - The ***cardiac functional reserve*:**
    - Blood pressure (BP)
    - Cardiac reserve (CR)
    - ECG
  - The ***pulmonary functional reserve:***
    - Maximal effective ventilation during effort (V_E)
  - The ***metabolic reserve (the gas exchange efficiency):***
    - Peak oxygen consumption (VO_2 max)
    - Anaerobic threshold (AT)
    - Respiratory exchange ratio (RER)

1. Blood pressure (BP)

- **PHYSIOLOGICAL variations:**

①During effort:
- SBP *increases progressively* during exercise up to values between 165 and 210 mmHg
- DBP remains *unchanged, decreases* or *increases* by a maximum of 10 mmHg as compared to initial values

②During recovery after exercise:
- SBP and DBP decrease progressively

- **PATHOLOGICAL variations**:
  - **Increase in SBP > 210 mmHg in men and > 190 mmHg in women** indicate**:**
    - in hypertensive patients = a high risk for AMI or malignant arrhythmias
    - in healthy individuals = a high risk for developing hypertension
  - **No increase in SBP <u>or</u> decrease of SBP ≥ 10 mmHg** during the exercise testing (after a previous increase) **<u>or</u> the paradoxal increase of SBP 3 minutes after exercise cessation** in patients with coronary heart disease (CHD) suggests:
    - latent left ventricular failure
    - extensive ischemia (plurivascular disease)
  - **A lack of increase in SBP <u>or</u> the impossibility of maintaining SBP ≥ 110 mmHg**, in a patient with coronary heart disease and AMI in his/her history indicates a reserved prognosis after the AMI

2. Cardiac reserve (CR)

- **Definition:** the difference between the target maximal heart rate and the maximal heart rate corresponding to the $VO_{2\ max}$ (the moment when the exercise test is stopped)
- **NORMAL value: CR < 15 b/min**
- **PATHOLOGICAL variations:**
  - A rapid *decrease/exhaustion of the CR* (i.e., the target maximal heart rate is achieved at lower exercise levels than predicted) is indicative of an increase in cardiac output via the increase in HR (not the systolic volume) and occurs in:
    - low physical condition
    - latent left ventricular failure
  - An *increase of CR > 15 b/min* (effort was stopped before having achieved the target MHR) occurs in:
    - *chronotropic heart incompetence (cardiac output increase is limited by the inadequate increase of HR) in*:
      - sinus node dysfunction
      - treatment with beta-blocking agents
    - *pulmonary diseases that limit physical effort*

3. Double product (DP):

- **Definition:** DP = maximal SBP x maximal HR
- **NORMAL value: ~ 35.000 (mmHg x b/min)**
- **CLINICAL significance:**
  - was the *most frequently used indicator of the heart's capacity for exercise adaptation* when equipments that performed only *cardiac* exercise testing were being used (equipments that still exist in some hospitals)

- allows the *indirect assessment of cardiac performance,* being a measure of the myocardial O_2 demand which correlates well with the peak oxygen consumption (VO_{2max})
- the DP reached at the moment when myocardial ischemia occurs (with or without pain) approximates the *angina* or *ischemic threshold:*
  - in angina pectoris, the angina threshold corresponds to a DP = 20.000 - 22.000
  - coronary patients with an angina threshold < 14.000 - 15.000 have indication for myocardial revascularization

4. Electrocardiogram (ECG)

- **PHYSIOLOGICAL variations:**
  - P wave amplitude increases
  - shortening of PR and QT intervals
  - narrowing of QRS complexes
  - right axis deviation
  - J point depression < 1 mm with a fast ascending ST segment (Figure 10.1A)
- **PATHOLOGICAL variations:** in patients with CHD (or other cardiac diseases) exercise may elicit changes of the ST segment, T and U waves, arrhythmias or conduction disturbances**.**

a) ST segment changes

Are the ECG changes *characteristic* for ischemia:

- **ST segment depression** indicates:
  - **subendocardial effort ischemia** – when it persists for at least 3 successive cardiac beats with a *horizontal or downslopping pattern*, amplitude ≥ 1 mm and duration of 0.06-0.08 sec. after the J point (Figure 10.1B)

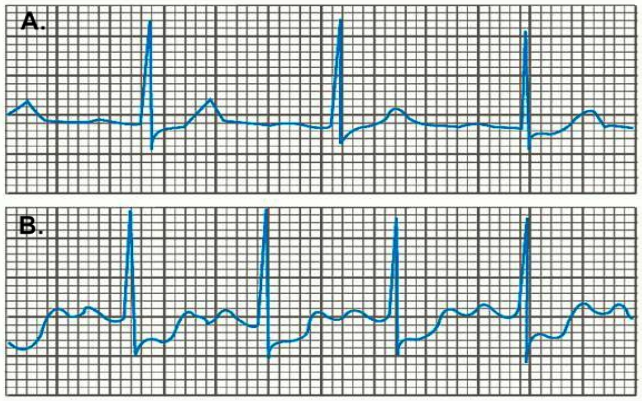


Figure 10.1 A – Normal exercise ECG. **B** - ST depression during exercise (subendocardial ischemia)
(*Modified after https://molconditions.wordpress.com*)

  - if the ST segment depression is already present at rest, a positive exercise test requires an additional
  - depression with the above mentioned characteristics to occur
  - ST depression that appears *early*, at low exercise levels and/or ST depression that lasts longer than 5-10 minutes after the end of exercise increases the specificity of the test and indicates *severe subendocardial effort ischemia*
- **ST segment elevation** indicates**:**
  - **transmural ischemia** – when it persists for at least 3 successive cardiac beats, has an amplitude ≥ 1 mm and a duration of 0.06-0.08 sec. after the J point. It is found in coronary patients with *severe transmural effort ischemia*.
  - occurs more rarely as compared to the ST depression.

b) T wave changes consist of:

- *flattened, isoelectrical, inverted or tall, positive symmetrical T waves.*
- these changes have diagnostic value **only in association** with ST segment or U wave changes

c) U wave changes consist of:

- ***inverted U wave* during exercise** (when visible on ECG prior to the test) is *highly specific for myocardial ischemia* even in the absence of other ECG changes (some authors believe that it is a good indicator of severe coronary stenosis, therefore having prognostic value

d) Arrhythmias and conduction disturbances:

Rhythm and conduction disorders that occur during the exercise test are used as diagnostic criteria for coronary heart disease (ischemic heart disease) and consist of:

- **Supraventricular arrhythmias** (e.g., premature atrial contractions, supraventricular tachycardia, atrial fibrillation):
  - they are *not specific* for patients with CHD, (i.e., can occur in healthy subjects too)
  - they indicate *myocardial ischemia* when present *in association with ST segment changes*
  - by increasing the HR they can impose effort cessation
- **Ventricular arrhythmias** (e.g., premature ventricular contractions, ventricular tachycardia)
  - have a negative prognosis due to the increased risk for sudden cardiac death (the lower the effort and HR at which they occur the higher the risk!)

5. Ventilation reserve (VR)

- **Definition:** the additional pulmonary ventilation theoretically available after the interruption of physical exertion:
  - **$RV = V_E / Vmax._{ind}$**

  Where:
  - *$V'max._{indirect}$* = the maximal indirect ventilation (L/min) calculated by means of spirometry ($V'max._{indirect} = 30 \times FEV_1$)
  - V_E = the maximal effective ventilation (L/min) or the maximal ventilation achieved during the exercise test (normally at $VO_{2\ max}$)
- **NORMAL values: VR > 0,3**

- **PATHOLOGICAL variations:**
  - VR is *decreased or rapidly exhausted* when exercise intolerance is due to *pulmonary* diseases
  - VR is normal or increased when exercise intolerance it is due to heart disease or other diseases, such as vasculopathy or anemia (Figure 10.2).

6. Peak O_2 consumption ($VO_{2\ max}$)

- **Definition:** the maximal O_2 consumption obtained during exercise, which depends on the intensity and duration of the physical effort
- **NORMAL values:** Current $VO_{2max} \geq$ **80%** of the predicted VO_{2max} (according to patient age, gender, height and weight)
- **PATHOLOGICAL variations:** Current $VO_{2max} <$ **80%** of predicted VO_{2max} signifies effort tolerance limitation due to low physical condition, heart disease, lung disease etc.
- **CLINICAL significance:**
  - defines the *maximal aerobic capacity* or the body's maximum capacity to adapt to effort
  - is indicative of the *maximal cardiac output* in both healthy individuals as well as patients with heart failure

7. Anaerobic Threshold (AT)

- **Definition:** the highest VO_2 value that can be attained during exercise without the occurrence of lactic acidosis (the level of VO_2 during physical exertion at which *aerobic* energy production is supplemented by *anaerobic* energy production, which causes the accumulation of lactic acid in the blood)
- **NORMAL values: AT> 40%** of the predicted VO_{2max}
  - *minimum: 40%* of the predicted VO_{2max} in sedentary individuals
  - *maximum: 80%* of the predicted VO_{2max} in trained individuals
- **PATHOLOGICAL variations**: **AT < 40%** of the predicted VO_{2max} occur in:
  - *low physical condition*
  - *cardiac disease*
  - *pulmonary disease*
- **CLINICAL significance: AT together with $VO_{2\ max}$** are used as indicators for the *exercise capacity in patients with heart failure* (Table 10.3)

Table 10.3. Classification of the exercise capacity in patients with heart failure (according to the *ACC/AHA Guide for exercise testing*)

CLASS	Degree of exercise dysfunction	$VO_{2\ max}$ (ml O_2/kg/min)	AT (ml O_2/kg/min)
A	Absent – Mild	> 20	> 14
B	Mild – Moderate	16-20	11-14
C	Moderate – Severe	10-16	8-11
D	Severe	< 10	< 8

VO_2 and T_A are measured in ml O_2/kg/min

8. Respiratory Exchange Ratio (RER)

- **Definition:** the ratio between CO_2 elimination (VCO_2) and O_2 consumption (VO_2)
- **NORMAL value:**
- **RER > 1.1** is the indicator of having performed **a maximal exercise test**
- **CLINICAL significance:**
- RER *is influenced by the respiratory quotient* (RQ) of the *energy substrates* used during the exercise test (RQ is 1 for carbohydrates and 0.7 for fatty acids):
  - ✓ RER < 1 indicates that a mix of fat and carbohydrates was used as a fuel source
  - ✓ RER > 1 indicates the exclusive use of carbohydrates as energy substrate
  - ✓ the VO_2 level when switching from a sub-unit RER (<1) to a supra-unit one (>1) corresponds to reaching the AT

Global interpretation of exercise testing:

From the analysis of the parameters directly measured or indirectly derived during the test, conclusions are formulated on the capacity of effort adaptation of the cardiovascular and pulmonary apparatus, respectively the cardiovascular and / or pulmonary functional deficit is determined.

A simplified algorithm for interpreting the results of cardiopulmonary exercise testing is shown in Figure 10.2.

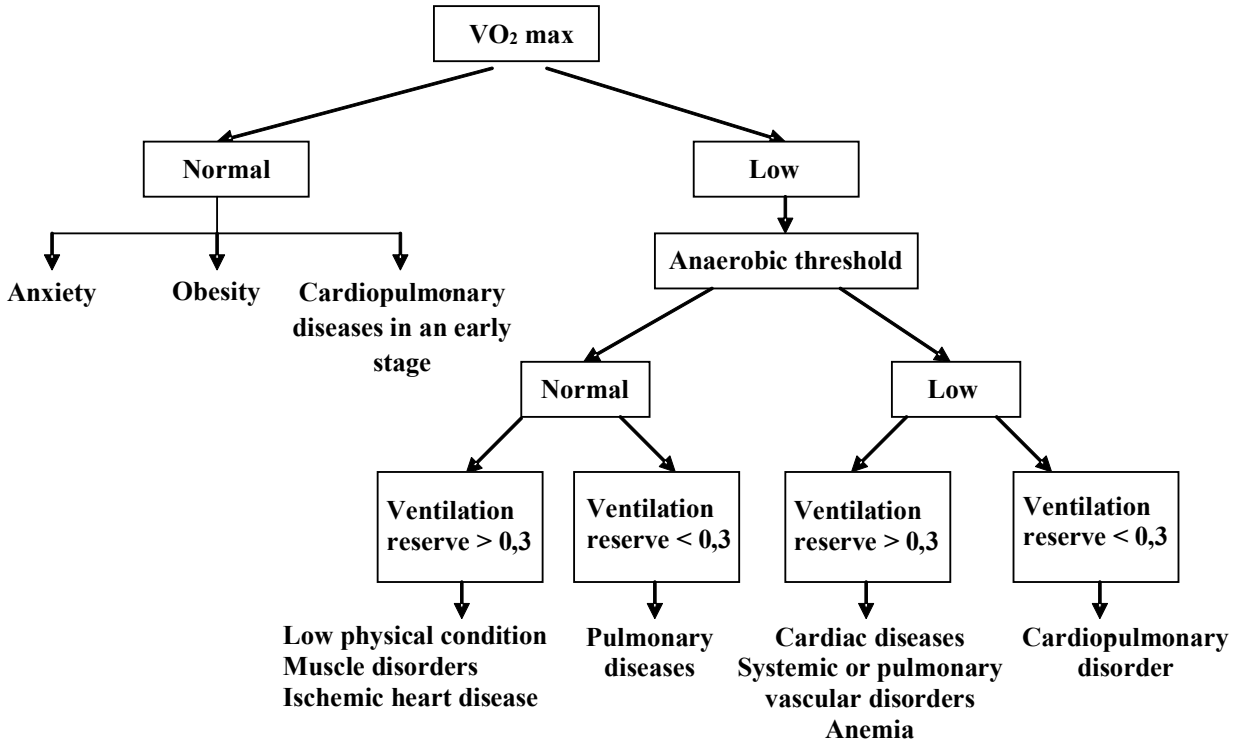


Figure 10.2. Diagnostic algorithm for factors that limit effort tolerance

CHECKPOINT!

