Paolo **POZZILLI**

HANDBOOK OF ENDOCRINOLOGY AND METABOLIC DISEASES

EDIZIONI MINERVA MEDICA

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FOREWORD

This Handbook of Endocrinology and Metabolic Diseases written by Professor Paolo Pozzilli and his collaborators at Campus Bio-Medico University of Rome is addressed to students of medical courses interested in consulting a concise and updated book in this specific field of medicine. It is worth noting that some of the clinicians and scientists who contributed to this book are well known internationally for their work in the field of diabetes, thyroid and osteometabolic diseases.

Of relevance for a rapid consultation by the reader are the tables summarising diagnostic and therapeutic approaches to endocrine diseases. Moreover, at the end of each chapter very recent key references are quoted for those interested in deepening their understanding of some specific fields of endocrinology. The book is also a helpful tool for those working in other areas of medicine and eager to learn the recent developments in the fascinating field of endocrinology. The book well reflects the enthusiasm of Professor Paolo Pozzilli who, after more than 40 years of passionate teaching, still enjoys sharing what he has learnt from his long-lasting experience.

Finally, I would like to congratulate Professor Pozzilli and his colleagues for their work and wish them success for their publications.

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PREFACE

It gives me great pleasure to present this endocrinology handbook aimed primarily, but not exclusively, at students attending courses in medicine and other related courses. It is a handbook entirely written by personnel who have worked in the past at the Endocrinology and Diabetes Unit of Campus Bio-Medico University of Rome and are still collaborating with us. Therefore, this handbook is written by colleagues, who were trained here with the same principles, a common synergy in teamwork, and in the relationship with the patient and with the students. There are many endocrinology texts available for students: why then another book? In the study of medicine, which is such a broad and articulated subject, it may be useful to have a handbook on endocrinology that is easy to consult, rich in tables, pictures, figures, diagrams, and synoptic tables for a more direct reading of the data and notions given. Thus, this book can facilitate the learning process and offer an easy-to-reference tool, especially when the information received needs to be correlated with other diseases. Due to its characteristic layout, we believe that this book can also represent a valuable tool for trainees in endocrinology and other specialist branches of medicine. It should be noted that at the end of each chapter there are up-to-date reference entries to deepen the reader's knowledge on the specific topic.

I would like to thank all colleagues at Campus Bio-Medico University of Rome who contributed to the writing of this handbook, which is the result of a joint effort aiming to explain the required notions in a modern

key. The creation of this work gives the reader the chance to deepen their knowledge of this fascinating and increasingly relevant branch of medicine.

I would like to express my deepest appreciation to Ms Daniela Petrelli for revising with thoroughness the final proofs of the handbook and also to extend my sincere thanks to Ms Jenny Cattermole for the extremely professional services provided for English editing. I am grateful to Roche Diabetes Care Italy SpA and Centro Internazionale Studi Diabete (CISD), a non-profit organization founded in 1983 and chaired in the past by my mentor, the late Professor Domenico Andreani, for their contributions.

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ABBREVIATIONS

AACE	American Association of Clinical Endocrinologists
ABI	ankle brachial pressure index
ACE	American College of Endocrinology
ACE	Angiotensin-converting enzyme
ACLY	ATP citrate lyase
ACTH	adrenocorticotropic hormone
AD	Addison's disease
ADA	American Diabetes Association
ADH	antidiuretic hormone
AEE	activity energy expenditure
AGEs	advanced glycation end products
AGHD	adult GH deficiency
AIDS	acquired immunodeficiency syndrome
AIRE	autoimmune regulator gene
AJCC	American Joint Committee on Cancer
ALT	alanine transaminase
AME	Apparent mineralocorticoid excess
AME	Association of Endocrinologist Physicians
anti-ZnT8	Anti-zinc transporter 8
APECED	Autoimmune-polyendocrine-candidiasisectodermal-dystrophy
APS	Autoimmune polyendocrine syndromes
ARH	autosomal recessive hypercholesterolemia
ARR	aldosterone/renin ratio
ART	assisted reproductive techniques
AST	aspartate aminotransferase
ATA	American Thyroid Association

BIA	bioelectrical impedance analysis
BMC	bone mineral content
BMD	bone mineral density
BMI	Body Mass Index
BUN	blood urea nitrogen
β-HCG	β human chorionic gonadotropin
CAH	Congenital adrenal hyperplasia
CAL	café-au-lait
CAL	calcitonin
CAT	Chronic autoimmune thyroiditis
CC	Chronic candidiasis
CEA	carcinoembryonic antigen
CGM	continuous glucose monitoring
CH	Chronic hypoparathyroidism
CNS	central nervous system
CNS	central nervous system
COPD	Chronic obstructive pulmonary disease
CRF	chronic renal failure
CRH	corticotropin-releasing hormone
CRP	C-reactive protein
CRPS	complex regional pain syndrome
CSII	continuous subcutaneous insulin infusion
CT	Computed Tomography
CTLA-4	cytotoxic T-lymphocyte-associated antigen-4
CV	cardiovascular
D1	deiodinase 1
D2	deiodinase 2
D3	deiodinase 3
DCCT	Diabetes Control and Complications Trial
DEXA	dual-energy X-ray absorptiometry
DHA	docosahexaenoic acid
DHEAS	dehydroepiandrosterone sulphate
DKA	Diabetic ketoacidosis
DN	Diabetic neuropathy
DOC	deoxycorticosterone
DPP-4	dipeptidyl peptidase-4

DR	Diabetic retinopathy
DSSMP	distal symmetric sensorimotor polyneuropathy
DTC	Differentiated thyroid carcinoma
ECG	electrocardiogram
ECL	enterochromaffin cell-like
EGD	esophagogastroduodenoscopy
eGFR	estimated glomerular filtration rate
ELISA	enzyme-linked immunosorbent assay
EMA	European Medicines Agency
EMG	electromyography
ENG	electroneurography
EPA	eicosapentaenoic acid
EPO	erythropoietin
ESC	embryonic stem cell
ESR	erythrocyte sedimentation rate
FDA	Food and Drug Administration
FDB	familial defective ApoB hypercholesterolemia
FGM	flash glucose monitoring
FNA	fine needle aspiration
FPG	fasting plasma glucose
FRAX	Fracture Risk Assessment Tool
FSH	follicle-stimulating hormone
FT3	free T3
FT4	free T4
GAD	glutamic acid decarboxylase
GADA	antiglutamic acid decarboxylase autoantibodies
GDM	gestational diabetes mellitus
GEP	gastroenteropancreatic
GER	Gastroesophageal reflux
GFR	glomerular filtration rate
GGT	gamma-glutamyl transferase
GH	growth hormone
GHRH	GH-releasing hormone
GHSR	growth hormone secretagogue receptor
GI	glycæmic index

GIP	gastric inhibitory peptide
GLP-1	glucagon-like peptide-1
GnRH	gonadotropin-releasing hormone
GPs	General Practitioners
2hPG	blood glucose levels 2 hours after an oral load of glucose
5-HT2c	5-hydroxytryptamine 2c
HbA1c	glycosylated hemoglobin
HDL	high-density lipoprotein
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HRT	hormone replacement therapy
IA-2	islet tyrosine phosphatase autoantibodies
IAA	insulin autoantibodies
ICA	pancreatic islet cell autoantibodies
IDF	International Diabetes Federation
IDL	Intermediate-density lipoproteins
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine
IFG	impaired fasting glucose
IGCNU	Intratubular germ-cell neoplasia undifferentiated
IGF-1	insulin-like growth factor-1
IGFBP	IGF-binding protein
IgG	polyclonal immunoglobulin G
IGT	impaired glucose tolerance
IIEF	International Index of Erectile Function
IL-2	interleukin-2
IL-5	interleukin-5
IL-6	interleukin-6
IOM	Institute of Medicine
IPSS	International Prostate Symptom Score
IR	insulin resistance
LADA	latent autoimmune diabetes in adults
LDH	lactate dehydrogenase
LDL	low-density lipoprotein
LDL-C	low-density lipoprotein cholesterol

LH	luteinizing hormone
LOH	late-onset hypogonadism
LPL	lipoprotein lipase
LT4	Levothyroxine
MAP	Medrossi Acetate Progesteron
MEN	multiple endocrine neoplasia
MESA	microepididymal sperm aspiration
MHC	major histocompatibility complex
MMT	mixed meal test
MNG	multinodular goiter
MODY	maturity onset diabetes of the young
MRI	magnetic resonance imaging
MS	metabolic syndrome
MSCs	mesenchymal stem cells
MTC	Medullary thyroid carcinoma
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
NK	natural killer
NSE	neuron-specific enolase
OCT	optical coherence retinal tomography
OGTT	oral glucose tolerance test
OSA	obstructive sleep apnea
OSAS	obstructive sleep apnea syndrome
PCOS	Polycystic ovary syndrome
PESA	percutaneous epididymal sperm aspiration
PET	Positron emission tomography
PHPT	Primary hyperparathyroidism
PIF	prolactin inhibiting factor
PLAP	placental alkaline phosphatase
POF	Premature ovarian failure/insufficiency
PP	pancreatic polypeptide
PPGLs	Pheochromocytomas and paragangliomas
PRA	plasma renin activity
PRL	prolactin

PSA	prostate-specific antigen
PTCA	percutaneous transluminal coronary angioplasty
PTH	parathyroid hormone
PTU	Propylthiouracil
PUFAs	polyunsaturated fatty acids
PYY	Peptide YY
QTC	quantitative computerized tomography
QUS	quantitative ultrasound
RANKL	Receptor activator of nuclear factor kappa-B ligand
REE	resting energy expenditure
RET_{gene}	rearranged during transfection$_{gene}$
rhGH	Recombinant human GH
RIA	radioimmunoassay
RNI	Reference Values for Nutrient and Energy Intake
ROS	reactive oxygen species
RVH	renovascular hypertension
SCORE	Systematic Coronary Risk Estimation
SD	standard deviation
SERM	selective estrogen receptor modulators
SGLT-2	sodium/glucose cotransporter 2
SHBG	sex hormone binding globulin
SIADH	syndrome of inappropriate antidiuretic hormone
SIOMMMS	Italian Society for Osteoporosis, Mineral Metabolism and Bone Diseases
SLE	systemic lupus erythematosus
SRS	somatostatin receptor scintigraphy
SSRI	selective serotonin reuptake inhibitor
SSRIs	selective serotonin reuptake inhibitors
T1D	type 1 diabetes
T2D	type 2 diabetes
T3	triiodothyronine
T4	tetraiodothyronine
TB	tuberculosis
TEE	Total energy expenditure

TESA	testicular sperm aspiration
TESE	testicular sperm extraction
TG	triglycerides
TGAb	antithyroglobulin antibodies
TIA	transient ischemic attack
TNF-α	tumor necrosis factor- α
TNM	tumor (T), nodes (N), and metastases (M)
TPOAb	antithyroid peroxidase antibodies
TRAb	TSH receptor-stimulating autoantibodies
TRH	thyrotropin-releasing hormone
TSH	thyroid stimulating hormone
UFC	urinary free cortisol
VFA	vertebral fracture assessment
VHL	Von Hippel-Lindau
VIP	vasoactive intestinal peptide
VLCKD	very low-calorie ketogenic diets
VLDL	very low-density lipoproteins
VSG	vertical sleeve gastrectomy
WHO	World Health Organization

DISEASES OF THE HYPOTHALAMUS AND ADENO-NEURO PITUITARY

1

Ernesto Maddaloni, Donatella Rausa, Gaia Tabacco, Silvia Egiddi

DIABETES INSIPIDUS

Definition

Diabetes insipidus is an increased excretion of hypotonic, dilute, and low-salt urine resulting from impaired antidiuretic hormone (ADH) secretion/action.

Classification and etiology

Main causes of hypotonic polyuria are listed in Table 1.I.

Clinical presentation

The main clinical manifestations are polyuria (>40 mL/kg/day in adults) and polydipsia occurring to compensate for it.

Diagnosis

To make the diagnosis of diabetes insipidus, it is necessary to:

- confirm polyuria with 24-hour urine collection;
- rule out diabetes mellitus and renal failure;
- rule out hypokalemia and hypercalcemia;
- rule out pregnancy;
- assess water restriction tests to confirm the diagnosis.

TABLE 1.I. Main causes of hypotonic polyuria.

Cause	Specific cause	Mechanism
Excessive fluid intake	Primary polydipsia: psychiatric disorders and rarely an organic basis	Physiological inhibition of ADH secretion due to plasma osmolality as a result of excessive fluid intake
	Fluid intake by parenteral route	
Inability to synthesize and/or secrete ADH (central diabetes insipidus)	Tumors: craniopharyngioma, pituitary metastases of distant tumors and lymphomas/Leukæmia	Infiltration of the hypothalamic-pituitary region
	Trauma or pituitary surgery	Transient and triphasic diabetes insipidus
	Granulomatous diseases: Langerhans cell histiocytosis (Letterer-Siwe diseases; Hand-Schuller-Christian disease; benign eosinophilic granuloma), tuberculosis and sarcoidosis	Infiltration of the hypothalamic-pituitary region
	Lymphocytic infundibulo-hypophysitis	Autoimmunity
	Pituitary ischemia: Sheehan Syndrome (*post-partum* pituitary necrosis) and ischemic encephalopathy	Pituitary necrosis
	Familial diabetes insipidus	Mutations of vasopressin gene
	Wolfram Syndrome	
	Familial hypothalamic adipsic diabetes insipidus	Altered osmoreceptor functionality. This causes a delayed ADH release in response only to the drop in blood pressure due to insensitivity to changes in plasma osmolality
Resistance to ADH action (nephrogenic diabetes insipidus)	Congenital	X-linked mutations in the V2 receptor gene
		Autosomal recessive mutations in the aquaporin-2 gene
	Acquired: renal disorders (polycystic kidney, renal infarction, sickle cell anæmia), drugs (lithium, demeclocycline) and hypocalcemia-hypercalcemia	Reduction in the ability of the kidney to concentrate urine due to either structural, or ischemic alterations or to reduced aquaporin expression
Increased vasopressin catabolism	Pregnancy	The oxytocinase produced during pregnancy also increases ADH catabolism, causing diabetes insipidus in some predisposed women; it usually resolves at the end of pregnancy

Water restriction test

Before starting the test, it is important to:

- weigh the patient;
- take a blood sample to determine plasma osmolality;
- perform urine test to measure urine osmolality.

Start water restriction (the patient can neither eat nor drink) and monitor weight, diuresis, and urine osmolality every hour.

When the patient has lost >2% of body weight or when two consecutive measurements of urine osmolality differ by less than 10%, take a sample to measure plasma osmolality and ADH levels. Subsequently administer desmopressin (synthetic analogue of ADH) 10 µg intranasally or 2 µg i.v. and measure urine osmolality for another 2 hours.

Interpretation

The interpretations of the results may include:

- primary polydipsia – increased urine osmolality with water restriction (500 to 600 mOsm/kg) and no response to the administration of desmopressin;
- partial central diabetes insipidus – urine osmolality increase with water restriction (600 mOsm/kg) + further increase of urine osmolality after the administration of desmopressin (50%). It presents low or non-measurable ADH;
- complete central diabetes insipidus – urine osmolality does not increase with fluid deprivation test, but desmopressin causes a significant increase in urine concentration (from 100% to 400%). It shows low or non-measurable ADH;
- nephrogenic diabetes insipidus – urine osmolality does not significantly increase either with fluid deprivation test or with the administration of desmopressin. It includes high ADH levels at the end of the test.

Treatment

The treatment includes:

- identification and elimination of secondary causes of diabetes insipidus;
- use of desmopressin (ADH analogue, effective in the treatment of central forms of diabetes insipidus);

- use of thiazide diuretics (effective in nephrogenic diabetes insipidus);
- use of amiloride (effective in lithium therapy-induced nephrogenic diabetes insipidus);
- use of indomethacin (prolongs the action of ADH and desmopressin and therefore has antidiuretic activity).

SYNDROME OF INAPPROPRIATE ANTIDIURETIC HORMONE SECRETION

Definition

Inappropriate antidiuretic hormone secretion is a clinical syndrome resulting from excessive ADH secretion and characterized by the presence of the following clinical criteria (Barrter and Schwartz criteria):

- low plasma osmolality (<275 mOsm/kg);
- inappropriate urine concentration (>100 mOsm/kg with normal kidney function);
- clinical euvolemia;
- increased Na concentration in the urine (>30 mEq/L);
- rule out other causes of euvolemic plasma hypo-osmolality (*e.g.*, hypothyroidism, adrenal insufficiency).

Etiology

The etiology and the main causes of the syndrome of inappropriate antidiuretic hormone secretion (SIADH) are expressed in Table 1.II.

TABLE 1.II. Main causes of SIADH.

Ectopic secretion	Carcinomas: the most frequent are lung carcinoma (most often small cell lung cancer), pancreatic, prostate, uterine carcinomas, thymomas, lymphomas and Leukæmias
Drugs	Chlorpropamide, carbamazepine, clofibrate, SSRIs, tricyclic antidepressants, etc.
Alterations of the baroreceptor system	Pulmonary diseases: pneumonia, TB, positive pressure ventilation, etc.
	CNS diseases: infections, trauma/surgery, hemorrhage, etc.
Other causes	AIDS, strenuous exercise, acute psychosis

SIADH: syndrome of inappropriate antidiuretic hormone secretion; SSRIs: selective serotonin reuptake inhibitors; TB: tuberculosis; CNS: central nervous system; AIDS: acquired immunodeficiency syndrome.

Clinical presentation

The SIADH clinical presentation is dominated by signs and symptoms of hyponatremia, which include:

- chronic hyponatremia (>120 mEq/L) – asymptomatic patient or presence of non-specific symptoms such as nausea, confusion, headache, difficulty concentrating or muscle cramps;
- rapid onset or severe hyponatremia (<120 mEq/L) – neurogenic pulmonary edema, seizures, stupor, coma, and respiratory arrest due to cerebral edema with brain stem herniation.

Diagnosis

The diagnosis of SIADH is based on the confirmation of the clinical criteria after assessment of plasma (pOsm) and urinary (uOsm) osmolality (uOsm/pOsm ratio >1), volume status, and sodium content in urine.

Treatment

The treatment includes:

- water restriction;
- vaptans (vasopressin receptor antagonists that allow the elimination of water without increasing natriuresis);
- demeclocycline (it causes nephrogenic diabetes insipidus, and therefore is used in SIADH treatment).

CENTRAL HYPERCORTISOLISM

Definition

Central hypercortisolism (Cushing's disease) is a clinical syndrome due to autonomous and uncontrolled adrenocorticotropic hormone (ACTH) hypersecretion from the anterior pituitary gland.

Clinical presentation

The clinical consequences of increased ACTH secretion are mainly due to the effects of excessive activity of the hormones secreted in excess by the adrenal cortex on their target organs (Table 1.III).

TABLE 1.III. Clinical presentation of hypercortisolism.

Clinical characteristics		Considerations
General	Obesity: the accumulation of fat in the supraclavicular and cervical–dorsal area causes "buffalo hump" sign; moon face	Central obesity present in 90% of Cushing's Syndrome patients
	Muscle weakness	Loss of muscle mass especially in the proximal muscles of the four limbs
	High blood pressure	Frequent elevated diastolic blood pressure (>100 mmHg)
Skin alterations	Skin atrophy (thin skin): facial plethora, striae rubrae and ecchymoses	Striae rubrae differ from the classic "stretch marks" in: color (red-purple), width (0.5-2.0 cm) and depth (below the skin surface)
	Slow wound healing	Frequent post-surgical dehiscence
	Mucocutaneous fungal infections	Oral candidiasis, onychomycosis, *tinea versicolor*
	Acne	
	Hirsutism	Face localization is the most common
Neuropsychiatric disorders	Emotional lability: irritability, anxiety, depression	Very frequent
	Insomnia	
	Psychosis, hallucinations, paranoia	Rare
Gonadal dysfunction	Decreased libido	
	Erectile dysfunction	
	Amenorrhea	
	Infertility	
	Clitoral hypertrophy	Specific, but not very sensitive, Cushing's sign
Metabolic disorders	Diabetes	
	Osteoporosis	
	Renal hypercalciuria and nephrolithiasis	
	Dyslipidæmia	
	Polyuria	Both from hyperglycæmia and from inhibition of ADH secretion due to excess cortisol
Ocular disorders	Glaucoma	
	Cataract	

TABLE 1.IV. Main causes of hypercortisolism.

ACTH-dependent	ACTH-secreting pituitary adenoma (Cushing's disease)	Most frequent cause of endogenous hypercortisolism (>80% of cases)
	Pituitary carcinoma	Rare
	Ectopic secretion of ACTH	In more than half of cases, ectopic secretion derives from bronchial carcinoids and lung small cell carcinomas
ACTH-independent	Iatrogenic	
	Adrenal neoplasia (adenoma or carcinoma)	
	Nodular adrenal hyperplasia	

Etiology

The most frequent cause of central hypercortisolism is an ACTH-secreting pituitary adenoma. Differential diagnosis of central hypercortisolism should be made with other causes of hypercortisolism, which can be classified into ACTH-dependent and ACTH-independent causes (Table 1.IV).

Diagnosis

The diagnosis can be made considering:

- the exclusion of iatrogenic causes of hypercortisolism;
- the confirmation of elevated cortisol levels through screening and confirmatory tests (dexamethasone 1 mg overnight test; 24-hour urinary free cortisol test; 11 p.m. salivary cortisol test; low dose Liddle test [2 mg x 2 days]);
- the confirmation of ACTH hypersecretion by measuring plasma ACTH compared to blood cortisol levels and possibly confirmed by dynamic tests (suppression test with high-dose dexamethasone, corticotropin-releasing hormone [CRH] test).

In presence of confirmed hypercortisolism:

- if ACTH<5 pg/mL = ACTH-independent;
- if ACTH>20 pg/mL = ACTH-dependent;
- if ACTH is 5 to 20 pg/mL perform CRH tests (see below);
- if CRH does not cause significant changes in ACTH and blood cortisol levels, it is likely an ACTH-independent cause.

After confirming the presence of ACTH-dependent endogenous hypercortisolism, it is necessary to determine whether ACTH has pituitary or ectopic origin:

- in the high-dose dexamethasone suppression test, blood cortisol measurement is performed in the morning, followed by the administration of dexamethasone 8 mg at 11 pm. A second blood cortisol sample is taken the next morning. If blood cortisol levels the morning after taking the tablets have not decreased by >50% compared to the previous morning, the diagnosis of ectopic ACTH secretion is plausible;
- pituitary magnetic resonance imaging (MRI) with contrast medium is useful for identifying any sellar region masses, possibly responsible for the ACTH secretion;
- the ACTH levels in blood sampling obtained through catheterization of the lower petrosal sinuses before and after stimulation with CRH and/or the suppression test with high-dose dexamethasone confirm or exclude the pituitary origin of ACTH hypersecretion.

Treatment

The treatment includes:

- surgical removal of any ACTH-secreting pituitary adenoma;
- use of pasireotide (somatostatin analogue, used in patients not eligible for surgery or with disease not controlled by surgery);
- the treatment of ACTH-secreting tumor, in case of ectopic ACTH secretion. If not treatable, use drugs that inhibit steroid synthesis (ketoconazole, metyrapone) and receptor antagonists such as spironolactone;
- bilateral adrenalectomy when Cushing's disease is not controllable by conventional therapies.

CENTRAL HYPOCORTISOLISM

Definition

Central hypocortisolism (or secondary adrenal insufficiency) is a syndrome characterized by decreased secretion of cortisol resulting from a deficit in the production and release of ACTH by the anterior pituitary gland.

Pathophysiology

In the absence of ACTH, the adrenal cortex is not adequately stimulated to secrete cortisol. ACTH deficiency does not also cause deficiency of mineralocorticoid hormones, which is different from what happens in most cases of primary adrenal insufficiency.

Clinical presentation and differential diagnosis

Clinical presentation is comparable to that of primary adrenal insufficiency. Differential diagnosis is possible due to skin hyperpigmentation as a result of the increased production of the ACTH precursor, pro-opiomelanocortin, which is present only in primary forms.

Causes

Secondary hypocortisolism is hardly an isolated finding. Much more frequently, ACTH deficiency is found in hypo-/panhypopituitarism. Please refer to later sections for causes of panhypopituitarism.

Diagnosis

The diagnostic steps for adrenal insufficiency are thoroughly presented in Chapter 10. In brief, cortisol deficiency is diagnosed by a blood cortisol test at 8:00 in the morning and confirmed by a blood cortisol test after stimulation with synthetic ACTH (cosyntropin). The plasma ACTH concentration will be elevated in primary hypocortisolism, that is in case of adrenal origin, in response to cortisol deficiency. Other tests that can be used in doubtful cases for the assessment of the ACTH pituitary reserve (for safety reasons too, to be performed always after the ACTH challenge test) are shown in Table 1.V.

Treatment

The treatment of secondary adrenal insufficiency, as well as the treatment of primary adrenal insufficiency (see the relevant section for reference), includes glucocorticoid replacement therapy. The minimum effective dosage should be used to avoid inducing iatrogenic Cushing's Syndrome. Unlike therapy for the primary form, treatment with mineralocorticoid drugs is usually unnecessary in secondary adrenal insufficiency.

TABLE 1.V. Tests to assess ACTH pituitary reserve.

Test	Rationale	Performance	Interpretation
Induced hypoglycæmia	Hypoglycæmia induces a counter-regulatory response characterized by, among other effects, increased ACTH, and GH secretion.	In fasting patient: intravenous insulin infusion in a dose sufficient to cause hypoglycæmia. Every 15 minutes, take blood sampling for glucose and cortisol levels. The test should always be performed under close medical observation as it can cause severe hypoglycæmia.	In healthy people, blood glucose levels <40 mg/dL cause an increase in blood cortisol levels >18 to 20 µg/dL, indicating that the ACTH reserve is maintained.
Metyrapone	Metyrapone inhibits cortisol biosynthesis by inhibiting the 11-beta-hydroxylase enzyme (last step). The inhibition of cortisol production in subjects with good ACTH reserve determines positive feedback with increase in ACTH secretion and subsequent increase in cortisol precursors. In subjects with ACTH deficiency, positive feedback will have no effect: accordingly, the concentration of cortisol precursors will not change.	The patient takes metyrapone 30 mg/kg at 11:00 p.m. The next morning at 8:00 a.m. a sample is taken to determine levels of cortisol and its precursor 11-deoxycortisol.	In healthy subjects the concentration of 11-deoxycortisol increases to levels >7 µg/dL. The measurement of blood cortisol is necessary to confirm adequate metyrapone inhibition of the 11-beta-hydroxylase enzyme (blood cortisol levels must be <10 µg/dL).
CRH	In healthy subjects CRH causes a peak of ACTH and blood cortisol levels.	CRH is given in i.v. bolus at a dosage of 1 µg/kg and blood samples to determine ACTH and blood cortisol levels are taken at 0, 15, 30 and 60 min.	In healthy subjects, blood cortisol levels after CRH stimulation rise to >10 µg/day. ACTH increases 15 minutes after CRH administration (threshold levels vary according to the assay used for measurement).

ACTH: adrenocorticotropic hormone; GH: growth hormone; CRH: corticotropin-releasing hormone.

PITUITARY GIGANTISM/ACROMEGALY

Definition

Pituitary gigantism and acromegaly are two clinical syndromes characterized by excessive growth hormone (GH) secretion before (gigantism) or after (acromegaly) the fusion of the long bone epiphyses.

Pathophysiology

Chronic GH hypersecretion causes increased liver production of the insulin-like growth factor-1 (IGF-1) hormone, which is the main mediator of GH effects. The main IGF-1 action on the target organs is to promote cell proliferation and growth. This action is carried out particularly on bones, cartilage, and soft tissues, resulting in the classical clinical manifestations of the disease, including:

- gigantism, if chronic GH hypersecretion starts in childhood and/or adolescence;
- non-linear bone enlargement, mainly localized in the skullcap and jaw, if chronic GH hypersecretion develops in adults;
- visceromegaly;
- insulin resistance and risk of/and diabetes mellitus;
- excess GH levels can have "prolactin-like" effects, therefore interfering with gonadal function.

Clinical presentation

The other clinical manifestations are consequent to the metabolic actions of GH (Table 1.VI).

Main differential diagnoses

Main differential diagnoses include ectopic GH or GH-releasing hormone (GHRH) secretion, that is rare complication of lung carcinomas, carcinoid tumors or pancreatic islet cell tumors. High GH levels not accompanied by increased IGF1 levels, and, therefore, without the classical clinical manifestations of gigantism/acromegaly. It can be found in various conditions, such as liver cirrhosis, renal failure, prolonged fasting (malnutrition, anorexia), etc.

Causes

Virtually all cases of gigantism/acromegaly are caused by a GH-secreting pituitary adenoma.

Diagnosis

The diagnosis of gigantism/acromegaly is strongly suggested by the above-mentioned clinical picture.

Clinical suspicion must therefore be confirmed by IGF-1 levels, which will be higher than the standardized range for age and gender. Subsequently, the glucose load suppression test can be performed since GH secretion is suppressed by the glucose load.

Procedure

The patient in fasting state takes glucose 75 g or 100 g orally. Blood samples are taken for GH measurement before glucose ingestion and 30 and 60 minutes after.

TABLE 1.VI. Clinical manifestations of GH hypersecretion.

Pathophysiological mechanism	Sign
IGF-1 action on musculoskeletal system and soft tissues	Skullcap thickening
	Prognathism
	Prominent superciliary arches
	Joint pain up to acromegaly debilitating arthritis
	Enlargement of hands and feet
	Enlargement of nose and lips
	Obstructive sleep apnea
IGF-1 action on other organs	Excessive sweating and oily skin
	Hypertrichosis
	Multiple cutaneous papillomas and fibroids
	Carpal tunnel syndrome
	Thyroid goiter
	Cardiomegaly
	Hepatomegaly
GH metabolic action	Insulin resistance and impaired carbohydrate metabolism; *achantosis nigricans*
	Decreased libido/impotence
	Alterations of menstrual cycle
	High blood pressure
Mass effect of GH-secreting adenoma	Hypopituitarism (hypothyroidism, adrenal insufficiency, hypogonadism)
	Headache
	Visual impairment (bitemporal hemianopsia)

GH: growth hormone; IGF-1: insulin-like growth factor-1.

Interpretation

GH values are suppressed at concentrations <1 ng/mL in healthy subjects. Once clinical and biochemical diagnosis has been made, pituitary MRI

with contrast agents will be performed to search for and localize the pituitary adenoma.

Treatment

Transsphenoidal surgery is the therapy of choice for removing GH-secreting pituitary adenoma. After surgery, the patient should be monitored over time through IGF-1 measurement and dynamic GH suppression testing with glucose load at predetermined intervals (12 weeks after surgery, then every 6 months for 2 years, and then annually).

Medical therapy is recommended in patients with persistent disease after surgery. Drugs that can be used in patients with GH hypersecretion are:

- somatostatin analogues (slow-release octreotide, lanreotide depot and pasireotide) – GH-secreting pituitary adenomas often express somatostatin receptors. Somatostatin analogues act on these receptors by decreasing GH secretion and causing a reduction in tumor mass;
- pegvisomant – a GH receptor antagonist. It acts by inhibiting IGF-1 secretion in response to GH, thus decreasing IGF-1-mediated effects. It has no effect on the tumor mass and therefore patients treated with pegvisomant should be monitored with pituitary MRI over time;
- cabergoline – dopaminergic agonist sometimes suggested as initial adjuvant medical therapy.

GROWTH HORMONE DEFICIENCY IN ADULTS

Definition

GH deficiency is a clinical condition resulting from decreased or absent GH secretion from the anterior pituitary gland. For a discussion of growth delay due to GH deficiency in children and adolescents, please refer to specific texts on pediatric endocrinology. The following section will deal only with adult GH deficiency (AGHD).

Pathophysiology

In adulthood, decreased GH secretion mainly has consequences on:

- adipose tissue – increased fat mass, reduced lean mass, and muscle mass reduction;

- bones – increased bone resorption, osteoid thickness and delayed mineralization, with a fivefold increase in the risk of fracture;
- cardiovascular system – an increase in cardiovascular risk secondary to endothelial dysfunction, increased low-density lipoprotein (LDL) cholesterol and apolipoprotein B100 and reduced high-density lipoprotein (HDL) cholesterol, and left ventricular dysfunction.

Clinical presentation

The classical clinical picture of GH deficiency in the adult patient is non-specific.

Causes

GH deficiency can have congenital causes when it occurs in pediatric age groups and persists in adulthood (genetic mutations, association with cerebral structural defects), acquired causes due to hypothalamic-pituitary disorders (hypothalamic and pituitary tumors, infections, trauma, surgery, irradiation, etc.). AGHD may also occur in idiopathic form.

Diagnosis

In addition to clinical and anamnestic evaluation of the patient, some laboratory tests can be helpful in AGHD diagnosis:

- IGF1 measurement (with standardized reference levels for age and gender). Low levels in the absence of other causes (advanced catabolic conditions such as decompensated diabetes mellitus, severe chronic developmental liver diseases or estrogen therapy) strongly suggest a GH deficiency, which in any case requires a dynamic confirmation test;
- GHRH + arginine test:
  - reason – GHRH directly stimulates somatotropic cells to produce and secrete GH. Arginine stimulates GH secretion by inhibiting the secretion of endogenous somatostatin, which is a potent inhibitor of GH secretion;
  - performance – GHRH is administered i.v. within 1 minute at a dosage of 1 μg/kg; it is followed by an infusion of arginine within 30 minutes at a dosage of 0.5 g/kg (maximum dose 30 g). Blood samples for GH measurement are taken at 0, 30, 60, 90 and 120 minutes;

- interpretation – in healthy subjects GH increases with a maximum peak within 30 to 60 minutes. Some authors suggest using correct cut-offs for Body Mass Index (BMI) as follows:
  - BMI<25 kg/m^2, normal GH levels >12 ng/L;
  - BMI 25 to 30 kg/m^2, normal GH levels >8 ng/L;
  - BMI>30 kg/m^2, normal GH levels >4.2 ng/L;
- induced hypoglycæmia:
  - reason – hypoglycæmia induces a counter-regulatory response characterized by, among other effects, increased ACTH, and GH secretion;
  - performance – in fasting patient, intravenous insulin infusion in a dose sufficient to cause hypoglycæmia must be carried out. Every 15 minutes, blood sampling for glucose, cortisol and GH levels are taken. The test should always be performed under close medical observation as it can cause severe hypoglycæmia;
  - interpretation – in healthy people, blood glucose levels <40 mg/dL cause an increase in blood cortisol levels >18 to 20 µg/dL, indicating that the ACTH reserve is maintained, and an increase in GH levels to >5 ng/mL.

Treatment

Recombinant human GH (rhGH) is approved for the treatment of GH deficiency in both pediatric and adult age groups. Treatment with rhGH is contraindicated in patients with cancer.

HYPERPROLACTINEMIA

Definition

Hyperprolactinemia is a clinical condition characterized by high concentrations of serum prolactin.

Pathophysiology

Prolactin is synthesized by the lactotroph cells of the anterior pituitary gland, the activity of which is mainly regulated by dopamine as an inhibitory stimulus (prolactin inhibiting factor [PIF]). Therefore, in addition to cases of functional autonomy of lactotroph cells (prolactinoma), the inter-

ruption of dopaminergic inhibition (by pituitary stalk rupture or by pharmacological inhibition) or the presence of secretory stimuli (among others, the increase of thyrotropin-releasing hormone [TRH] levels in hypothyroid patients) can also cause hyperprolactinemia. High prolactin levels stimulate lactation, a mechanism responsible for the classic sign of galactorrhea that accompanies many cases of hyperprolactinemia. Although it does not seem that prolactin has a physiological role in regulating gonadal function, hyperprolactinemia causes hypogonadism, probably by altering the hypothalamic–pituitary control of gonadotropin secretion.

Clinical presentation

The classical clinical presentation of hyperprolactinemia is characterized by:

- galactorrhea (present in the majority of women with prolactinoma, much less common in men);
- amenorrhea, oligomenorrhea and infertility (in women);
- decrease in testosterone and spermatogenesis (in men);
- headache, bilateral hemianopsia and hypopituitarism in cases of prolactinoma.

Main differential diagnoses

In cases of confirmed hyperprolactinemia, the physiological and pharmacological causes enter differential diagnosis with the pathological causes, as described below.

Causes

There are many causes of hyperprolactinemia. These can be grouped into three main categories (physiological, pathological, and pharmacological) as reported in Table 1.VII.

Diagnosis

The diagnosis of hyperprolactinemia includes 4 steps:

- step 1 – measurement of blood prolactin levels. Serial prolactin level measurement in three stages (0, 15 and 30 min) could be also considered. In fact, prolactin levels may increase in cases of strong stress. Prolactin levels usually correlate with the size of the prolactinoma;

TABLE 1.VII. Causes of hyperprolactinemia.

Etiological categories		Cause
Physiological Coitus Exercise Stress Sleep		Pregnancy, lactation
Pathological	Disorders of hypothalamus or hypothalamic-pituitary stalk	Granulomas, infiltrative disorders
		Rathke's pouch cyst
		Trauma or surgery
		Tumors (including craniopharyngioma)
	Pituitary gland disorders	Prolactinoma (the most common cause)
		Other pituitary adenomas displacing the hypothalamus–pituitary stalk
		Empty sella syndrome
		Lymphocytic hypophysitis
	Systemic disorders	Primary hypothyroidism
		Chronic renal failure
		Liver cirrhosis
		Polycystic ovary syndrome
		Neurogenic: chest wall injuries (thoracotomy, herpes zoster, burns, etc.); spinal cord injury
		Seizures
		Ectopic production (rare)
Drugs		Antipsychotics (typical and atypical) and dopamine agonists and antagonists
		Antidepressants (tricyclic antidepressants, monoamine oxidase [MAO] inhibitors, SSRIs)
		Antihypertensive (verapamil, labetalol, reserpine, methyldopa)
		Phenytoin
		Metoclopramide, domperidone
		Anesthetics
		Estrogens
		Antihistamines
		Substances of abuse (including alcohol)

- step 2 – exclusion of physiological and pharmacological causes by thorough medical history;
- step 3 – exclusion of systemic disorders that can cause a rise in prolactin through a careful medical history, physical examination, and blood chemistry (thyroid, liver and kidney function, gonadal panel);
- step 4 – MRI with contrast media in the hypothalamic-pituitary region. In asymptomatic patients, exclude macroprolactinemia: about 15% of prolactin exists in polymeric forms known as "macroprolactin." Some measurements may be distorted in cases of a preponderance (>60%) of the prolactin polymeric forms over the monomeric ones.

Treatment

Drug therapy with dopamine agonists (cabergoline or bromocriptine) is the therapeutic strategy of choice in patients with prolactinoma to lower prolactin levels, reduce tumor size and restore gonadal function in patients with macroadenoma or symptomatic microadenoma. Current recommendations suggest not treating patients with non-symptomatic acroprolactinoma. In cases of hyperprolactinemia from etiology other than prolactinoma, the resolution of the triggering cause (*e.g.,* drug suspension, treatment of hypothyroidism, etc.) will also lead to the resolution of the hyperprolactinemia.

CENTRAL HYPOTHYROIDISM AND HYPERTHYROIDISM

Definition

Thyroid function disorders due to alterations in thyroid stimulating hormone (TSH) secretion by the thyrotropic cells of the anterior pituitary gland.

Etiology

Main causes of central hypothyroidism and hyperthyroidism are expressed is Table 1.VIII.

Clinical presentation, differential diagnosis, and treatment

Clinical presentation, differential diagnosis and therapy are comparable to those of primary thyroid disorders.

TABLE 1.VIII. Main causes of central hypothyroidism and hyperthyroidism.

Cause		Specific cause
Primary anterior pituitary gland disorders	Central hyperthyroidism	TSHoma, rare TSH-secreting pituitary adenoma
	Central hypothyroidism	Causes similar to those of hypopituitarism
Dysfunctions in TRH production and secretion		

TSH: thyroid-stimulating hormone; TRH: thyrotropin-releasing hormone.

Diagnosis

The diagnosis includes the assessment of TSH, FT4 and FT3 blood levels (Table 1.IX).

TABLE 1.IX. Assessment of TSH, FT4 and FT3 blood levels.

Central hypothyroidism	TSH inappropriately low or within the low range
	Low FT3 and FT4 levels
Central hyperthyroidism	TSH inappropriately high or within the high range
	High FT3 and FT4 levels

TSH: thyroid-stimulating hormone; FT3: free T3; FT4: free T4.

CENTRAL HYPOGONADISM

Definition

Central hypogonadism is a pathological condition due to impaired production of gonadotropins (follicle-stimulating hormone [FSH] and luteinizing hormone [LH]).

SPECIFIC CLINICAL CONDITIONS

Hypopituitarism

Definition

Hypopituitarism is a deficiency of one or more pituitary hormones; if the deficit involves the entire gland, the condition is called panhypopituitarism.

Etiology

The main causes of hypopituitarism are listed in Table 1.X.

Clinical presentation

Clinical presentation depends on:

- etiological cause;
- patient's age;
- onset time;
- hormones involved;
- diabetes insipidus in panhypopituitarism, especially in cases of hypothalamic or post-surgical etiology.

TABLE 1.X. Main causes of hypopituitarism.

Cause	Specific cause
Idiopathic and genetic	Hypothalamic or pituitary deficiency
	Hormone synthesis deficiency
Acute	*Post-partum* necrosis (Sheehan Syndrome)
	Internal carotid aneurysm
	Cranial trauma
	Lymphocytic hypophysitis
	Iatrogenic causes (post-surgical, radiation)
Chronic	Pituitary adenomas (more frequent)
	Infectious and granulomatous diseases (sarcoidosis, histiocytosis, tuberculosis, syphilis, haemochromatosis)
	Metastasis
	Hypothalamic tumors

Diagnosis

The diagnosis includes two steps:

- step 1 – pituitary reserve screening: measurement of blood levels of TSH, PRL, LH, FSH, IGF-1, cortisol, estradiol/testosterone. The basal measurements can be followed by challenge tests, if appropriate;
- step 2 – pituitary MRI with contrast agents.

Treatment

Administration of hormones that are deficient. It is important to underline that in cases of adrenal insufficiency and hypothyroidism, adrenal insufficiency must be treated first and therapy for hypothyroidism introduced subsequently, to avoid an adrenal crisis.

Empty sella syndrome

Definition

No evidence of pituitary gland in the sella turcica on X-ray.

Etiology

Main causes of empty sella syndrome are listed in Table 1.XI.

TABLE 1.XI. Main causes of empty sella syndrome.

Cause	Specific cause
Primary empty sella	Due to incomplete development of the sellar diaphragm associated with other factors: intracranial hypertension, obesity, high blood pressure and pregnancy
Secondary empty sella	Due to pituitary gland atrophy associated with: Infarction, surgery, radiotherapy and macroadenoma with compression effect

Clinical presentation

Clinical presentation includes:

- absence of specific symptoms. In most cases, the finding is occasional;
- headache, the most common symptom, followed by other neurological and ophthalmological disorders;
- specific hormone deficiencies (GH deficiency is the most common);
- hyperprolactinemia due to impaired dopamine release further to the pituitary stalk stretching.

Differential diagnosis

Differential diagnosis shows:

- intracranial hypertension;
- radiologically similar cystic expansive lesions, such as arachnoid cysts and Rathke's pouch cyst.

Diagnosis

The diagnosis presents two steps:

- step 1 – pituitary reserve screening, that is measurement of blood TSH, PRL, LH, FSH, IGF-1, cortisol, estradiol/testosterone levels. The basal measurements can be followed by challenge tests, if appropriate;
- step 2 – pituitary gland MRI with contrast agents.

Treatment

Treatment choices include:

- no therapy, if asymptomatic;
- surgical treatment, in case of rhinoliquorrhea or visual field changes;
- therapy recommended in case of hypopituitarism and hyperprolactinemia.

TUMORS OF HYPOPHYSIS AND HYPOTHALAMUS

CRANIOPHARYNGIOMA

DEFINITION

Craniopharyngioma is a benign suprasellar tumor of epithelial origin that develops along the craniopharyngeal duct. Typical of young subjects.

CLINICAL PRESENTATION

Clinical presentation of craniopharyngioma is listed Table 1.XII.

TABLE 1.XII. Clinical presentation of craniopharyngioma.

Symptoms of intracranial hypertension	Headache
	Nausea
	Vomiting
	Stuporous state
Compression symptoms of the optic pathways	Visual and field defects
Symptoms of pituitary and hypothalamic compression	Diabetes insipidus
	Hypopituitarism

DIAGNOSIS

The diagnosis considers:

- absence no tumor markers;
- hormonal assessment to diagnose hypopituitarism;
- brain MRI;
- brain CT (the presence of calcifications is pathognomonic).

TREATMENT

Treatment of craniopharyngioma includes:

- surgical removal by transsphenoidal or transcranial route;
- if there are contraindications to surgery: radiotherapy.

Prior to or after therapy, deficiencies in hypothalamic-pituitary secretion may occur and they can influence the need for hormone replacement therapy.

PROGNOSIS

Prognosis data indicates that:

- prognosis is established in relation to the tumor size and location;
- the 5-year survival rate is 55 to 85%;
- relapses occur more frequently within 1 year from surgery.

Pituitary adenomas

Definition

Pituitary adenomas are benign epithelial tumors originating from the cells of the anterior pituitary gland. They are the most common lesions of the sellar region, accounting for 10 to 15% of intracranial tumors (Table 1.XIII).

Clinical presentation

Clinical presentation of pituitary adenomas is shown in Table 1.XIV.

Diagnosis

Diagnosis can be made through:

- pituitary MRI with contrast agents;
- study of pituitary function (TSH, FSH, LH, PRL, IGF-1, ACTH, cortisol) – both hormonal hyperfunction and hypopituitarism may occur.

Differential diagnosis

Differential diagnosis regards other tumors or masses within the sella turcica.

Treatment

Treatment of pituitary adenomas includes:

TABLE 1.XIII. Classification of pituitary adenomas.

Dimensions	Microadenomas (<1 cm)	Macroadenomas (>1 cm)
Localization	Intrasellar	Extrasellar
Secretory activity	Secretory	Non-secretory

TABLE 1.XIV. Clinical presentation of pituitary adenomas.

Secretory adenomas	Endocrine manifestations related to the secreted hormone: the most frequent are prolactinomas.
Non-secretory adenomas or adenomas secreting non-active substances	Compressive effects such as visual/field disturbances
	Headache
	Hypopituitarism
MEN1: multiple endocrine neoplasia 1 syndrome.	

- transsphenoidal surgical resection for:
  - functional adenomas (except for prolactinomas) – treatment of choice;
  - macroadenomas and adenomas that cause visual or neurological symptoms;
- postoperative complications (rhinorrhea, third cranial nerve palsy and pan-hypopituitarism);
- medical therapy, for example for prolactinomas;
- stereotactic radiotherapy – in presence of surgical contraindications.

In case of pituitary incidentaloma, after ruling out the presence of symptoms, straightforward serial MRI monitoring (6 to 12 months) can be performed to evaluate any growth.

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THYROID DISEASES

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2

In normal physiological conditions both synthesis and secretion of thyroid hormones are regulated by the hypothalamus-pituitary-thyroid system. Thyrotropin-releasing hormone (TRH) from the hypothalamus stimulates the release of thyrotropin-stimulating hormone (TSH) at the pituitary level. In its turn, TSH induces the production of thyroid hormones (triiodothyronine or T3 and tetraiodothyronine or T4). The hypothalamus-pituitary-thyroid system is regulated mainly by:

- thyroid hormone synthesis, particularly influenced by iodine intake;
- sympathetic nervous system activity;
- mechanisms that induce the extrathyroid production of iodothyronines.

The effects of TSH on the thyroid gland are:

- stimulation of iodine uptake and organification processes;
- cell hyperplasia and increased glandular vascularization;
- synthesis and regulation of the secretion of thyroid hormones.

Under physiological conditions, the thyroid is able to provide up to 80% of T4 and up to 20% of T3 synthesis. The remaining T3 hormone levels are due to peripheral T4 deiodination. There are three types of deiodinases:

- deiodinase 1 (D1) and deiodinase 2 (D2) – are responsible for most of the circulating T3 production and are involved in the cellular activation of T4;
- deiodinase 3 (D3) – provides the metabolically inactive inverse T3.

Most thyroid hormones circulate in the blood, bound to transport proteins such as albumin and thyroid-binding globulin and enter the cells *via*

specialized transporters. Despite being very low, the free T4 and T3 levels (FT3 and FT4) determine the biological action of thyroid hormones. Some of the circulating iodothyronines are metabolized in the liver, secreted in the enteric system and partially reabsorbed in the distal ileum into the systemic circulation.

SIMPLE OR NON-TOXIC GOITER

Definition

"Goiter" indicates an increase in the volume of the gland not induced by inflammatory or neoplastic processes: this pathology is usually more diffuse in women. From the point of view of function, it is possible to distinguish between:

- simple goiter, characterized by euthyroidism;
- toxic goiter, characterized by hyperthyroidism.

From an anatomopathological point of view, goiter can be classified as:

- diffuse;
- nodular.

Finally, from an epidemiological point of view, goiter is classified as:

- familial goiter that occurs frequently in several members of the same family;
- sporadic goiter occurring in less than 10% of the general population;
- endemic goiter, when present in more than 10% of the general population in a given geographical area. To date, iodine deficiency represents the most common cause of endemic goiter.

With the spread of iodized salt and the introduction of iodates in fertilizers, animal foods and food preservatives, iodine deficiency has become relatively rare in developed countries. According to the new Recommended Nutrient Intake Levels, the iodine daily requirement in adults is 150 μg; in children and adolescents it is between 90 μg and 120 μg; while the requirement increases to 220 μg *per* day during pregnancy and up to 290 μg *per* day during breastfeeding. Please note that in the latter two cases, a possible iodine deficiency increases the risk of abortion and of cognitive impairment in the child.

Etiology

The etiology and the main causes of goiter are:

- iodine deficiency (most common cause);
- goitrogenic foods – sprouts, cabbage, broccoli, cassava;
- drugs – antithyroid agents, lithium, iodized contrast agents, amiodarone, expectorants, iodine tinctures, iodopovidone;
- dyshormonogenesis – thyroid hemiagenesis, thyroglossal duct cyst, Pendred Syndrome (an autosomal recessive disease caused by a defect in iodine organification due to pendrin enzyme deficiency; clinically it is characterized by simple goiter or hypothyroidism and sensorineural hearing loss);
- benign and malignant neoplasms;
- other – acromegaly, TSH-secreting pituitary adenoma, hydatidiform mole and choriocarcinoma.

Clinical presentation

The clinical picture may be silent or characterized by compression disorders (dysphagia, dyspnea, dysphonia). Pemberton's sign may be noticed (facial congestion while keeping both arms elevated, also accompanied by dizziness and syncope) in patients with more voluminous goiters.

Diagnosis

The diagnosis can be made considering:

- the measurement of thyroid function to exclude the presence of a toxic goiter;
- antibody levels – antithyroglobulin (TGAb) and antithyroid peroxidase (TPOAb) antibodies (to exclude the presence of autoimmune thyroiditis in euthyroid status);
- imaging – color Doppler ultrasonography to define the size or the possible presence of thyroid nodules;
- neck-thorax computed tomography (CT) scan – only in case of submerged goiter with possible tracheal deviation.

Therapy

Treatment consists of:

- for asymptomatic patients – clinical and ultrasound monitoring over time (promote the use of iodized salt);

- for symptomatic patients with signs and/or symptoms of compression – thyroidectomy or I-131 radiometabolic therapy (only if there is hyperfunction) or thermoablation in patients with high surgical risk.

HYPOTHYROIDISM

Definition

Hypothyroidism is a clinical syndrome characterized by the insufficient action of thyroid hormones (triiodothyronine and tetraiodothyronine) at tissue level, leading to a generalized downregulation of most of the body's metabolic processes.

Epidemiology notes

Clinically overt hypothyroidism ranges from 0.1% to 1% in the general population. Women are generally the most affected. Furthermore, positive antithyroid peroxidase antibodies are present in about 5% of adults and 15% of postmenopausal women in the general population.

Etiology overview in relation to pathophysiology

Hypothyroidism can be divided into:

- primary – if the damage that leads to hypothyroidism is of thyroid origin;
- secondary – if the organ damage is primarily in the pituitary;
- tertiary – if the primary organ damage is in the hypothalamus;
- peripheral – if there is a receptor alteration in various organs and tissues that blocks or reduces the action of iodothyronines;
- hypothyroidism due to primary alterations of iodothyronine transport proteins.

Different clinical conditions (hepatitis, cirrhosis, nephrotic syndromes or protein-losing diseases) or drugs (estrogens, glucocorticoids, mitotane, methadone, selective estrogen receptor modulators [SERM]) can alter the bioavailability of iodothyronine transport proteins with subsequent altered bioavailability of iodothyronines. The first four types of hypothyroidism include congenital and acquired causes (**Table 2.I, 2.II**).

TABLE 2.I. The most common forms of congenital hypothyroidism according to pathophysiological picture.

Primary	Secondary/tertiary	Peripheral resistance to hormones
Agenesis/dysgenesis	TRH deficit	Generalized
Hormone genesis defects	Isolated TSH deficit	
Iodine deficiency	Hypopituitarism	
Transplacental passage of antibodies/drugs		

TABLE 2.II. The most common forms of acquired hypothyroidism in relation to pathophysiological picture.

Primary	Secondary	Alterations of transport proteins
Autoimmune chronic thyroiditis	Hypothalamic tumor	Estrogens
Thyroidectomy	Pituitary tumors	Glucocorticoids
Radiometabolic treatment	Hypothalamus/pituitary region surgery	SERM
External radiotherapy	Hypophysitis	Methadone
Riedel thyroiditis	Radiation	Mitotane
Iodine deficiency	Cranial trauma	Cirrhosis
Drugs and natural goitrogenic agents		Nephrotic syndrome

Primary hypothyroidism

Primary hypothyroidism accounts for 90-95% of hypothyroidism patients. It includes congenital (congenital hypothyroidism) or acquired (acquired hypothyroidism) thyroid disease which causes a reduced secretion of thyroid hormones with a consequent compensatory increase in TSH levels.

Congenital primary hypothyroidism

This condition of hypothyroidism has an incidence of about 1/4000 newborns (**Table 2.I**). Thyroid agenesis and dysgenesis are the most frequent conditions and can be induced by cytotoxic insults (infections) that have affected the mother in the early stages of gestation, compromising adequate thyroid differentiation during fetal development. More than 50% of cases of congenital/infantile hypothyroidism can be caused by partial

or total ectopy of the thyroid tissue (of which lingual ectopy is the most common). There are also hormonogenesis defects of autosomal recessive inheritance, which can compromise:

- sodium/iodide symporter action;
- peroxidase action, involved in iodine organification (Pendred Syndrome);
- conversion of mono- and diiodothyronine to T3 and T4;
- deiodination of iodotyrosine.