*__1. Which of the following represents a physiological ST segment change during the exercise test?__
A. Downslopping depression
B. Horizontal depression
C. Slow ascending depression
D. Fast ascending depression
E. ST elevation

*__2. Which of the following has a high specificity for myocardial ischemia observed during exercise testing, even in the absence of other ECG changes?__
A. Increased P wave amplitude
B. Progressive right axis deviation
C. Inverted U wave
D. Flat T waves
E. Supraventricular arrhythmias

*__3. Which of the following parameters defines the maximal aerobic capacity?__
A. Cardiac reserve
B. Ventilation reserve
C. Maximal effective ventilation during effort
D. Peak oxygen consumption
E. Double product

*__4. An increase of SBP > 220 mmHg during exercise testing in a healthy male patient is suggestive for:__
A. Increased risk for AMI
B. Increased risk for malignant arrhythmia
C. Increased risk for developing hypertension
D. Latent left ventricular failure
E. Myocardial ischemia

*__5. Which of the following parameters is an indicator of having performed a maximal exercise test?__
A. Cardiac reserve < 15 b/min
B. Decreased ventilation reserve
C. Current $VO_{2\,max}$ < 80% of maximum predicted VO_2
D. AT < 40% of maximum predicted VO_2
E. RER > 1.1

__6. Which of the following are pathological changes of blood pressure during the exercise test?__
A. Decrease in DBP value by 10 mmHg as compared to the rest value
B. Increase in DBP value by 10 mmHg as compared to the rest value
C. No increase in SBP value
D. No increase in DBP value
E. Increase in SBP value > 210 mmHg

__7. Which of the following are true about the double product?__
A. Represents the product between the maximal HR and the maximal diastolic blood pressure reached during the exercise test
B. It indirectly assesses cardiac performance
C. Is an indicator of the maximal O_2 consumption
D. Can be used to assess the ischemic threshold
E. Is useful to identify patients that require myocardial revascularization

__8. An increase of the cardiac reserve > 15 b/min is suggestive for:__
A. Low physical condition
B. Latent left ventricular failure
C. Sinus node dysfunction
D. Treatment with beta-blockers
E. Respiratory diseases

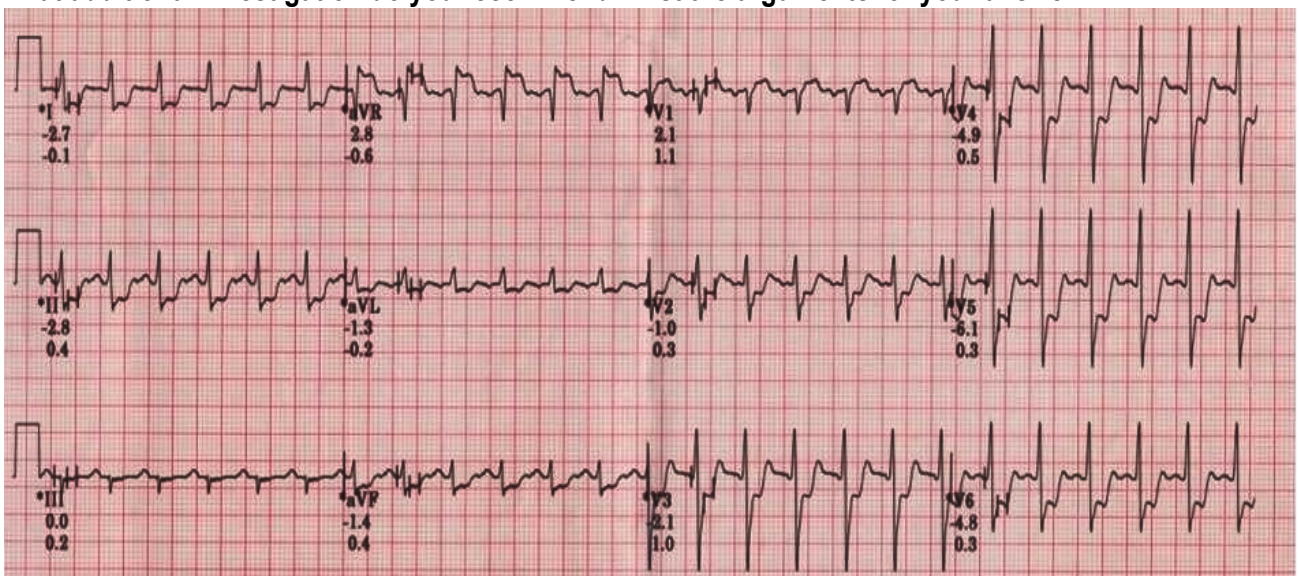
__9. Which of the following ECG changes are characteristic for myocardial ischemia during the exercise test?__
A. Increase in P wave amplitude
B. Short PQ interval
C. Horizontal ST segment depression
D. Isolated inverted T waves
E. Persistent ST segment elevation

__10. RER:__
A. Is the highest VO_2 value attained during exercise without the occurrence of lactic acidosis
B. RER values > 1 indicate the exclusive use of carbohydrates as energy substrate during effort
C. Together with VO_2 is used to grade the exercise capacity of patients with cardiac failure
D. Is influenced by the respiratory quotient
E. Increases in respiratory diseases

CASE STUDIES

1. A 57-year-old male with a personal history of type I diabetes mellitus and 2nd degree HT presents with chest pain that started after stair climbing. Resting ECG is normal. Cardio-pulmonary exercise testing is performed. During the 4th minute of the effort testing HR =150 b/min, BP = 180/90 mmHg and patient indicated chest pain.

Analyze the ECG changes. What diagnosis do you suspect?
What additional investigation do you recommend? List the arguments for your answer!



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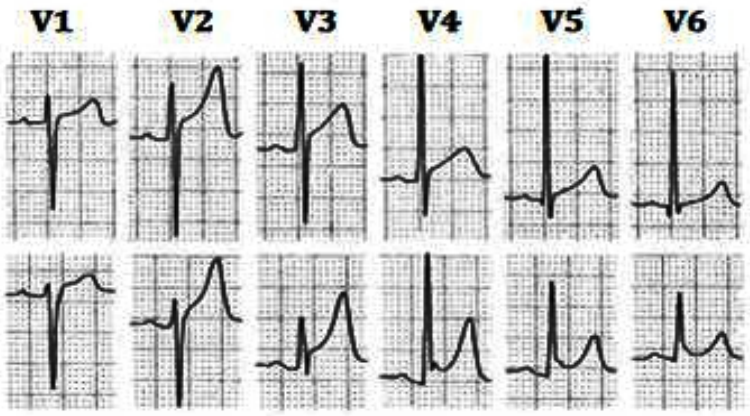
2. A 37-year-old male with no history of cardiovascular disease presents with chest pain after an intense physical effort. Resting ECG is normal. A cardio-pulmonary exercise test is performed. During the 6th minute of the exercise test HR=143 b/min, BP=160/90 mmHg and the ECG shows the changes depicted below.

Analyze the ECG changes.
Should the exercise test be stopped or not?
List the arguments for your answer!

Resting ECG

ECG at the 6th min. of exercise



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3. During cardiopulmonary exercise testing, a patient with COPD had a VO_2max = 73% of the predicted value, the anaerobic threshold was 45% of the predicted $VO_{2\ max}$ and the ventilation reserve 0.35.

Analyze the exercise tolerance of this patient using the diagnostic algorithm in Figure 10.2.
List the arguments for your answer!

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NOTES

11. INVESTIGATION OF MAJOR ENDOCRINE DISORDERS

LEARNING OBJECTIVES:

At the end of this chapter students must be able to:

1. *Request the diagnostic investigations to confirm the excess of adrenal cortical hormones and to identify its etiology.*
2. *Know the diagnostic algorithm of Cushing's syndrome and adrenal cortical insufficiency.*
3. *Request the necessary laboratory and paraclinical investigations to confirm hyperthyroidism and identify its etiology.*
4. *Request the necessary laboratory and paraclinical investigations to confirm hypothyroidism and identify its etiology.*
5. *Know the diagnostic algorithm in hypo- and hyperthyroidism.*

I. INVESTIGATION OF ADRENAL CORTEX DISORDERS

- The adrenal cortex (AC) is the outermost layer of the adrenal gland, comprising three different areas:

–***zona glomerulosa,*** that produces **mineralocorticoid** hormones (aldosterone)

–***zona fasciculata,*** that produces **glucocorticoid** hormones (cortisol)

–***zona reticularis,*** that produces **sex hormones, mainly androgens** (dehydroepiandrosterone).

! Observation: **zona fasciculata and reticularis function as a whole under the control of corticotropine (ACTH), whereas the glomerulosa** (that produces aldosterone) **functions independently from the influence of ACTH.**

A. ADRENAL CORTEX HYPERFUNCTION

•**Definitions:**

a.Hypercorticism - is defined as an excess of glucocorticoid hormones (mainly cortisol) from the level of the zona **fasciculata**.

b.The Adrenogenital Syndrome: an excess of androgen sex hormones – due to a **zona reticularis hyperplasia/tumor**

c.Conn Syndrome (primary hyperaldosteronism): excess of mineralocorticoid hormones due to a **zona glomerulosa hyperplasia/tumor**.

CUSHING Syndrome

•**Definition:** clinical expression, regardless of the cause, of the **chronic excess** of **glucocorticoid hormones (i.e., hypercortisolism),** which sometimes associates with **excess androgens.**

•**Classification:**

<u>ACTH-independent</u> Cushing syndromes:

- **Benign** (adenoma) or **malignant** (carcinoma) adrenal cortex **tumors**
- **Prolonged corticotherapy** (iatrogenic Cushing)

<u>ACTH-dependent</u> Cushing syndromes:

- **Cushing disease** – due to a *pituitary* adenoma, that overproduces ACTH;
- **Ectopic ACTH Syndrome** – due to malignant tumors that produce „ACTH-like" substances: bronchial, pancreatic or thymic tumors.

Consequences of hormone hypersecretion:

1. Cortisol excess causes**:**

–Central obesity (android type)

–Secondary hypertension

–Effects on protein (proteolysis), lipid (lipolysis) and carbohydrate metabolism (hyperglycaemia and risk of diabetes mellitus)

–Round, „moon-like" face

–Purple stretch marks on the abdomen, buttocks, thighs, breasts
–Bruises that appear after minor trauma
–Central nervous system: euphoria, insomnia, psychosis
–Growth inhibition in children
–Osteoporosis in adults
–Susceptibillity to infections and delayed wound healing
–Hypercoagulability with risk of venous thrombosis.

2. Androgen excess causes**:**
– Virilizing effect (acnea, seborrhea, excessive body hair- hirsutism) in women, early onset of puberty in children
– Gonadotropic inhibition with amenorrhea in women, disorders of sexual dynamics in men

- **Paraclinical diagnosis:**

a) Confirmation of hypercortisolism - ***at least 2 positive tests are required;***
b) Exclusion of functional hypercortisolism (pseudo-Cushing);
c) Establishing the cause;
d) Establishing the location of the tumor.

A. Confirmation of hypercortisolism:

- **Principle:** to demonstrate the existence of increased cortisol secretion that is NOT inhibited by exogenous glucocorticoids (dexamethasone) - at least 2 screening tests are required to identify Cushing's syndrome (free urinary cortisol, nocturnal salivary cortisol and/or dexamethasone suppression test):

o Normal screening tests mean an unlikely Cushing's syndrome

o Positive screening tests mean a probable Cushing's syndrome that needs to be confirmed by a low-dose dexamethasone inhibition test for 48 hours

1. Free urinary cortisol:

- **NORMALvalue** = 100-379 nmol/24 h;
- **Interpretation:** an increased value is **suggestive** for Cushing syndrome
- **CLINICAL Value:**
  –the rate of urinary excretion of cortisol **is NOT influenced** by the diurnal variations of its secretion (as opposed to basal serum cortisol)
  –although the test has **decreased sensitivity** and **specificity**, it is a **useful screenig test** for **Cushing's syndrome**

2. Circadian cortisol secretion rhythm:

- **Principle:** after 48 hours of hospitalization, blood samples are collected at 9:00 in the morning and at 24.00 (without warning the patient) from which the level of cortisolemia is determined
- **PHYSIOLOGICAL variations**: cortisol levels are at a maximum in the **morning** (between **7-10 a.m.**) representing basal serum cortisol (NV = 172-497 nmol/L), then **gradually decreases**, reaching the **minimum value at night** at **24.00** (< 100 nmol/L)
- **PATHOLOGICAL variations**: the rhythm is disturbed in Cushing's syndrome, cortisolemia having high values > 100 nmol/L during the night.

3. Nocturnal salivary cortisol:

• **Principle:** the patient chews a tampon for 2 minutes until it is soaked with saliva and then inserts it into the Salivette Cortisol tube until the next day when the nocturnal salivary cortisol is determined.

- **NORMALvalue** < 10 nmol/L
- **CLINICAL value:**
  –free plasma cortisol, the biologically active form of this hormone, is in **balance** with salivary cortisol, a form that is NOT affected by the rate of production of saliva
  –an increase in plasma cortisol is immediately followed by the increase in salivary cortisol levels.
  –is a **screening test** for Cushing's syndrome, with very high sensitivity and specificity and **does not require hospitalization** (determined at the patient's home)
  – useful for **monitoring the treatment response**

4. a. Dexamethasone suppression test

- **Principle**: 1 mg of Dexamethasone is administered orally at 23.00 and cortisolemia is determined in the blood sample taken the next day at 9.00 in the morning
- **Interpretation:**
  - The test is normal if cortisol levels decrease < 100 nmol/L
  - The test is positive if the cortisol level remains elevated > 100 nmol/L
- CLINICAL value: represents **a screening test** for Cushing's syndrome that highlights the inadequate suppression of cortisol secretion by dexamethasone (Figure 11.1).

b. Low-dose dexamethasone suppresion test for 48 hours

- **Principle:** 8 doses of 0.5 mg Dexamethasone are administered orally every 6 hours, starting at 9.00 a.m. on day 0 of the test**.** Cortisol level is determined at 9.00 a.m. on day 0 and day +2 of the test, respectively.
- Interpretation:
  - The test is normal: if cortisol level **decreases < 50nmol/L -> the Cushing's syndrome is excluded**
  - The test is **positive** if the cortisol level in sample +2 > **50 nmol/L -> the Cushing's syndrome is confirmed**

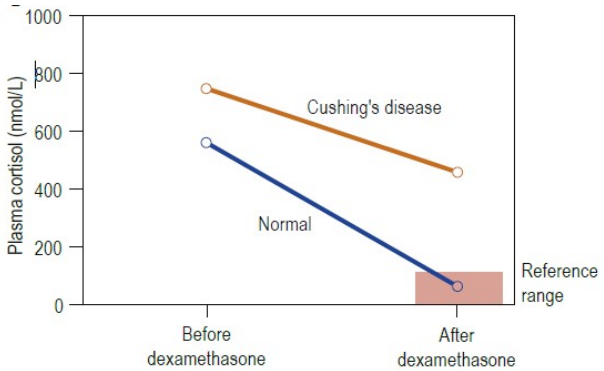

Figure 11.1 Dexamethasone suppresion test in a normal subject and a patient with Cushing's syndrome shows inadequate suppression of cortisol secretion in Cushing's syndrome (*after Kumar and Clark, Clinical Medicine, 10th Anniversary Ed., 2021*)

B. Exclusion of functional Cushing's Syndrome (pseudo-Cushing)

- **Principle:** certain clinical situations can associate a functional hypercortisolism with increased basal cortisol values, that must be distinguished from Cushing syndrome:
  - Obesity
  - Chronic alcoholism
  - Severe depression
- **The differential diagnosis** is based on screening tests (free urinary cortisol, nocturnal salivary cortisol and dexamethasone nocturnal suppresion test) which are **normal in pseudo-Cushing's** and positive in Cushing's syndrome.

C. Establishing the etiology of Cushing syndrome

- **Principle:** after hypercortisolism was confirmed, one must differentiate *ACTH-independent* forms from *ACTH-dependent* ones (Figure 11.2)

1. Determination of basal plasma ACTH levels (7-10 am) on two or more occasions

- **NORMAL values:** 10 - 80 ng/L
- **PATHOLOGICAL findings:**
  - the basal ACTH level is **very low** or **undetectable**–- In **ACTH independent** Cushing syndromes (TUMORAL Cushing and IATROGEN Cushing)
  - In **ACTH-dependent** Cushing's syndromes - the basal ACTH level is:
    - –**moderately increased -> Cushing's disease**
    - –**very increased (>200 ng/L) -> in ectopic ACTH syndrome**

A high-dose dexamethasone suppresion test for 48 hours is required to identify the pituitary or **ectopic** origin of increased ACTH secretion.

High-dose dexamethasone suppresion test for 48 hours

- **Principle:**

o 8 doses of 2 mg Dexamethasone are administered orally every 6 hours, starting at 9.00 on day 0 of the test

o cortisolemia is determined at 9.00 in the morning on day 0 and +2 of the test, respectively

- **Interpretation:**
  - the test is **positive** if the serum cortisol level in sample +2 is **<50%** of the serum cortisol level in sample 0 and is interpreted as **Cushing's disease**
  - the test is **negative** if the serum cortisol level in sample +2 is **>50%** of the serum cortisol level in sample 0 and is interpreted as **ectopic ACTH syndrome** (ectopic ACTH sources are not inhibited even by high-dose dexamethasone)

D. Establishing the location of the tumor

- **In *Cushing disease* (a pituitary tumor that overproduces ACTH):**

− **magnetic resonace imaging (MRI)** visualises pituitary adenomas that overproduce ACTH, but only if their dimension is > 5 mm

− frequently, these tumors are microadenomas (less than 5 mm) so they cannot be analysed through MRI and require investigation in a specialized center in invasive imaging, through ***inferior petrosal sinus catheterization (IPS)***

− ***selective inferior petrosal sinus catheterization (IPS)***:

o ***Principle****:* for each pituitary half, venous blood is drained into the ipsilateral inferior petrosal sinus. The levels of *ACTH in the plasma* and *in the two inferior petrosal sinuses* will be determined simultaneously.

o ***Clinical value:*** IPS catheterization ***can differentiate between an ectopic ACTH source and a pituitary one.***

✓**Cushing's disease (pituitary tumor that overproduces ACTH)** - the value of ACTH at the level of the petrosal sinus is much higher than the one in the plasma (the ratio between **$ACTH_{IPS}$/ $ACTH_{PLASMA}$ > 2**)

✓**Ectopic ACTH secretion** - the value of ACTH at the level of the petrosal sinus is comparable to the one in the plasma (the ratio between **$ACTH_{IPS}$/$ACTH_{PLASMA}$ < 1.4).**

- **In the *ectopic ACTH Syndrome***: whenever an ectopic secretion suspicion exists, the source must be identified. In most cases, a ***pulmonary tumor*** (or mediastinal/ abdominal) is the cause, which requires radiological and imaging evaluation (thoracic X-ray, CT/MRI).
- **In the *adrenal cortex tumors*:** in order to localise such tumors, an abdominal ultrasound, CT and/or abdominal MRI may be useful (but only for tumors greater than 10 mm).