Finally, there is a particular condition of transient congenital hypothyroidism, which may be due to:

- transplacental passage of maternal TSH receptor-blocking autoantibodies. This condition usually persists for 1 to 3 months (maternal antibody clearance time);
- the mother's intake of thyrostatic agents;
- iodine deficiency due to inadequate maternal dietary intake;
- exposure to high iodine concentrations, which can occur in newborns whose mothers are treated with amiodarone, iodine-containing disinfectants or iodinated contrast media. These may cause a functional inhibition of the thyroid gland.

Acquired primary hypothyroidism

Acquired primary hypothyroidism in its most common forms is reported in Table 2.II.

Chronic autoimmune thyroiditis (Hashimoto's thyroiditis)

Chronic autoimmune thyroiditis (CAT) is the most common cause of hypothyroidism in areas with sufficient iodine and is caused by cellular destruction of thyroid tissue. Over 90% of patients with chronic autoimmune thyroiditis have high levels of antithyroid peroxidase antibodies (TPOAb) and/or antithyroglobulin antibodies (TGAb). Less frequently, there are antibodies that block TSH action on its receptor (TRAb). These antibodies have little or no functional activity: thyroid cell destruction is thought to be mediated primarily by cytotoxic CD8+ T lymphocytes. In most cases, the result is permanent hypothyroidism. The disease is charac-

terized by a marked genetic susceptibility and its best-known association is with human leukocyte antigen (HLA) genes. There are two main variants of Hashimoto's thyroiditis:

- goiter-associated;
- atrophic.

These conditions share the same pathophysiological aspect, but differ according to lymphocytic infiltrate, fibrosis and thyroid follicular cell hyperplasia. Atrophic thyroiditis is the final stage of Hashimoto's thyroiditis.

Iodine

Hypothyroidism can be caused by both a deficiency and an excess of iodine. Iodine deficiency is the most common cause of hypothyroidism associated with goiter. Low dietary intake refers to less than 100 µg/day (particularly low in mountain areas). The Wolff-Chaikoff effect explains why excess iodine can also cause hypothyroidism. It is responsible for the inhibition of iodine organification as well as T4 and T3 synthesis. While normal subjects develop an efficient "escape" mechanism from such effects of iodine, this does not happen in patients with thyroid disease. These patients may develop hypothyroidism if their iodine intake is higher even for short periods. Patients at risk for iodine-induced hypothyroidism are those with chronic autoimmune thyroiditis, those subjected to subtotal thyroidectomy and radiometabolic therapy or those with *post-partum* and subacute thyroiditis. In addition, excess iodine can be caused by the use of drugs (amiodarone, betadine) or other products with a high concentration of iodine (iodinated contrast agents).

Drugs

Drugs to be used are:

- thyrostatic agents – propylthiouracil, methimazole, potassium perchlorate;
- alpha-interferon;
- interleukin 2 (IL-2);
- tyrosine kinase inhibitors (*e.g.* sunitinib);
- immune checkpoint inhibitors (*e.g.* nivolumab);

- drugs that reduce L-thyroxine absorption in patients treated with LT4 – sucralfate, aluminium hydroxide, proton pump inhibitors, cholestyramine;
- drugs that increase L-thyroxine clearance – carbamazepine, rifampicin, phenytoin.

Central (secondary/tertiary) hypothyroidism

This condition is due to reduced TSH secretion from the anterior pituitary gland (secondary hypothyroidism) or reduced TRH secretion from the hypothalamus (tertiary hypothyroidism). It accounts for less than 1% of cases of hypothyroidism.

Hypothyroidism due to generalized resistance to thyroid hormones

This occurs due to a reduced or null response of the target tissues to the action of thyroid hormones.

Diagnosis

The diagnosis of hypothyroidism should be based on clinical suspicion and subsequently confirmed by blood tests for TSH and FT4. In fact, most of the symptoms are non-specific and a TSH level over the reference range, together with a normal FT4 level, would allow hypothyroidism (subclinical hypothyroidism) to be diagnosed at an earlier stage (Table 2.III). TSH levels alone in some conditions may not be useful for a clear diagnosis of hypothyroidism (*i.e.*, secondary/tertiary hypothyroidism; with the use of drugs that can reduce the TSH level [glucocorticoids, somatostatin analogues, dopamine, phenytoin]; and drugs that can increase TSH level [amiodarone, domperidone, dopamine agonists]). Testing for TGAb and TPOAb levels is fundamental to understand the potential autoimmune nature of hypothyroidism (see chronic autoimmune thyroiditis).

TABLE 2.III. Classification of hypothyroidism according to the biochemical/clinical picture.

Type	TSH	FT3-FT4	Clinical picture
Primary subclinical	▲	=	Negative or indistinct
Primary clinical	▲	▼	Manifest
Secondary/tertiary	=/▼	▼	Indistinct/manifest
Peripheral	=/▲	=/▲	Variable

Clinical notes

Congenital hypothyroidism

Congenital hypothyroidism is the most common cause of treatable intellectual disability. In this condition, the symptoms manifest around the fourth month of extrauterine life, when the thyroid hormone intake *via* breast milk becomes insufficient. Other signs/symptoms are drowsiness, sucking and breathing difficulties, hypothermia, prolonged neonatal jaun-

TABLE 2.IV. Congenital hypothyroidism.

Organs and systems	Pathophysiology	Signs and symptoms
Endocrine system	▼Growth hormone ▼Adrenal and gonadal steroids ▼Basal metabolism ▲Prolactin ▼Testosterone levels	Low stature (children) Alterations of menstrual cycle and anovularity Erectile dysfunction and/or delayed ejaculation Hypothermia Alteration of lipid profile + xanthelasma Asthenia
Skin and adnexa	Vasoconstriction Accumulation of glycosaminoglycans Water retention in dermis Decreased sweat gland and sebaceous secretion Early arrest in the anagen phase of hair growth	Pale, dry, rough and finely peeling skin Thin and fragile capillaries Skin cold to the touch Brittle, dry, thin hair, prone to falling out and thinning Loss of the lateral part of the eyebrows Rarefaction of axillary and pubic hair Myxedema[1,*] Carotenoderma[2,*] Thin, brittle and streaked nails Delayed wound healing Eyelid oedema Thyroid acropachy[3]
Cardiovascular	Bradycardia ▼Systolic output Peripheral vasoconstriction Cardiomegaly* Pericardial effusion* Heart failure*	▼Systolic blood pressure ▲Diastolic blood pressure
Hematopoietic	Normochromic normocytic anæmia due to reduced bone marrow red cell production	Asthenia

To be continued

TABLE 2.IV. Congenital hypothyroidism (*continues*).

Organs and systems	Pathophysiology	Signs and symptoms
Respiratory	▼Ventilatory capacity due to respiratory muscle weakness and reduced ventilatory response to hypoxemia	Dyspnœa
Renal	▼Glomerular filtrate ▼EPO production	Oliguria Hypodipsia (reduction of thirst sensation) Anæmia
Nervous system	Not well known but likely influenced by: Hyponatremia Demyelination	Apathy Mental torpor Easy irritability Bradypsychism Bradylalia Bradyphagia Drowsiness Coma*
Gastrointestinal	Motility slowing	Constipation of varying degrees
Muscular skeletal	▼Muscle protein synthesis	Arthralgia Tenderness of muscle masses Rigidity

* In severe states.
[1] Oedema of the subcutaneous area, with water retention and tissue thickening, which causes the skin to become pale and yellowish due to alteration of the functionality of the sebaceous and sweat glands.
[2] Yellow–orange colour due to the accumulation of carotene in the stratum corneum.
[3] Pathological enlargement (clubbing) of the fingers and toes with a drumstick-like shape, also known as Pierre-Marie pneumatic hypertrophic osteoarthropathy.
EPO: erythropoietin.

dice, growth deficit and nervous and muscular system development disorders (Table 2.IV).

Myxedema coma

This rare medical emergency is characterized by severe hypothyroidism with hypothermia, mental status deterioration and eventually coma. It is the final stage of an untreated long-standing severe hypothyroidism and may be precipitated by an acute event (myocardial infarction, infection, opiate use). Urgency requires levothyroxine treatment to be started on the basis of clinical suspicion without waiting for the results of lab tests.

Therapy notes

Synthetic analogue of levothyroxine

Levothyroxine is the natural levorotatory isomer of thyroxine, an amino acid containing iodine that is incorporated in thyroglobulin. The main indications of levothyroxine therapy are:

- treatment of all types of hypothyroidism;
- TSH-suppressive therapy of non-toxic nodular goiter (an increasing obsolete indication due to the potential occurrence of side effects affecting the cardiovascular and skeletal systems).

The average daily replacement dose for maintenance in adults is 1.5-2.0 µg/kg/day.

Subclinical hypothyroidism

The debate on the intervention threshold in this diagnostic category is still open. The L-thyroxine treatment goal is to prevent conversion to overt hypothyroidism, which occurs at a rate of around 5% *per* year and doubles in the case of antibody positivity (TPOAb and TGAb). The introduction of L-thyroxine is recommended for TSH values >10 µU/mL; for TSH levels lower than 10 µU/mL, it is necessary to integrate the blood chemistry panel test with the clinical picture. More specifically, age, family history, antibody profile, thyroid volume and metabolic structure must be considered.

THYROTOXICOSIS AND HYPERTHYROIDISM

Definition

Thyrotoxicosis refers to a clinical picture characterized by an excess of blood thyroid hormones regardless of the cause, while hyperthyroidism refers to conditions where the excess of thyroid hormones derives from their increased production by the thyroid gland.

Causes

The causes of hyperthyroidism and thyrotoxicosis without hyperthyroidism are listed in Table 2.V.

TABLE 2.V. Causes of hyperthyroidism and thyrotoxicosis.

Hyperthyroidism	Thyrotoxicosis without hyperthyroidism
Flajani-Basedow-Graves' disease	Iatrogenic thyrotoxicosis
Toxic multinodular goiter	Thyrotoxicosis factitia
Autonomous nodule	Hashitoxicosis
Iodine excess	Subacute thyroiditis
TSH-secreting pituitary adenoma	Postpartum thyroiditis
Thyroid tumor metastasis	
Struma ovarii	
Choriocarcinoma	
Hydatidiform mole	

TABLE 2.VI. Symptoms of Basedow's disease and multinodular goiter.

Clinical signs	Basedow's disease	MNG and autonomous nodule
Hyperactivity and psychomotor restlessness	++	+
Tachycardia	+++	+++
Atrial fibrillation	0/+	+
Weight loss	+++	++
Asthenia	++	+
Hyperhidrosis	++	++
Intolerance to heat	++	++
Pruritus	+	-
Tremors	++	++
Loose stools	++	+
Polyphagia (increased appetite)	+	-
Irregular periods	+	+
Ophthalmopathy	++	-
Damp and fine skin	++	++
Decreased libido	+	+

MNG: multinodular goiter.

Symptoms

The symptoms of Basedow-Graves' disease and multinodular goiter are reported in Table 2.VI.

Flajani-Basedow-Graves' disease

Graves' disease is a condition characterized by diffuse goiter and hyperthyroidism with potential association of a typical form of ophthalmopathy. Graves' disease is the most frequent cause of hyperthyroidism under 40 years of age. It has a higher frequency in women (female:male ratio of 5:7.1). The autoimmune origin of the disease is confirmed by the presence of TSH receptor-stimulating autoantibodies (TRAb): they are responsible for receptor activation with subsequent increased synthesis and secretion of T3 and T4, and for thyroid growth. HLA system-related genetic susceptibility and cigarette smoking are the most predisposing factors for disease onset and severity. Infections, stress

and drugs (containing large amounts of iodine) are also considered to be precipitating factors for Graves' disease. From a clinical point of view, patients with Graves' disease can present with typical "basedowian faces:"

- anxious expression on the face;
- exophthalmos associated with potential positivity of certain ocular signs, such as eyelid edema, Graefe's sign (failure to lower the upper eyelid during downgaze), Stellwag's sign (rarity of blinking), Dalrymple's sign (widening of the eyelid rims), conjunctival hyperemia, Moebius sign (associated ocular motility disorders: convergence insufficiency of the eyeballs when looking at nearby objects), Sattler's sign (spontaneous nystagmus jolts), Sainton's sign (nystagmus caused by lateral direction of the gaze), Suker's sign (divergent strabismus with rapid change of gaze direction in the lateral-frontal direction). Furthermore, the difficulty in completely closing the eyelids often leads to keratitis, in association with chemosis and conjunctival hyperemia;
- diffused goiter.

On palpation, the thyroid appears parenchymatous and uniformly increased in volume. The vessels of the overlying skin may be dilated with potential signs of red dermographism on the anterior neck region.

Diagnosis

Weight loss, menstrual cycle alterations, psychomotor restlessness, extrasystoles and/or the onset of atrial fibrillation and myopathies are signs and symptoms that should indicate hyperthyroidism. Lab tests will detect a condition of hyperthyroidism (TSH secretion suppressed and high hormonal free fractions) usually with T3>T4, due to an increased peripheral conversion of T4. Furthermore, most Basedow patients show a marked positivity for TRAb. These belong to the IgG1 subclass (they are therefore oligoclonal, unlike anti-TG and anti-TPO antibodies which are polyclonal) and are specific for Graves' disease. Thyroid ultrasound is used to assess the glandular volume (usually increased) and vascularization (increase in vascularization, which causes the classic picture of thyroid inferno). Thyroid scintigraphy (no longer employed) after an adequate pharmacological wash-out of iodized substances, shows an intense increase in I-131 uptake.

Scintigraphy may be useful for the differential diagnosis of other forms of thyrotoxicosis, such as subacute thyroiditis and hashitoxicosis (low uptake).

Toxic multinodular goiter

Toxic or hyperfunctioning multinodular goiter is the most frequent cause of hyperthyroidism in the elderly. The persistence of multiple nodular formations may cause some of these to have an autonomous capacity for hormone production over time (the so-called "hot" nodules on scintigraphy), together with others that have poor biosynthetic capacity and therefore will have normal or poor function ("cold" on scintigraphy).

Diagnosis

Cardiovascular symptoms (tachycardia, atrial fibrillation) and apathy are predominant: these conditions are definitely more typical in the elderly. In addition, the patient may report compression symptoms due to the increase in the thyroid gland volume. Blood tests show hyperthyroidism in the absence of TRAb positivity. Ultrasound shows a clear increase in the gland volume with numerous nodular formations. However, scintigraphy may potentially reveal the presence of nodular areas of increased uptake alternating with areas of low uptake.

Toxic adenoma

Thyroid adenoma is the most frequent benign neoplasm affecting the thyroid gland. It derives from regional glandular proliferation separated from the remaining thyroid parenchyma by a fibrous connective capsule. It is prevalent in women and rarely associated with clinical signs of hyperthyroidism. When such signs are present, the adenoma is referred to as toxic adenoma or Plummer's disease. Generally, toxic adenomas are almost always follicular adenomas with a very low probability of malignancy.

Diagnosis

The patient reports symptoms of hyperthyroidism and the absence of ocular impairment. Thyroid scintigraphy usually shows a high uptake nodule with suppression of the remaining glandular parenchyma (warm nodule).

On ultrasound, the nodule appears predominantly solid, with clear margins and with a type III vascular (peri- and intralesional) pattern.

Therapy notes

The treatment goal is to reduce thyroid hormone activity: it may be achieved through surgical, I-131 radiometabolic and pharmacological treatment (Table 2.VII).

TABLE 2.VII. Therapy for hyperthyroidism.

Parameters	Thyroid-ectomy	I-131	Pharmaco-logical treatment
Large goiter	x		
Relapse after medical therapy	x	x	
Inability to follow medical therapy	x	x	
Basedow's disease + thyroid carcinoma	x		
High surgical risk		x	x
Poor control during pregnancy	x*		x
Ophthalmopathy	x		x

*In special cases only in the second trimester of pregnancy.

Surgery

Thyroidectomy or near total thyroidectomy definitively solves the problem of thyrotoxicosis. However, it should not be recommended when there is a high surgical risk. Post-surgical hypoparathyroidism and recurrent nerve damage are complications to be considered.

Radiometabolic treatment with I-131

The goal of treatment is to destroy the hyperfunctioning thyroid tissue by administering radioactive iodine. Elderly patients and/or those at high surgical risk or young patients with small toxic goiters are the main candidates for this treatment. Plummer's toxic adenoma greatly benefits from radioiodine treatment if there are not mass formations of substantial volume. Hypothyroidism is the most frequent side effect. The ophthalmopathy is a relative contraindication to the treatment.

Pharmacological treatment

A large part of the medical and scientific community still considers pharmacological treatment to be the first therapeutic approach for a patient

with hyperthyroidism. Antithyroid drugs can also be used as a preparatory treatment for therapy with I-131 or during pregnancy. The most commonly used drug classes are thioamides and propylthiouracil. Methimazole, the main member of the thioamide group, blocks thyroid hormone synthesis by inhibiting iodide oxidation to organic iodine. Iodine organification is thus inhibited in the tyrosine residues of thyroglobulin. In addition, iodine organification impairment causes inhibition of the production of anti-TSH receptor antibodies. Propylthiouracil (PTU), a compound derived from thiouracil, inhibits the thyroid peroxidase enzyme, normally involved in thyroid hormone synthesis by oxidizing the iodide anion and facilitating the incorporation of iodine into the tyrosine residues of thyroglobulins. PTU is also able to inhibit the 5'-deiodinase enzyme which peripherally converts T4 into the active form T3. For both compounds the occurrence of side effects is low. In rare cases, episodes of agranulocytosis, leukopenia, aplastic anæmia and thrombocytopenia have been described.

THYROIDITIS

Acute bacterial thyroiditis (or suppurative or pyogenic)

Bacterial thyroiditis is a rather rare manifestation, usually caused by diffusion through the blood, lymphatic system or by contiguity of a Gram-positive bacterial infection (*Staphylococci*, *Streptococci*) (Table 2.VIII). The symptoms are those typical of an infection, with swelling in the anterior neck region: it will be painful and sore to the touch but soft. The skin above it is often hot and red. Usually, thyroid function is within the normal range, often associated with a predominantly neutrophilic leukocytosis and an increase in inflammation markers. Ultrasound may show a purulent collection (significant hypoechoic/anechoic areas). Treatment is based on the use of broad-spectrum antibiotics possibly associated with surgical drainage of the lesion.

Subacute thyroiditis (Quervain's or granulomatous)

Subacute thyroiditis is an inflammatory condition, possibly secondary to viral infection. The disease usually occurs in the weeks following an upper

TABLE 2.VIII. Thyroiditis.

Parameters	Acute	Subacute (De Quervain's)	Transient or silent lymphocytic	Chronic lymphocytic (Hashimoto's)	Fibrosing (Riedel's)
Etiology	Bacterial	Viral	Autoimmune	Autoimmune	Idiopathic
Clinical signs	Pain, swelling, skin rash, fever	Pain, previous upper respiratory tract infection, low-grade fever, signs of hyperthyroidism	Painless goiter, possible initial thyrotoxicosis, sometimes hypothyroidism	Painless goiter of elastic consistency, frequent definitive hypothyroidism, sometimes initial thyrotoxicosis	Goiter stony hard in consistency, possible compressive symptoms, sometimes hypothyroidism
Diagnosis	Leukocytosis, TSH=	ESR▲, TSH ▲/▼/=, TPOAb–, scintigraphy with abolished uptake	ESR=, TSH ▲/▼/=, low TPOAb+ with low titer, scintigraphy with abolished uptake	Laboratory data compatible with hypothyroidism (TSH▲), high + TPOAb titer	Possible hypothyroidism (TSH▲), TPOAb–, non-uptake scintigraphy
Therapy	Antibiotics and/or drainage	Corticosteroids, β-blockers	β-blockers ± corticosteroids, in case of hypothyroidism levothyroxine	Substitutive therapy with levothyroxine	Surgery or levothyroxine therapy

respiratory infection. Histological alterations include the destruction of the thyroid parenchyma and the presence of many multinucleated giant cells (granulomas). Subacute thyroiditis usually manifests with fever, malaise, and pain in the anterior neck region that can extend to the jaw angle or to the earlobe on one or both sides. Laboratory data may initially show a high erythrocyte sedimentation rate (ESR) and a blood chemistry of thyrotoxicosis with increased free fractions and suppressed TSH (transient thyrotoxicosis phase). However, scintigraphy is mute, and the thyroid parenchyma is focally and markedly non-homogeneous on ultrasound. At a later stage, the inflammation tends to subside and is followed by a functional phase of euthyroidism or hypothyroidism (in case of permanent damage). Treatment is mostly symptomatic and involves the use of glucocorticoids, which usually cause the pain to disappear after the first 24 hours from the beginning of the treatment. Furthermore, β-blockers (propanol) can also be used in presence of significant tachycardia.

Lymphocytic, silent or postpartum thyroiditis

It is a condition of autoimmune origin that usually occurs 2-6 months *postpartum* in women with antithyroid antibodies in the first trimester of pregnancy. Usually, the higher the antibody concentration, the greater the risk of developing thyroiditis. The patient may show a transient phase of mild or subclinical hyperthyroidism, followed by euthyroidism/hypothyroidism. The thyroid is not painful, but only increased in volume due to both inflammation and potential hypothyroidism, further to TSH stimulation. I-131 uptake is low, and ultrasound shows diffuse inhomogeneity. In case of hypothyroidism, it must be corrected with levothyroxine.

Chronic lymphocytic thyroiditis (or Hashimoto's)

See the "Hypothyroidism" section.

Riedel thyroiditis

A very rare condition characterized by severe diffuse intra- and extraglandular fibrosis. Physical examination shows a diffuse goiter of hard, wooden consistency associated with compression symptoms such as dysphagia, dysphonia or hoarseness. One third of patients experience hypothyroidism. On ultrasound, the destruction of thyroid parenchyma enters the differential diagnosis with thyroid neoplasms to the point of needing a biopsy. Neck surgery is the only treatment for disease-related compression disorders.

THYROID NODULES

Epidemiology notes

Nodular thyroid disease is a clinical condition with 60% prevalence in the general population. In about 7-15% of cases there are nodules with malignant characteristics.

Clinical and diagnostic approach

Following the occasional finding of nodular formations or reported episodes of dysphagia, dyspnea, irritating cough or dysphonia, the first steps to be taken for a correct diagnosis are as follows:

- anamnesis (family history of thyroid disease or thyroid cancer, exposure to ionizing radiation, intake of iodine-containing products, sudden or gradual symptoms over time, etc.);
- objective examination (neck inspection and superficial and deep palpation *e.g.*, parenchyma consistency, tenderness on palpation, mobility when swallowing, identification and localization of one or more nodular formations – which should be examined for consistency, surface characteristics, mobility and possible tenderness – lymphadenomegaly, etc.);
- blood tests (TSH and, in specific cases, calcitonin);
- thyroid ultrasound.

Thyroid scintigraphy with a contrast medium is a diagnostic investigation only in conditions of thyrotoxicosis with TSH below the normal limits. A "hot" (increased uptake) nodule is considered an expression of a low risk of malignancy, while "cold" (low uptake or non-uptake) nodules in reduced percentages (10-15%) may have increased malignancy characteristics.

Specifications on ultrasound assessment

Thyroid ultrasound is usually recommended in the presence of certain clinical features (**Table 2.IX**). Nodular formations identified by ultrasound must be described in detail, highlighting characteristics that can be of different degrees of malignancy (low, medium, high; **Table 2.X**). Elastography, which

TABLE 2.IX. Clinical and prognostic features of malignant nodular thyroid disease that requires further diagnostic investigation with thyroid ultrasound.

Parameters	Variables
Age <14 years or >70 years with the following characteristics	Persistent dysphagia, dyspnoea and/or dysphonia
Male gender	Nodule not mobile on palpation or of hard consistency
Family history of medullary thyroid carcinoma, papillary thyroid carcinoma (1st degree), MEN-2	Increased nodule size
Previous head and/or neck radiation exposure	Suspected cervical lymphadenopathy

TABLE 2.X. Ultrasound features of the thyroid nodule at high risk of malignancy.

Parameters
Marked hypoechogenicity
"Taller-than-wide" shape
Irregular margins
Microcalcifications
Extrathyroid expansion or deformation of the thyroid capsule
Pathological lymphadenopathy

is not a substitute for ultrasound, may provide additional information on nodules with indeterminate ultrasound or cytological characteristics.

Cytological exam through fine needle aspiration (FNA)

After finding one or more ultrasound features listed in **Table 2.X**, and also considering the size of the nodule (as the procedure is not recommended for nodules <5 mm in size), diagnostic investigation is carried out in order to evaluate the cytological characteristics of the nodule. Needle aspiration is usually recommended in the presence of certain clinical and ultrasound features (**Table 2.XI**). In 2016, the American Thyroid Association (ATA) and the American Association of Clinical Endocrinologists (AACE), the American College of Endocrinology (ACE) and the Association of Endocrinologists Physicians (AME) published an ultrasound classification of nodules to identify the useful characteristics for further fine needle aspiration (FNA) investigation. The cytological outcome is based on a classification divided into five diagnostic subclasses (SIAPEC 2014; **Table 2.XII**).

TABLE 2.XI. Features requiring further diagnostic examination by ultrasound-guided thyroid needle aspiration.

Parameters	ATA	AACE/ACE/AME
Benign nodule	Purely cystic nodule	
Very low risk nodule	Spongiform or cystic nodule	
	No features from the other three categories	
Low risk nodule	Isoechoic or hyperechoic (also partially cystic) nodule	Purely cystic or mainly cystic nodule
	No: irregular margins, microcalcifications, extrathyroid extension, "taller-than-wide" morphology	Spongiform isoechoic nodule
Intermediate risk nodule	Hypoechoic nodule	Isoechogenic nodule + one of the following: intranodular vascularization, macrocalcifications, internal hyperechoic spots, high rigidity to elastosonography
	No: irregular margins, microcalcifications, extrathyroid extension, "taller-than-wide" morphology	
High risk nodule	Hypoechoic (even partially cystic) nodule + one of the following: irregular margins, microcalcifications, extrathyroid extension, "taller-than-wide" morphology	Nodule + one of the following: hypoechogenicity, microcalcifications, irregular margins, "taller-than-wide" morphology, extracapsular growth, lymph node involvement

Other diagnostic procedures on the nodule

Ultrasound-guided cutting-needle thyroid biopsy is recommended for in-depth diagnosis of solid nodules with persistently non-diagnostic FNA cytological results.

Other instrumental diagnostic imaging procedures

Magnetic resonance imaging (MRI) and computed tomography (CT) are recommended for nodular goiter (to take measurements that cannot otherwise be documented with ultrasound), any compression of the airways, mediastinum extension and lymph node localization that cannot be assessed with ultrasound. Positron emission tomography (PET) and PET-CT are instrumental investigations that provide additional information on the malignancy characteristics expressed by the nodule.

DIFFERENTIATED THYROID CARCINOMA

Prevalence

Differentiated thyroid carcinoma (DTC) derives from thyroid follicular epithelial cells and includes the vast majority of thyroid cancers (Table 2.XIII, 2.XIV, 2.XV).

TABLE 2.XII. Thyroid nodule cytological classification – Italian Consensus for Thyroid Cytology (SIAPEC 2014).

Classification	
TIR 1	Non-diagnostic (inadequate or insufficient material)
TIR 1c	Cystic, non-diagnostic
TIR 2	Benign
TIR 3A	Indeterminate lesion at low risk
TIR 3B	Indeterminate lesion at high risk
TIR 4	Suspected of malignancy
TIR 5	Malignant

TABLE 2.XIII. The main features of differentiated thyroid cancer.

Papillary carcinoma	Follicular carcinoma
Most common form (80%)	10-20% of thyroid cancer
Sporadic or genetic	Sporadic or genetic
In regions with high iodine content	In regions with deficit in iodine content
2.9-3.8 times more common in women	1.9-3.6 times more common in women
Higher incidence between 25 and 50 years old	Late age (40-60 years old)
Well/poorly differentiated	Invasive/minimally invasive
Lymphatic diffusion	Lymphatic and vascular diffusion
Optimal prognosis	Conditioned by the invasion site/stage

TABLE 2.XIV. Common ultrasound features of differentiated thyroid cancer.

Characteristics	Papillary thyroid cancer papilliferous	Follicular thyroid cancer
Echogenicity	Solid and hypoechoic	Tends to be isoechoic Subtle peripheral halo
Microcalcifications	Present	Not present
Margins	Irregular	NA
Vascularization	Increased	NA

TABLE 2.XV. Clinical/cytological features of differentiated thyroid carcinoma.

Papilliferous carcinoma	Follicular carcinoma
Papillary structure	Follicular structure
Psammoma bodies	Capsule invasion
Non-encapsulated	More or less encapsulated
Multifocal	Almost always unifocal
30% already have lymph node metastasis at diagnosis	Distant (lung, bone) metastasis

TABLE 2.XVI. Main risk factors of differentiated thyroid cancer.

Risk factors
Radiation in the head and neck region – the only documented risk factor
Exposure to environmental radiation
Family predisposition
Age <14 years or >70 years
Genetic factors
Other risk factors
Previous history of multinodular goiter
Family history of Hashimoto's thyroiditis
Female gender for the follicular form
Male gender for the papillary form
Asian race

The main differentiated tumors include:

- the papillary variant, representing about 85% of cases. It includes various subtypes:
  - well-differentiated less aggressive variants (follicular, oxyphilic, cribriform-morular variant) and highly aggressive variants (tall cell, columnar cell, solid and diffuse-sclerosing variant);
  - poorly differentiated variants (insular and large cell variant);
- the follicular variant (it comprises about 12% of cases). Within it there are various subtypes, in particular:
  - Hürthle cell tumor (also called oncocyte tumor or oxyphilic cell carcinoma), considered a subtype of thyroid follicular carcinoma, which originates from the follicular epithelium and is associated with a worse outcome compared to other differentiated tumor types.

Etiology

Within differentiated thyroid carcinoma there are sporadic and genetic forms (Table 2.XVI). The genetic forms of follicular carcinoma are:

- hereditary, including:
  - adenomatous polyposis;
  - Cowden Syndrome;
  - Werner Syndrome;
- familial non-medullary thyroid cancer (its criteria are having ≥2 first-degree relatives with DTC and absence of other syndromes; autosomal dominant inheritance with incomplete penetrance. This cancer is a more aggressive tumor compared to sporadic DTC due to greater local invasion, multicentricity, lymph node metastases and relapse).

Acquired forms, include:

- *PAX8-PPAR* gamma gene rearrangements (effects of *PAX8-PPAR* gamma rearrangement on intracellular signaling are unclear);
- point mutations of *RAS* gene (due to the constitutive activation of the RAS protein, resulting in oncogenesis).

The genetic forms of papillary carcinoma can also be:

- hereditary, such as:
  - Gardner Syndrome (familial adenomatous polyposis);
  - Cowden Syndrome;
  - Werner Syndrome;
  - familial non-medullary thyroid cancer;
- acquired, including:
  - point mutations of the *BRAF* gene;
  - rearrangements of *RET/PTC* genes (common in small multifocal papillary thyroid carcinomas);
  - point mutations of the *RAS* gene (common in poorly differentiated papillary thyroid cancer).

Clinical notes

Staging

The TNM system is among the most commonly used staging systems employed to predict the risk of mortality and to identify the extent of the

disease. It allows disease risk stratification, which is used as a guideline for initial prognosis, disease management and follow-up strategies. An accurate postoperative risk assessment is a crucial element in the management of patients with DTC.

Primary tumor

The primary tumor (T) can be classified as:

- Tx primary tumor cannot be assessed;
- T0 no evidence of primary tumor;
- T1 tumor size ≤2 cm in its greatest dimension, limited to the thyroid;
  - T1a tumor size ≤1 cm, limited to the thyroid;
  - T1b tumor size >1 cm but ≤2 cm in its greatest dimension, limited to the thyroid;
- T2 tumor size >2 cm but ≤4 cm in its greatest dimension, limited to the thyroid;
- T3 tumor size >4 cm limited to the thyroid or extension invading only the extrathyroidal muscles;
  - T3a tumor size >4 cm limited to the thyroid;
  - T3b gross extrathyroidal extension that invades only the muscles (sternohyoid, thyrohyoid or omohyoid muscles) from a tumor of any size;
- T4 includes gross extrathyroidal extension;
  - T4a gross extrathyroidal extension that invades the subcutaneous soft tissues, larynx, trachea, esophagus or recurrent laryngeal nerve from a tumor of any size;
  - T4b gross extrathyroidal extension that invades the prevertebral fascia or encloses the carotid artery or the mediastinal vessels from a tumor of any size.

Regional lymph nodes

Regional lymph nodes (N) can be classified as:

- Nx regional lymph nodes cannot be assessed;
- N0 no evidence of locoregional lymph node metastasis;
  - N0a one or more cytologically or histologically confirmed benign lymph nodes;

- N0b no radiological or clinical evidence of locoregional lymph node metastasis;
- N1 metastasis to regional lymph nodes;
  - N1a metastasis to level VI or VII (pretracheal, paratracheal and prelaryngeal or upper mediastinal) lymph nodes; this can be unilateral or bilateral disease;
  - N1b metastasis to unilateral, bilateral or contralateral lateral neck lymph nodes (levels I, II, III, IV or V) or retropharyngeal lymph nodes.

Distant metastasis (M)

Distant metastasis (M) can be classified as:

- M0 no distant metastases (no M0 pathology; use clinical M to complete stage group);
- M1 distant metastases (lung, bone, central nervous system).

Therapy

General goals of DTC therapy

The general goals of DTC therapy are:

- to improve the overall and specific survival of the disease;
- to reduce the risk of persistent/recurrent disease and associated morbidity;
- to allow for accurate staging and risk stratification;
- to minimize treatment-related morbidity.

Treatment

Total thyroidectomy

This enables diagnostic confirmation and allows the removal of the primary tumor and of the clinically relevant lymph node metastases. In the preoperative phase, neck ultrasound for the cervical (neck central and especially lateral) lymph nodes is recommended in all patients who must undergo thyroidectomy.

Radioiodine therapy

Post-surgical thyroid ablation with radioiodine (I-131) is performed to destroy residual thyroid tissue and/or known or residual (metastatic) disease. It is associated with a decrease in the risk of locoregional recurrence and

distant metastasis over the next 10 years. Radioiodine therapy (RAI) has real indications for most patients with more advanced stages of the disease. The preparation for treatment includes a low iodine diet and elevated TSH>30 milliunits/L to improve the response to therapy.

Levothyroxine

In the postoperative period, suppressive therapy with levothyroxine is recommended to reduce the risk of relapse after thyroid surgery and radioiodine remnant ablation. Supraphysiological doses of levothyroxine are used to suppress the production of thyroid hormone (TSH).

External radiation

Is the management of metastatic lesions responsible for potential fractures (bone lesions), pain, neurological effects or compression symptoms that cannot be resolved by surgery.

Follow-up

The follow-up includes:

- timing – in the postoperative period, every 6 months in the first 2 years and then annually;
- blood tests with plasma thyroglobulin (the only true tumor marker), antithyroglobulin antibodies (TGAb), thyroid hormone (TSH) levels;
- neck and lymph node ultrasound;
- whole body scintigraphy – for patients with I-131 ablation, it can identify residual lesions or persistence and/or recurrence of the disease.

TABLE 2.XVII. Prognostic classification of the American Joint Committee on Cancer (AJCC), 8th edition, based on TNM staging.

Stage		T	N	M
<55 years	I	T	N	M0
	II	T	N	M1
≥55 years	I	T1 T2	NO/NX NO/NX	M0 M0
	II	T1 T2 T3a/3b	N1 N1 N	M0 M0 M0
	III	T4a	N	M0
	IVA	T4b	N	M0
	IVB	T	N	M1

Prognosis

Generally, stage-by-stage, the prognoses for papillary and follicular cancer are similar. The relapse rate is quite low (7.9% for pap-

illary and 13% for follicular carcinoma) and usually one third of relapses occur within 10 years of surgery and usually only locally. At 10 years, the mortality rate is 2.5% for the papillary form and 1.9 to 3% for the follicular form. Hürthle cell carcinoma is generally associated with a worse prognosis than follicular or papillary carcinoma (Table 2.XVII).

MEDULLARY THYROID CARCINOMA

Definition

Medullary thyroid carcinoma (MTC) is a rare neuroendocrine tumor that originates from parafollicular cells or C-cells (responsible for calcitonin production). MTC can occur in adults in sporadic form (75% of cases) and in children and adolescents in hereditary/familial form (25% of cases). The familial form may either be isolated (familial medullary thyroid carcinoma) or included in multiple endocrine neoplasia (MEN) syndromes such as MEN2A and MEN2B, with autosomal dominant transmission. The clinical manifestations of the tumor vary between the sporadic and familial forms.

Diagnosis

Generally, MTC patients have a palpable thyroid nodule and in 50% of cases, associated neck lymph node metastases since diagnosis (may represent the first element of disease). In 20% of patients, metastases outside the neck region can be localized in the liver, the lungs or the bones. The diagnosis can be made considering:

- cytological diagnosis;
- lab tests – serum levels of calcitonin (CAL) and carcinoembryonic antigen (CEA);
- genetic assessment to search for any mutations of the RET proto-oncogene (to exclude the hereditary/familial form).

Lab tests

CAL

CAL is the most sensitive diagnostic tool for MTC and C-cell hyperplasia. Other conditions with increased CAL levels are: pregnancy, post-exercise,

some cases of renal failure, thyroiditis or follicular tumor, other neuroendocrine tumors of the pancreas or respiratory tract, liver or kidney carcinoma, laboratory artefact, drugs and smoking.

CEA

CEA is produced by neoplastic C-cells and measured together with CAL. A rapid/significant increase in CEA is strongly indicative of disease progression. Other conditions with increased CEA levels are: smoking, inflammatory diseases of the gastrointestinal tract, benign lung tumors, other non-thyroid malignancies.

Thyroid needle aspiration

The sporadic form of MTC usually manifests as a solitary, unilateral nodule, while the hereditary/familial form of MTC manifests as a multinodular and bilateral thyroid disease: thyroid needle aspiration is a useful and safe tool for diagnosing nodular thyroid disease. All suspected thyroid lesions must be investigated with fine needle aspiration. In cases of suspected MTC it is recommended to also perform CAL measurement in washing liquid, as in MTC cytology can be extremely variable and may limit the orientation of preoperative management. The American Thyroid Association (ATA) Guidelines recommend immunohistochemical analysis of any suspected MTC lesion, in order to determine the presence of markers such as CAL, chromogranin, CEA, absence of thyroglobulin. In case of a diagnostic cytological test for MTC, patient management before surgery consists of physical examination, genetic analysis for the evaluation of the *RET* gene mutation and neck ultrasound for the evaluation of possible metastases. In case of suspected metastasis, neck CT examination is the most sensitive imaging technique for presurgical diagnostics of any lung and lymph node metastases in the mediastinum. MRI is a complementary diagnostic technique. Bone scan represents a valid diagnostic tool for the assessment of bone metastases.

Treatment

The gold standard treatment of MTC (regardless of form) without evidence of metastasis is total thyroidectomy and central compartment

lymphadenectomy. In case of preoperative evidence of laterocervical compartment metastasis, neck laterocervical compartment lymphadenectomy should be considered. In case of extensive or metastatic disease, external beam radiotherapy and chemotherapy can accompany total thyroidectomy to obtain local control of the disease. The benefits of external beam radiotherapy as an "add-on" to thyroidectomy for MTC are difficult to evaluate as no prospective studies have been carried out by randomizing patients to radiotherapy or observation. Neck and mediastinum radiotherapy should be evaluated in patients at high risk for local recurrence and in those at risk of airway obstruction. After surgery, TSH suppression is not necessary as it is not a follicular tumor, so therapy with L-thyroxine is indicated in order to keep TSH within the euthyroid range. Radioactive iodine therapy is not indicated in MTC. Palliative therapy, including surgery, external beam radiotherapy or chemotherapy should be considered in patients with metastasis resulting in pain, mechanical compression or signs and symptoms of hormonal excess. Cytostatic chemotherapeutics are used with a single agent or in combination. The most effective regimens are doxorubicin or 5-fluorouracil and dacarbazine.

Follow-up

Three months after surgery, it is recommended to assess whether the procedure was curative or not by measuring CAL, which should be undetectable, and CEA. If the levels are undetectable or within the normal range, monitoring is recommended every 6 months for one year and annually thereafter. In case of increased postoperative CAL levels, less than 150 pg/mL, ATA guidelines recommend performing neck ultrasound. If negative, a follow-up with physical examination, CAL and CEA measurement and neck ultrasound every 6 months should be performed. In case of increased postoperative CAL levels over 150 pg/mL, ATA Guidelines recommend level II investigations, which include neck ultrasound, chest CT, MRI/CT with liver-specific contrast agents, bone scintigraphy, pelvic and skeleton MRI.

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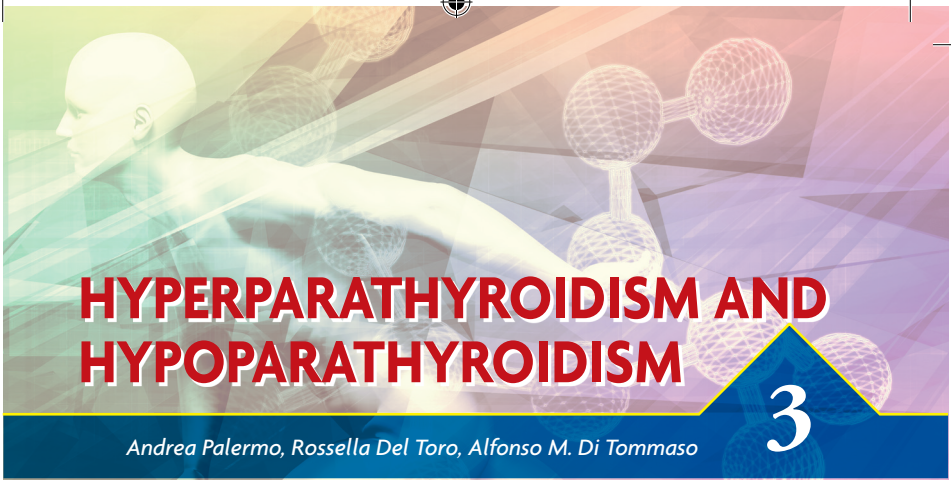

HYPERPARATHYROIDISM AND HYPOPARATHYROIDISM

3

Andrea Palermo, Rossella Del Toro, Alfonso M. Di Tommaso

The parathyroid glands are normally four in number and typically located posterior to the thyroid gland, although ectopic localizations (mainly neck and mediastinum) are not uncommon. Calcium and phosphorus homeostasis is regulated and guaranteed by the action of parathyroid hormone (PTH), produced by the parathyroid glands, and vitamin D. The main effects of PTH are to:

- stimulate bone and renal reabsorption of calcium;
- increase 1,25-dihydroxy vitamin D synthesis in the kidney;
- inhibit renal reabsorption of phosphorus.

Hypercalcemia activates the parathyroid receptor which reduces the release of PTH-containing secretory granules; conversely, in the case of hypocalcemia the receptor is inactivated, stimulating PTH release. In acute hypocalcemia, PTH is rapidly released into the bloodstream, while in chronic hypocalcemia there is an increase in PTH synthesis.

HYPERPARATHYROIDISM

Hyperparathyroidism is an endocrine condition characterized by high blood PTH levels. Excessive PTH secretion is typically classified into:

- primary hyperparathyroidism;
- secondary hyperparathyroidism;
- tertiary hyperparathyroidism.

TABLE 3.I. Causes of primary hyperparathyroidism.

Causes	%
Sporadic PHPT, single parathyroid gland adenoma	80%
Sporadic PHPT, multiple parathyroid gland adenoma	5%
PHPT associated with	14%
Type 1 or type 2A MEN syndrome	
Mandible ossifying tumor	
Severe neonatal hyperparathyroidism	
Familial hypocalciuric hypercalcemia	
Isolated familial hyperparathyroidism	
Parathyroid carcinoma	<0.5%

PHPT: primary hyperparathyroidism; MEN: multiple endocrine neoplasia.

TABLE 3.II. Causes of secondary hyperparathyroidism.

Causes
Reduced intestinal absorption of calcium
Vitamin D deficiency (serum levels of 25-OH vitamin D <20 ng/mL)
Bariatric surgery
Malabsorption syndromes
Low calcium intake
Renal failure – eGFR <60 mL/min
Hypercalciuria
Loop diuretics
Hungry bone syndrome*
Pseudohypoparathyroidism
Drugs
Lithium in short-term use
Hydrochlorothiazides
Anticonvulsants, occasionally when associated with vitamin D deficiency
Antiresorptive drugs (*e.g.*, bisphosphonates and denosumab)

Parathyroidectomy for severe symptomatic primary hyperparathyroidism may be followed by a period during which the skeletal system needs to recall calcium and phosphorus, reducing their blood availability.
eGFR: estimated glomerular filtration rate.

Primary hyperparathyroidism (PHPT) is an intrinsic pathology of the parathyroid glands characterized by increased (or inappropriately high) PTH levels associated with hypercalcemia. Hypophosphatemia and hypercalciuria are also frequently associated. PHPT may occur sporadically in about 85% of cases (Table 3.I). In the hereditary forms of PHPT, diffuse hyperplasia of the parathyroid glands is usually the most frequent clinical condition.

Secondary hyperparathyroidism includes all those conditions in which stimuli are established that could increase PTH values. Table 3.II summarizes the numerous causes. Tertiary hyperparathyroidism may occur in patients with long-standing chronic secondary hyperparathyroidism. Over time, this condition could cause secondary hyperplasia of the parathyroid glands with loss of the capacity to regulate in response to calcium levels and consequent autonomization. It is usually found in patients with severe chronic renal failure (patients with end-stage renal disease or on dialysis).

Clinical manifestations of primary hyperparathyroidism

Nowadays, PHPT is diagnosed quite early in subclinical form. The main PHPT-related changes are as follows:

- nephrolithiasis – in PHPT, both the direct action of PTH in the bone and its indirect actions in the intestine may lead to calcium levels high enough to overcome the primary PTH action in the kidney (this condition is manifested by hypercalciuria and a consequent increased risk of kidney stones);
- nephrocalcinosis – clinical manifestation due to the deposition of calcium salts in the renal tissue, most frequently in the renal papillae;
- chronic renal failure – the reduction in glomerular filtration rate (GFR) may be a consequence of nephrolithiasis or directly of hypercalcemia;
- osteitis fibrosa cystica – X-Ray images show the subperiosteal bone resorption of the phalanges, the salt-and-pepper appearance of the skull and the presence of bone cysts and brown tumors typically located in the ribs, clavicle, pelvis and mandible;
- osteoporosis – in most patients this represents the first clinical manifestation of PHPT; unlike post-menopausal osteoporosis, in primary hyperparathyroidism the bone mineral density deficit is greater at the cortical level with a tendency to lumbar sparing (the presence of osteoporosis and/or osteoporotic fractures is one of the criteria for referring the patient for parathyroidectomy);
- cardiovascular manifestations – arterial hypertension, cardiac arrhythmias, metabolic changes and functional alterations (electrocardiogram alterations – QT shortening, prolonged PQ, J waves), cardiac structural alterations (hypertrophy, diastolic dysfunction, valvular calcifications) and vascular changes (vascular elasticity and endothelial dysfunction), most of which are due to hypercalcemia;
- gastrointestinal manifestations – constipation, nausea, vomiting and abdominal pain;
- neuropsychiatric manifestations – severe hypercalcemia may be accompanied by clinical pictures of psychosis, confusion, lethargy, and coma (when calcemia is less high, the clinical manifestations may be milder and non-specific – depression, anxiety, irritability, memory impairment and mood swings).

Diagnosis

PHPT diagnosis is biochemical (Table 3.III). X-Ray imaging tests are important to localize the disease. The diagnosis of hyperparathyroidism is therefore based on the presence of hypercalcemia, associated with elevated or inappropriately normal PTH levels. Urine calcium level is elevated in half of the patients; serum phosphorus is in the low normal range, while phosphaturia is increased. In case of high PTH levels and normal calcium levels, it is necessary to measure serum creatinine and 25-OH vitamin D to rule out any secondary hyperparathyroidism. In case of hypovitaminosis D (<30 ng/mL), PTH levels and calcemia should be re-evaluated after vitamin D supplementation.

Once the diagnosis of primary PHPT has been made, neck ultrasound and/or parathyroid scintigraphy in dedicated centers are indicated in order to localize the hyperfunctioning tissue. If neck ultrasound or scintigraphy do not localize the source of disease, PET/TC with 11C-Metionina or 18F-o 11C-Colina represent the most accurate techniques to identify the cause of primary hyperparathyroidism.

TABLE 3.III. Hyperparathyroidism: diagnosis.

Laboratory tests	
Mineral homeostasis	Serum (corrected for albumin) or ionized calcium
	PTH
	Phosphate
	25-OH vitamin D
Renal function	Serum creatinine and blood urea nitrogen (BUN)
	eGFR
24-hour urine collection test	Urine calcium level/24 h
	Urine phosphate level/24 h
	Urine creatinine level/24 h
	An assessment of the risk of kidney stones must be performed if the urinary calcium level is >300 mg *per* day (oxalate, sodium, uric acid, sulphate, and citrate levels)
Bone formation markers	Specific bone alkaline phosphatase in serum
	CTX, P1NP (rarely required in clinical practice)
Imaging	
Localization of hyperfunctioning parathyroid tissue (neck ultrasound, parathyroid scintigraphy) PET or PET/TC	
DEXA (dual-energy X-Ray absorptiometry) bone mineral density analysis of lumbar spine, femoral neck, total femur and distal third of the radius	
Vertebral fracture assessment by X-Ray evaluation or by DEXA (morphometry)	
Abdomen imaging for kidney stones or nephrocalcinosis (ultrasound, CT or abdominal radiography)	

Treatment

PHPT therapy aims to restore normal calcemia levels. The first-choice therapy is parathyroidectomy, which must be offered to all symptomatic patients or to patients with disease complications. The indications for surgery are summarized in Table 3.IV.

TABLE 3.IV. Recommendation of parathyroidectomy in symptomatic and asymptomatic patients who meet at least one of the following criteria.

Criteria
Age <50 years
Calcium levels at least 1 mg/dL above the normal upper limit
T-score equal to or less than -2.5 at the lumbar spine and/or femoral neck and/or total femur and/or distal third of the radius and/or presence of vertebral fracture by X-Ray assessment
Creatinine clearance <60 mL/min, increased risk of kidney stones by biochemical assessment, renal stones or nephrocalcinosis assessed by abdominal imaging calciuria >400 mg/24 h

Therapy with cinacalcet, a calcium mimetic, is indicated for patients with hypercalcemia who meet surgical criteria but in whom parathyroidectomy is unsustainable or clinically inappropriate. In patients who do not undergo surgery, bisphosphonate therapy, in particular antiresorptive agents (*e.g.*, bisphosphonates), may be initiated in order to inhibit bone resorption.

HYPOPARATHYROIDISM

Hypoparathyroidism is a rare disease characterized by insufficient or inappropriate PTH secretion or action; from a laboratory point of view, it is characterized by hypocalcemia associated with hyperphosphatemia. Hypoparathyroidism may be subdivided into:

- primary, due to rare intrinsic defects of the parathyroid glands that are usually recognized to have genetic causes;
- secondary (or acquired), due to damage that negatively affects the function of parathyroid glands.

TABLE 3.V. Causes of hypoparathyroidism.

Causes
Post-surgical (frontal neck surgery)
Autoimmune (isolated, polyglandular syndrome)
Functional (changes in magnesium metabolism, infants born to mothers with hyperparathyroidism)
Infiltrative disorders (haemochromatosis, Wilson's disease, tumor metastases)
Genetic alterations (Di George Syndrome, agenesis)

Anterior neck surgery and thyroidectomy or parathyroidectomy are the most common causes of acquired hypoparathyroidism (about 75% of patients). The next most common acquired cause in adults is an autoimmune disease affecting the parathyroid glands, usually in association with other endocrine glands. The remaining cases of acquired hypoparathyroidism are secondary to rare infiltrative diseases where the parathyroid glands are affected by metastatic disease or iron or copper overload or ionizing radiation exposure (Table 3.V).

Clinical manifestation

The symptoms depend on the severity of hypocalcemia and the time it occurs. A rapid decrease in blood calcium levels results in perioral and upper extremity paresthesia followed by muscle spasms, laryngospasm, and tetany. Latent tetany may be highlighted by:

- Chvostek's sign (rapid contraction of the facial muscles on one side of the face in response to percussion over the facial nerve on the same side);
- Trousseau's sign (the occurrence of the muscle spasm known as "obstetrician's hand" after 2 minutes of compression-induced ischemia in the arm using the inflation of a sphygmomanometer cuff to >20 mmHg).

TABLE 3.VI. Hypocalcemia symptoms.

System	Hypocalcemia symptoms
Central nervous system	Depression, irritability, seizures
Neuromuscular	Acral paranesthesia, spasms, contractions, cramps
Cardiovascular	Heart failure symptoms
Gastrointestinal	Abdominal cramps
Respiratory	Dyspnea, rales, laryngospasm

Hypocalcemia symptoms are summarized in Table 3.VI.

Diagnosis

The diagnosis of hypoparathyroidism is based on the following biochemical data:

- hypocalcemia corrected for the albumin level and/or the ionized calcium concentration confirmed in two different circumstances, at least 2 weeks apart;
- undetectable or inappropriately low PTH levels associated with hypocalcemia detected in two tests at least 2 weeks apart;

- medium to high or very high phosphorus levels.

Hypoparathyroidism may be subdivided into:

- chronic hypoparathyroidism: >6 months;
- transient hypoparathyroidism: <6 months.

Treatment

Acute hypocalcemia is an endocrine emergency that requires calcium salts in intravenous infusion: 1-2 vials of 10% calcium gluconate i.v. over 10 minutes. Treatment may be repeated in case of inadequate rise in blood calcium, subsequently followed by an i.v. solution containing 10 vials of calcium gluconate in 1 L of 0.9% NaCl normal saline solution infused at a starting rate of 50 mL/h. The goals of therapy for chronic hypoparathyroidism are:

- to prevent the signs and symptoms of hypocalcemia;
- to maintain the calcium concentration slightly below the lower limit of the normal range or the lower limits of the normal range;
- to maintain the calcium to phosphate product below 55 mg/dL (4.4 mmol/L);
- to avoid hypercalciuria;
- to avoid hypercalcemia;
- to avoid extraskeletal calcifications, particularly renal (nephrocalcinosis/nephrolithiasis).