The diagnostic algorithm for Cushing syndromes is described in Figure 11.2.

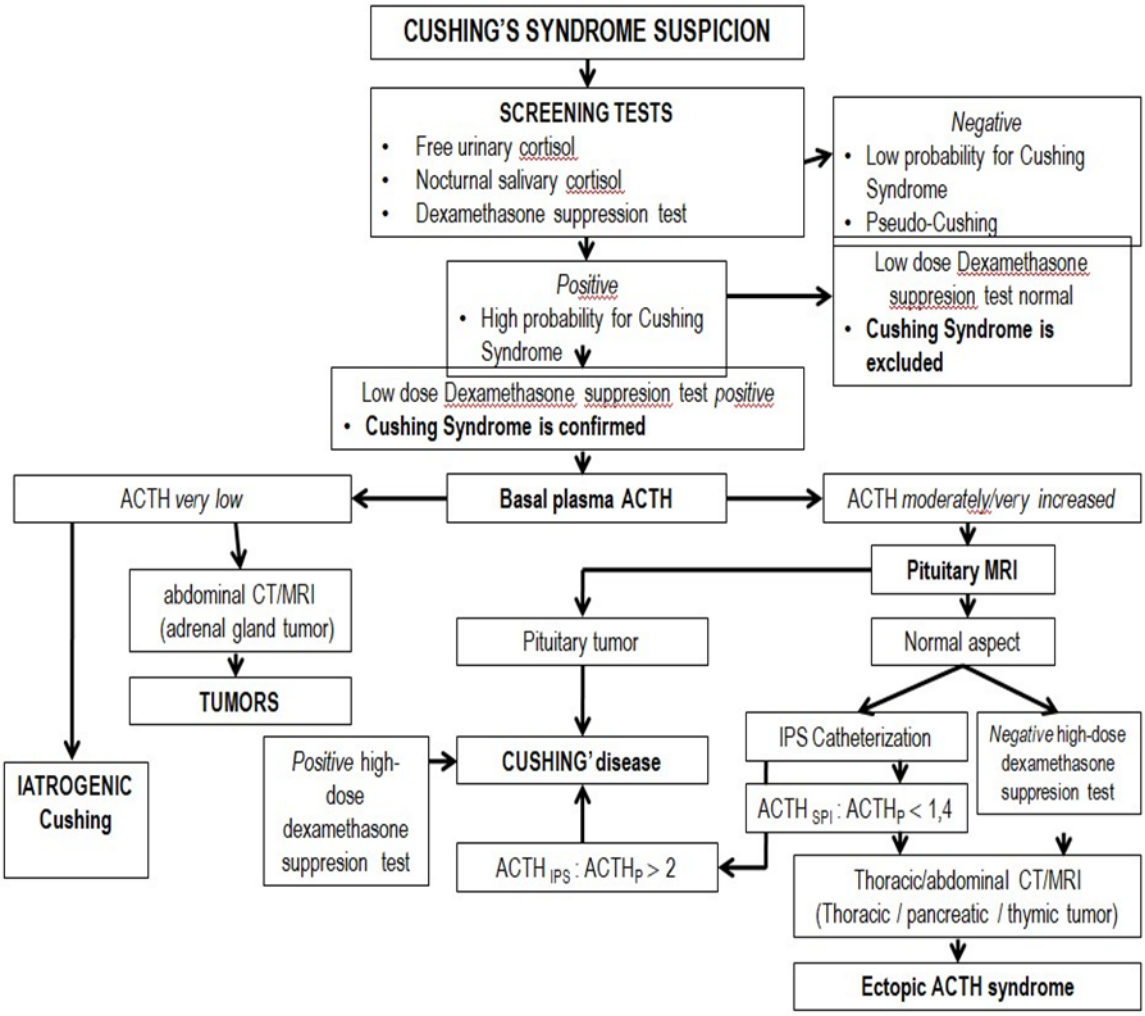

Figure 11.2. Diagnostic algorithm in Cushing syndromes.
(IPS = inferior petrosal sinus, $ACTH_{IPS}$= ACTH in the inferior petrosal sinus, $ACTH_P$ = ACTH in the plasma)

B. ADRENAL CORTEX INSUFFICIENCY

- **Definition:** reduction of corticosteroid hormone synthesis.
- **Classification** – 2 major forms:
  - **Primary** - **Addison's disease**: the lesion is at the level of CSR: autoimmune, tuberculosis, hemorrhage (anticoagulant therapy), tumors, surgical excision, heart attack, etc.
  - **Secondary:**
    - o by **inadequate production of ACTH** as a result of a pituitary lesion
    - o **prolonged corticosteroid therapy** that results in suppression of pituitary ACTH production and consequent atrophy of adrenal gland
- **Consequences of low hormone secretion:**
  - **asthenia/adynamia** (muscle weakness due to trophic and metabolic changes determined by a decrease in cortisol secretion)
  - **melanodermia** (generalized pigmentation of the skin with hyperpigmentation of normally pigmented areas: mammary areola, scrotum, labia major (vulva), abdominal white line, palmar creases and friction areas - elbows, knees) – occurs in primary adrenal cortex insufficiency

- **arterial hypotension** (systolic pressure drops below 80 mmHg, dyastolic pressure drops below 50 mmHg) and **ECG changes** (flattened T, prolonged PQ and QT intervals), as a consequence of mineralocorticoid deficit.
- **digestive symptoms:** loss of appetite, nausea, vomiting, intestinal transit disorders, weight loss
- **urinary symptoms:** oliguria, late elimination of the water ingested during daytime (opsiuria)
- **neuromotor impairment:** muscular atrophy, mixed sensitive deficiencies
- **neuropsychiatric symptoms:** asthenia, adynamia, decreased reactivity, difficult or absent initiative, depression, anxiety
- **sexual dysfunction**:
  - In men: sexual asthenia, decreased spermatogenesis
  - In women: irregular menstrual periods, frigidity, diminished fertility

- **Paraclinic diagnosis:**
  a)USUAL investigations
  b)HORMONE tests
  c)Tests for the ETIOLOGICAL diagnosis

a) USUAL investigations

- ***Blood count*** shows, regardless of the type of insufficiency:
- normocytic anemia (rarely, macrocytic - 4% of cases)
- neutropenia
- lymphocytosis, eosinophillia
- **Blood glucose**: is decreased regardless of the type of insufficiency (decreased hepatic glycogenolysis)
- ***Serum ionogram:***
  - *primary insufficiency*: hypoNa^+ + hyperK^+, metabolic acidosis (Aldosterone deficit)
  - *secondary insufficiency*: hypoNa^+ + normal K^+ (reflects normal aldosterone secretion that does not depend on ACTH levels)

b) HORMONE tests:

1. UNIQUE determinations of serum cortisol at any time of the day

• CLINICAL value:
- cortisol levels < 100 nmol/L are highly suggestive of adrenal cortex insufficiency, but need to be confirmed by a rapid ACTH stimulation test
- cortisol levels > 550 nmol/L are interpreted as unlikely adrenal cortex insufficiency

2. Determination of basal plasma ACTH levels (7-10 am) on two or more occasions

• CLINICAL value:
- an increased value > 80 ng/L of ACTH, associated with a low serum cortisol level, is interpreted as **primary adrenal cortex deficiency** = Addison's disease
- a normal / low value < 10 ng/L of ACTH, associated with a low serum cortisol level, is interpreted as **secondary adrenal cortex insufficiency**

3. DYNAMIC tests

a. Rapid ACTH stimulation test $\Rightarrow$ plasmatic cortisol is measured before, at 30, respectively 60 minutes after the administration of synthetic ACTH (0,25 mg tetracosactide i.v. or i.m.).

- ***Interpretation*:**
- the test is normal if the cortisol level increases > 600 nmol/L and the adrenal cortex insufficiency is excluded
- the test is negative if the cortisol level remains < 400 nmol/L and is interpreted as a confirmed adrenal cortex insufficiency

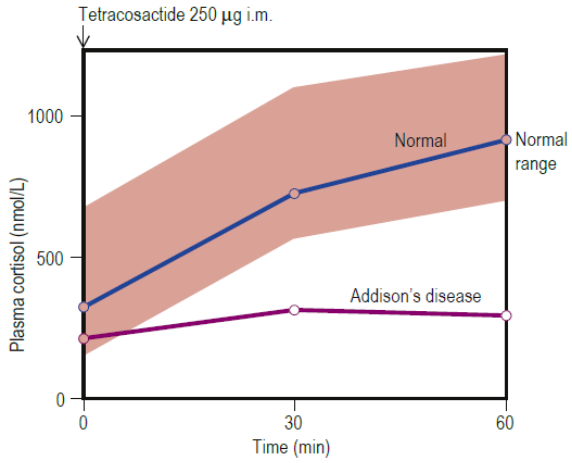

Figure 11.3. Rapid ACTH stimulation test shows a normal response in the healthy subject and a low response (negative test) in the patient with Addison's disease (*After Kumar and Clark, Clinical Medicine, 10th Anniversary Ed., 2021*)

CLINICAL value:
- the test has no clinical utility in the differential diagnosis between primary and secondary adrenal cortex insufficiency
- may, however, highlight two types of response in the context of confirmed secondary adrenal cortex deficiency:
  - an absent response (< 100 nmol/L) in **secondary CSR insufficiency with a prolonged total ACTH deficiency**, which causes **adrenal cortex atrophy** – they cannot respond by increasing cortisolemia, to a single dose of ACTH
  - **a present but inadequate response** ("borderline" stimulated cortisolemia = 400-600 nmol/L) in **secondary CSR insufficiency with partial ACTH deficiency**, which does not cause adrenal cortex atrophy – they may respond to single doses of ACTH, but will not be able to ensure an adequate increase in cortisolemia to ensure the body's adaptation to stress.

b. The insulin tolerance test:

- **Principle:** test that can be used in case of suspected secondary adrenal cortex insufficiency. It evaluates the response of the hypothalamic–pituitary– adrenal gland axis to hypoglycemia (< 40 mg/dL). Rapid Insulin (0.15 U/kg body) is administered and the serum cortisol is measured at 30, 45, 60, 90 and 120 minutes.
- **Interpretation:**
  - the test is normal if the stimulated cortisolemia increases ≥ 550 nmol/L and is interpreted as an adequate reserve of ACTH in the anterior pituitary gland, which excludes a secondary CSR deficiency
  - the test is negative if stimulated cortisolemia is < 550 nmol/L and is interpreted as an inadequate reserve of ACTH in the anterior pituitary gland, which corresponds to a secondary adrenal cortex insufficiency
- **CLINICAL value**: useful diagnostic test for secondary adrenal cortex insufficiency

3. Tests for the ETIOLOGICAL DIAGNOSIS

- **Abdominal CT** reveals:
  - Adrenal gland hypertrophy with/without calcification (in case of tumors, haemorrhages, infections eg. TB)
  - Adrenal gland atrophy (in autoimmune adrenal cortex insufficiency)
- **Immunological tests: Anti-adrenal cortex and anti-21 α-hydroxilase antibodies –** positive in autoimmune adrenal cortex insufficiency

The adrenal cortex insufficiency diagnosis algorithm is depicted in Figure 11.4.

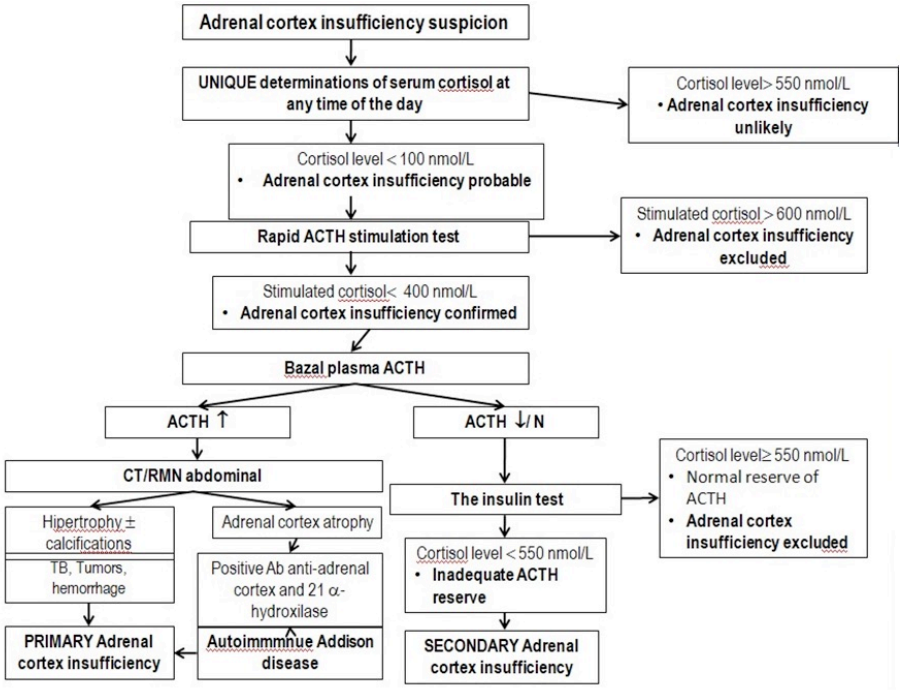

Figure 11.4. Diagnostic algorithm in chronic cortico-adrenal insufficiency.

II. INVESTIGATION OF THYROID GLAND DISORDERS

Normal thyroid gland physiology implies a normal function of the hypothalamic-pituitary-thyroid axis and a normal thyroid hormone synthesis with multiple effects on cellular metabolism and peripheral tissues.
Thyrocytes are responsible for thyroid hormone synthesis, ***T4 - thyroxine*** (97%) and ***T3 - triiodothyronine*** (3%), respectively.