Therapeutic interventions in patients with hypoparathyroidism:

- calcium supplementation – calcium carbonate/calcium citrate;
- supplementation of vitamin D – calcitriol and cholecalciferol;
- thiazide diuretics;
- phosphorus chelators, only if the hyperphosphatemia is >6.5 mg/dL;
- PTH analogues – teriparatide, recombinant human (rh) PTH (1-34)/rh PTH (1-84).

Such therapies are indicated when, to maintain adequate calcium levels, very high doses of calcium supplements are added: they predispose to hypercalciuria, renal stones, nephrocalcinosis and in some patients also to impaired renal function and possible ectopic calcifications; however, in other patients the prescription of high amounts of calcium salts and vita-

min D are not sufficient to avoid the fluctuations of calcium levels and the symptoms of hypocalcemia.

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DIABETES AND ITS COMPLICATIONS

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4

DIABETES

Diabetes: framework

Diabetes is a chronic syndrome due to absolute or relative insulin deficiency, characterized by increased blood glucose levels in association with complex alterations in glucose, lipid, and protein metabolism.

The commonly used classification system identifies five types of diabetes (Table 4.I):

- type 1 diabetes (T1D), with autoimmune pathogenesis due to the destruction of pancreatic β-cells, with acute onset and prevalent onset before 25 years of age. These patients have β-cell antibodies and often other autoimmune diseases such as Hashimoto's thyroiditis, Addison's disease, vitiligo, pernicious anæmia and celiac disease;
- type 2 diabetes (T2D), characterized by insulin resistance in peripheral tissues and a defect in β-cell insulin secretion. This is the most common form of diabetes, and is strictly associated with family history, old age, obesity, and inadequate lifestyle. It is more common among women, especially those with a history of gestational diabetes, and Afro-American, Hispanic and Native American women;
- latent autoimmune diabetes in adults (LADA) – adult-onset autoimmune diabetes, characterized by a slower progression towards insulin dependence. It affects 5 to 12% of patients initially diagnosed as type 2

TABLE 4.I. Etiological classification of diabetes.

Type 1 diabetes
Caused by β-cell destruction, on an autoimmune or idiopathic basis, and characterized by complete insulin deficiency (the variant LADA, latent autoimmune diabetes in adults, has a slow course and occurs in adults)
Type 2 diabetes
Caused by a partial deficit of insulin secretion, which generally progresses over time but never leads to complete hormone deficiency. It is often based on a multifactorial condition of more or less severe insulin resistance.
Other types of diabetes
Genetic β-cell defects (MODY, neonatal diabetes, mitochondrial DNA)
Genetic insulin action defects (type A insulin resistance, leprechaunism)
Exocrine pancreas diseases (pancreatitis, pancreatectomy, tumors, cystic fibrosis)
Endocrine disorders (acromegaly, Cushing's, pheochromocytoma, glucagonoma)
Diabetes induced by drugs or toxic substances (glucocorticoids, other immunosuppressive agents, thiazides, diazoxide, drugs for the treatment of HIV/AIDS) or infections (congenital rubella)
Rare genetic syndromes associated with diabetes (Down, Klinefelter, Turner, Wolfram, Friedreich)

diabetic. It is more heterogeneous than juvenile autoimmune diabetes and shares clinical and metabolic characteristics with both type 2 and type 1 diabetes. It generally occurs after 30 years of age and does not require insulin treatment for at least 6 months after diagnosis, despite being characterized by positivity for antiglutamic acid decarboxylase autoantibodies (GADA) and/or other autoantibodies typical of type 1 diabetes. Recent guidelines have paved the way for the use of innovative drugs such as SGLT-2 inhibitors and glucagon-like peptide-1 (GLP-1) receptor agonists for this form of autoimmune diabetes as well, as they have established how an ideal therapeutic intervention should not only promote an early and long-lasting maintenance of blood glucose compensation, but also have a protective effect on the residual β-cell pool, and show cardiovascular safety and efficacy in reducing the progression towards micro- and macrovascular complications, as already evidenced in patients with T2D (**Table 4.II**);

- the fourth class includes various forms of diabetes with different causes, grouped together under the name of 'other specific types': genetic defects of β-cell function (maturity onset diabetes of the young, MODY);

TABLE 4.II. Clinical differences between T1D, T2D and LADA.

	T1D	LADA	T2D
Clinical characteristics			
Onset age	Childhood/adolescence	30-50 years	>50 years
Onset symptoms	Frequent (acute)	Subclinical (rarely acute)	Subclinical/absent
Insulin therapy	At diagnosis	>6 months from diagnosis	Unnecessary/after many years of disease
Insulin resistance	No variations	Increased/no variations	Increased
BMI (Body Mass Index)	<25 kg/m^2 (often <18 kg/m^2)	>25 kg/m^2 (often <25 kg/m^2)	>25 kg/m^2
Risk of complications/ chronic diagnosis	Low	Low	High
Biochemical characteristics			
Detection of autoantibodies	High titer (rarely low)	High/low titer	Absent
C-peptide on diagnosis	Non-measurable	Low but measurable	Normal/high
Pathophysiological characteristics			
MHC association	High	High/moderate	Moderate
Family history of diabetes	Negative/positive	Negative/positive	Frequently positive
Family history of autoimmune disorders	Frequently positive	Frequently positive	Negative (no correlation

T1D: type 1 diabetes; LADA: latent autoimmune diabetes in adults; T2D: type 2 diabetes; BMI: Body Mass Index; MHC: major histocompatibility complex.

genetic insulin action defects; diseases of the exocrine pancreas (pancreatitis or cystic fibrosis); endocrine disorders (acromegaly); and dysfunctions caused by drugs, toxins or infections;

- gestational diabetes mellitus (GDM) identifies women who develop diabetes mellitus during gestation; it includes women who develop T1D during pregnancy and women with undiagnosed asymptomatic T2D that is discovered during pregnancy.

Diagnostic criteria

Diagnosis of diabetes can be made by analyzing fasting plasma glucose (FPG) or blood glucose levels 2 hours after an oral load of glucose (75 g) (2hPG) or HbA1c (glycosylated hemoglobin) levels (Table 4.III).

TABLE 4.III. Criteria for diabetes diagnosis (American Diabetes Association 2018).
In the absence of typical disease symptoms, the diagnosis of diabetes must be made by detection, confirmed on at least two separate occasions of:
Blood fasting glucose levels ≥126 mg/dL (7.0 mmol/L). Fasting means at least 8 hours of abstaining from food
Blood glucose levels ≥200 mg/dL 2 hours after oral glucose load
HbA1c ≥6.5% (48 mmol/mol), provided that the HbA1c measurement is standardized, International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)-aligned and considering factors that may interfere with the test
In case of typical disease symptoms (polyuria, polydipsia, weight loss), the diagnosis of diabetes is made with the finding, even on only one occasion, of random blood sugar levels ≥200 mg/dL (regardless of food intake)

Disglicæmia

Alterations in blood glucose parameters that identify subjects at risk of developing diabetes and cardiovascular disease (formerly referred to as 'prediabetes'):

- impaired fasting glucose (IFG): fasting glucose levels between 100 mg/dL and 125 mg/dL;
- impaired glucose tolerance (IGT): blood glucose levels 2 hours after oral glucose load between 140 mg/dL and 199 mg/dL;
- HbA1c 42-48 mmol/mol (6.00-6.49%) (only with an IFCC aligned assay).

Technologies in diabetes

Blood glucose monitoring systems

Blood glucose monitoring is an essential component in the treatment of patients with both type 1 and type 2 diabetes. Blood glucose monitoring is performed by using:

- traditional meters (glucose meters) that store in their memory capillary blood glucose measurements performed with finger stick tests: many of these devices can download the data to a PC for statistical processing (*e.g.*, average blood glucose levels at various times of the day, trend over time, etc.) or send data to the diabetes center. There are also blood glucose meters that can give suggestions on the insulin bolus to be given at mealtimes;

- continuous glucose monitoring (CGM) systems, whether in combination or integrated with the pump, consisting of a subcutaneous sensor for detecting the glucose concentration in the dermal interstitial fluid. The most modern systems are provided with alarm functions and warnings that signal the achievement of preset blood sugar levels (indicating the risk of hypo- and hyperglycæmic events) and suggest the need to perform the therapeutic actions agreed with the diabetologist, as well as indicating the trend and the rate of change in blood glucose levels.
- flash glucose monitoring systems (FGM): novel devices, similar to CGM, which measure interstitial glucose levels for 14 days on demand, by a quick scan with a reader, even through clothing.

Insulin pump or continuous subcutaneous insulin infusion

The microinfusion device or insulin pump is a device that allows 24-hour continuous insulin infusion into the subcutaneous tissue. It is currently used in the treatment of T1D patients or insulin treated T2D patients. It has demonstrated the potential to achieve optimal glycæmic control without increasing the risk of hypoglycæmia, thus reducing the risk of chronic diabetes complications. The distinctive feature of modern insulin pumps is the capability to perform continuous subcutaneous insulin infusion (CSII) with varying infusion rates during the day, according to individual needs; pumps can reproduce the physiological action of insulin more precisely, as it varies during the day. Pumps can deliver insulin into the subcutaneous tissue in the following two ways.

Continuous basal infusion

This is needed to maintain normal blood sugar levels during fasting by delivering a micro-bolus of rapid insulin every few minutes, within 24 hours.

Rapid bolus

An insulin bolus regulated and activated directly by the person wearing it, according to current needs (meal or too high blood glucose levels). An internal motor and an infusion set injects the insulin contained in a special cartridge into the subcutaneous tissue. The infusion set consists of a small plastic nee-

dle, the cannula, which is placed under the skin together with a small tube, also made of plastic. Every 2 to 3 days the set must be replaced to avoid infections. Possible rare side effects secondary to insulin pump treatment can be:

- mild skin infections in the infusion area that subside with moist warm compresses;
- abscesses requiring antibiotic therapy or surgical drainage;
- contact dermatitis due to the infusion set components and the adhesive plasters, which regresses after changing them.

Type 1 diabetes

Epidemiology

In Italy, there are about 300,000 people with T1D, with an incidence of about 15 cases x 100,000 inhabitants *per* year under the age of 15. The incidence of T1D differs according to geographical area, ethnicity, age, gender, family history and Body Mass Index (BMI). It has been widely demonstrated that the incidence of T1D starts to increase around the age of 9 months, continues to increase up to 12-14 years and then decreases.

Pathogenetic factors

The etiology of the disease involves an interaction between genetic predisposition, immunological factors, and environmental factors: it should be therefore considered a multifactorial disease. The decline in insulin secretion during the first years after diagnosis has been described as biphasic: more rapid during the first year than during the second year after diagnosis. The loss of insulin secretion may continue for years after diagnosis if there is little or no insulin production. However, in most patients, low C-peptide levels may still be detectable after 30 years of disease.

Genetic factors

Studies carried out in monozygotic twins have demonstrated that the risk of both twins developing T1D, if one is diagnosed with it, is 30 to 40%, while it drops to 5 to 10% in non-twin brothers and 2 to 5% in children of subjects already suffering from T1D: diabetes should therefore be included in the group of polygenic diseases. Disease onset results from the

interaction of several affected genes with the significant contribution of predisposing environmental factors.

HLA genes

The association of T1D with the human leukocyte antigen (HLA) system has been known for more than 20 years. The *HLA* gene complex extends for about 3500 kb on the short arm of chromosome 6. In Caucasian populations, 90-95% of diabetics have DR3 or DR4 antigens; in the Japanese population, on the other hand, DR3 is rare and DR4 associated with DR9 prevails, while in the Black population the DR9 allele is also present. This emphasizes how HLA alleles vary according to the ethnicity of the population. Considering the individuals carrying these genes, the risk of becoming ill with T1D can be calculated in comparison to a person without these alleles. The absolute risk for subjects with DR3/DR4 is about 6-7%. Table 4.IV expresses the risk percentages in relation to HLA. A significant correlation was observed between the amino acid residue at position 57 of the DQβ chain and disease susceptibility or resistance. In fact, most haplotypes not associated with the disease have aspartic acid in this position (Asp 57), while T1D-associated haplotypes have neutral amino acids (serine, valine, alanine).

Non-HLA genes

T1D is a polygenic disease and HLA contributes only 40-50% to susceptibility. New sources that can be employed as gene markers are small vari-

TABLE 4.IV. HLA and risk of developing type 1 diabetes.

Individual risk compared to general population	0.3%
Individual risk compared to general population with HLA DQB1*0201/0302 alleles	1.7%
First-degree relative risk (not considering *HLA* typing)	3-6%
First-degree relative risk not sharing any HLA allele with the affected subject	≤1%
First-degree relative risk sharing any HLA allele with the affected subject	6%
First-degree relative risk sharing 2 HLA alleles with the affected subject	16%
Non-DR3/DR4 identical monozygotic twins	35%
DR3/DR4 identical monozygotic twins	70%

ations in DNA sequences, called microsatellites. They are short nucleotide sequences (from 1 to 6 bp) found uniformly throughout the genome and repeated together a variable number of times. To date, 10 T1D susceptibility loci have been identified. The association between T1D and the polymorphism of the gene encoding cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4), located on chromosome 2q33, has also been confirmed.

Immunological factors

Insulitis is the T1D histopathological marker, and it is characterized by pancreatic islet infiltration with B and T lymphocytes, both CD4+ and CD8+, NK cells and to a lesser extent by macrophages. It is important to emphasize that the autoimmune events and the development of insulitis can begin several years before the clinical manifestation of the disease; in fact, for the first clinical signs to occur, many pancreatic β-cells must be destroyed. While individuals with a single positive autoantibody frequently become negative, this disappearance is rare in patients with multiple autoantibodies.

The following may be identified among the different classes of autoantibodies:

- pancreatic islet cell autoantibodies (ICA) – a heterogeneous class of polyclonal immunoglobulin G (IgG) directed against different antigenic determinants such as glutamic acid decarboxylase (GAD), insular tyrosine phosphatase IA-2 and zinc transporter 8. They are detected through radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA). They can precede the clinical onset of the disease by several years. When present, they indicate an increased risk of developing diabetes; in fact, ICA are detectable in 70-95% of patients at the time of diagnosis;
- glutamic acid decarboxylase (GAD) autoantibodies – this antibody pattern is found in about 80% of subjects with diabetes at clinical onset;
- insular tyrosine phosphatase autoantibodies (isoforms IA-2 and IA-2β) – these autoantibodies are highly predictive of future disease occurrence in first-degree relatives of T1D patients;
- insulin autoantibodies (IAA) – unlike other autoantibodies, these have an inverse correlation both with age (in diabetic children under the age

of five the antibody titer is very high) and with preclinical phase duration (the higher the IAA levels, the more rapid the progression towards the disease seems to be); for this reason, they have proved to be the most specific in terms of early diagnosis in subjects under 10 years;

- anti-ZnT8 antibodies – these were detected in 60 to 80% of patients with recent onset T1D. More precisely, these antibodies were identified in 26% of diabetic subjects classified as negative for anti-islet autoantibodies;
- antibodies to oxidized insulin – this is the most recent class of antibodies studied in T1D patients. The test developed at the Campus Bio-Medico University for measuring antibodies to oxidized insulin is highly sensitive (84%) and specific (99%) for T1D diagnosis.

Environmental factors

It is assumed that a precipitating event of an environmental (viral, toxic, dietary, etc.) or a mutational nature is necessary to initiate the autoimmune process, hence determining the loss of immune tolerance towards self-molecules of pancreatic β-cells:

- infections – the most accredited hypotheses support the theory that enterovirus infection, in particular the Coxsackie B virus, can cause direct β-cell infection with functional alteration and cell lysis. The seasonal variation in diabetes-associated antibody occurrence, higher during the cold season, also provides some (weak) evidence regarding an association between viral infections (more frequent in winter) and T1D;
- gut microbiota – some of the T1D candidate environmental factors (caesarean delivery, early childhood diet and antibiotic use) are intertwined with the development and function of the human microbiome. Gut microbes influence lipid and glucose metabolism, as well as immunity and systemic inflammation outside the intestine and could therefore modulate the T1D risk;
- solid food/grains – the DAISY study has demonstrated that the timing of introducing any type of grains (gluten-containing and gluten-free) is associated with an increased risk of autoimmunity with the nadir at introduction at age 4–6 months; On the other hand, the BABYDIET

primary prevention study was designed to investigate whether delaying dietary gluten introduction was able to prevent the development of autoimmunity in infants with a first-degree relative suffering from T1D; however, no benefit has been found in delaying gluten exposure in regard to the autoimmunity associated with diabetes or coeliac disease;

- breastfeeding and cow's milk – in many subjects with T1D, antibodies against cow's milk proteins used during formula feeding have been identified. It has been hypothesized that breastfeeding could provide maternal antibodies to prevent infections that lead to diabetes. However, a prospective study conducted from birth in T1D-predisposed subjects, with non-breastfeeding mothers and fed bovine hydrolyzed milk, did not show a positive effect on the development of T1D;
- vitamin D – some data in the literature have shown low levels of vitamin D at the onset of T1D. Vitamin D levels can in fact influence the immune response by modulating pro- and anti-inflammatory levels of cytokines;
- accelerator hypothesis – some studies have documented a greater T1D risk among people with a higher body weight as children, strengthening the hypothesis which suggests that weight gain in pediatric age induces insulin resistance and promotes the underlying autoimmunity in predisposed subjects.

Clinical picture

T1D onset can be:

- acute with ketoacidosis, often related to intercurrent stress (infection, surgery, etc.);
- subacute with clinical symptoms and signs that are more indistinct and difficult to recognize;
- more nuanced in adults, since the β-cell loss of function is more gradual.

The most common clinical symptoms at the onset of T1D are polyuria, thirst, polydipsia, polyphagia, asthenia and weight loss, acetone breath (with fruity smell of rotten apples), and dullness to lethargy. When glycæmic levels exceed the renal threshold for glucose (180 mg/dL), there is glycosuria (glucose in the urine), which, if considerable, causes osmotic diuresis with consequent loss of water and glucose.

The lab picture is generally characterized by:

- blood glucose >250 mg/dL;
- ketonuria;
- arterial pH<7.35 with arterial bicarbonate <15 mEq/L and increased anion gap;
- variable serum osmolality, usually above normal levels.

The clinical picture is often sufficient for classification; however, in some cases the assessment of autoimmunity markers (IAA, GADA, ICA, IA-2) may be necessary.

Treatment of type 1 diabetes

Insulin therapy is the treatment of choice for T1D since β-cell damage progressively leads to absolute and/or relative deficiency of endogenous insulin secretion. The administration of insulin is essential to maintain glucose homeostasis to ensure adequate blood glucose compensation. An inadequate dosage of insulin therapy entails serious risks for the patient's safety. On one hand, an overdose of insulin can lead to the onset of severe hypoglycæmia, while reduced insulinization increases the risk of developing diabetic ketoacidosis with exposure to persistent high blood glucose levels, which are directly related to the onset and progression of chronic diabetes complications. As is well documented in the largest epidemiological study carried out in T1D, the Diabetes Control and Complications Trial (DCCT), incidence and progression of chronic T1D complications are directly related to blood glucose control. On this basis, strict blood glucose levels as near as possible to the normal range and HbA1c<7.0% are recommended as treatment target for T1D patients. However, a large proportion of T1D patients show suboptimal blood glucose control and 60% of them perform less than the three blood glucose tests *per* day recommended by national and international scientific societies. Blood glucose monitoring is recommended as a key component in the management of T1D patients. Optimal blood glucose control can be achieved by intensive insulin treatment, with multiple daily subcutaneous insulin administrations. What best mimics physiological insulin secretion is multiple subcutaneous insulin therapy characterized by three preprandial subcutaneous insulin

TABLE 4.V. Insulin types and main characteristics.

Insulin	Onset	Peak	Duration of action	Clinical intent
Rapid acting				
Lispro	15 to 30 minutes	1 to 2 hours	3 to 6 hours	Mimics bolus insulin and is taken prior to meals.
Aspart	15 to 30 minutes	1 to 2 hours	3 to 6 hours	It acts quickly to minimize the rise in blood sugar levels which follows eating
Glulisine	15 to 30 minutes	1 to 2 hours	3 to 6 hours	
Long acting				
Degludec	30 to 90 minutes	No peak time	36 to 45 hours	Provides insulin coverage for about 24 hours.
Glargine	1 to 1.5 hours	No peak time	19 to 24 hours	Often combined with rapid-acting insulin in basal-bolus regimen
Detemir	1 to 2 hours	6 to 8 hours	19 to 20 hours	
Short-acting				
Regular	30 minutes to 1 hour	2 to 4 hours	3 to 6 hours	Not commonly used nowadays
Intermediate-acting				
NPH	2 to 4 hours	8 to 10 hours	10 to 18 hours	Not commonly used nowadays

administrations (fast-acting analogue or regular human insulin) and a slow-acting insulin injection (Table 4.V). Currently, the most common method of insulin delivery is a mealtime insulin analogue (aspart, lispro, glulisine) and a long-acting basal analogue usually given in the evening or before going to bed (*i.e.,* glargine, detemir or degludec). The preprandial insulin bolus exhausts its action in 4 hours, whereas basal insulin has a duration of action of approximately 24 hours. Continuous subcutaneous insulin infusion (CSII) using a pump integrated with continuous glucose monitoring is used in approximately 5-50% (depending on the country) of patients with T1D.

Type 2 diabetes

Epidemiology

T2D is the most common form of diabetes, accounting for approximately 90% of all cases. It should be noted that its prevalence has increased

dramatically over the past few decades, from 30 million cases in 1985 to 425 million in 2018. In fact, based on the current trend, the International Diabetes Federation (IDF) estimates that in 2045 there will be 629 million people with T2D, which represents one of the chronic diseases with the greatest socio-economic impact. Generally, type 2 diabetes starts after the age of 40 – often in overweight individuals – and it is more common after the age of 65.

Natural history of type 2 diabetes

T2D patients have two fundamental pathophysiological characteristics:

- insulin resistance due to a decreased number of receptors on the target tissues and a post-receptor defect;
- impaired insulin secretion, from hyperinsulinemia to relative secretory deficit.

There are three phases that can be recognized in the course of disease:

- a first phase with normal blood sugar despite the presence of high insulin levels, a sign of peripheral insulin resistance;
- a second phase in which there is increased insulin resistance;
- a third phase in which there is reduced insulin secretion, which causes the onset of fasting hyperglycæmia and frank diabetes.

Genetic factors

Genetic susceptibility to TD2 is dependent on changes in the structure of genes, called polymorphisms. In terms of pathogenesis, the importance of the genetic component is demonstrated by the greater concordance of disease occurrence in monozygotic compared to dizygotic twins. It is estimated that the lifetime risk of developing this form of diabetes is about 40% if one of the parents is affected by the disease, a percentage that increases if the other parent is also diabetic.

Environmental factors

Obesity

Among the factors that have a fundamental role in diabetes pathogenesis, excess body weight (estimated by assessing the BMI=kg/m^2), plays the

most important role. In fact, obesity contributes to the development of diabetes in about 55% of cases. Obesity alone explains by itself the increased incidence of this disease in children and adolescents.

Diet and exercise

A diet rich in simple sugars or foods with a high glycæmic index and high in saturated fats can promote T2D onset. Furthermore, muscle tissue represents the main site of peripheral insulin resistance; however, during physical activity this tissue loses its insulin dependence and glucose enters the muscle cells even in the presence of particularly low blood insulin levels.

Dyslipidæmia and metabolic syndrome

Various defects in lipoprotein metabolism are observed in T2D, particularly hypertriglyceridemia. This is caused by decreased activity of endothelial lipoprotein-lipase due to a defect in insulin secretion and action. The consequence is reduced removal of triglycerides associated with chylomicrons and very low-density lipoproteins (VLDL) in capillaries.

Furthermore, insulin inhibits liver ApoB100 production by inducing an increase in VLDL synthesis. Due to insulin resistance-induced lipotoxicity, structural changes are also observed in circulating low-density lipoproteins (LDL), which become smaller and denser because they are rich in esterified fatty acids.

Metabolic syndrome also recognizes insulin resistance as a common pathogenetic factor and includes visceral obesity with increased abdominal circumference, hypertriglyceridemia, low levels of high-density lipoprotein (HDL) cholesterol, high blood pressure and fasting hyperglycæmia.

Clinical picture

Onset of symptoms is gradual: hyperglycæmia is often an occasional finding. Clinical onset may be fatigue or weight loss and otherwise unexplainable polyuria and polydipsia. These patients usually do not develop ketoacidosis (rare event), while hyperosmolar non-ketotic coma may occur in case of decompensation. **Table 4.VI** lists the main clinical differences between the two main forms of diabetes (T1D, T2D).

Initial assessment

The patient must be assessed by a multidisciplinary team consisting of a physician, nurse, and dietician, but also a psychologist and a podiatrist.

The initial assessment includes:

- anamnesis – family history, early and distant medical history, diabetic history: onset age and characteristics, lifestyle survey, previous and current pharmacological therapies, history of glycæmic control, results of glycæmic monitoring and the presence of any complications.
- physical examination – anthropometry: weight, height, waist circumference; blood pressure, heart rate, evaluation of carotid and lower limb pulses, liver and thyroid palpation, foot inspection, skin assessment.
- lab tests – blood glucose fasting HbA1c, blood creatinine and glomerular filtrate, albuminuria, total cholesterol, HDL, LDL, triglycerides and transaminases.
- interview with the dietician – nutrition survey, nutrition education, interview with the psychologist (if present and if indicated).
- instrumental tests – *fundus oculi*, electrocardiogram (ECG), ABI (ankle brachial pressure index) and sensitivity to monofilament. If any of these tests have altered results, the diagnosis of late diabetes complications will be further investigated.

TABLE 4.VI. Clinical differences between type 1 and type 2 diabetes.

	T1D	T2D
Prevalence	0.3%	5.3%
Onset age	More common in children or young adults (<30 years)	More frequent >40 years
Onset symptoms	Present, at sudden onset	Moderate or absent
Weight	Normal or reduced	Generally increased
C-peptide	Low or non-measurable	Normal or high
Autoimmunity	Present	Absent
Chronic complications	Years after diagnosis	Often present at the time of diagnosis
Tendency to ketoacidosis	Present	Absent

Treatment of type 2 diabetes

Today many drugs are available for blood glucose control in patients with type 2 diabetes, and the treatment of hyperglycæmia is becoming a com-

TABLE 4.VII. Therapeutic strategy.

Pharmacological class	Drug	Mechanism of action	Side effects
Biguanide	Metformin	Increases insulin sensitivity by: inhibiting liver glucose production (gluconeogenesis); stimulating muscle tissue and other insulin-dependent tissues to capture and use glucose	Intestinal disorders (diarrhea, abdominal pain)
Sulphonylureas	Glibenclamide	Stimulate pancreatic β-cells to produce more insulin	Hypoglycæmia
	Glimepiride		
	Gliclazide		
Glinide	Repaglinide	Stimulates pancreatic β-cells to release greater amounts of insulin (the effect is generally faster and less lasting than with sulphonylureas)	Hypoglycæmia
α-glucosidase inhibitor	Acarbose	Slows the intestinal absorption of monosaccharides (*e.g.*, glucose), by blocking a specific enzyme (α-glucosidase) that breaks down disaccharides	Intestinal disorders (flatulence, diarrhea, abdominal pain)
Glitazone (or thiazolidinedione)	Pioglitazone	Increases insulin sensitivity more than metformin, especially in adipose tissue and skeletal muscle	Increases body weight or causes fluid retention
Dipeptidyl peptidase-4 (DPP-4) inhibitors	Sitagliptin	Slow down the breakdown of hormones (GLP-1 and GIP) that are produced by the intestine and stimulate the secretion of insulin and inhibit glucagon secretion from the pancreas	
	Vildagliptin		
	Saxagliptin		
	Linagliptin		
	Alogliptin		
Glucagon-like peptide 1 (GLP-1) analogues	Exenatide	Stimulate insulin secretion and inhibit pancreatic glucagon secretion, also causing moderate weight loss	Intestinal disturbances (nausea, vomiting)
	Liraglutide		
	Lixisenatide		
Inhibitor of the renal sodium-glucose cotransporter 2 (SGLT-2)	Canagliflozin Dapagliflozin Empagliflozin	Partially reduce reabsorption of glucose filtered by the kidney, lowering blood sugar through an increase in urine glucose; they also cause an appreciable drop in body weight and blood pressure due to their powerful diuretic effect	Genital and urinary infections

plex therapeutic strategy. Table 4.VII lists the main drugs currently used in clinical practice and their mechanism of action.