A. HYPERTHYROIDISM

○ **Definition:**
- **Hyperthyroidism:** is the hyperfunction of the thyroid parenchyma defined by the excessive production of thyroid hormones
- **Thyrotoxicosis** is an excess of thyroid hormones at the receptor level

○ **Classification:**

a. PRIMARY hyperthyroidism:

- AUTOIMMUNE MECHANISM
  - *Graves-Basedow disease*
- AUTONOMOUS THYROID SECRETION
  - *Single toxic adenoma* (Solitary thyrotoxicosis)
  - *Toxic multinodular goiter* (Multinodular thyrotoxicosis)
- ACUTE INFLAMMATORY PROCESS (Transient thyrotoxicosis) - *Viral subacute thyroiditis* (De Quervain)

b. SECONDARY hyperthyroidism – *TSH-secreting pituitary adenoma*

• **Consequences of hormonal HYPERSECRETION:**

a) Symptoms of THYROTOXICOSIS

- nervous: irritability, restlessness, insomnia, fine tremors of the extremities
- heat intolerance: sweating, warm skin
- weight loss with preserved appetite, accelerated intestinal transit (diarrhea)
- palpitations

b) Signs of THYROTOXICOSIS

- **Goitre:**
  - diffuse, homogenous in Basedow-Graves and of soft consistency (on palpation) in viral subacute thyroiditis
  - a nodular - in the single toxic adenoma
  - a multinodular - in the toxic multinodular goiter
- uni-/bilateral **ophthalmopathy** or **thyroid orbitopathy** - in Graves-Basedow disease
- **CARDIOVASCULAR** manifestations:
  - Rhythm disorders: constant sinus tachycardia (100-130 BPM), supraventricular tachyarrhythmias (atrial flutter, atrial fibrillation, PAC)
  - Secondary hypertension

- **Paraclinical diagnosis comprises:**

a) USUAL investigations
b) HORMONE tests
c) OTHER investigations

a) USUAL investigations:

1. Blood count: microcytic anemia, moderate leukopenia
2. Blood glucose: hyperglycaemia (increased hepatic glycogenolysis)
3. Serum lipid profile: hypolipidemia with hypocholesterolemia (increased liver uptake of LDLc)
4. Bone alkaline phosphatase: increased (increased bone remodeling rate)
5. Calcemia: hypercalcemia (by bone demineralization)
6. Total serum proteins (proteinemia): hypoproteinemia (increased protein catabolism)
7. General inflammatory tests (CRP, ESR): positive in subacute viral thyroiditis

b) HORMONE tests:

1. Thyroid-stimulating hormone dosing (TSH):

- **NORMAL values: 0,3 - 3,5 mU/L**
- **CLINICAL value (Figure 11.5):**
  - is the **most sensitive test** of thyroid dysfunction, representing the **initial test** for the diagnosis of thyroid disease
  - even small changes in thyroid hormone concentration will determine opposite (and much more significant) changes of TSH concentration.
  - TSH is **suppressed (< 0.05 mU/L)** in all causes of **primary hyperthyroidism**, for its confirmation it is necessary to simultaneously determine the fractions FT4 and FT3 (Table 11.1)
  - Low TSH values, with FT4 and FT3 within normal limits, define **subclinical hyperthyroidism**
    - TSH is a **sensitive** and **specific** parameter, for the diagnosis/exclusion of **hypothalamic-pituitary-thyroid** axis disorders
  - Elevated TSH values, associated with elevated FT4 and FT3 values, define **secondary hyperthyroidism**
    - is a specific and sensitive parameter for the control of thyroid function, with clinical utility in treatment monitoring

Table 11.1. Differential diagnosis in hyperthyroidism

Causes	TSH	FT4	FT3
• Primary hyperthyroidism	suppressed	↑*	↑
• Subclinical hyperthyroidism	↓	N	N
• Secondary hyperthyroidism	↑	↑	↑

***FT4 is normal in T3 toxicosis**

2. Thyroid hormone dosing:

❑ FT4 (free T4, free thyroxine)

- **NORMAL values**: **10 - 25 pmol/L**
- **CLINICAL value**:
  - diagnosis - FT4 has elevated values in all forms of hyperthyroidism, except T3 toxicosis
  - FT4 dosing is also useful in thyroid supression therapy monitoring, because it has the advantage of being independent of the concentration and the

- binding properties of proteins that transport thyroxine, ***thus closely correlating with the clinical status of the patient.***

❑ FT3 (free T3, free triiodothyronine)

- **NORMAL values**: **3,5 – 7,5 nmol/L**
- **CLINICAL value**:
  - ***Identification of an isolated triiodothyronine (T3) secretion***, seen in about 10% of hyperthyroidism cases
  - ***Prognosis evaluation*** in patients with Graves-Basedow disease (an increased FT3 level before the beginning of therapy suggests a high rate of recurrence)
  - For ***recurrent diagnosis*** in patients with hyperthyroidism (FT3 increase can be an early sign)

c) OTHER investigations:

1. Thyroid Ultrasound/Doppler:

- quantifies diffuse hyperplasia and hypervascularization in Graves-Basedow disease
- highlights single or multiple thyroid adenomas

2. Thyroid scintigraphy (with I^{131}or Tc^{99}), allows the differential diagnosis of nodular and diffuse forms of goiter in hyperthyroidism.

Hypersecreting tissue areas will always have an **intense uptake ofI^{131}/Tc^{99}** („warm" areas).

- **In Graves-Basedow disease –** *diffuse intense uptake* (the whole thyroid has a " warm" aspect)
- **in toxic adenoma** – *single "warm" nodule with intense uptake surrounded by "cold"perinodular thyroid tissue with decreased uptake* (hormone synthesis at this level is decreased)
- **in multinodular toxic goitre** (multiple nodular hypersecreting areas) – *multiple "warm" nodules with increased uptake*, surrounded by*"cold", low-uptake tissue areas*
- **in subacute viral thyroiditis** – the uptake is minimal

3. ECG: sinus tachycardia, sometimes supraventricular tachyarrhythmias (atrial fibrillation, atrial flutter, premature atrial contractions)

4. Bone osteodensitometry (DEXA): osteopenia, osteoporosis

5. Ophthalmologic examination for the evaluation of thyroid ophthalmopathy in Basedow-Graves (exophthalmometer).

6. Immunological tests: assessement of auto-antibodies (autoAb) anti-TSH receptors (TRAB - TSH-R-Antibodies) that are increased in most patients with Graves-Basedow disease

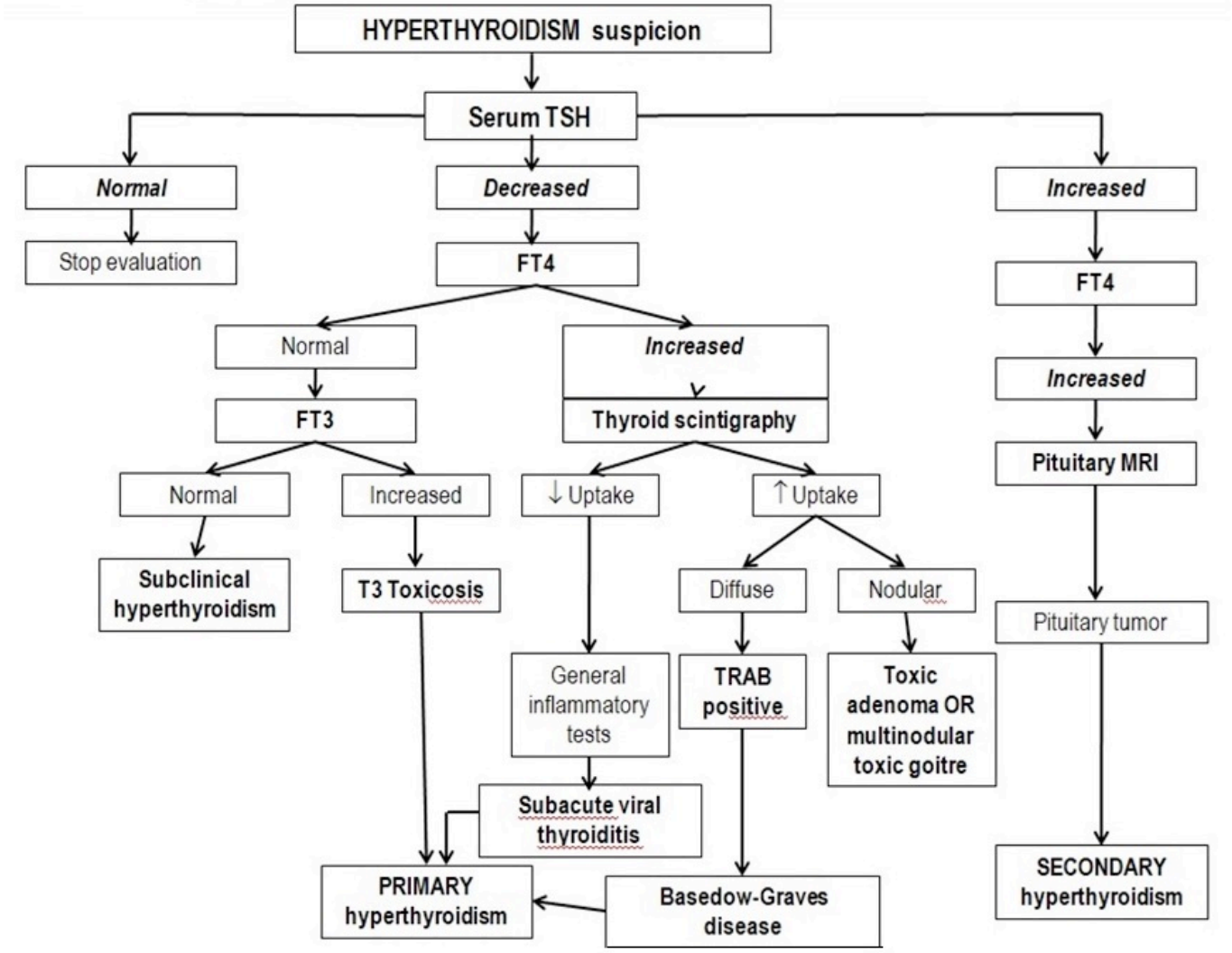

Figure 11.5. Diagnostic algorithm in HYPERTHYROIDISM.

B. HYPOTHYROIDISM

- **Definition:**
  - **Hypothyroidism =** the hypofunction of the thyroid parenchyma defined by inadequate secretion of thyroid hormones
  - **Myxedema=** is the clinical syndrome caused by a deficiency of thyroid hormones in peripheral tissues

- **Classification:**

a. PRIMARY hypothyroidism

o AUTOIMMUNE MECHANISM – chronic autoimmune thyroiditis (Hashimoto)

o THYROID ABLATION:

-postthyroidectomy,

- post irradiation with radioactive iodine

o HORMONAL SYNTHESIS DEFECTS – iodine deficiency, drugs (e.g., lithium, amiodarone)

b. SECONDARY hypothyroidism - pituitary

o Pituitary diseases with isolated TSH deficiency

c. TERTIARY hypothyroidism - hypothalamic

o Hypothalamic disorders with isolated TRH deficiency

d. Peripheral thyroid hormone resistance syndrome

- **Consequences of hormonal HYPORSECRETION:**
  - Can be of variable intensity, according to the severity of thyroid hormone deficiency.
  - **the clinical presentation of myxedema** is characterized by:
    - myxedematous generalised skin infiltrate – white, tough, painful, non-pitting edema
    - weight increase
    - goiter (iodine deficiency)
    - cold intolerance
    - rough, dry, cold skin with trophic changes
    - inexpressive, edematous facies
    - rough, scarce, dry, friable hair
    - bradylalia, bradypsychia
    - bradycardia
    - secondary hypertension
    - constipation

- **Paraclinical diagnosis:**

a) USUAL investigations
b) HORMONE tests
c) OTHER investigations

a) USUAL investigations

- **Complete blood count**:
  - normocytic anemia
  - macrocytic anemia (associated Addison-Biermer pernicious anemia)
  - microcytic anemia (ferritin deficiency, associated celiac disease)
- **Blood glucose**: decreased (decreased hepatic glycogenolysis)
- **Lipid profile**: hyperlipidemia with hypercholesterolemia (decreased liver LDLc uptake)
- **Serum proteins:** decreased (decrease of serum proteins sensitive to thyroid hormones, like fibronectin, osteocalcin, ferritin, angiotensin converting enzyme, etc.)
- **Enzymatic dosing:** AST, CPK, elevated (with muscular origin - myopathy)

b) HORMONE tests (Table 11.2)

1. Thyroid-stimulating hormone dosing (TSH):

• **CLINICAL value:** represents the **initial** and **most sensitive test** in the diagnosis of **hypothyroidism** (Figure 11.6):
- TSH is increased (>10 mU/L) in **primary hypothyroidism (thyroid)**
- TSH is low or lower than normal (<0.3 mU/L) in **secondary hypothyroidism (pituitary)** and **tertiary hypothyroidism (hypothalamic)**
- TSH is slightly elevated (5-10 mU/L), with FT4 and FT3 within normal limits in **subclinical hypothyroidism** (compensated euthyroidism)
- TSH is normal (0.3-3.5 mU/L), with increased FT4 and FT3, in **thyroid hormone resistance syndrome**

Table 11.2. Differential diagnosis in hypothyroidism

Causes	TSH	FT4	FT3
• **Primary hypothyroidism**	↑	↓	↓
• **Subclinical hypothyroidism** (compensated euthyroidism)	Slightly ↑	N	N
• Secondary and Tertiary hypothyroidism	↓/N	↓/N	↓/N
• Thyroid hormone resistance syndrome	N	↑	↑

2. Thyroid hormone dosing:

• **CLINICAL value:**
- diagnosis: FT4 and FT3 decrease in all forms of hypothyroidism
- assessment of the severity of primary hypothyroidism
- monitoring levothyroxine treatment to prevent overdose

3. DYNAMIC tests

- **TRH STIMULATION test**
- **Principle:** in subjects with basal TSH within normal limits, i.v TRH (protirelin) is administered and serum TSH is determined after 15, 30, 60 and 120 minutes.
- **Interpretation:**
  - a prompt increase (after 15-30 minutes) in TSH levels may signify primary hypothyroidism or peripheral thyroid hormone resistance syndrome
  - a lack of increased TSH levels means secondary hypothyroidism
  - a delayed increase (after 60-120 minutes) means a tertiary hypothyroidism
- **CLINICAL value:**
  - is occasionally used for the differential diagnosis of **secondary and tertiary hypothyroidism**

c) OTHER investigations:

1. Thyroid Ultrasound/Doppler: highlights an intense hypoechoic image of the thyroid (caused by autoimmune changes), coexisting with benign or malignant nodules in chronic autoimmune thyroiditis Hashimoto.

2. CT and/or thyroid MRI: in suspicion of malignancy of thyroid nodules from chronic autoimmune thyroiditis Hashimoto.

3. Immunological tests: Anti-thyroid antibodies: anti-thyroglobulin (anti-TG), anti-thyroid peroxidase (anti-TPO) – positive in chronic autoimmune thyroiditis (Hashimoto).

4. Brain MRI: useful in exploring the hypothalamic-pituitary axis in secondary or tertiary hypothyroidism.

5. ECG (in severe myxedema): sinus bradycardia, hypovoltage, flattened or negative T, prolongation of the PR interval (grade I AV block), QRS complex and QT interval.

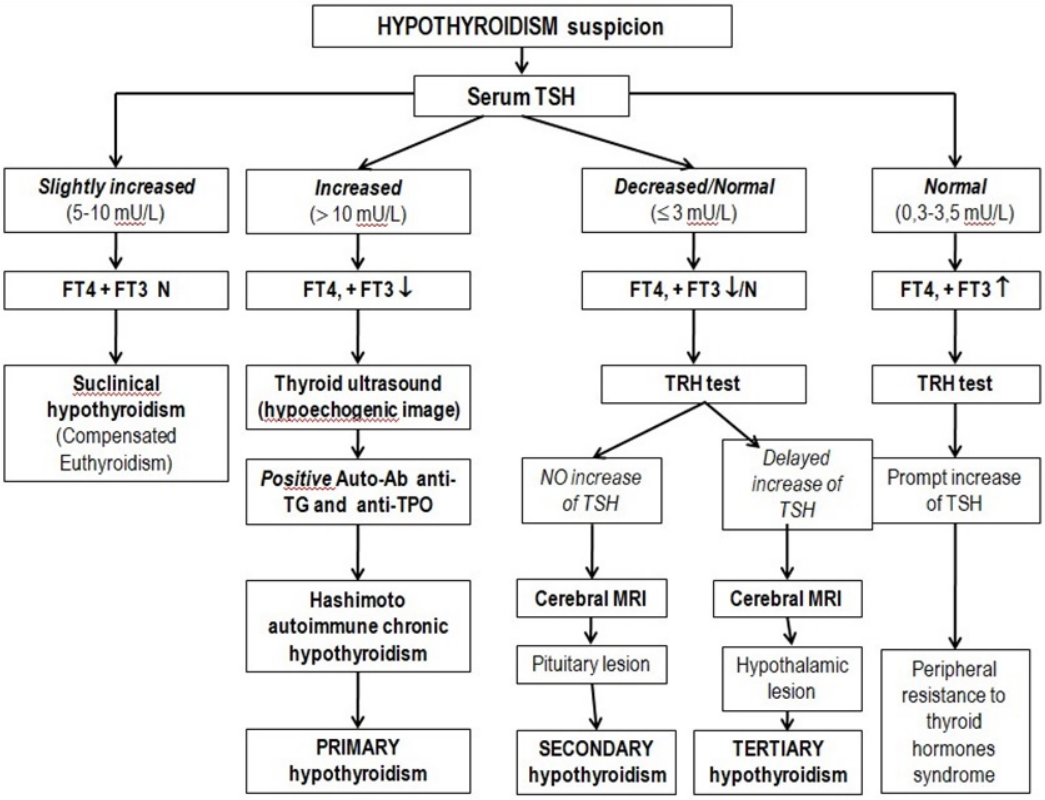

Figure 11.6. Diagnostic algorithm in HYPOTHYROIDISM.

CHECKPOINT!

1. Which of the following are screening tests for hypercortisolism?

A. Nocturnal salivary cortisol
B. Basal plasma ACTH
C. ACTH stimulation test
D. Nocturnal inhibition test with dexamethasone
E. High-dose dexamethasone inhibition test for 48 hours

***2. Which of the following statements about nocturnal salivary cortisol is true?**

A. It is affected by the secretion rate of saliva
B. It is a useful tool only for Cushing syndrome diagnosis
C. It is a useful tool only for monitoring the response to treatment
D. It is in balance with free cortisol in the blood
E. It is increased in pseudo-Cushing

***3. In which endocrine disorder are autoantibodies detected?**

A. Toxic multinodular goiter
B. Single toxic adenoma
C. Graves-Basedow disease
D. Cushing's disease
E. Cushing's syndrome

4. Which of the following changes are present in hyperthyroidism?

A. Moderate leukocytosis
B. Hyperlipidemia
C. Hypercholesterolemia
D. Increased bone alkaline phosphatase
E. Anemia

***5. What is the most likely diagnosis if TSH is low, FT3 and FT4 are increased, and thyroid scintigraphy reveals a diffuse uptake?**

A. Subclinical hyperthyroidism
B. Thyrotoxicosis factitia
C. Single toxic adenoma
D. Multinodular toxic goiter
E. Gaves-Basedow disease

6. Which of the following may occur in primary adrenal cortex insufficiency?

A. Hyperglycemia
B. Hypotension
C. Decreased basal plasma ACTH
D. Positive anti-CSR and anti-21 α-hydroxylase antibodies
E. A low (negative) response to the rapid ACTH stimulation test

7. Which tests are needed to determine the etiology of Cushing's syndrome?

A. Rapid stimulation test with ACTH
B. Abdominal ultrasound, CT and / or abdominal MRI for the diagnosis of Cushing's disease
C. Determination of basal ACTH plasma levels (7-10 am) on two or many occasions
D. Low-dose dexamethasone inhibition test for 48 hours
E. Selective Catheterization of the Inferior Petrosal Sinus (IPS) for the diagnosis pituitary tumors

***8. Which is the most likely diagnosis if serum TSH is slightly elevated (5-10 mU / L) and FT3 and FT4 are normal?**

A. Primary hypothyroidism
B. Subclinical hypothyroidism
C. Secondary hypothyroidism
D. Subclinical hyperthyroidism
E. Secondary hyperthyroidism

9. Which of the following statements about TSH dosing are correct?

A. Represents the initial test in the diagnosis of thyroid disease
B. It is increased in primary hyperthyroidism
C. It is low in secondary hyperthyroidism
D. It is increased in primary hypothyroidism
E. It is low in secondary hypothyroidism

10. Which of the following statements about pseudo-Cushing are correct?

A. Basal serum cortisol is elevated
B. Urinary free cortisol is increased
C. Nocturnal salivary cortisol is elevated
D. Occurs in morbid obesity
E. It is a functional hypercortisolism

CASE STUDIES

1. A 40-year-old male presents to the doctor's office for asthenia, marked muscle weakness, skin hyperpigmentation. The followingobservations were made during the clinical examination: melanodermia – especially obvious at the level of the white abdominal line, BP= 100/50 mmHg, HR= 90 b/min. The patient is referred to the endocrinology clinic, where the following evaluations are performed:

- Hb = 10.9 g/dL **(N.V: 15,5± 2g/dL)**, Ht = 34.1 % **(N.V: 42-52%)**, RBC = 3.82 x 10^6 /mm^3 **(N.V.: 4,2-5,9/mm^3)**, MCV = 89.1 fL **(N.V.: 80-100 fL)**, MCH = 27.3 pg **(N.V.:27-33 pg)**, MCHC = 33 g/dL **(N.V.:32-36 g/dL),**

WBC = 8,2x 10^9/L **(N.V.: 4-11)**, WBC formula: Ne= 1.28 x 10^9/L **(N.V.: 2 – 7,5),** Ly= 5,5 x 10^9/L **(N.V.: 1,5-4)**, Mo= 0,7x 10^9/L **(N.V.: 0,2-0,8),** Ba= 0,1 x 10^9/L **(N.V.: 0,01-0,1),** Eo=0,5x 10^9/L (**N.V.:0,04-0,4)**, Platelets = 210.000/mm^3 **(N.V.: 150.000-450.000/mm^3)**,

- Fasting blood glucose = 60 mg/dL **(N.V.:70-110 mg/dL)**
- ALT = 20 U/l **(N.V.:<30 U/l),** AST = 18 U/l **(N.V.:<40 U/l)**
- Creatinine = 0.84 mg/dL **(N.V.:0,8-1,3 mg/dL)**
- Total cholesterol = 110 mg/dL **(N.V.:<200 mg/dL),** LDLc = 65 mg/dL **(N.V.:<130 mg/dL)**
- Na^+ = 115 mmol/L **(N.V.:135-145 mmol/L),** K^+ = 6.25 mmol/L **(N.V.:3,5-5 mmol/L)**, Total Ca^{2+} = 9.5 mg/dL **(N.V.:8,5-10,5 mg/dL)**
- Alkaline phosphatase = 63 U/l **(N.V.:<120 U/l)**
- Morning ACTH = 650 pg/dL **(N.V: 10-80 nmol/L)**
- Plasma cortisol at 8 a.m.= 3 nmol/L **(N.V.: 172-497 nmol/L)**
- Free urinary cortisol = 2 nmol/24h **(N.V: 100-379/24 h)**
- TSH= 3 mu/l **(N.V. 0.3-3.5),** FT4 = 14pmol/L **(N.V.: 10-25)**

What diagnosis do you suspect? Provide arguments.
Which complementary investigations are required?

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2. A 51-year-old patient with prior thyroid disease (has been operated a month ago for a massive multinodular goitre that was extending in the mediastinum and associated thyrotoxicosis symptoms – subtotal thyroidectomy has been performed) presents to the doctor's office for: cold, rough, dry skin, asthenia, facial edema, constipation. The followinginvestigations are perfomed:

- Hb = 13.9 g/dL **(N.V: 14 ± 2 g/dL)**, Ht = 40.1 % **(N.V: 37-47%)**, RBC = 4,52 x 10^6/mm^3 **(N.V.: 3,7-5,5/mm^3),** MCV = 87.1 fL **(N.V.: 80-100 fL),** MCH = 26.7 pg **(N.V.:27-33 pg)**, MCHC = 32 g/dL **(N.V.:32-36 g/dL)**, WBC = 7,98x 10^9/L **(N.V.: 4-11)**, WBC formula: NE = 5 x 10^9/L **(N.V.: 2 – 7,5)**, LY = 3,9 x 10^9/L **(N.V.: 1,5-4)**, MO = 0,8 x 10^9/L **(N.V.: 0,2-0,8)**, BA= 0,1 x 10^9/L **(N.V.: 0,01-0,1)**, EO = 0,2 x 10^9/L **(N.V.: 0,04-0,4)**, Platelets = 261.000 /mm^3 **(N.V.: 150.000-450.000/mm^3)**
- Fasting blood glucose = 90 mg/dL **(N.V.:70-110 mg/dL)**
- Total cholesterol = 290 mg/dL **(N.V.:<200 mg/dL)**, LDLc = 175 mg/dL **(N.V.:<130 mg/dL)**, HDLc = 44 mg/dL **(N.V.: >50 mg/dL)**, TG = 200 mg/dL **(N.V.:<150 mg/dL)**
- Creatinine = 0.74 mg/dL **(N.V.: 0,6-1 mg/dL)**
- Alkaline phosphatase = 58 U/l **(N.V.:<120 U/l)**
- Na^+ = 141 mmol/l **(N.V.:135-145 mmol/L)**, K^+ = 4.3 mmol/l **(N.V.:3,5-5 mmol/L)**, Total Ca^{2+} = 8.1 mg/dL **(N.V.:8,5-10,5 mg/dL)**
- TSH = 11.5 mUI/l **(N.V.:0.3-3.5)**
- FT4 = 15.5 ng/mL **(N.V.: 10-25)**
- FT3 = 0,4 nmol/L **(N.V: 3.5-7.5)**

What diagnosis do you suspect? Provide arguments.
Which complementary investigations are required?

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NOTES

12. INVESTIGATION OF CALCIUM AND PHOSPHATE METABOLISM ABNORMALITIES

LEARNING OBJECTIVES

At the end of this chapter, students are expected to:

1. *Request and interpret the static and dynamic tests that assess the phosphate-calcium balance.*
2. *Request and interpret the usual and complementary investigations used for the diagnosis of metabolic bone disorders.*
3. *Know the main steps for the diagnosis of hypercalcemia.*

Know the main steps for the diagnosis of hypocalcemia

I. REGULATION OF CALCIUM AND PHOSPHATE BALANCE – Brief physiology overview

- **Definition:** calcium and phosphate balance is defined as *the abillity to maintain the serum concentration of these minerals in the normal range* as an interplay among their dietary intake, digestive absorption and renal excretion. The calcium-phosphate homeostasis is closely related with the skeletal metabolism, being regulated by four hormones: parathormone, calcitonin, vitamin D and fibroblast growth factor 23.

  a. Parathormone (PTH) - is produced by the chief cells of the **parathyroid glands** being mainly regulated by the serum calcium levels. PTH secretion is stimulated directly by *hypocalcemia* and indirectly by *hyperphosphatemia* (the excess of phosphate in the serum precipitates with calcium with the formation and tissue storage of insoluble calcium phosphate complexes and subsequent hypocalcemia).

  PTH induces **hypercalcemia** and **hypophosphatemia** by acting at 3 levels:

- ***at bone level:*** *increase in bone resorption by stimulating the bone osteoclastic activity*, followed by increase in calcium levels in the extracellular fluid (ECF)
- ***at renal level:***
  - *increase in calcium reabsorption and phosphate excretion*
  - *increase in synthesis of active vitamin D by stimulating 1 α-hydroxylase*
- ***at intestinal level:*** *increase in calcium absorption* - indirectly, by inducing the synthesis of active vitamin D in the kidney

b. Calcitonin - is produced by the *parafollicular C cells* of the **thyroid gland** under the influence of *hypercalcemia* or *hyperphosphatemia*; induces **hypocalcemia** and **hypophosphatemia** by acting:

- ***at bone level:*** decreased bone resorption by inhibiting the osteoclastic activity
- ***at renal level:*** decreased renal reabsorption of calcium and phosphate

c. Vitamin D - is a liposoluble vitamin that comes from foods of *animal* origin, under the form of *vitamin D_3* (cholecalciferol) or of *plant* origin, under the form of *vitamin D_2* (ergocalciferol). Vitamin D_3 is synthesized in the skin under the action of ultraviolet rays. Both vitamin D_2 and vitamin D_3 have a low biological activity and must be activated by **2 successive hydroxylation:**

- *the first hydroxylation* occurs in *liver* with the formation of ***25-hydroxy-cholecalciferol*** or ***calcidiol (25-OH-D)***
- *the second hydroxylation* (activated by PTH) is performed mainly in *kidneys* under the action of 1 α - hydroxylase, causing the formation of ***1,25 dihydroxy-cholecalciferol*** or ***calcitriol (1,25 $(OH)_2$-D)***

! Observation: *The reaction may occur in pathologically-infiltrated tissues in sarcoidosis or lymphomas with subsequent hypercalcemia.*

Vitamin D induces ***hypercalcemia*** and ***hyperphosphatemia*** through the following mechanisms:

- ***at intestinal level:*** *increases the absorption of calcium and phosphate*
- ***at bone level:*** the effects are dependent on the level of *serum calcium and 1,25 $(OH)_2$-D*:
  - if the level of calcium and 1,25$(OH)_2$-D are *normal*, *bone mineralization is stimulated*
  - if the level of *1,25$(OH)_2$-D is high* or the *calcium level is low, bone demineralization occurs.*

The most recent hormone of bone origin that has been systematically involved in the regulation of phosphatemia and vitamin D metabolism is:

d. Fibroblast growth factor 23 (FGF23) – an important regulator of serum phosphate concentration, along with PTH. It is secreted by *osteocytes* in response to hyperphosphataemia and acts on the proximal convoluted tubules where it *decreases phosphate reabsorption (independently of PTH) with phosphaturia*. It also *reduces the synthesis of 1,25 $(OH)_2$-D by inhibiting 1α-hydroxylase*.

In patients with **chronic kidney disease** (CKD), phosphate retention leads to *increased FGF-23 synthesis* with a consequent decrease in 1,25 $(OH)_2$-D concentration and impaired calcium absorption; hyperphosphatemia and hypocalcemia are responsible for the occurrence of *secondary hyperparathyroidism* in CKD.

II. INVESTIGATION OF CALCIUM AND PHOSPHATE ABNORMALITIES

II.1. STATIC INVESTIGATIONS

A. USUAL tests

1. TOTAL SERUM CALCIUM (CALCEMIA) and IONIZED serum calcium

- **Definition:** calcemia is the total concentration of calcium in the serum and comprises **3 fractions:**
  - *Ionized calcium (50%)* - free, *unbound* calcium; *the biologically active fraction* which participates in the negative feedback mechanism for regulating PTH secretion
  - *Calcium bound to plasma proteins,* mostly to albumins (45%)
  - *Calcium bound to anions* – e.g., calcium citrate, phosphate, carbonate, sulphate (5%)
- **Factors that INFLUENCE serum calcium:**

a) TOTAL serum calcium is influenced by the ALBUMINS level (mean value = 4 g/dL):

- ***hypoalbuminemia*** causes a transient ***decrease in total serum calcium (false hypocalcemia)*** due to a decrease of the protein-bound fraction, with *no effect on ionized calcium*
- ***hyperalbuminemia*** causes a transient ***increase of total serum calcium (false hypercalcemia)*** due to an increase of the protein-bound fraction, with *no effect on ionized calcium*

Observation!

Changes in albumin concentration require the determination of **corrected calcemia**, as follows:

$$Calcemia_{corrected}\ (mg/dL) = Measured\ calcemia\ (mg/dL) + [0{,}8\ (4 - Measured\ albuminemia\ (g/dL)]$$

b) IONIZED serum calcium depends on PLASMA pH:

- **an increased pH or alkalosis** causes a **decrease in ionized calcium**, due to protein-binding of calcium, with *no change in total serum calcium*
- **a decreased pH or acidosis** causes **an increase in ionized calcium**, due to the release of calcium from albumins, with *no change of total serum calcium*

- **NORMAL values**:
  - **Total serum calcium***:* **8,5-10,5 mg/dL** (2,2-2,6 mmol/L)
  - **Ionized calcium** = **1,1-1,3 mmol/L** (50% of total serum calcium)
- **PATHOLOGICAL values:**
  - **Hypercalcemia: total calcium > 10,5 mg/dL**
  - **Hypocalcemia: total calcium < 8,5 mg/dL**

① HYPERcalcemia

- **Causes:**

a) PTH HYPERsecretion in:

- ***Primary hyperparathyroidism***: *autonomous* hyperfunction of the parathyroid glands due to:
  - Parathyroid adenoma (85% of cases)
  - Parathyroid carcinoma or diffuse hyperplasia of the parathyroid glands (15% of cases)
- ***Paraneoplastic hyperparathyroidism:*** increased secretion of *PTH-like substances* ('PTH related protein') in various *malignant tumors* (bronchial, breast, kidney, pancreatic, liver)

b) Non–PTH–mediated hypercalcemia in:

- Osteolysis:
  - **Malignant hypercalcemia -** *bone metastases* in breast, pulmonary or kidney

cancer, multiple myeloma, lymphomas
  - **Hyperthyroidism**
- Increased intestinal absorption of calcium: in ***hypervitaminosis D***
- Increased renal reabsorption of calcium: in ***prolonged treatment with thiazides***

- **CLINICAL manifestations:**

✓ *Renal:* lithiasis, nephrocalcinosis
✓ *Cardiac:* shortening of the QT interval, sinus tachycardia, premature ventricular contractions
✓ *Digestive:* gastric ulcers, acute pancreatitis
✓ *Neurological:* fatigue, depression
✓ *Bone:* osteoporosis, bone lesions - cysts, brown tumors, pathological bone fractures

②Hypocalcemia

- **Causes:**

a) PRIMARY hypoparathyroidism: due to thyroidectomy, radiotherapy or autoimmune diseases

b) Pseudo-hypoparathyroidism (Albright hereditary osteo-dystrophy) – deficit in PTH recognition by peripheral receptors accompanied by increased PTH secretion

c) Non-PTH mediated hypocalcemia – *most frequent in **vitamin D deficiency***.