Therapeutic recommendations of the Italian Society of Diabetes and the Italian Association of Medical Diabetologists, published recently, reaffirm a series of valid key points in the T2D treatment of hyperglycæmia: these are articulated within an algorithm for a rational therapeutic approach, with a variety of progressive steps in the choice of the various pharmacological options.

Remember that diet, exercise, and weight control are the starting points for any T2D treatment program. In the absence of specific contraindications, metformin is still considered the first-line therapeutic option. If the HbA1c target is not reached with single metformin therapy, further pharmacological options can be chosen between one of the following four treatments: DPP-4 inhibitors, GLP-1 analogues, SGLT-2 inhibitors, and pioglitazone. The choice of one of the various options available will be based on the drug characteristics and especially on the patient profile, in particular the comorbidities (cardiovascular events, chronic renal failure, etc.).

MODY

In the classification of diabetes, Maturity Onset Diabetes of the Young (MODY) represents a class of its own as it is characterized by autosomal dominant family inheritance. Very often, MODY is erroneously classified as T1D or T2D based on the patient's age at the time of diagnosis. Numerous genetic mutations underlie the development of MODY; however, 80-90% of patients have mutations referable to classes 1, 2 and 3 (Table 4.VIII).

TABLE 4.VIII. Maturity onset diabetes of the young (MODY) genetic mutations.

Class	Gene
MODY 1	Hepatocyte nuclear factor 4 α (*HNF4A*)
MODY 2	Glucokinase (GCK)
MODY 3	Hepatocyte nuclear factor 1 α (*HNF1A*)
MODY 4	Insulin promoter factor (*IPF1*)
MODY 5	Hepatocyte nuclear factor 1 β (*HNF1B*)
MODY 6	Neuro transcription factor D1 (*NEUROD1*)
MODY 7	Kruppel-like factor 11 (*KLF11*)
MODY 8	Carboxyl ester lipase (*CEL*)
MODY 9	Paired Homeobox 4 (*PAX4*)
MODY 10	Insulin (*INS*)
MODY 11	B Lymphocyte Kinase (*BLK*)

MODY diagnosis is usually made at a young age, between the ages of 20 and 40 years. Clinically, these patients will present considerable phenotypic variability, in the absence of typical metabolic syndrome characteristics and particularly those related to insulin resistance. The T1D-specific autoantibody pattern is negative, and the clinical course of the disease is generally benign and does not worsen. The finding of impaired fasting glycæmia or reduced carbohydrate tolerance to the oral glucose tolerance test (OGTT) with a positive family history of diabetes, C-peptide levels within normal range and negative autoantibodies makes it necessary to consider MODY for differential diagnosis in young and normal weight subjects.

A genetic analysis is needed to identify the different mutations and individualize the MODY class, since each class is characterized by peculiar phenotypic aspects and clinical progression. Subjects affected by MODY 2 show moderately altered fasting glycæmia and/or reduced tolerance to carbohydrates at the standard OGTT exam with 75 g of glucose. Rarely, even many years after diagnosis, will patients develop macro- or microvascular diabetic complications.

Therapy is mainly aimed at modifying the patient's lifestyle, encouraging strict adherence to diet and regular exercise. Insulin therapy may be required in pregnant patients. On the other hand, MODY 1 and 3 have a less favorable clinical course, often undergoing both macro- and microvascular complications and in particular diabetic nephropathy. Pharmacological therapy involves the administration of sulphonylureas, glinides and sometimes insulin. In the event of poor glycæmic compensation further to pharmacological therapy, the patient may experience real metabolic emergencies such as hyperosmolar glycæmic syndrome which, if not properly treated, can lead to death.

Pregnancy and diabetes

Diagnostic framework

Gestational diabetes mellitus (GDM) is defined as carbohydrate intolerance of varying degrees that is firstly diagnosed during pregnancy and usually resolves shortly after delivery. GDM screening is indicated in

TABLE 4.IX. Oral glucose tolerance test (OGTT) performance stage.

Between 16th and 18th week of pregnancy (high risk, to be repeated between 24th and 28th week if normal)	Previous gestational diabetes
	BMI ≥30 kg/m^2
	Altered fasting glucose levels
Between 24th and 28th week of pregnancy (moderate risk)	Age >35 years
	First-degree relative with diabetes
	BMI>25 kg/m^2
	Previous fetal macrosomia (>4500 g)
	Ethnicity at risk (Asia, Middle East, Caribbean)

TABLE 4.X. Threshold values in OGTT for diagnosis of gestational diabetes

Time (min)	Plasma glucose levels
0	≥92 mg/dL
60	≥180 mg/dL
120	≥153 mg/dL

all women at medium and high risk of developing glucose intolerance (Table 4.IX).

The GDM diagnostic glucose levels for a 75-g glucose loading curve are shown in Table 4.X. Even a single blood glucose reading above the reported limits is enough to confirm the diagnosis.

Follow-up

Blood glucose self-monitoring and morning ketonuria for assessing the adequate dietary intake of carbohydrates and biometric ultrasound determinations for the assessment of fetal abdominal circumference are the most effective tools for monitoring women with gestational diabetes. The capillary glycæmic targets are as follows: <90 mg/dL at fasting, <130 mg/dL after 60 minutes and <120 mg/dL 120 minutes after a meal.

Therapy

A proper diet includes three main meals and two snacks. The daily calorie requirement in the first trimester of pregnancy is comparable to that of a non-pregnant patient; in the second and third trimester it increases by about 350 and 450 calories *per* day, respectively. Weight gain during gestation should be proportional to weight prior to pregnancy. When the glycæmic target is not reached with diet alone, it is advisable to resort to insulin therapy. The ultimate goal of optimizing blood glucose control in pregnancy is to avoid obstetric (macrosomia, preterm birth, gestosis) and

fetal complications. After delivery, blood glucose levels generally return to within normal limits, although the risk of developing T2D is increased in these women: consequently, it is useful for these patients to have a further OGTT with 75 g of glucose 6 to 12 weeks after delivery and then every 1 to 3 years, according to outcome.

CHRONIC AND SEVERE COMPLICATIONS OF DIABETES

CHRONIC COMPLICATIONS

Chronic complications depend on the persistence of metabolic alterations caused by diabetes over time. They generally appear years after the disease onset (10–15 years); however, they may already be present at the time of diagnosis, as this is often delayed compared to the onset of the hyperglycæmic state.

MACROANGIOPATHIC COMPLICATIONS

Diabetic macroangiopathy is the main cause of morbidity and mortality in diabetes: it manifests as ischemic heart disease, central vascular disease, and peripheral vascular disease. Atherosclerotic disease in diabetes does not differ from non-diabetic disease, but it occurs earlier and is more severe. Furthermore, the pathogenesis of macrovascular complications is made more complex by the frequent coexistence of other risk factors such as high blood pressure, dyslipidæmia (characterized by increased triglycerides and LDL and reduced HDL), obesity and cigarette smoking.

Ischemic cardiac disease

Ischemic heart disease, although it does not differ in its clinical manifestations from that in non-diabetic patients (angina pectoris, myocardial infarction, coronary insufficiency) is 2 to 4 times more frequent than in the general population and has a worse prognosis. It should also be mentioned that myocardial infarction can be asymptomatic (silent) in diabetic patients due to coexisting neuropathy. Many studies have shown that adequate blood glucose control (HbA1c levels <7%) can significantly influence the incidence of ischemic heart disease.

Diagnostic tests

Level I

Level I includes:

- annually performed electrocardiogram (ECG);
- basal echocardiogram within the first 3 years from diagnosis.

Level II

Level II includes:

- ergometric test (strain ECG);
- pharmacological stress myocardial perfusion scintigraphy;
- coronary angiography (when a high-risk condition of ischemic heart disease is found through the functional tests performed).

Cerebral and peripheral vascular disease

The risk of stroke and transient ischemic attack (TIA) is 2 to 5 times higher among diabetic patients than in the non-diabetic population. Peripheral arterial occlusive disease in diabetic patients is asymptomatic in 65% of cases, is manifested by claudication in 35% of cases and is already present as critical ischemia in 10% at diagnosis.

Diagnostic tests

Level I

Level I includes:

- Winsor's Ankle/Brachial Index (ABI);
- Color-Doppler ultrasonography of supra-aortic trunks and lower limbs.

Level II

Level II includes

- computed tomography (CT) angiography;
- magnetic resonance imaging (MRI) angiography.

Microangiopathic complications

Diabetic microangiopathy is a vascular complication that affects the small vessels (capillaries, arterioles, venules). The most frequently involved or-

gans are retina, kidney, and nervous system. The pathogenesis is closely related to the duration of disease and chronic hyperglycæmia. The main biochemical mechanisms involved in hyperglycæmia-induced microangiopathic damage are non-enzymatic protein glycation and formation of advanced glycation end products (AGEs), activation of the polyol pathway, activation of protein kinase C isoforms and oxidative stress.

Diabetic retinopathy

Definition

Diabetic retinopathy (DR), a microvascular complication of diabetes, is an eye disease caused by retinal vessel damage. In developed countries it represents the main cause of blindness in people of working age.

Epidemiology and pathophysiology

The prevalence of DR is around 30% and incidence is about 1% *per* year. Several factors promote its onset, particularly diabetes duration, low

TABLE 4.XI. Clinical and diagnostic features of diabetic retinopathy.

Diagnosis	Clinical signs	Therapy/follow-up
No signs of diabetic retinopathy	No visual disturbances	Screening after 12/24 months
Mild non-proliferative retinopathy	No visual disturbances	Screening at 12 months
	Isolated microaneurysms	Blood control monitoring
Moderate non-proliferative retinopathy	No visual disturbances	Screening at 6 to 12 months
	Hemorrhages/microaneurysms/exudates that do not involve the macular area	Blood control monitoring
	Cottony nodules	
Severe non-proliferative retinopathy	No visual disturbances	Urgent eye treatment (photocoagulation/ pharmacological treatment)
	Hemorrhages/microaneurysms/ exudates/multiple cottony nodules	
	Macular oedema or ischemic macular disease	
Proliferative retinopathy	Neo-vessels in the retina or optic disc with preretinal hemorrhages	Urgent eye treatment (photocoagulation/ pharmacological treatment)
	Retinal detachment	

glycæmic compensation, and high blood pressure. Although pathogenesis is multifactorial, blood flow increase, sorbitol accumulation within retinal cells and the presence of AGEs are the main causes.

There are two different types of retinopathies: non-proliferative retinopathy (early stage) and proliferative retinopathy, which represents its progressive stage, although it can also be present at onset (**Table 4.XI**). A dreadful complication of DR is macular œdema, which can occur in any stage of retinopathy.

Clinical presentation

Like other microvascular complications, retinopathy has no symptoms except in the advanced stages or when the macula is involved (with subsequent vision reduction) (**Table 4.XI**).

Diagnosis

The first-level test is the dilated-pupil fundus examination. The second-level tests, particularly in cases of suspected macular oedema, are fluorescein angiography and optical coherence retinal tomography (OCT).

Therapy notes

The most important modifiable factor is blood glucose control. Optimizing blood glucose control delays and improves DR over the long term. Systemic blood pressure monitoring is also recommended according to the standards indicated by international guidelines. Specific treatments for proliferative retinopathy or macular oedema are as follows:

- laser photocoagulation – this counteracts macular oedema and neoangiogenesis. It is indicated in all cases of proliferative retinopathy or in the case of macular oedema.
- intravitreal drugs – these are anti-VEGF or cortisone drugs with intravitreal administration, capable of reducing inflammation and neoangiogenesis.
- vitrectomy – in case of pathological adhesions between the vitreous body and the retina with a retinal traction effect, it is necessary to remove the vitreous-macular traction, thus preventing retinal detachment.

Diabetic neuropathy

Definition

Diabetic neuropathy (DN) represents a set of clinical syndromes that can affect both the peripheral nervous system (sensory and/or motor) and the autonomic nervous system, in the form of mono- or polyneuropathy. The most frequent form is symmetrical sensorimotor peripheral polyneuropathy, which mainly involves the lower limbs. The most used classification is the one presented in the "Toronto Expert Panel on Diabetic Neuropathy Classification," which distinguishes three different groups of DN:

- distal symmetric sensorimotor polyneuropathy (DSSMP), further divided into typical (chronic, symmetrical neuropathy) and atypical (characterized by acute or subacute symptoms) forms;
- autonomic neuropathy;
- focal and multifocal neuropathy: this is further divided into cranial or limb mono-neuropathy (radial, ulnar, femoral, etc.), trunk mono-neuropathy, multiplex mono-neuropathy.

Epidemiology and pathophysiology

DN is a very frequent chronic complication of diabetes and represents the most common form of neuropathy in developed countries, accounting for 50 to 75% of non-traumatic amputation cases. The pathogenesis is multifactorial, resulting from the combination of microangiopathy glucotoxicity, intracellular metabolic alterations and chronic inflammation. The main risk factors for DN are reported in (Table 4.XII).

TABLE 4.XII. Risk factors for diabetic neuropathy.

Risk factors	Non-influenceable	Influenceable
Strongly associated	Age (particularly >70 years)	Low blood glucose monitoring (HbA1c levels)
	Disease duration ≥10 years	High blood pressure
	Height	Dyslipidæmia, hypertriglyceridemia
	Genetic profile	Other cardiovascular risk factors
Hardly associated	Autoimmunity	Smoking, alcoholism, obesity

Clinical presentation

Clinically, DN manifestations are highly variable, depending on DN type and the affected region (Table 4.XIII). DN can be silent and unnoticed while causing significant damage, or it can be present with slowly progressive, non-specific, and insidious clinical symptoms and signs which mimic those of other diseases.

TABLE 4.XIII. Main clinical manifestations of diabetic neuropathy.

Peripheral DN	Autonomous DN
Burning, pinprick pain that worsens during night-rest	Cardiovascular disorders
	Orthostatic hypotension
	Resting tachycardia
	Arrhythmias (QT prolongation on ECG)
	Reduced tolerance to exercise
Cramps	Gastrointestinal alterations
	Delayed gastric emptying
	Gastroparesis
	Bowel movement alterations (constipation, diarrhea, fecal incontinence)
Numbness	Genitourinary alterations
	Bladder dysfunction
	Neurogenic bladder
	Erectile dysfunction
	Vaginal dryness
Paresthesia and muscle weakness	Altered thermoregulation
	Night sweating
	Sudomotor dysfunction (increased or decreased sweating)
Hypoesthesia (more frequently with a "stocking-glove" distribution)	Asymptomatic hypoglycæmia (lack of awareness of the hypoglycæmic event)
Allodynia	

Diagnosis

DN screening is recommended in all T2D patients following diagnosis and in T1D patients 5 years after diagnosis and annually thereafter. Screening is also recommended for patients with prediabetes if they report specific symptoms. Initial evaluation should include medical history, general and neurological examination (sensory, motor, and reflex evaluation), and assessment of orthostatic hypotension and heart rate variability by the CAN test (deep breathing, Valsalva maneuver and lying-to-standing). DSSMP level I diagnostic procedures include electromyography (EMG), electroneurography (ENG), and somatosensory potentials.

Therapy notes

DN therapy is based on three fundamental aspects: adequate blood glucose control; pathogenetic and symptomatic pharmacological therapy; and re-

habilitation. Prevention remains the best DN therapy. The first-line drugs indicated for the treatment of painful forms are pregabalin and duloxetine.

Diabetic kidney disease

Definition

Diabetic kidney disease (or diabetic nephropathy) is the impairment of renal function in diabetes patients; typically, it firstly manifests with albuminuria and causes progressive renal damage.

Epidemiology and pathophysiology

Diabetic kidney disease is the second leading cause of end-stage renal failure and the most frequent cause of chronic kidney damage. It occurs in approximately 25% of T1D patients and up to 40% of T2D patients. Hyperglycæmia, hyperlipidæmia and high blood pressure are predisposing factors for disease progression, but a genetic basis for the development of this complication is assumed. The different stages of diabetic nephropathy are summarized in Table 4.XIV.

TABLE 4.XIV. Stages of diabetic kidney disease according to the anatomopathological classification.

Stage	Description
Class I	Thickening of the mesangial membrane
Class II (IIa and IIb)	Increased thickness of the mesangial membrane and microalbuminuria
Class III	Presence of at least one Kimmelstiel-Wilson lesion. Mesangial hyperplasia
Class IV	>50% of glomerules have glomerulosclerosis

Clinical presentation

The initial stages of diabetic nephropathy are silent; therefore, screening for diabetic nephropathy in accordance with the guidelines is very important. Chronic kidney disease is classified as shown in Table 4.XV.

Diagnosis

Screening for diabetic nephropathy is recommended annually in all T1D patients with a disease duration >5 years and in T2D patients starting from

TABLE 4.XV. Stages of chronic kidney disease.

Stage	GFR (mL/min/1.73 m^2)	Description
I	≥90	Albuminuria with normal/increased GFR
II	60-89	Albuminuria with reduced GFR
IIIa (moderate)	45-59	Reduction of moderate GFR
IIIb (severe)	30-44	Reduction of severe GFR
IV	15-29	Severe GFR reduction
V	<15-dialysis	Chronic renal failure

GFR: glomerular filtration rate.

diagnosis. Screening in pregnant diabetic women is also recommended as the presence of albuminuria is associated with an increased risk of pre-eclampsia. The diagnosis is made after the detection of microalbuminuria in at least two out of three tests performed over a period of 6 months. Serum creatinine levels and renal filtrate estimation complete the screening and allow monitoring of the disease stage.

Therapy notes

Angiotensin-converting enzyme (ACE) inhibitors or sartans have shown efficacy in reducing albuminuria and slowing the progression of kidney disease. In any case, the therapeutic approach is multifactorial, and the aforementioned specific therapy is complemented by optimization of blood glucose compensation and blood pressure control.

Acute complications and diabetic emergencies

This section includes hypoglycæmia and hypoglycæmic coma, diabetic ketoacidosis, and hyperosmolar hyperglycæmic syndrome.

Hypoglycæmia

Definition

Hypoglycæmia is defined as blood glucose levels <70 mg/dL. Severe hypoglycæmia is defined by levels <55 mg/dL.

Epidemiology and pathophysiology

Incidence varies according to the type of diabetes and the therapy in place. It is greater in case of insulin therapy followed by treatment with sulphonylureas. In case of hypoglycæmic crisis, the brain is the first organ to suffer damage from hypoglycæmia as it is not able to store glucose in the form of glycogen (neuroglycopenia). The main causes of hypoglycæmia are:

- inappropriate diet (skipping or delaying meals, eating less than prescribed);
- overdose of hypoglycæmic drugs or insulin;
- over exercising;
- excess alcohol intake;
- serious infections (sepsis);
- chronic diseases (example: renal failure or adrenal failure);
- insulinomas (rare).

Clinical presentation

Whipple's triad includes:

- low blood glucose levels.
- symptoms suggestive of hypoglycæmia.
- relief of symptoms after normal blood glucose levels are restored.

Symptoms and severity of clinical picture (stages of hypoglycæmia) can be:

- mild – presence of adrenergic symptoms alone (tremors, palpitation, sweating and hunger) caused by the release of counter-regulatory hormones (catecholamines, cortisol, growth hormone). The patient can manage the situation.
- moderate – autonomic symptoms and neuroglycopenia symptoms (weakness, blurred vision, irritability, drowsiness, confusion). The patient is still able to manage hypoglycæmia independently.
- serious – altered consciousness state; requires third-party intervention.

Patients with autonomic neuropathy may not be able to sense low glucose levels and are consequently at greater risk of experiencing seizures or coma. The perception of hypoglycæmic symptoms may also be reduced by concomitant therapy with β-blockers.

Diagnosis

Capillary blood glucose measurement.

Therapy notes

Therapy of patient in alert state must include:

- oral administration of 15 g of simple sugars and re-evaluation of blood glucose levels after 15 minutes (15/15 rule).

Therapy of unconscious patient must include:

- in hospital setting – infusion of 15-20 g glucose in 20% or 33% hypertonic solutions (*e.g.*, 80 mL of 20% glucose, or 50 mL of 33% glucose) within 1 to 3 minutes;
- at home – administration of glucagon intramuscularly (1 mg in adult patients) or intranasally (3 mg) *via* a pre-prepared kit, by third parties.

Reactive hypoglycæmia

Definition

Reactive hypoglycæmia is a low blood glucose episode that occurs within 5 hours after a meal.

Epidemiology and pathophysiology

More frequent cases:

- insulin resistance.
- gastrectomy (hyperinsulinism due to rapid intestinal glucose absorption).

Rare cases:

- increased insulin receptor sensitivity;
- enzyme deficiencies such as hereditary fructose intolerance, galactosemia and hypersensitivity to leucine.

Clinical presentation

As in all other cases of hypoglycæmia, symptoms are due to the adrenergic activation and subsequently to the onset of neuroglycopenia symptoms.

Diagnosis

Diagnostic criteria of the Endocrine Society for hyperinsulinemic hypoglycæmia:

- plasma glucose levels <55 mg/dL (3.1 mmol/L);
- plasma C-peptide ≥0,6 ng/mL (0.02 nmol/L);

- plasma insulin ≥3 μU/mL (18 pmol/L);
- plasma pro-insulin >5 pmol/L;
- β-OH-butyrate ≤2.7 mmol/L;
- negativity for anti-insulin antibodies and screening for sulfonylurea intake.

Diagnostic confirmation includes oral glucose tolerance test (OGTT) protracted for 5 hours or MMT (mixed meal test), with serial samples for blood glucose and insulin levels.

Therapy notes

Therapy of hypoglycæmia after bariatric surgery includes:

- dietary therapy (meal subdivision; diet based on polysaccharides and fibers that slow down gastric emptying and intestinal absorption of glucose).

Therapy of insulin resistance/prediabetes includes:

- dietary therapy (sugar-free diet and fractionated diet).

Diabetic ketoacidosis

Definition

Diabetic ketoacidosis (DKA) is an acute complication of diabetes, more common in T1D and rarer in T2D. It is characterized by a state of marked insulin deficiency aggravated by subsequent hyperglycæmia, dehydration, and acidosis. In extreme situations, and if not promptly treated, it can be fatal. From a laboratory point of view, DKA is characterized by:

- hyperglycæmia (>300 mg/dL);
- ketonemia and ketonuria;
- metabolic acidosis (pH <7.30);
- plasma bicarbonates <15 mEq/L.

Epidemiology and pathophysiology

The incidence of DKA varies according to the case series and is approximately 0.4-1.0 episodes x 100 T1D patients *per* year.

The main causes are represented by:

- clinical onset of type 1 diabetes (the more frequent);

- infections, mainly of the urinary tract;
- discontinuation of insulin therapy.

Other causes may be:

- myocardial infarction;
- cerebrovascular ischemic events;
- surgery;
- trauma;
- stress;
- complicated pregnancies;
- medication intake (corticosteroids, clozapine, olanzapine, epinephrine, pentamidine, SGLT2 inhibitors).

Pathogenesis includes insulin deficiency, which leads to the inability of cells to take up glucose, and use of fatty acids instead of glucose for energy production, which leads to the production and release of ketone bodies (acetoacetic acid, acetone, β-hydroxy-butyric acid), and, therefore, to metabolic acidosis.

At the same time, the lack of intracellular sugar causes the paradoxical increase in counter-regulatory hormones that further stimulate the release of glucose into the circulation with consequent worsening of hyperglycæmia.

Clinical presentation

Clinical presentation includes acute onset (within 24 hours)

Initial symptoms are represented by thirst, polydipsia, polyuria-nocturia, nausea, vomiting, asthenia, muscle pain and muscle cramps, anorexia, or bulimia.

Typical signs (physical examination) generally include: dry skin and mucous membranes, tachycardia, low blood pressure, Kussmaul's breathing, acetone breath (smell of rotten fruit), hypothermia, fever (in case of infection), stuporous state or coma (more severe cases).

Diagnosis

The diagnostic triad is represented by: 1) hyperglycæmia (usually >300 mg/dL); 2) blood or urine ketones; and 3) metabolic acidosis in blood gas analysis (generally <7.30: bicarbonates (HCO3-) <15 mEq/L).

Further diagnostic tests/clinical monitoring:

- complete blood count, renal function (creatinine, blood urea nitrogen), electrolytes (sodium, potassium, and chlorine), C-reactive protein, amylase, lipase.
- ECG monitoring.

Therapy notes

Therapy must include:

- rehydration – infusion of saline solution (Na 0.90%) at a rate capable of allowing a decrease in blood sodium levels between 0.5 and 1.0 mEq/hour;
- correction of electrolyte imbalance – in case of hypokalemia (plasma potassium <5.5 mEq/L) add 20 to 40 mEq of potassium chloride for each liter of saline solution; in case of hyperkalemia, insulin should be infused simultaneously with hydration, with potassium monitoring every 1 to 2 hours;
- correction of hyperglycæmia – intravenous insulin infusion in bolus of 0.1 to 0.15 U/kg aiming to reduce blood sugar by about 80 mg/dL every hour. In the presence of hypokalemia and hypotension, it is advisable to not administer insulin before the appropriate corrections. When blood glucose levels are below 200 mg/dL, administer 5% glucose solution in saline solution to prevent dangerous hypoglycæmia. Subsequently, blood glucose monitoring every hour for the first 4 hours is recommended.

Hyperosmolar hyperglycæmic syndrome

Definition

Insulin deficiency associated with inadequate fluid intake, typical of type 2 diabetes, often occurring in the context of stress.

Main characteristics:

- High plasma osmolality (>320 mOsm/kg).
- Severe hyperglycæmia (blood glucose levels >600 mg/dL).
- Marked dehydration.
- Absence of acidosis (plasma pH always >7.3 and bicarbonate >15 mEq/L).

Epidemiology and pathophysiology

Hyperosmolar hyperglycæmic syndrome is characteristic of T2D patients: it generally occurs after a period of symptomatic hyperglycæmia, during which fluid intake is not sufficient to prevent the extreme dehydration caused by hypoglycæmia-induced osmotic diuresis.

It is mainly characterized by:

- incidence is 10 to 17 cases x 100,000 population *per* year;
- more frequent in the elderly;
- poor prognosis in 20% of cases;
- triggering factors are infections, intercurrent disorders such as acute myocardial infarction, stroke, pancreatitis, acute renal failure, pharmacological therapies (diuretics, β-blockers, phenytoin, cimetidine), administration of hyperosmolar solutions (enteral or total parenteral nutrition), surgery, relative deficiency of insulin or inadequate/discontinued hypoglycæmic therapy and social care issues (poor home care).

Clinical presentation

Symptoms may include:

- initial – polydipsia, polyuria, intense asthenia. In 50% of cases: nausea, vomiting, abdominal pain.
- late – neurological symptoms with progressive alteration of consciousness (confusion and disorientation up to lethargy, stupor, and coma).
- other factors – focal or generalized seizures and transient hemiplegia (absent in DKA).

Clinical signs (physical examination) are represented by:

- dehydrated skin and mucous membranes;
- sunken eyes (enophtalmos);
- tachycardia;
- low blood pressure;
- any signs of infection.

Diagnosis

The diagnosis is generally characterized by hyperglycæmia (generally >600 mg/dL), high plasma osmolality (>320 mosm/kg), absence of acidosis on blood gas analysis, and generally negative ketonemia and ketonuria.

The state of severe dehydration leads to marked increase in blood urea nitrogen and creatinine and a significant risk of developing acute prerenal kidney failure. Serum potassium levels are generally normal (unlike DKA) while the finding of sodium alterations due to the state of dehydration (pseudohyponatremia) is more frequent (Table 4.XVI).

TABLE 4.XVI. Lab tests during hyperosmolar hyperglycæmic syndrome.

Blood glucose	>600 mg/dL
Na^+	Variable
K^+	Variable
pH	>7.3
Bicarbonates	>20 mEq/L
Plasma osmolality	>320 mOsm/kg
Anion gap	<12 mEq/L
Ketones	Absent or traces
Blood count	Hemoconcentration, possible neutrophilic leukocytosis from dehydration
Blood urea nitrogen/ creatinine ratio	Hemoconcentration, high levels for pre-renal failure
Amylase, transaminase, creatine phosphokinase (CPK)	Sometimes increased (non-specific increase or due to triggering conditions)
Lipase	Increased in pancreatitis
Triglycerides	Increased due to metabolic decompensation

Etiological diagnosis includes:

- markers of liver cytolysis and cholestasis, amylase and lipase, heart and muscle enzymes, coagulation panel, culture tests;
- instrumental tests, such as ECG and chest X-ray (research for any foci of infection).

Therapy notes

The therapy goals are to correct dehydration to restore the circulating volume and osmolality, to normalize blood sugar, to restore electrolyte balance and to treat the triggering cause.

- rehydration – infusion of 2-3 liters of saline solution (0.9% NaCl) within the first 3 hours. The water deficit must be corrected slowly, within 36 to 72 hours and must always be associated with potassium administration, even in case of target K^+ levels;
- correction of hyperglycæmia – regular insulin administration *via* intravenous boluses of 5 to 10 units followed by continuous infusion of 3

to 7 units/hour. In case of hypokalemia, delaying the start of insulin therapy until reaching a potassium level >3.5 mEq/L may be considered. As per ketoacidosis, 5% glucose solution in saline solution should be administered after reaching blood glucose levels below 200 mg/dL at a rate of 100 mL/hour;

- hourly monitoring of hemodynamic status, water balance, neurological status, blood glucose levels, electrolytes and kidney function, ECG, and temperature every 2 to 3 hours.

PATIENT EDUCATION AND NUTRITION

Many studies demonstrate that receiving an adequate education in self-managing the disease at the time of diagnosis is associated with reduction of HbA1c levels, weight loss, reduction of complications and improvement in quality of life. This educational process plays a fundamental role in the effectiveness and efficacy of the treatments provided by the diabetes team and therefore it must also include reinforcements further to diagnosis, to maintain the skills acquired.

Notes on nutritional therapy in diabetes

Medical nutrition therapy is recommended in all people with diabetes as an integral part of care and should be managed by a nutrition expert such as a dietician/nutritionist. Its role in improving metabolic compensation with subsequent reduction in glycated hemoglobin (HbA1c) levels and the complications associated with diabetes has been widely highlighted in clinical studies, which have also recognized its strong impact on reducing the health costs of the disease.

The goals of medical nutrition therapy are to:

- obtain and maintain blood glucose levels close to the normal range;
- obtain optimal blood lipid levels;
- guarantee an adequate caloric intake;
- promote normal growth and normal development;
- prevent, delay or treat risk factors or complications related to nutrition;
- improve or maintain the state of health.

Medical nutrition therapy must be personalized, considering individual needs, willingness to change, metabolic targets, type of diabetes, pharmacological therapy, and lifestyle, as well as the economic possibilities and access to healthy food. It must also respect individual religious beliefs and cultural traditions, maintaining the pleasure of eating by promoting a healthy diet without judging individual choices.

Weight management and loss

Weight loss is recommended for both overweight/obese patients with prediabetes to slow T2D progression, while improving metabolic parameters in those with overt diabetes. Lifestyle management must be continuous with frequent check-ups to ensure lifestyle changes. Weight reduction is correlated with a 0.3 to 2% reduction in HbA1c and pharmacological therapy, with a definite improvement in quality of life. We recommend a weight loss of at least 5% of the initial weight, as minor losses have been shown to have no positive effects on metabolic parameters. Weight loss can be achieved with a moderate reduction of calorie intake and increasing energy expenditure by exercising. A fiber-rich diet from vegetables, fruit, and unrefined grains, low in animal fats, such as the Mediterranean diet, is recommended.

Carbohydrate management

According to the ADA (American Diabetes Association), there is no ideal carbohydrate intake for people with diabetes; therefore, it is advisable to monitor intake and to evaluate the postprandial blood glucose response. The 2016 Italian Standards for Diabetes recommend a diet based on vegetables and greens, legumes, fruit, and whole grains as source of carbohydrates for people with type 1 and type 2 diabetes. It is, however, advisable not to reduce the total dietary amount of carbohydrates below 130 g/day. Low glycæmic index (GI) foods are recommended, as they can lead to improved blood glucose control and help to reduce the risk of hypoglycæmia. ADA's position on this issue is more cautious, as there are no studies comprehensively confirming the role of GI. Results concerning the role of low-carb diets are also unclear. There is still no clear definition of what is meant by a low-carb diet, although studies have shown that diets with a

carbohydrate content below 45% of total energy are superior to those with higher carbohydrate content in settling blood glucose levels.

Some authors have demonstrated the benefits of very low-calorie ketogenic diets (VLCKD), with less than 50 g of carbs daily. According to ADA, this type of dietary approach can be followed for a short period of time (about 3 to 4 months) if the patient wishes to. Furthermore, there is evidence that people fail to change their dietary pattern sustainably and generally tend to restore their original macronutrient distribution after a short period of time. It is therefore recommended to individualize the dietary plan keeping the distribution of macronutrients as close as possible to normal.

People with type 1 and type 2 diabetes on insulin therapy must learn to dose their insulin in relation to the carbohydrate content of each meal. An excellent tool can be applying the carb count, which allows adjustment of the dose of insulin to be administrated at a meal, to the carbohydrate content of that meal. It is essential that the patient learns to recognize carbohydrate-containing foods and to count their content in grams.

It is advisable to reduce the intake of refined carbohydrates or sugars, especially as drinks or light products, that are often high in sugars. Simple sugars can be consumed to replace other carb-rich foods; if consumed in addition to other foods, they must be considered to adapt insulin therapy or therapy with oral hypoglycæmic agents.

Protein management

According to the 2016 Italian Standards for Diabetes, dietary proteins must comprise about 10 to 20% of the total daily energy intake, while subjects with kidney disease are advised to reduce the protein intake to 0.8 g/kg body weight.

Some authors have demonstrated that dietary schemes with higher protein content, around 30% of total energy intake, can increase the sense of satiety and adherence to the diet. Therefore, the ADA recommends individualizing the protein requirement in relation to the patient's dietary pattern. It is also recommended to avoid carbohydrate-based and high-pro-

tein foods for treating or preventing hypoglycæmia, as studies have shown that proteins can increase the insulin response.

Lipid management

Italian and American guidelines agree in recommending a dietary fat amount of 20 to 35% of the total daily energy intake. The percentage of saturated fats must be less than 10%, to be further reduced to <8% in the case of high LDL cholesterol levels.

Consumption of trans fatty acid-containing foods must be drastically reduced. According to the Italian guidelines, it is important that dietary cholesterol does not exceed 300 mg *per* day, reduced to 200 mg *per* day in case of hypercholesterolemia. These limits were not set by the 2015 American guidelines as they declare the need for major scientific evidence to set the exact dose-response of dietary cholesterol compared to blood cholesterol.

The 2016 Italian Standards for Diabetes recommend consuming fish, preferably omega-3-rich fatty fish, at least twice weekly. However, when considering the therapeutic goals and the cardiovascular risk, the quality of fats is more important than the total quantity, and it is recommended to limit the percentage of saturated fat. Some studies have shown that a Mediterranean diet, rich in polyunsaturated and monounsaturated fatty acids, can improve both blood glucose and lipid control. A systematic review of the literature concluded that dietary supplements with omega 3 fatty acids do not improve blood glucose control in individuals with type 2 diabetes.

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5 DYSLIPIDÆMIA

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DEFINITION

Dyslipidæmia is a group of lipid metabolism disorders characterized by an increase in blood lipid levels over the 90th to 95th percentile compared to the mean values of the reference population (epidemiological definition) or by an increased cardiovascular risk in the individual patient (clinical definition).

CLINICAL PRESENTATION

High blood cholesterol levels are mostly asymptomatic and, in most cases, found in blood tests occasionally performed for screening purposes or for defining the cardiovascular risk of the patient. Therefore, the first clinical manifestation of dyslipidæmia is often its most frequent complication, that is, an atheromatous vessel disease (such as coronary artery disease, cerebral stroke, or peripheral vascular disease).

Xanthomas (especially tendinous, but also plantar and tuberous), xanthelasmas and *arcus corneae* are clinical signs found in patients with very high levels of low-density lipoprotein (LDL) cholesterol. On the other hand, eruptive skin xanthomas are manifestations of very high levels of very low-density lipoprotein (VLDL) and chylomicrons (hypertriglyceridemia).

Lipemia retinalis is a characteristic sign of severe hypertriglyceridemia, characterized by a creamy discoloration of retinal vessels that can be explored upon examination of the eye fundus.

Acute pancreatitis can sometimes be the first manifestation of particularly high blood triglyceride levels.

ETIOLOGY

Dyslipidæmias can have primary (genetic basis) and secondary (lifestyle, drugs, other diseases) causes and can be classified based on the lipid pattern. Sometimes the secondary causes of dyslipidæmia can add to and aggravate primary lipoprotein metabolism disorders.

Monogenic familial hypercholesterolemia

Monogenic familial hypercholesterolemia includes defects localized on a single gene with autosomal recessive or dominant inheritance:

- familial hypercholesterolemia, due to LDL receptor gene mutation is caused by a mutation in the short arm of chromosome 19 that determines a functional defect of the LDL receptor. It has an autosomal dominant inheritance and involves a heterozygous variant, much more prevalent in the general population, and a homozygous one, characterized by significantly elevated LDL levels with severe clinical repercussions;
- familial hypercholesterolemia due to the *PCSK9* gene mutation is caused by a mutation of the pro-protein convertase able to bind to the LDL receptor;
- familial defective ApoB hypercholesterolemia (FDB) is due to a mutation of the gene encoding the apolipoprotein B100 that has a reduced binding affinity for the LDL receptor, which is therefore catabolized more slowly;
- autosomal recessive hypercholesterolemia (ARH) is due to a mutation of the gene located on chromosome 1 – called *LDLRAP1* – that promotes endocytosis of the LDL receptor–LDL complex by liver cells;
- sitosterolemia is due to a mutation of genes encoding for steroline-1 and 2 that stimulate sterol secretion, both from the apical portion of enterocytes in the intestinal lumen and from the biliary pool of liver cells in the bile.

Monogenic hypertriglyceridemia

Monogenic hypertriglyceridemia is represented by:

- familial hypertriglyceridemia, due to an unknown mutation that causes an autosomal dominant inherited disease. This leads to a significant increase in triglycerides levels with normal LDL levels and reduced high-density lipoprotein (HDL) levels;
- chylomicronemia syndrome, an autosomal recessive condition due to a deficiency in the enzyme responsible for the catabolism of triglycerides, chylomicrons and VLDL (lipoprotein lipase, LPL). Diagnosis is made at the pediatric stage. There are low HDL and LDL blood levels and

TABLE 5.I. Fredrickson classification.

Type	Lipoprotein pattern	Method of diagnosis		Frequency
		Plasma appearance	Blood levels	
I	↑ Chylomicrons	Formation of a milky layer on clear undernatant	↑ ↑ ↑ Triglycerides (TG)	<1%
	Normal VLDL		Cholesterol from normal to ↑	
			Total cholesterol/TG <0.2	
IIa	↑ LDL	Clear	↑ ↑ LDL cholesterol	10%
	Normal VLDL		Normal triglycerides	
			Total cholesterol/TG >1.5	
IIb	↑ LDL	Clear to turbid, without milky layer on the surface	↑ ↑ LDL cholesterol	40%
	↑ VLDL		↑ ↑ Triglycerides	
			Total cholesterol/TG variable	
III	↑ IDL	Turbid with a faint milky layer on the surface	↑ Total cholesterol	<1%
			↑ ↑ Triglycerides	
			Total cholesterol/TG 0.3-1	
IV	↑ VLDL Chylomicrons and LDL within range	Clear to turbid, without milky layer on the surface	↑ ↑ Triglycerides	45%
			↑ Cholesterol (or normal)	
			Total cholesterol/TG 0.2-1	
V	↑ VLDL	Formation of a milky layer on turbid undernatant	↑ ↑ ↑ Triglycerides	5%
	↑ Chylomicrons		↑ ↑ Cholesterol	
			Total cholesterol/TG 0.15-0.6	

VLDL: very low-density lipoprotein; TG: triglycerides; LDL: low-density lipoprotein.

significant increases in triglyceride blood levels.

Hypertriglyceridemia with complex genetics

Hypertriglyceridemia with complex genetics includes:

- atherogenic dyslipidæmia, characterized by hypertriglyceridemia, reduction in HDL blood levels and high concentration of small LDL and increased VLDL synthesis; it is associated with metabolic syndrome.

DIAGNOSIS

The diagnosis of dyslipidæmia considers:

- medical history and physical examination;
- measurement of serum lipid profile (total cholesterol, HDL cholesterol, triglycerides, LDL cholesterol) after at least 10 hours of fasting;
- determination of the phenotypic pattern of dyslipidæmia according to the Fredrickson classification (Table 5.I);
- identification of the main secondary forms of dyslipidæmia.

TABLE 5.II. Dutch Lipid Clinic Score.

Criteria	Points
Family history	
First-degree relatives with premature coronary artery disease (<55 years in men, <60 years in women)	1
First-degree relatives with total cholesterol ≥310 mg/dL	1
First-degree relatives with tendinous xanthomas and/or arcus corneae	2
Children <18 years with total cholesterol ≥230 mg/dL	2
Clinical history	
Subject with premature coronary artery disease (<55 years in men; <60 years in women)	2
Subject with premature cerebral or peripheral vascular disease (<55 years in men; <60 years in women)	1
Physical examination	
Tendinous xanthomas	6
Arcus corneae in subjects aged <45 years	4
LDL cholesterol levels (not in pharmacological therapy)	
325 mg/dL	8
251-325 mg/dL	5
191-250 mg/dL	3
155-190 mg/dL	1
DNA analysis	
Causative mutations in known genes	8
Diagnosis	Total score
Certain	>8
Probable	6 to 8
Possible	3 to 5
Improbable	0 to 2

LDL: low-density lipoprotein.

TABLE 5.III. Expected lipid targets according to ESC/EAS 2019 Guidelines.

Clinical type	Risk level	Therapeutic aim LDL-C
Diabetes mellitus with three major risk factors or organ damage or early onset type 1 diabetes with disease duration ≥20 years	Very high	<55 mg/dL and/or LDL-C reduction ≥50%
Diabetes mellitus without organ damage with disease duration ≥10 years or one additional risk factor	High	<70 mg/dL
Ischemic heart disease, previous heart attack, previous aortocoronary bypass, previous PTCA	Very high	<55 mg/dL and/or LDL-C reduction ≥50%
Previous ictus cerebri, previous TIA	Very high	<55 mg/dL and/or LDL-C reduction ≥50%
Peripheral arterial disease	Very high	<55 mg/dL and/or LDL-C reduction ≥50%
Severe chronic renal disease (eGFR<30 mL/min)	Very high	<55 mg/dL and/or LDL-C reduction ≥50%
Moderate chronic renal disease (eGFR<60 mL/min)	High	<70 mg/dL

LDL-C: LDL cholesterol; PTCA: percutaneous transluminal coronary angioplasty; TIA: transient ischemic attack; eGFR: estimated glomerular filtration rate.

- identification of any primary forms of dyslipidæmia using the Dutch Lipid Clinic Score (Table 5.II), a system that allows the clinician to diagnose or suspect the presence of familial hypercholesterolemia, the most common form of primary dyslipidæmia after combined familial hyperlipidæmia.

THERAPY

Intervention strategies to be implemented in individual patients to achieve the recommended lipid targets (Table 5.III) should be guided by the total cardiovascular risk and LDL levels and include both lifestyle modification and drugs (Table 5.IV).

LIFESTYLE MODIFICATION

NUTRITIONAL THERAPY AND WEIGHT LOSS

Saturated fats should not exceed 10% of total calories (<7% if LDL>100 mg/dL) with daily cholesterol intake less than 300 mg (<200 mg if LDL>100 mg/dL). An increase in monounsaturated fats (oleic acid in olive oil) and polyunsaturated fat (omega 3 and omega 6) intake is recommended. It is advisable to increase daily fiber intake (20-25 g/day) as

TABLE 5.IV. Intervention strategies in primary prevention related to total cardiovascular risk and LDL levels (ESC/EAS 2019 guidelines). In patients with previous major cardiovascular event, statin therapy should be considered irrespective of total cholesterol levels.

	Untreated LDL-C levels					
Total CV risk (SCORE) %	<55 mg/dL, <1.4 mmol/L	55 to <70 mg/dL, 1.4 to <1.8 mmol/L	70 to <100 mg/dL, 1.8 to <2.6 mmol/L	100 to <116 mg/dL, 2.6 to <3.0 mmol/L	116 to <190 mg/dL, 3.0 to <4.9 mmol/L	≥190 mg/dL, ≥4.9 mmol/L
<1	No drug intervention	No drug intervention	No drug intervention	No drug intervention	Lifestyle intervention, consider drug if uncontrolled	Lifestyle intervention and concomitant drug intervention
Class[a]/level[b]	I/C	I/C	I/C	I/C	IIa/A	IIa/A
≥1 to <5	No drug intervention	No drug intervention	No drug intervention	Lifestyle intervention, consider drug if uncontrolled	Lifestyle intervention, consider drug if uncontrolled	Lifestyle intervention and concomitant drug intervention
Class[a]/level[b]	I/C	I/C	IIa/A	IIa/A	IIa/A	IIa/A
≥5 to <10	No drug intervention	No drug intervention	Lifestyle intervention, consider drug if uncontrolled	Lifestyle intervention and concomitant drug intervention	Lifestyle intervention and concomitant drug intervention	Lifestyle intervention and concomitant drug intervention
Class[a]/level[b]	IIa/A	IIa/A	IIa/A	I/A	I/A	I/A
≥10 or very high risk	No drug intervention	Lifestyle intervention, consider drug if uncontrolled	Lifestyle intervention and concomitant drug intervention	Lifestyle intervention and concomitant drug intervention	Lifestyle intervention and concomitant drug intervention	Lifestyle intervention and concomitant drug intervention
Class[a]/level[b]	IIa/B	IIa/A	I/A	I/A	I/A	I/A

CV: cardiovascular; LDL-C: low-density lipoprotein cholesterol; SCORE: Systematic Coronary Risk Estimation.
[a]Class of recommendation; [b]level of evidence.

well as to reduce use of alcohol. Fish should be preferred. The intake of phytosterols should be increased (about 2 g/day) since they are able to break down the circulating LDL by about 10%.

Exercise

This leads to increased fibrinolysis and lipolysis, improved endothelial function, reduced small and dense LDL levels, and reduced sympathetic tone. Furthermore, exercise has the potential to reduce total cholesterol by about 6% and LDL cholesterol by 10%, causing an increase in HDL cholesterol of about 5%. These results can be achieved by practicing at least 30 minutes of moderate aerobic exercise daily. Mortality appears to be favorably influenced by regular aerobic physical activity.

Drugs

Statins

These inhibit the synthesis of endogenous cholesterol by acting on the enzyme hydroxymethylglutaryl-CoA reductase. This enzyme converts the molecule 3-hydroxy-3-methylglutaryl-CoA into mevalonic acid, a precursor of cholesterol. Statins are effective in reducing LDL cholesterol levels, in preventing the cardio-cerebral-vascular damage caused by atherosclerosis in subjects at risk and in preventing cardio-cerebral-vascular damage in all those subjects who have already had an event. The choice of the type of statin is related to its ability to reduce the LDL levels (Table 5.V).

TABLE 5.V. Statins (from G Ital Cardiol, 2019).

Atorvastatin	Simvastatin	Pravastatin	Fluvastatin	Rosuvastatin	LDL-C reduction*
	10 mg	20 mg	40 mg		25-30%
10 mg	20 mg	40 mg	80 mg		31-35%
20 mg	40 mg			5 mg	36-40%
40 mg				10 mg	41-50%
80 mg				20 mg	51-55%
				40 mg	56-60%

*High intensity statin + ezetimibe: estimated LDL reduction 65%.

Ezetimibe

This is a triphenyl azetidinone derivative able to selectively inhibit intestinal absorption of dietary and bile cholesterol without causing the typical side effects of bile acid binding resins. Concomitant administration of statins is possible and combination pills are available on the market. The molecular target of ezetimibe is a sterol transporter, present both on the brush border of intestinal epithelial cells and in the canalicular membrane of liver cells: it is responsible for cholesterol and phytosterol uptake.

Fibrates

Amphipathic carboxylic acids used in the treatment of hypertriglyceridemia. They increase LPL activity with hydrolysis of triglycerides. Fibrates are indicated in the treatment of severe hypertriglyceridemia and hyperlipidæmia as second or third choice treatment, alone or in combination with statins, omega-3, niacin, or bile acid sequestrants.

Bile acid sequestrants

Cholestyramine is the best-known representative of this class. It is a hydrochlorinated anion exchange resin which binds bile acids in the intestine, forming an insoluble complex excreted with feces.

LDL apheresis

This treatment is reserved for severe cases (*e.g.,* homozygous familial hypercholesterolemia). A single apheresis procedure can reduce LDL cholesterol and lipoprotein(a) levels by 70-80%, with proven efficacy in reducing mortality and major cardiovascular events.

TABLE 5.VI. Expected LDL-C reduction with anti-PCSK9 antibodies alone or in combination (from ESC/EAS 2019 Guidelines).

Ab *vs.* PCSK9	LDL-C reduction
Alone	60%
With high intensity statin	75%
With high intensity statin and ezetimibe	85%

Anti-PCSK9 antibodies

These block *PCSK9* causing a reduction in LDL levels by up to 64% of the baseline levels (**Table 5.VI**).

Long-chain polyunsaturated fatty acids n-3

N-3 PUFA, also referred to as "omega-3," have been identified as the molecules likely responsible for the cardiovascular benefits of consuming large quantities of fish. Particularly, the supplementation of 1-3 grams *per* day of eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA), the two main n-3 PUFAs contained in fish, can reduce blood triglyceride levels by about 25-30%.

Bempedoic acid

This inhibits the enzyme ATP citrate lyase (ACLY), which is involved in cholesterol synthesis upstream of the statin target, in the liver. In addition to statins, it allows a further reduction in LDL cholesterol levels (up to 28% compared to placebo). Furthermore, bempedoic acid is not active in the skeletal muscle, thus reducing the risk of muscle-related adverse events.

Supplements

Numerous naturally derived compounds, some classified as supplements, have also been shown to have a positive effect on the control of hypercholesterolemia. However, currently none of them has reached sufficient evidence to be recommended as gold standard treatment of the disease. They include inositol, red yeast rice extracts (source of monacolin K), artichoke and dandelion, spirulina, omega-3 and glucomannan. In case of failure to reach the target with lifestyle changes, pharmacological treatments should be started.

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6 OSTEOPOROSIS

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BIOLOGY AND PHYSIOLOGY OF BONE TISSUE

Organization

Bone tissue is a highly specialized connective tissue characterized by an extracellular matrix consisting of an organic component (collagen, and mucopolysaccharides and glycoproteins to a lesser extent) and an inorganic component, represented by calcium and phosphate deposited as hydroxyapatite mineral. The inorganic component is the site of Ca^{2+} deposits, which are continuously mobilized both for bone remodeling and for homoeostatic control of calcium blood levels. The organic component is the scaffolding, which acts as a guide for mineralization. Collagen fibers give elasticity and mechanical strength. The mucopolysaccharide and glycoprotein fraction is called the extrafibrillary matrix. It constitutes a homogeneous liquid mass. Among various glycoproteins, the main one is osteopontin, which, due to its net negative charge, binds the Ca^{2+} in the already formed hydroxyapatite crystals, blocking their further mineralization.

Bone tissue cells

There are three bone cell types: osteoblasts, osteocytes, and osteoclasts. The first two derive from the differentiation of mesenchymal stem cells (MSCs) that are also precursors of many other cell types (*e.g.*, adipocytes).

Osteoclasts derive from the differentiation of monocytes, also the precursors of macrophages. Osteoblasts are secretory mononuclear cells responsible for the deposition of both organic and inorganic bone matrixes. Osteocytes are the most abundant cells in bone: they communicate with each other and with the surrounding environment through the extension of their plasma membrane. Following erosion of the adjacent matrix or in the event of a fracture, osteocytes can re-differentiate into osteoblasts and act as mechanical sensors, instructing the osteoclasts on where to reabsorb the matrix and the osteoblasts on where to reattach it. Finally, osteoclasts perform an erosive function through the secretion of lysosomal hydrolase enzymes. Osteoclasts appear as multinucleated giant cells derived from the fusion of several newly formed osteoclasts, rich in lysosomes, with a ruffled border whose projections trap apatite particles, derived from the hydrolysis of hydroxyapatite, in their clefts. Osteoclasts are also involved in the homoeostatic control of serum calcium levels and in the remodeling of bone microarchitecture.

Regulation of bone metabolism: parathyroid hormone, vitamin D, and calcitonin

Parathyroid hormone

Parathyroid hormone (PTH) is a peptide with hormonal action produced by the main parathyroid cells. Following cleavage and processing, the form with biological activity is obtained, *i.e.,* the "intact PTH," consisting of 84 amino acids. Its secretion is regulated by plasma calcium levels and 1,25-dihydroxycholecalciferol (vitamin D). Serum calcium levels control PTH secretion by interacting with a calcium-sensitive receptor coupled to the G-protein. Increased serum calcium levels reduce PTH release; conversely, a reduction in calcium induces PTH release. Vitamin D inhibits PTH secretion, which is stimulated instead by hyperphosphatemia. The secretion of this hormone in a healthy adult is pulsatile, showing a circadian rhythm with a nocturnal peak. PTH regulates blood calcium levels by acting in a coordinated manner on three main target organs: bone, intestinal mucosa, and kidney. In the bone, it stimulates the reabsorption of calcium and phosphates and, by stimulating the synthesis of osteoblast RANKL,

it is responsible for the formation of mature osteoclasts; by reducing the synthesis of osteoprotegerin, it can activate osteoclastogenesis. In the renal tubule, it stimulates instead the reabsorption of calcium and magnesium, reduces phosphorus reabsorption, increases urinary secretion of cAMP, and stimulates alpha-hydroxylation of 25-hydroxycholecalciferol, a precursor of 1,25-dihydroxyvitamin D. Lastly, in the intestine it indirectly increases calcium reabsorption through 1,25-dihydroxyvitamin D.