- **CLINICAL manifestations:**
  - tetany
  - osteomalacia in hypovitaminosis D
  - secondary hyperparathyroidism in pseudo-hypoparathyroidism and hypovitaminosis D

Tetany

Is a clinical and electrical syndrome of **neuro-muscular hyperexcitability** manifested as:

- *paresthesia* (of extremities, perioral)
- *carpal spasm* - flexion of the elbow, fist and metacarpophalangeal joints, with the extension of the interphalangeal joints and forced adduction of the thumb
- *pedal spasm* - forced adduction of the thigh and calf, with flexion of the foot and toes
- *Chvostek sign* - contraction of the upper lip, elicited by tapping at the middle of the line joining the earlobe with the labial commissure
- *Trousseau sign* - the appearance of the carpal spasm after the sphygmomanometer cuff is placed on the arm and inflated to an above systolic pressure value, maintained for at least 3 minutes
- *cramps* in the GI tract
- *spontaneous repetitive electrical activity* on the EMG

Remember!

Alkalosis precipitates the onset of a tetany crisis *by decreasing the level of the ionized calcium fraction, e.g., after bicarbonate administration, vomiting, hyperventilation.*

2. PHOSPHATEMIA

- **Definition:** the serum phosphates ($H_2PO_4^-$, HPO_4^{2-}) concentration; it represents 30% of the total phosphorus concentration in the blood (named phosphoremia).
- **NORMAL values:**
  - adults: **3-4,5 mg/dL** (0,8-1,4 mmol/L)
- **PATHOLOGICAL values:**
  - **Hyperphosphatemia > 4,5 mgd/L**
  - **Hypophosphatemia < 3 mg/dL**

① HYPERphosphatemia

- **Causes:**
  - **Primary hypoparathyroidism**
  - **Non PTH-mediated hyperphosphatemia in:**
    - ***hypervitaminosis D*** (increased renal reabsorption)
    - renal failure (decreased glomerular filtration)
    - release of phosphate from damaged tissues: osteolysis, rhabdomyolysis, tumor lysis syndrome
- **Clinical manifestations:** are those induced by hypocalcemia, to which the secondary metastatic calcifications (excess phosphate causes the formation of calcium phosphate which precipitates in the tissues) are added.

②Hypophosphatemia

- **Causes:**
  - **Primary hyperparathyroidism**
  - **Non PTH-mediated hypophosphatemia** in:
    - ***hypovitaminosis D*** (decreased renal reabsorption)
    - increased urinary elimination: dysfunction of the proximal convoluted tubules
    - intracellular migration: insulin administration in patients with diabetic ketoacidosis
- **Clinical manifestations:** muscle weakness, bone demineralisation

3. CALCIURIA

Definition: calcium level measured in 24 hour-urine.

- **NORMAL values: 100-300 mg/day**
- **PATHOLOGICAL values:**
  ①**Hypercalciuria** - may appear in:
  - primary hyperparathyroidism
  - paraneoplastic hyperparathyroidism
  - hypervitaminosis D
  - malignant hypercalcemia
  - sarcoidosis
  - multiple myeloma

  ②**Hypocalciuria** – may appear in:
  - hypovitaminosis D
  - prolonged treatment with thiazide diuretics

4. PHOSPHATURIA

- **Definition:** phosphate level measured in 24 hour-urine.
- **NORMAL values**:
  - adults**: 400-800 mg/day**
- **PATHOLOGICAL values**:
  ①**Hyperphosphaturia** – may appear in:
  - primary hyperparathyroidism
  - decreased levels of vitamin D

  ②**Hypophosphaturia** – may appear in:
  - primary hypoparathyroidism
  - pseudohypoparathyroidism
  - increased levels of vitamin D

B. COMPLEMENTARY tests

1. Serum PTH

- **NORMAL Values: 10-65 ng/L**
- **CLINICAL value:** is useful in the diagnosis of parathyroid gland diseases or other pathologies that impair the calcium-phosphate balance.

2. Vitamin D metabolites

a) Serum 25-hydroxy vitamin D (calcidiol)

- **NORMAL values: 30-80 ng/mL**
- **PATHOLOGICAL variations:**
  - **Insufficient level: 21-29 ng/mL**
  - **Deficiency: ≤ 20 ng/mL**
- **CLINICAL value**: represents the **main laboratory test** that assesses in clinical practice the **vitamin D reserve** of the body, being useful in:
  - the *diagnosis of decreased serum vitamin D* due to: insufficient sun exposure, inadequate intake/absorption, impaired renal activation. The following conditions are encountered in practice:
    - *mild or moderate deficiency* - associated with osteoporosis and secondary hyperparathyroidism
    - *severe deficiency* - associated with osteomalacia and primary hyperparathyroidism
  - differential diagnosis of the causes for *rickets and osteomalacia* (Table 12.1)
  - monitoring of vitamin D therapy
  - diagnosis of hypervitaminosis D

b) Serum 1,25 $(OH)_2$-D (calcitriol)

- **Normal values**: **20-75 ng/mL**
- **CLINICAL value:** ***complementary lab test*** that evaluates vitamin D status (Table 12.1) and is useful:
  - in the diagnosis of hypovitaminosis D in patients with renal diseases
  - to differentiate hypercalcemia due to hyperparathyroidism from vitamin D intoxication

Table 12.1. Clinical value of abnormal vitamin D metabolites in the serum.

Cause	25 (OH)D	1,25 $(OH)_2$D
Exogenous hypovitaminosis D (insufficient intake or sun exposure)	↓	↓
Endogenous hypovitaminosis D (chronic renal failure, primary hypoparathyroidism)	↑	↓
Exogenous hypervitaminosis D (vitamin D intoxication)	↑	↑
Endogenous hypervitaminosis D (primary hyperparathyroidism)	↓	↑

3. Urinary cyclic adenosine monophosphate (cAMP)

- **CLINICAL value:** urinary cAMP consists of the *filtered and unreabsorbed cAMP* plus the *cAMP which is secreted by the renal tubular cells* in the presence of PTH. Its measurement is useful for the diagnosis of ***paraneoplastic hypercalcemia*** - tumors are able to release PTH-like peptides (that stimulate the renal cAMP secretion). In these

conditions the serum PTH is normal or decreased whereas the urinary cAMP increases.

II.2. DYNAMIC INVESTIGATION

1. Cortisol test

- **Principle:** administration of 40 mg of cortisol (decreases hepatic vitamin D synthesis) 3 times a day for 10 days, after which the doses are progressively decreased over a period of 5 days. The decrease in serum calcium is assessed at 5, 8 and 10 days after cortisol administration.
- **Interpretation:** the test is considered positive if there is a decrease in serum calcium by > 1.0 mg/dL, signifying hypervitaminosis D

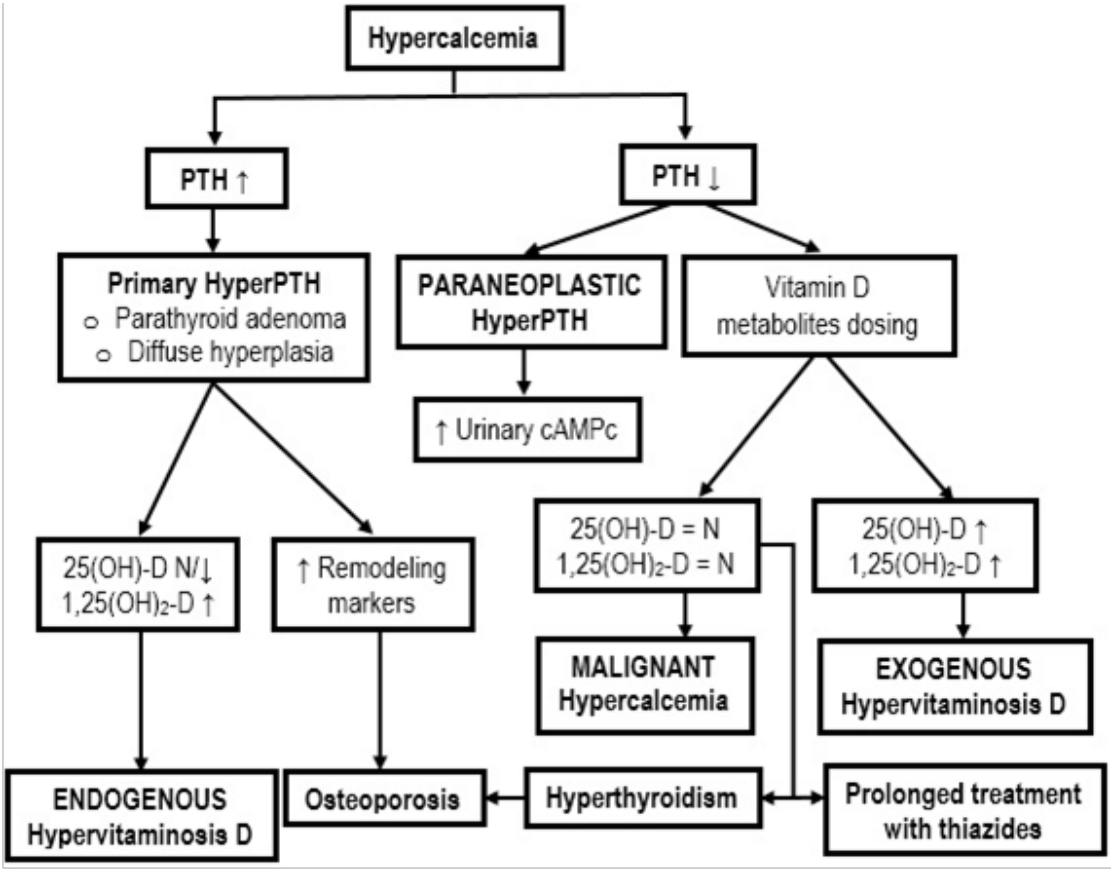

Figure 12.1. Diagnostic algorithm of HYPERCALCEMIA.

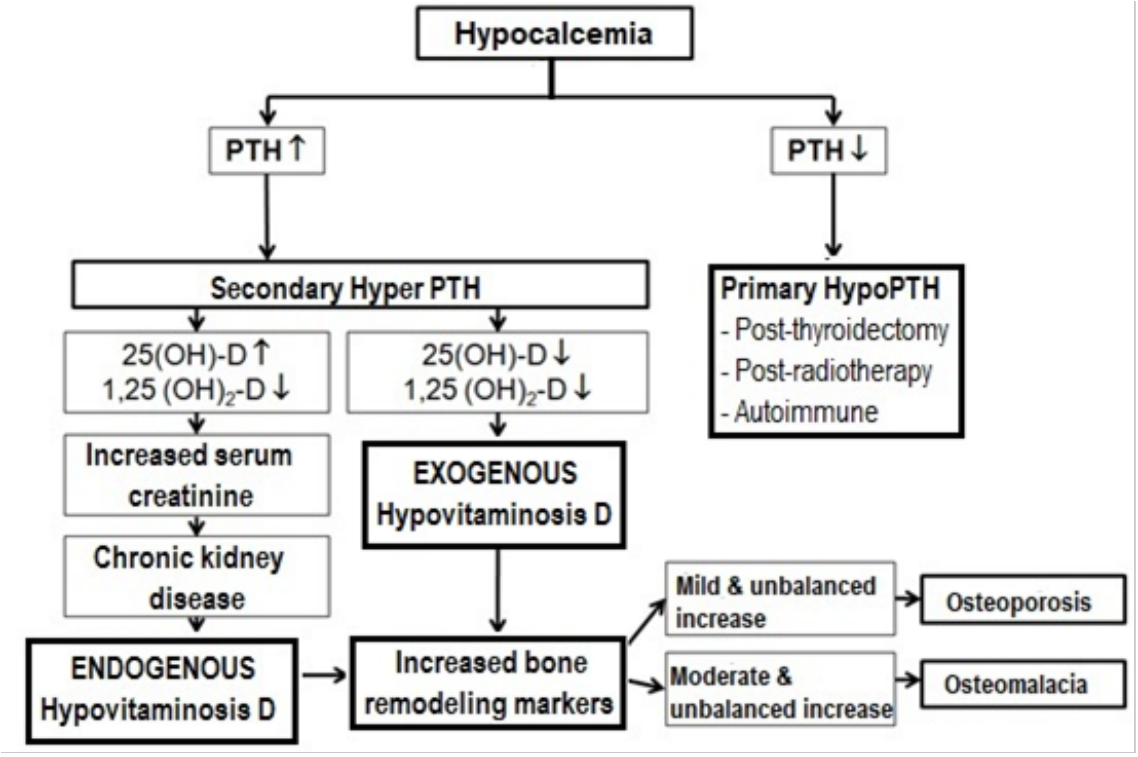

Figure 12.2. Diagnostic algorithm of HYPOCALCEMIA.

II.3. BONE REMODELING MARKERS

Bone remodeling reflects the permanent replacement of the old bone with newly formed one. It begins around the age of 20-30 years and has *two main phases*:

- **bone RESORPTION**, with increased **osteoclastic activity**. Its main role is to reabsorb the old bone.
- **bone FORMATION**, with increased **osteoblastic activity**, is responsible for the synthesis of new bone.

The two phases are *linked* in order to ensure that old bone resorption is always followed by new bone formation. The speed by which the two phases follow one another is called ***bone remodeling rate or bone turnover.*** The efficacy of this phenomenon is reflected by the **preservation of bone mass and bone microstructure** and defines the **bone remodeling equilibrium.**

The bone metabolic disturbances are caused by alterations of the *rate and equilibrium of bone remodeling* and comprise:

- osteoporosis
- osteomalacia
- Paget's Disease

- **Osteoporosis** is the most frequent metabolic disturbance of the bone, characterized by ***loss of bone mass*** and ***bone microstructure deterioration***, that leads to a decrease in bone resistance and *increased risk for fractures*. Bone mass is maximum at the age of 20-30 years. After the age of 30-40 years, the bone mass decreases *slowly*, due to an imbalance of bone remodeling, that shifts the metabolic processes towards the resorptive phase. This disturbance will worsen with age and leads to a *rapid* loss of bone mass with increased bone turnover, because bone resorption cannot be compensated adequately through bone formation.

Clinical forms:

- **primary:**
  - *after menopause*, caused by a decrease of estrogen levels
  - *age-related (in the elderly),* caused by a decrease in the proliferative and secretory capacity of osteoblasts, together with calcium and vitamin D deficiencies (responsible for secondary hyperparathyroidism)
- **secondary**: caused by diseases that accelerate bone turnover:
  - *endocrine diseases*: hyperparathyroidism, hyperthyroidism, Cushing syndrome, acromegaly
  - *malignant tumors and metastases*
  - *drug-induced* (> 3 months): corticotherapy, excess of thyroid hormones, chemotherapy, anti-seizure medication.

- **Osteomalacia** is a metabolic disorder of the bone caused by the hypocalcemia and hypophosphatemia that result from ***vitamin D deficiency***. It can lead to ***inadequate mineralization of the newly formed bone*** together with ***deformities of the long bones*** (mainly). Secondary hyperparathyroidism is responsible for the acceleration of bone turnover with associated osteoporosis.
- **Paget disease** is a metabolic disorder of the bone, in which the causes are yet unknown (an infection of the osteoclasts caused by the Paramyxovirus has been presumed) that causes ***excessive bone remodeling***. The increased bone resorption is *linked* to bone formation, but the entire bone remodeling process develops chaotically, with multiple areas of remodeling that find themselves in different phases of the process. The severe alteration of the bone's microstructure leads to ***size, form and density abnormalities***.

1. Markers of bone FORMATION (osteoblastic activity)

Are secreted by the osteoblasts, and can be measured in the **SERUM**:

a. Bone alkaline phosphatase is an isoform of the alkaline phosphatase (ALP) that ensures the *mineralization of the newly formed bone (osteoid)*, via the lysis of pyrophosphate (an inhibitor of bone mineralization)

b. Osteocalcin is a non-collagen protein found in the structure of the osteoid, produced exclusively by *osteoblasts* and with a role in *osteoid mineralization* (because it represents the binding site for hydroxyapatite crystals in the bone matrix).

- **PHYSIOLOGICAL variations:**
  - serum levels depend on the *age* (increased in rapid growth periods in children) and the *gender* of the patient (increased in men)
  - a *physiological increase* can be seen during fracture healing processes and in postmenopausal women.
- **PATHOLOGICAL variations**:
  - increased levels can be found in association with **accelerated bone turnover** in: primary and secondary osteoporosis, osteomalacia, Paget's disease
  - decreased levels can be found in alterations associated with *low bone turnover*: hypoparathyroidism, hypothyroidism, growth hormone deficiency
- **CLINICAL value:**
  - ***usual tests*** for the diagnosis and severity assessment of metabolic disorders of the bone associated with *accelerated bone turnover*
  - for the evaluation of therapy efficiency in **osteoporosis** and **Paget's disease** - the treatment is considered efficient if the first evaluation after three months shows a decrease in serum bone alkaline phosphatase of at least 25%.

Observation!

Serum levels of bone ALP have a *superior clinical value* as compared to the total ALP. However, bone ALP is not used in primary osteoporosis screening, since its values remain unchanged in the early stages of the disease.

Experimental research from the last 2 decades on murine (mouse) models has shown that osteocalcin, released into the circulation, exerts pleiotropic effects, being involved in the control of insulin secretion, in male fertility (testosterone secretion), in adaptation to effort and recently, in cancer prevention.

2. Markers of bone RESORPTION (osteoclastic activity)

Represent degradation products of the osteoid (90% type I collagen) that can be measured in the **24 h URINE**:

a. Urinary hydroxyproline – results from collagen degradation and is the main component of type I collagen, with an important role in maintaining the triple helix structure

b. Urinary N-telopeptide (NTx) – also results from collagen degradation and is the N-terminal fragment of type I collagen, with the role of binding to the C-terminal fragment of a neighboring collagen molecule (Figure 12.3)

- **PHYSIOLOGICAL variations:** *increased* values can be seen in children during growth spurts and during the healing process of fractures. The values are *mildly* increased and *correlated* with the bone formation markers and have *no diagnostic value*.

- **PATHOLOGICAL variations:** increased values can be seen in metabolic diseases of the bone, reflecting a high bone turnover. Low values are associated with low bone turnover.

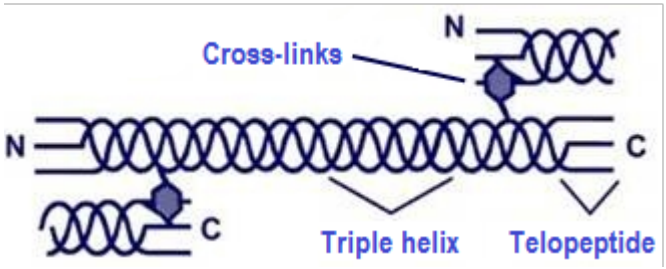

Figure 12.3. Type I collagen molecule: triple helix structure and the crossed links at the N and C-terminal fragments (*Adapted after emedicine.medscape.com*)

In order for these markers to have clinical value, the increased levels must be evaluated by comparison with those of the osteoblastic activity markers (Table 12.2):

- ***osteoporosis:*** *mild increase (up to 1-2 times the normal value) and unbalanced, with the predominance of bone resorption*
- ***osteomalacia:*** *moderate increase (up to 2-4 times the normal value) and unbalanced, with the predominance of bone resorption*
- ***Paget's disease****: severe increase (up to 10-25 times the normal value), but balanced in correlation with the markers of osteoblastic activity*

- **CLINICAL value:**
  - **diagnostic:**
    - ***complementary*** tests for the diagnosis of diseases associated with a *high bone turnover*
    - useful in the *differential* diagnosis of *osteoporosis* from *osteomalacia*
    - indicate the *presence of bone metastasis in breast and prostate cancer*, even in the absence of radiological proof
  - **therapeutic:**
    - monitoring the antiresorptive treatment response in *osteoporosis* and *Paget's disease* – the treatment is efficient if after the first monitoring done at 3 months, the blood level of NTx drops by at least 50% as compared to the base level.

Table 12.2. Bone remodeling markers' increase in METABOLIC BONE DISEASES

Metabolic bone disease	Bone formation markers	Bone resorption markers
Osteoporosis	N /↑	↑
Osteomalacia	↑	↑↑
Paget's disease	↑↑↑	↑↑↑

Note: ↑ = 1-2 x increase, ↑↑ = 2-4 x increase, ↑↑↑ = 10-25 x increase

II.4. OTHER INVESTIGATIONS

1. Electromyogram (EMG)

- **Definition:** represents the ***gold standard diagnostic method in tetany.*** Consists of a recording of the global electrical activity of the muscle in the second interosseous space of the hand. The EMG recording (with a surface or a needle electrode) has 3 steps:
  - *Muscle relaxation*
  - *Triggering of tetany via provoked ischemia over 10 minutes* (sphygmomanometer cuff is placed on the arm and is inflated to a pressure above the systolic pressure) followed by 5 minutes of postischemic rest
  - *Triggering of tetany via voluntary hyperventilation for 5 minutes* (respiratory alkalosis lowers the ionized calcium fraction)
- **Interpretation:** the characteristic electrical change in tetany is the *spontaneous repetitive activity* as seen in the form of patterns of bursts: double, triple or multiple biphasic electrical discharges (Figure 12.4):
  - If the electrical activity is seen at rest – ***manifest tetany***
  - If the electrical activity is seen during or after provoked ischemia or hyperventilation – ***latent tetany***

! Remember

Spasmophilia or **normocalcemic** tetany is a

particular type of ***neuro-muscular hyperexcitability***, with symptoms similar to tetany, triggered by emotions, exertion, fatigue. *The parameters of phosphate and calcium balance and parathyroid function are within normal limits*, but the **EMG shows spontaneous electrical activity**

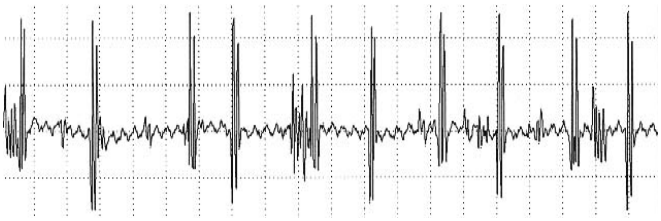

Figure 12.4. Double and multiple muscular bioelectrical discharges seen in TETANY.

2. Electrocardiogram (ECG)

ECG may show alterations determined by hypocalcemia (long QT interval) or hypercalcemia (short QT interval).

3. Imaging tests

Are used for localization of parathyroid adenomas (high-resolution ultrasound of the cervical region, scintigraphy with Tc99m Sestamibi, computed tomography, magnetic resonance imaging.

4. Bone densitometry

- **Principle:** consists of the tissue absorption of two photonic waves generated by an X-ray source. The difference in absorption between the two waves approximates the mineral bone density over a surface unit (g/cm^2)
- **CLINICAL value:** currently represents the ***investigation of choice*** for the ***diagnosis of osteoporosis*** and the assessment of the ***fracture risk***, respectively, based on the evaluation of the **bone mineral density (BMD).** The *„gold standard"* used in current medical practice is represented by **Dual-Energy X-ray Absorptiometry test**, also known as the **DEXA test.**

DEXA test allows the evaluation of **3 parameters:**

a) BMD (g/cm^2) at the level of the *lumbar spine/hip:*

- **A low BMD** even at a single evaluation, regardless of the localization, **is of diagnostic value for osteoporosis**, even if this disease is asymptomatic at the moment of diagnosis
- BMD values are decreased in other diseases with high bone turnover: osteomalacia, multiple myeloma etc.

b) T score – the standard deviation from the mean value of BMD corresponding to the healthy young adult of the same gender and race as the patient under examination. The T score is currently used for the ***screening of osteoporosis*** (Table 12.3) in high risk patients:

- Postmenopausal women
- Old patients, regardless of gender
- Patients with a personal history of fractures, especially if these occurred after minimal trauma
- Patients with a family history of severe osteoporosis
- Patients at risk for secondary osteoporosis

Table 12.3. T score interpretation.

Value	Interpretation
Between –1 and 1	No risk for osteoporosis
Between –1 and – 2,5	Osteopenia
- 2,5 and lower	Osteoporosis

Observation!

The T score is less reliable as compared to the bone remodeling markers for the monitoring of osteoporosis antiresorptive treatment. An increase in BMD can be seen only after 1-2 years of therapy, while a significant drop in bone remodeling markers occurs even after 3-6 months.

c) Z score – the standard deviation from the mean BMD value corresponding to a subject of the same gender, race and age as the patient under examination. The Z score is used in the evaluation of the ***fracture risk*** according to the following formula: **fracture risk = $2^{Z\ score}$.** If a patient has a Z score = -2 the fracture risk will be of $2^2 = 4$ (4 x greater than the average for other people of the same age, gender and race). A score lower than -2 usually indicates a secondary cause of osteoporosis.

CHECKPOINT!

***1. Which of the following is a cause of hypocalcemia?**

A. Primary hyperparathyroidism
B. Long-term treatment with thyazides
C. Paraneoplasic hyperparathyroidism
D. Vitamin D deficiency
E. Bone metastasis

***2. All of the following are causes of HYPERPHOSPHATHEMIA, EXCEPT for:**

A. Renal impairment
B. Primary hypoparathyroidism
C. Rhabdomyolysis
D. Hypovitaminosis D
E. Tumor lysis syndrome

***3. Which of the following is true about fibroblast growth factor 23 (FGF-23):**

A. It is secreted in response to hypophosphatemia
B. Increases the synthesis of the active form of vitamin D
C. Increases renal phosphate reabsorption
D. It is responsible for the occurrence of secondary hyperparathyroidism in CKD
E. Activates 1 α-hydroxylase

4. Which of the following are the markers of bone resorption?

A. Bone alkaline phosphatase
B. Hydroxyproline
C. Osteocalcin
D. Calcitriol
E. N-telopeptide

***5. How do you interpret a T score = -3?**

A. No risk of osteoporosis
B. At risk of osteoporosis
C. Osteoporosis
D. Osteopenia
E. Osteomalacia

6. Which of the following are true about the usual tests used for the investigation of the calcium and phosphate disturbances?

A. Total calcemia must be corrected to the serum albumin level if the latter is modified
B. Total calcemia is increased in secondary hyperparathyroidism
C. Ionized serum calcium depends on blood pH
D. Alkalosis increases the serum ionized calcium level
E. Phosphatemia is increased in primary hypoparathyroidism

7. Which of the following are true about bone alkaline phosphatase?

A. Is an isoform of alkaline phosphatase
B. Is a non-collagen protein in the bone structure
C. Is a marker of osteoblastic activity
D. Is a marker of osteoclastic activity
E. Raised levels can be seen in the early stages of primary osteoporosis

8. Which of the following are responsible for low 25 (OH)-D levels?

A. Low vitamin D intake
B. Insufficient sun exposure
C. Primary hyperparathyroidism
D. Chronic kidney disease
E. Primary hypoparathyroidism

9. Which of the following investigations are used in the case of hypocalcemia?

A. Electromyogram
B. Cortisone test
C. Electrocardiogram
D. ACTH test
E. Urinary cAMP

10. Which of the following are true about bone densitometry?

A. It is the test of choice for the diagnosis of osteomalacia
B. Low BMD at a single evaluation is diagnostic for osteoporosis
C. The T score is a screening parameter for the diagnosis of osteoporosis
D. The T score is superior to the bone remodeling markers in the monitoring of bone antiresorptive treatment for osteoporosis
E. The Z score is used for fracture risk evaluation in patients with osteoporosis

CASE STUDIES

1. A-48-year-old female presents to the doctor's office for bone pain and muscular fatigue.
The following lab test are available:

- calcemia = 13,96 mg/dL
- calciuria = 450 mg/day
- phosphatemia =1,63 mg/dL
- phosphaturia = 1000 mg/day

Which is the most likely diagnosis? Which investigations are necessary for a final diagnosis?

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2. A-19-year-old female patient presents for a check-up due to a feeling of tightness in her throat, palpitations, sleep disorders. The Chvostek sign is positive. The ECG shows a prolonged QT interval.
The following lab tests are available:

- calcemia = 6,9 mg/dL
- calciuria = 80 mg/day
- phosphatemia = 2 mg/dL
- phosphaturia = 880 mg/day

Which is the most likely diagnosis? Which investigations are necessary for a final diagnosis?

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NOTES

13. INVESTIGATION OF THE BODY'S RESPONSE TO STRESS. THE BURNOUT SYNDROME

LEARNING OBJECTIVES

At the end of this lab, students are expected to:

1. *Define the types of stress and list the effects of acute and chronic stress*
2. *Present the characteristics of the local and general adaptation syndrome to stress*
3. *Evaluate the stressors in the case of medical students using the MSSQ test*
4. *Know and interpret the result of the MBI test used to assess the burnout syndrome in doctors*

I. THE BODY'S RESPONSE TO STRESS

- **Definition:** stress represents a *general, non-specific reaction of the body* triggered by the activation of *neuroendocrine and immune control systems* under the action of various stress factors or *stressors* (physical, chemical, biological, and psychological).
- **The hormones** and neurotransmitters released during the stress response have the role of redirecting the body's energy by enhancing cardiovascular and metabolic activity and reducing the activity of other systems that are not immediately necessary to ensure proper management of the incriminated stressor. Stress hormones and their physiological effects are presented in Table 13.1.

Table 13.1. Hormones involved in the stress response

Hormone	Effects
Catecholamines	○ ↓ insulin secretion and ↑ glucagon secretion ○ ↑ inotropism, heart rate ○ Vasoconstriction ○ Bronchodilation
Cortisol (secreted under the influence of CRH - ACTH)	○ Enhances the effect of catecholamines ○ Inhibits TSH release / action ○ Suppresses the immune response
Aldosterone	○ ↑ renal reabsorption of sodium
ADH (antidiuretic hormone, vasopressin)	○ ↑ renal reabsorption of water ○ Vasoconstriction ○ Stimulates ACTH release

- **Classification:**
  - **According to the *origin* of stressors:**
    - *endogenous stress*
    - *exogenous stress*
  - **According to the *nature* of stress:**
    - *somatic stress* - induced by physical, chemical, biological agents
    - *psychogenic stress* - induced by psychological, social and cultural factors
  - **According to the *duration* of stress:**
    - *acute stress*
    - *chronic stress (intermittent or sustained)*
  - **According to its *consequences* on the body:**
    - *eustress* – elicits *positive* consequences on the body (adaptation) if the stress is *acute*, *low/moderate and of short duration, under controllable conditions*
    - *distress* – elicits *negative* consequences on the body (the occurrence of the disease) if the stress is *chronic*, *intense and prolonged, under uncontrollable conditions*

- **Stress-induced modifications** have been systematically studied by **Hans Selye** (1907-1982, recognized as the "father of stress") and include:

- two **stress adaptation syndromes:** local and general
- a **triad of manifestations:** *hypertrophy of the adrenal glands' cortex, atrophy of the thymus and lymphoid structures and gastroduodenal hemorrhagic ulcers* associated with the **general adaptation syndrome to stress (GAS).**

These manifestations were induced by **elevated cortisol levels** and were present in **all types of <u>chronic</u> stress** to which the experimental animals were subjected.

A. The LOCAL adaptation syndrome to stress (LAS)

- **Characteristics:**

– takes place on a *short-term* basis
– is limited to the *site of action* of the stressor
– represents a *non-specific local defense reaction* manifested in the form of an *acute inflammatory response* characterized by the 5 classic signs: *redness (rubor), heat (calor), edema (tumor), pain (dolor) and functional impotence (functio laesa)*.

B. The GENERAL adaptation syndrome to stress (GAS)

- **Characteristics:**

– takes place on a *long-term* basis
– involves the *whole body* through the **sympathetic vegetative nervous system (SNVS)** and the **hypothalamic-pituitary-adrenal axis (HPA)**
– includes *3 stages: alarm, resistance and exhaustion* (the exhaustion stage is not mandatory)
– ensures the *restoration of homeostasis* (resistance stage) or causes its *progressive alteration*, with the *appearance / aggravation of the disease or even death* (exhaustion stage).

1) The ALARM stage

– begins with the action of the stressor which activates the *SNVS* and the *HPA axis*
– the subsequent changes induced by the high levels of *catecholamines* and *cortisol* are responsible for the *"fight or flight"* reaction
– the resistance of the body to the action of the stressor is *low*
– the changes in homeostasis induced by the stressor *tend to exceed normal limits*

! Observation: The *"fight or flight" reaction* (or *acute stress syndrome*) was described in 1920 by the great American physiologist Walter Cannon who demonstrated the major role of the sympathetic system/adrenal medulla in orchestrating the response to acute stress, being included later as the first stage of the GAS.

2) The RESISTANCE stage

– in which the activity of the *SNVS and HPA axis return to normal* with the normalization of the plasma levels of catecholamines and cortisol and the disappearance of the *"fight or flight"* reaction, respectively.
– the resistance of the body to the action of the stressor is *high*
– the body has selected the most *effective* and *economical ways of ADAPTING to stress* and has restored homeostasis, but mobilized the energy resources of the whole body

3) The EXHAUSTION stage

– persistence of the stress stimulus leading to a new increase in *cortisol* secretion
– the appearance of the *triad of cortisol-induced manifestations* (adrenal hypertrophy, thymus and lymphoid structures' atrophy, gastroduodenal ulcerations)
– the resistance of the body to the action of the stressor is *collapsed*
– the energetic resources of the body are *exhausted*
– systemic signs of functional impairment (*"wear and tear"*) occur and trigger the onset/worsening of stress disorders (see below) or even death.

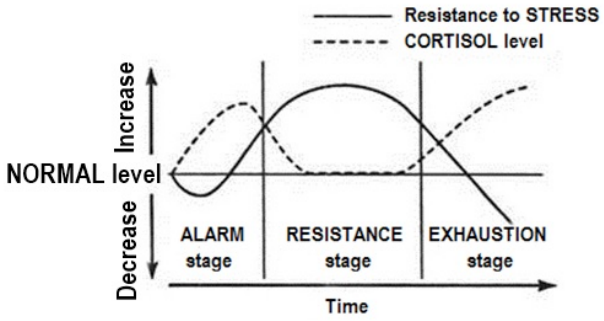

Figure 13.1. Stages of GAS to stress.
(Modified after http://deansomerset.com/recovery-adaptation-missing-piece-training-programs/)

• The effects of stress

A. The effects of ACUTE STRESS

- **Characteristics:** it is associated with the manifestations caused by the *excessive activation of the SNVS* and the increase of catecholamines within the *"fight or flight" reaction* (GAS alarm stage)
- **Consequences:**

– *in healthy subjects*: induces *positive effects* (eustress) if the stressor is not severe
– *in subjects with chronic diseases*: induces *negative effects* (distress) consisting in the onset of *acute events* (e.g., myocardial infarction in patients with coronary heart disease) or in the *decompensation*

of chronic diseases (e.g., marked increase in blood sugar in patients with diabetes mellitus, decompensation of heart failure).

B. The effects of CHRONIC STRESS

- **Characteristics:** it is associated with the manifestations caused by the *excessive and prolonged activation of the HPA axis* and the increase of the stress hormones level within the *wear and tear* reaction
- **Consequences:** the appearance of the ***burnout syndrome*** and **stress-induced disorders/ diseases:**

- onset / worsening of addictions: smoking, drug use
- migraine, depression, anxiety
- lowering of the painful threshold
- sleep disorders (insomnia) and eating disorders
- obesity, hyperglycemia, diabetes mellitus
- gastritis, ulcerative colitis
- irritable bowel syndrome
- arrhythmias, arterial hypertension, coronary heart disease (angina attacks or heart attack)
- immunosuppression with increased risk of recurrent/prolonged infections, exacerbation of pre-existing autoimmune diseases
- alopecia, eczema, psoriasis, neurodermatitis
- menstrual disorders, impotence.

Post-traumatic stress disorder (PTSD)

- **Characteristics:** particular form of chronic stress, consequence of the prolonged response of the body, lasting **at least 6 months**, to a **deeply traumatic *physical or mental event***
- **Manifestations:**
  - *intrusive symptoms* - persistent reliving of the traumatic event through: memories, nightmares, flashbacks, intense emotional reactions
  - *avoidance reactions of the conditions associated with the traumatic event* (e.g., places, objects, people) *and emotional paralysis* (e.g., loss of interest in normal activities)
  - *neurophysiological hyperactivity* - restless sleep, irritability, anger / nervous outbursts

<u>Assessment of CHRONIC STRESS in medical students</u>

Several clinical studies showed that persistent intense stress can be associated with mental and physical health problems in medical students: *low self-esteem, anxiety and depression, difficulties in resolving interpersonal conflicts, sleep disorders, increased alcohol and drug consumption, cynicism, reduced attention and concentration, academic dishonesty, etc.*
The most common **tool** used to identify and evaluate these effects is the **MSSQ test (Medical Student Stressor Questionnaire).** It measures the stress factors pertaining to **6 areas:**

I. Academic related stressors (ARS)
II. Intrapersonal and interpersonal related stressors (IRS)
III. Teaching and learning-related stressors (TLRS)
IV. Social related stressors (SRS)
V. Drive and desire related stressors (DRS)
VI. Group activities related stressors (GARS)

The *Medical Student Stressor Questionnaire (MSSQ)*

1. COMPLETING the MSSQ test

The assessment of the stress level for each of the 40 points included in sections A and B is made according to the following score:**0 – causing no stress at all, 1 – causing mild stress, 2 – causing moderate stress, 3 – causing high stress, 4 – causing severe stress**

- ***Of note!***
  - *If you are not in a clinical year yet, please answer based on how you think you would face this situation
  - verbal abuse is defined as speaking insultingly, harshly and unjustly about a person
  - physical abuse is defined as treating a person harmfully or offensively

Section A Items		I	II	III	IV	V	VI
1.	Tests/examinations						
2.	Talking to patients about personal problems*						
3.	Conflicts with other students						
4.	Grading system in examinations						
5.	Verbal or physical abuse by other student(s)						
6.	Parental wish for you to study medicine						
7.	Need to do well (self-expectation)						
8.	Not enough study material						
9.	Conflicts with university personnel(s)						
10.	Heavy workload						
11.	Participation in class discussion						
12.	Falling behind in reading/studying						
13.	Classes attendance						
14.	Lack of guidance from teacher(s)						
15.	Feeling of personal incompetence						
16.	Uncertainty of what is expected from me						
17.	Not enough medical practice*						
18.	Lack of time for family and friends						
19.	Competitiveness in relation to colleagues						
20.	Teacher - lack of teaching skills						
Total score SECTION A							

Section B Items		I	II	III	IV	V	VI
21.	Unable to answer questions from patients*						
22.	Assignment of inappropriate tasks						
23.	Having difficulty understanding the content materials						
24.	Facing illness or death of the patients*						
25.	Getting poor marks						
26.	Poor motivation to learn						
27.	Lack of time to review what has been taught						
28.	Verbal or physical abuse from teacher(s)						
29.	Frequent interruption of work/studying by others						
30.	Unable to answer the questions posed by the teachers						
31.	Conflicts with teacher(s)						
32.	Unwillingness to study medicine						
33.	Large amount of content to be learnt						
34.	Need to do well (expectations imposed by others)						
35.	Not enough feedback from teacher(s)						
36.	Unjustified grading process						
37.	Lack of recognition for work done						
38.	Working with computers						
39.	Verbal or physical abuse from university personnel(s)						
40.	Family responsibilities						
Total score SECTION B							

2. CALCULATING the MSSQ score

Stage I	I	II	III	IV	V	VI
Total A						
Total B						
TOTAL						

Stage II	I	II	III	IV	V	VI
TOTAL						
Divided by	**13**	**7**	**7**	**6**	**3**	**4**
SCORE						

3. INTERPRETING the MSSQ score

SCORE	0,00 - 1,00	1,01 – 2,00	2,01 - 3,00	3,01 - 4,00
STRESS level	**MILD**	**MODERATE**	**HIGH**	**SEVERE**
I. Academic related stressors	Indicating that it is not stressful to you, or even if it is causing stress, it is a mild one.	Indicating that it causes you a reasonable stress that you can manage well.	Indicating that it causes you a high stress. Your emotional reactions seem to be affected by stress. Your daily activities are easily compromised because of this.	Indicating that it causes you a severe stress. Your emotional reactions are highly affected by stress. Your daily activities are totally compromised because of this.
II. Intrapersonal and interpersonal related stressors				
III. Teaching and learning-related stressors				
IV. Social-related stressors				
V. Drive and desire-related stressors				
VI. Group activities-related stressors				

II. THE BURNOUT SYNDROME

- **Definition:** the ***burnout syndrome***, also known as the ***chronic fatigue syndrome***, represents the physical, emotional, and mental exhaustion due to **chronic occupational stress exposure.**
- The burnout syndrome is specific to several professional categories such as *medical doctor, medical assistant, psychologist, professor* and is a multifactorial illness caused by the combination of causes such as the working place, life style, and personality type.

Assessment of the BURNOUT SYNDROME in medical doctors

The ***Maslach Burnout Inventory (MBI) test*** is the most used **instrument** for identifying the risk of burnout syndrome in medical doctors. It comprises 22 items exploring **3 components**: *Emotional exhaustion; Depersonalization; Personal achievement.*
These items assess a series of feelings in terms of how often the doctor experiences them, on a scale from 0 to 6 (where 0 means *never* and 6 means *every day*).

The *Maslach Burnout Inventory (MBI) test*

Section A – EMOTIONAL exhaustion

Questions	Never	A few times per year	Once a month	A few times per month	Once a week	A few times per week	Every day
	0	1	2	3	4	5	6
1. I feel emotionally drained by my work.							
2. Working with people all day long requires a great deal of effort.							
3. I feel like my work is breaking me down.							
4. I feel frustrated by my work.							
5. I feel I work too hard at my job.							
6. It stresses me too much to work in direct contact with people.							
7. I feel like I'm at the end of my rope.							
Total score SECTION A							

- **Interpretation:**
  - ***Total*** $\leq$ ***17***: Low-level burnout
  - ***Total between 18 and 29***: Moderate burnout
  - ***Total*** $\geq$ ***30***: High-level burnout

Section B – DEPERSONALIZATION

Questions	Never	A few times per year	Once a month	A few times per month	Once a week	A few times per week	Every day
	0	1	2	3	4	5	6
1. I look at certain patients impersonally, as if they were objects.							
2. I feel tired when I get up in the morning and have to face another day at work.							
3. I have the impression that my patients make me responsible for some of their problems.							
4. I feel I am at the end of my patience by the end of my work day.							
5. I don't care about what happens to some of my patients.							
6. I have become more insensitive to people since I've been working.							
7. I'm afraid that this job is making me uncaring							
Total score SECTION B							

- **Interpretation:**
  - ***Total* $\leq$ *5***: Low-level burnout
  - ***Total between 6 and 11***: Moderate burnout
  - ***Total* $\geq$*12***: High-level burnout

Section C – PERSONAL ACHIEVEMENT

Questions	Never	A few times per year	Once a month	A few times per month	Once a week	A few times per week	Every day
	0	1	2	3	4	5	6
1. I feel I have accomplished many worthwhile things in this job.							
2. I feel full of energy.							
3. I am easily able to understand what my patients feel.							
4. I look after my patients' problems very effectively.							
5. In my work, I handle emotional problems very calmly.							
6. Through my work, I feel that I have a positive influence on people.							
7. I am able to create a relaxed atmosphere with my patients/clients.							
8. I feel refreshed after having been close to my patients at work.							
Total score SECTION C							

- **Interpretation:**
  - ***Total* $\leq$*33***: High-level burnout
  - ***Total between 34 and 39***: Moderate burnout
  - ***Total* $\geq$*40***: Low-level burnout

Remember!

A high score in the first two sections and a low score in the last section may indicate burnout. The MBI questionnaire cannot be used with diagnostic value, regardless of the results. Its goal is to make you aware that you may be on the verge of chronic fatigue.

Observation!

The large workload to be performed in a relatively limited time, the associated stress and insufficient organizational support in the context of the current COVID-19 pandemic are the main factors responsible for the increased risk of chronic fatigue syndrome among health professionals in the past years. The burnout syndrome is a gradual and cumulative process that severely impacts performance in the workplace by decreasing alertness and the desire/motivation to perform current tasks.

On the other hand, fatigue is one of the most common symptoms of presentation among patients infected with COVID-19. Moreover, recent studies show that fatigue is evident (and in some cases debilitating) even 10 weeks after confirmation of healing, with about a third of patients not being able to resume their duties at work. In the case of medical staff, this has dramatic consequences for global health systems, given the growing number of healthcare professionals infected with COVID-19. Thus, the early detection of fatigue syndrome is an important goal in combating this situation.

One of the most widely used methods of assessing fatigue among both patients and occupational groups is the ***Chalder Fatigue Scale questionnaire***. It has the advantage of being a short questionnaire that assesses both physical and mental fatigue and has results comparable to longer multidimensional tests.

The *Chalder Fatigue Scale questionnaire*

	Less than usual	**No more than usual**	**More than usual**	**Much more than usual**
	0	**1**	**2**	**3**
1. Have you noticed having problems with tiredness				
2. Do you need to rest more				
3. Do you feel sleepy				
4. Do you have problems starting things				
5. Do you lack energy				
6. Do you have less muscle strength				
7. Do you feel weak				
8. Do you have difficulties concentrating				
9. Do you make slips of the tongue when speaking				
10. Do you find it more difficult to find the right word				
	Better than usual	**No worse than usual**	**Worse than usual**	**Much worse than usual**
	0	**1**	**2**	**3**
11. How is your memory				

- **Interpretation:**
  - **Maximum possible score = 33**
  - In general, the **healthy population** registers values of the **score between 11-14**
  - Patients with **chronic fatigue** usually have **score values > 24**
  - Performing the test pre- and post-therapy allows the **evaluation of the effectiveness of the treatment.**

CHECKPOINT!

***1. Mild / moderate acute stress, of short duration, under controllable conditions is called:**
A. Endogenous stress
B. Somatic stress
C. Exogenous stress
D. Eustres
E. Distress

2. Which of the following represent effects of chronic stress?
A. Fight or flight reaction
B. Wear and tear reaction
C. Excessive activation of the HPA axis
D. Excessive activation of sympathetic vegetative nervous system
E. Immunosuppression

3. Select the correct statements about the state of resistance of the general adaptation syndrome to stress:
A. It begins with the action of the stressor
B. The body's resistance to the action of the stressor is high
C. Catecholamine secretion is increased
D. Cortisol secretion is increased
E. HPA axis activity returns to normal

4. Which of the following are characteristics of the alarm stage of the general adaptation syndrome to stress?
A. Normal cortisol levels
B. Increased plasma catecholamines
C. Fight or flight reaction
D. High resistance of the body to the action of the stressor
E. The occurrence of post-traumatic stress syndrome

***5. For the evaluation of chronic stress in the case of medical students, the following is used:**
A. The Chalder Fatigue Scale questionnaire
B. The Maslach Burnout Inventory questionnaire
C. The MSSQ questionnaire
D. Serum vitamin D
E. Serum catecholamines

CASE STUDIES

A student at the Faculty of Medicine presents to the family doctor for mild recurrent respiratory viral infections, affirmatively associated with the exams session periods. In addition to the laboratory investigations, the student also completed the Medical Student Stressor questionnaire. The MSSQ score for each of the 6 sections is as follows: I - 3.5; II - 2.3; III - 3.8; IV - 2.3; V - 1.4; VI - 2.5.

How do you interpret the MSSQ questionnaire scores?

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NOTES

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