Vitamin D

The vitamin D-group includes five different fat-soluble vitamins, the biosynthesis of which occurs from the "7-dehydro-" precursors of endogenous sterols. Chemically these are molecules with a steroid nucleus with one broken ring bond. This group of vitamins has two main forms: vitamin D2 or ergocalciferol, and vitamin D3 or cholecalciferol. Ergocalciferol is of plant origin, while cholecalciferol, synthesized from 7-dehydrocholesterol, is of animal origin. The other three forms of vitamin D are of lesser relevance in Ca^{2+} metabolism. Vitamin D levels >30 ng/mL are optimal for mineral metabolism in adults. Vitamin D deficiency is defined by levels <10 ng/mL, while levels in the range of 10 to 30 ng/mL indicate a state of insufficiency. Toxicity states are defined by levels >100-150 ng/mL, mainly leading to hypercalciuria and hypercalcemia.

Calcitonin

Calcitonin, a peptide hormone released by thyroid C-cells, is a PTH antagonist. Its secretion is stimulated by increased calcium and magnesium levels, while it is inhibited by 1,25-dihydroxyvitamin D. Through mechanisms not fully understood, calcitonin in bone interferes with osteoclast differentiation and fusion, and their release of lysosomal hydrolases. In the kidneys, calcitonin increases calcium and phosphorus clearance and facilitates the expression of 1a-hydroxylase, a catalyst of the hydroxylation reaction of 25-hydroxycholecalciferol to 1,25-dihydroxyvitamin D. Finally, at gastrointestinal level, calcitonin reduces exocrine and endocrine pancreatic secretion, gastric secretion, and sodium, potassium, and chlorine secretion in the ileus.

OSTEOPOROSIS

Osteoporosis is a systemic skeletal disease characterized by quantitative and qualitative bone alterations, accompanied by an increased risk of fracture. It is often caused by bone loss, but it can also occur if the person does not reach the optimal peak of bone mass during adolescence.

Epidemiology

Bone mass reduction and the prevalence of osteoporosis increase with age. There are more than 200 million people affected by osteoporosis worldwide. In 2010, about 22 million women and 5.6 million men were estimated to have osteoporosis in the five largest European countries. In Italy, 3.5 million women and 1 million men are estimated to have osteoporosis, mostly affecting the population over 80 years of age.

Osteoporotic fractures and mortality

Osteoporosis is an important risk factor for fragility fractures. Worldwide, it causes about 8.9 million fractures annually. About 3.5 million new fragility fractures occur every year in Europe. The lifetime risk for a subject with osteoporosis to undergo a site-specific fracture is 15%, while the overall risk is 40%. In Italy, the number of femur fractures in subjects over 50 is greater than 90,000 every year. Patients with proximal femur fracture have a mortality rate of 15 to 30%, as well as a significant reduction in quality of life. In the elderly, mortality due to fractures is comparable to that from stroke and breast cancer.

Risk factors for osteoporosis

Osteoporosis leads to a reduction in bone resistance to traumas resulting in an increased risk of fracture. There are two forms: primary and secondary.

Primary osteoporosis includes the forms:

- juvenile;
- idiopathic in adulthood;
- postpregnancy;
- postmenopausal;
- senile.

TABLE 6.I. Characteristics of the two main forms of primary osteoporosis.

Postmenopausal (Type I)	Senile (Type II)
Women 55 to 75 years old	Women >70 years old; men >80 years old
Rapid and short-lasting bone loss	Slow and long-lasting bone loss
Calcium and phosphorus levels within range	Calcium and phosphorus levels within range
Increased alkaline phosphatase levels in the presence of fractures	Increased alkaline phosphatase levels in the presence of fractures
Increased urine calcium levels	Urine calcium levels within range
Reduced PTH activity and intestinal calcium absorption	Increased PTH activity and intestinal calcium absorption
Associated with decreased estrogen secretion	Not associated with decreased estrogen secretion
Prevalence of 5-29% in postmenopausal women	Prevalence of 6% in elderly population
Appears within the first 20 years from onset of menopause	Affects both genders after the age of 70
Yearly bone loss up to 5%	
Mainly affects the trabecular bone, with apparent effects on the vertebral column	Bone mass loss both cortical and trabecular
Vertebral fractures are the most common	Fractures affect both vertebrae and long bones (femoral neck, distal end of the radius, humerus)

Of these, the two main types are the postmenopausal and senile types, the characteristics of which are summarized in Table 6.I.

Secondary osteoporosis can be systemic or regional. Possible causes of the systemic form are diseases of a different nature, summarized in Table 6.II, and the intake of certain drugs (corticosteroids, antiepileptic drugs, heparin) (Table 6.III). The main causes of secondary regional osteoporosis are trauma, lack of use (long periods of bed rest) and algoneurodystrophy also known as complex regional pain syndrome (CRPS).

Menopause

Menopause is one of the most common causes of osteoporosis. Menopause is characterized by a decline in ovarian estrogen production that cause physical symptoms that may be debilitating, including bone loss. Estrogens have a protective action on bone by acting on growth and mat-

TABLE 6.II. List of diseases associated with osteoporosis.

Secondary osteoporosis	
Endocrine diseases	Renal diseases
Hypogonadism	Renal idiopathic hypercalciuria
Hypercortisolism	Renal tubular acidosis
Hyperparathyroidism	Chronic renal failure
Hyperthyroidism	
Hyperprolactinemia	
Type 1 and 2 diabetes	
Acromegaly	
Growth hormone deficit	
Hematological diseases	Neurological diseases
Myelo- and lymphoproliferative diseases	Parkinson's
Multiple myeloma	Multiple sclerosis
Systemic mastocytosis	Paraplegia
Thalassemia	Evidence of previous stroke
Monoclonal gammopathies	Muscle dystrophies
Falciform anæmia	
Hemophilia	
Gastrointestinal disorders	Genetic disorders
Chronic liver disease	Osteogenesis imperfecta (brittle bone disease)
Primary biliary cirrhosis	Ehlers-Danlos Syndrome
Celiac disease	Gaucher's Syndrome
Gastrointestinal chronic inflammatory disease	Glycogenosis
Gastrointestinal resection	Hypophosphatasia
Gastric bypass	Hemochromatosis
Lactose intolerance	Homocystinuria
Intestinal malabsorption	Cystic fibrosis
Pancreatic insufficiency	Marfan Syndrome
	Menkes Syndrome
	Porphyria
	Riley-Day Syndrome
Rheumatic diseases	Other diseases
Rheumatoid arthritis	COPD
Systemic lupus erythematosus	Anorexia nervosa
Ankylosing spondylitis	AIDS/HIV
Psoriatic arthritis	Amyloidosis
Scleroderma	Sarcoidosis
Other connectivitis	Depression

uration of bone as well as in the regulation of bone turnover. During the menopause, estrogen deficiency leads to more bone resorption and in turn less bone formation. Increased bone resorption is caused by increased osteoclast numbers and activity leading to a reduction in bone mass and increased risk of fractures.

TABLE 6.III. List of drugs that induce osteopenia.

Iatrogenic secondary osteoporosis	
Pharmacological class	**Mechanism of action**
Glucocorticoids	Inhibition of osteoblastic activity/osteocyte apoptosis
Aromatase inhibitors	High turnover-hypogonadism
SSRI	Inhibition of osteoblast proliferation, RANKL activation
Proton pump inhibitors	Reduction of calcium intestinal absorption
H2 inhibitors	Reduction of calcium absorption
Thiazolidinediones	Inhibition of neoformation and osteoblast differentiation
Thyroid hormones (excess)	Bone turnover increased
Anticoagulants	Reduction of osteocalcin activity
Anticonvulsants	Interference with vitamin D metabolism
GnRH	High turnover-hypogonadism
Loop diuretics	Calciuric effect
Antiretroviral agents	Interference with vitamin D metabolism
	Renal phosphate depletion
	Inhibition of osteoblastogenesis/RANKL increased
Inhibitors of calcineurin	Increased bone turnover Increased expression
	RANKL

SSRI: selective serotonin reuptake inhibitor; GnRH: gonadotropin-releasing hormone.

Primary hyperparathyroidism

Primary hyperparathyroidism (PHPT) is a disorder of calcium and phosphorus metabolism caused by the autonomous and uncontrolled secretion of parathyroid hormone (PTH) by one or more hyperfunctioning parathyroid gland(s). This results in hypercalcemia due to increased bone resorption and therefore reduced bone mass.

Male hypogonadism

Testosterone stimulates longitudinal growth and periosteal bone apposition in childhood, while estrogens induce epiphyseal closure. Adequate testosterone levels are needed upon reaching a normal bone mass peak. In adulthood this hormone continues to stimulate periosteal growth, while

estrogens are important for maintaining the trabecular bone mass. Testosterone and estrogens indirectly stimulate osteoclast apoptosis, reducing their proliferation and activity. Sex hormones inhibit the expression of IL-6 and TNF-α, which activate the RANK/RANKL system, leading to a further reduction in bone resorption. Therefore, men with primary or secondary gonadal insufficiency have significantly lower bone mineral density (BMD) for their age and gender compared to healthy controls.

Diabetes mellitus

Diabetes impairs bone health through an imbalance of:

- bone formation and reabsorption;
- collagen formation;
- production of inflammatory cytokines;
- the incretin system;
- bone marrow adiposity;
- calcium metabolism.

Such alterations lead to an increased risk of fracture.

Type 1 diabetes (T1D) may lead to:

- increased risk of hip and vertebral fracture;
- reduction in femoral BMD.

Type 2 diabetes (T2D) may lead to:

- increased risk of hip fracture;
- BMD levels within range or higher.

The difference between these two bone phenotypes could reflect the pathophysiological background of the two types of diabetes.

Hyperglycæmia has several negative effects on both osteoblastogenesis and osteoclastogenesis:

- reduction of MSC vitality and clonogenicity with consequent stimulation of adipogenic differentiation, to the detriment of osteoblastogenic differentiation;
- impaired bone resorption;
- alteration of the embryonic stem cell (ESC) differentiation into osteoclasts.

Advanced glycation end products (AGEs) produced due to chronic hyperglycæmia reduce the expression of genes that have a crucial role in osteo-

blastic differentiation and activity, as well as in increasing their apoptotic death. Finally, AGEs, reactive oxygen species (ROS) and TNF-α in osteocytes increase the production of sclerostin, an antagonist of the regular Wnt/β-catenin pathway.

Chronic renal failure

Fragility fractures are among the most frequent complications in patients suffering from chronic renal failure (CRF) who have undergone organ transplantation. Mortality from hip fractures in these subjects is double that of the general population. These data agree with several factors typical of many subjects with end-stage CRF and renal osteodystrophy, characterized by bone damage and extreme tissue fragility.

Drug intake

There are many drugs associated with osteoporosis and fragility fractures. The different pharmacological classes and the mechanisms of action by which they affect bone health are summarized in **Table 6.III**.

Diagnosis

In order to diagnose osteoporosis, both instrumental and biohumoral assessment techniques are indispensable, especially in patients with forms of osteoporosis or osteopenia that are decidedly more severe than those expected for a person of that age. From a symptomatic point of view, osteoporosis does not manifest itself except as a result of wrist, femoral or vertebral fragility fractures, which can emerge from anamnestic evaluation.

Imaging

Bone densitometry

Bone densitometry techniques (or computerized bone mineralometry) allow b*one* m*ineral* d*ensity (BMD) evaluation*, predicting the risk of osteoporotic fractures. The densitometry report is based on the comparison, expressed in standard deviations (SD), between the patient's BMD value and the T-score (BMD of subjects of the same gender and aged 25-30

years = peak bone mass) or Z-score (mean BMD value of subjects of the same age and gender). Data from dual-energy X-ray absorptiometry (DEXA) allow diagnosis according to the gender- and age-related variable criteria established by the World Health Organization (WHO) (Table 6.IV).

TABLE 6.IV. WHO criteria for diagnosis and use of T-score and Z-score in densitometry reporting.

T-Score	Z-Score
Postmenopausal women	Premenopausal women
Men >50 years	Men <50 years
Serum protein fraction levels	Serum parathyroid hormone levels
Criteria	
Osteopenia ≤1 SD	Inferior to the age- related range ≥2 SD
Osteoporosis ≤2.5 SD	Within the age- related range ≥2 DS

BMD assessment techniques

BMD assessment techniques are the following:

- DEXA – this is the gold standard for bone mass assessment. It allows to assess *bone mineral content* (BMC) and bone mineral density (BMD) (projected BMC for an area) for each skeletal segment. A reduction of 1 SD in BMD increases the risk of fracture by 1.5 to 3 times. Sites where measurement can predict both the risk of each type of fracture and the risk related to the specific site are lumbar spine, proximal femur, proximal and distal radius, and calcaneus (heel). Recommendation: examination of the lumbar spine and proximal femur, neck and total femur should be requested. If >65 years old, central evaluation of the lumbar spine may be inaccurate and at least two lumbar vertebrae should be examined. The lowest value among the three sites should be considered for diagnosis. In some cases (*e.g.,* severe obesity or hyperparathyroidism), radius BMD can be assessed;
- quantitative ultrasound (QUS) – it provides two indirect parameters of bone mass and structural integrity predictive of fracture risk in postmenopausal women and in men. It has lower costs than DEXA and is therefore useful for first level screening;
- quantitative computerized tomography (QTC) – it allows the direct measurement of true bone density, at the sub-structural level (cortical

and trabecular component) and in the sectional area; however, it exposes the patients to ionizing radiation about 300 times higher than that of DEXA. QTC can predict the risk of vertebral but not femoral fractures, only in postmenopausal women.

Indications and monitoring

It is essential to assess bone mass change both in subjects with rapid BMD loss and to monitor ongoing therapies (Table 6.V). This examination is never justified before a 1-year period and should be repeated every 1.5 to 2 years.

TABLE 6.V. Densitometry indications and monitoring.

Indications	
Women >65 years	Men >70 years
Postmenopausal women	Men <60 years with risk factors
Subjects of any age with fragility fractures, risk factors or X-ray findings with osteoporosis	
Monitoring	
Vertebral DEXA	Every 18 months
Femoral DEXA	Every 18 to 24 months

Diagnosis of vertebral fractures

Non-traumatic vertebral fractures are often asymptomatic and only rarely are there suspicious symptoms: consequently, diagnostic imaging is fundamental. A vertebral fracture is defined by the semiquantitative method as a 20% reduction in one of the vertebral body heights; an osteoporotic fracture may be defined as mild, moderate, or severe (Genant's criteria).

TABLE 6.VI. Indications for densitometry test.

Presence of symptoms	Absence of symptoms
Always	Women >70 years
	Men >80 years
	T-Score ≤1.5 in:
	Women 65 to 69 years old
	Men 70 to 79 years old
	Postmenopausal women and men >50 years old with the following risk factors:
	Previous fragility fractures
	Height reduction >4 cm compared to young age or >2 cm compared to the last visit
	Marked reduction in densitometry values (T-score ≤3)
	Cortisone therapy equivalent to >5 mg of prednisone *per* day for >3 months.
	Concomitant disorders associated by themselves with an increased risk of vertebral fractures

Techniques for assessing vertebral fractures

DEXA-VFA. DEXA can be used with VFA (vertebral fracture assessment) software: it allows the acquisition of dorsal and lumbar spine views on which vertebral morphometry may be applied after semiquantitative evaluation. To perform a differential diagnosis between osteoporosis and non-osteoporotic fractures, it is advisable to implement X-ray methods such as computed tomography (CT) for visualization of the fractured vertebra or magnetic resonance imaging (MRI) for multiple vertebral fractures.

Indications for diagnosing vertebral fractures

The guidelines of the Italian Society for Osteoporosis, Mineral Metabolism and Bone Diseases (SIOMMMS) provide such indications (**Table 6.VI**).

Fracture risk assessment

Risk factors may be divided into individual (positive history of previous falls, conditions of compromised muscle strength and mass, lower limb function etc.) and environmental factors (obstacles, lighting, surfaces, and footwear).

There are several methods for fracture risk assessment:

- BMD – BMD measurement is fundamental to assessing fracture risk; the preferred site for predicted risk is the proximal femur;
- FRAX – is a tool developed to calculate the risk of fractures in women and men with many risk factors. It can be used both with and without proximal femur BMD measurement and provides the 10-year probability of a hip fracture and the 10-year probability of a major osteoporotic fracture (spine, forearm, hip, or humerus). It is applicable to people in the 40- to 90-year-old age range;
- DeFRA – this method has been developed in Italy and overcomes some of the intrinsic limitations of FRAX, providing a more accurate risk estimation;
- QFracture calculates the 10-year risk of hip or major osteoporotic fracture without measuring BMD. It is applicable to people in the 30- to 85-year-old age range.

Biohumoral diagnostics

By undertaking a laboratory assessment, it is possible to perform a differential diagnosis with other metabolic diseases that are characterized as

TABLE 6.VII. Level I and II tests.

Level I tests	Level II tests
Erythrocyte sedimentation rate (ESR)	TSH
Complete blood count (CBC)	Ionized calcium
Serum protein fraction levels	Serum parathyroid hormone levels
Serum calcium levels	Serum 25-OH-vitamin D
Serum phosphate	Serum cortisol after overnight suppression test with 1 mg dexamethasone
Total alkaline phosphatase	Male total testosterone
Serum creatinine	Serum and/or urinary immunofixation
24-h urine calcium levels	Antitransglutaminase antibodies (+ total Ig and gluten-free diet)
	Specific tests for associated diseases (*e.g.*, ferritin and transferrin saturation rate, tryptase, etc.)

TSH: thyroid stimulating hormone; Ig: immunoglobulin.

having an osteoporosis-like profile, diagnose secondary osteoporosis, and help guide therapeutic choice and monitoring of the disease (Table 6.VII). Levels of bone turnover markers should be closely monitored in selected cases to assess the therapeutic response and correct diagnostic framework (Table 6.VIII).

TABLE 6.VIII. Specific markers of bone turnover.

Bone neoformation markers
Bone alkaline phosphatase isoenzyme
Osteocalcin
Type 1 collagen propeptide
Pyridinoline
Deoxypyridinoline
N- or C-terminal telopeptides of type I collagen

Diagnosis

In males, DEXA is recommended in the following cases:

- presence of major risk factors (*e.g.,* corticosteroid therapies or history of fragility fractures);
- age of 50 to 69 years with two or more minor risk factors;
- age over 70 years.

The same diagnostic criteria are used also in females. The presence of possible vertebral fractures is detected by dorsal lumbar spine X-ray in subjects over 50 years of age with a positive history of fragility fractures, height loss

>4 cm compared to height at 20 years of age, and glucocorticoid therapy. It is recommended in subjects aged 70 to 79 years of age if vertebral or femoral T-score is ≤1.5 or if they are over 80 years old with a T-score ≤1.

NUTRITION AND BONE HEALTH

Calcium

Proper calcium intake is essential to ensure and maintain a healthy bone mineralization status. Adequate daily calcium intake values are recommended by the Reference Values for Nutrient and Energy Intake (RNI) and vary according to age and different conditions (Table 6.IX). The composition of a specific food type and that of a full meal can compromise calcium bioavailability. The main items that affect it are summarized in Table 6.X. Accordingly, cow's milk, which contains 120 mg of Ca/100 mL as per its composition, represents the reference standard for calcium bioavailability.

TABLE 6.IX. Reference values of calcium for the Italian population.

Years	Daily calcium requirement (mg/die)
1-3 years	600
4-6 years	900
7-10 years	1100
11-14 years	1300
Women	
15-17 years	1200
18-59 years	1000
>60 years	1200
Pregnancy	1200
Breastfeeding	1000
Men	
15-17 years	1300
18-59 years	1000
>60 years	1200

PRIs: population reference intakes.

TABLE 6.X. Factors affecting calcium bioavailability.

	Intestinal uptake	Urinary excretion
Increase	Lactose	Protein
	Carbohydrate	Sodium
	Protein	Caffeine
	Vitamin D	Alcohol
Decrease	Fiber	Phosphorus
	Folates	Potassium
	Uric acid	
	Oxalates	
	Steatorrhea	

Vitamin D

Vitamin D is closely involved in calcium homoeostasis, influencing its bioavailability by regulating intestinal absorption, and acting synergistically with parathyroid hormone. However, vitamin D is poorly rep-

resented in food and therefore the normal diet is completely insufficient to achieve the vitamin D requirement of 15 μg/day in children and adults and 20 μg/day in the elderly (same for both genders). The main source of vitamin D in humans is derived from sunlight followed by a synthesis reaction that occurs in the skin. In cases where sun exposure and vitamin D intake are insufficient to ensure adequate serum levels, the use of supplements is necessary (see the paragraph "Therapeutic solutions," below).

Phosphorus

Phosphorus from foods affects calcium bioavailability. An optimal Ca/P ratio (2:1) promotes intestinal calcium absorption, while excess phosphorus intake leads to increased calcium excretion in the feces. It is therefore important to keep in mind that milk, dairy products, and vegetables are the only foods that can guarantee a higher calcium content than phosphorus, and that the Western diet, which is characterized by a high consumption of meat, fish, and cereals, always tends to have a calcium/phosphorus ratio of <1.

Sodium and potassium

Renal excretion of sodium contributes to calcium elimination in the urine. It has been demonstrated that for each gram of sodium excreted in urine, 26.3 mg of calcium/day is excreted. This quantity represents a 1% reduction in bone mineral mass in 1 year. The amount of sodium contained in 1 g of table salt (NaCl) is 0.4 g; therefore, to achieve the daily sodium reference intake of about 1.5 g, 4 g of salt/day is sufficient. However, only 5% of men and 15% of women follow the daily salt dose recommended by WHO of <5 g/day. The hypercalciuric effect of a high sodium diet can be counterbalanced by the intake of potassium citrate (90 mmol/day), which prevents urine calcium excretion caused by excess sodium.

Proteins

Proteins promote the intestinal absorption of calcium, although for each gram of metabolized protein, there is a loss of >1.75 mg/day of calcium in the urine. Data on the role of proteins in osteoporosis are not yet completely clear; however, protein malnutrition promotes the onset of osteo-

porosis as, besides reducing the available amount of nitrogen and amino acids essential for organic bone matrix synthesis, it also compromises effective calcium absorption.

Antinutrients

Although food is the primary source of an adequate calcium intake, it is also a vehicle for many antinutrients that compromise its bioavailability. These are dietary fiber, uronic acid and phytic acid (found in cereals, legumes, and nuts), which form insoluble complexes with calcium, and oxalic acid (found in spinach, beets, tomatoes, and chocolate). Due to its direct toxicity to osteoblasts and the alteration of proper calcium absorption, alcohol is an antinutrient and should be consumed in the amounts suggested by the guidelines (<10-12 g/day for women or the elderly and <20-24 g/day for men).

THERAPEUTIC SOLUTIONS

Various therapeutic solutions are proposed for osteoporosis and should be chosen by assessing individual cases. The single purpose is to reduce the risk of fracture to which patients with this condition are exposed to.

Calcium and vitamin D

Proper calcium intake since childhood is a fundamental requirement for preventing and treating osteoporosis. The main recommendation is therefore to achieve sufficient calcium intake, especially through a correct diet. In case of deficiency, the appropriate daily doses do not exceed 500-600 mg. The most common supplements cover two different types of calcium: calcium carbonate and calcium citrate. Calcium carbonate, which should be taken on a full stomach, contains 40% elemental calcium while calcium citrate, which can be taken away from meals, contains 21% calcium; therefore, considering the equivalent bioavailability of the two types of calcium, the cost-benefit analysis favors carbonate products that are less expensive and more effective. Calcium absorption can be compromised by several factors, including age, hypochlorhydria, low estrogen levels,

high-fiber diet, and low vitamin D levels: an adequate vitamin D intake is therefore critical. This vitamin is mainly of endogenous origin (skin level synthesis following exposure to UVB rays): it is found in foods in limited quantities (20%), the major source being animal fat contained mainly in fatty fish and dairy products. Vitamin D level is assayed by serum levels of 25-hydroxyvitamin D; by global consensus, these levels have been defined as an indicator of vitamin D status. The combined intake of vitamin D and calcium in the elderly plays a protective role against fractures, especially non-vertebral fractures. The vitamin D form that should be used for supplements is mainly cholecalciferol, except for certain conditions such as liver or renal disease, in which the already hydroxylated forms of the vitamin must be administered.

The supplementation dose of vitamin D is expressed as a daily dose; however, at the same cumulative dose vitamin D can also be given in weekly bolus (50,000 IU/week). Alternatively, to improve the adherence to treatment it is also possible to use monthly bolus (200,000 IU/month). In order to maintain proper bone health and calcium homoeostasis, the Institute of Medicine (IOM, 2011) recommends a daily intake of vitamin D of 600-800 IU/day.

Bisphosphonates

Bisphosphonates are a class of drugs that can block the activity of osteoclasts, interfering with their recruitment, differentiation, and action, as well as promoting their apoptosis. The main ones are alendronate (used orally at a weekly dose of 70 mg) and risedronate (used orally at a dose of 35 mg weekly or 150 mg monthly). The same category includes ibandronate (used orally, at a dose of 150 mg monthly, or intravenously, at a dose of 3 mg every 3 months) and zoledronic acid or zoledronate (used intravenously, at a dose of 5 mg every 12 months). The non-nitrogenous bisphosphonates are etidronate and clodronate, which are incorporated into osteoclasts and cause apoptosis through the production of intracellular cytotoxic analogues of ATP.

Calcium (about 1 g/day) and vitamin D (about 800 IU/day) should be combined with all bisphosphonates.

Denosumab

Denosumab is a humanized monoclonal antibody that can inhibit osteoclast activity by blocking the RANKL system receptor. Denosumab, by binding to RANKL, inhibits the activation and therefore the action of osteoclasts. It is given subcutaneously in a dose of 60 mg every 6 months. Treatment can lead to severe hypocalcemia, so it is necessary to correct any possible risk situations first.

Replacement therapy

Female sex hormones, especially estradiol, are important modulators of bone metabolism. The reduction in estrogen levels that occurs in menopause is critical for bone integrity, both by compromising calcium absorption in the gut and by modulating bone sensitivity to parathyroid hormone (PTH). Estrogen administration in postmenopausal women (hormone replacement therapy, HRT) can increase bone mass and compensate for the natural decline of these hormones during menopause. The protective effect on fractures is counterbalanced by an increased risk of breast cancer, stroke, ischemic heart disease and thromboembolic events, with an unfavorable risk/benefit ratio especially for long-term treatment. Considering these data, hormone replacement therapy is no longer indicated as cure for osteoporosis.

Parathyroid hormone or parathormone

Teriparatide, the biologically active part of the hormone, is given subcutaneously at a daily dose of 20 μg for 24 months. It stimulates the bone tissue anabolic window by promoting its neoformation. The side effects of the drug are mild (nausea, lower limb cramps) and hypercalcemia is rare.

Strontium ranelate

On the one hand, strontium ranelate stimulates appositive osteoblast capacity, while also reducing osteoclast resorption. The drug is administered orally in a daily dose of 2 g in postmenopausal, senile, and steroidal osteoporosis.

Calcitonin

This has a marked inhibitory effect on the bone resorption processes mediated by osteoclasts. This drug has been widely used in the past but nowadays is rarely used. It is mainly administered as nasal spray (200 IU/day).

SERMs

Selective estrogen receptor modulators (SERMs) are compounds of synthetic origin that are distinguished from estrogen by their ability to interact with the estrogen receptor both as agonists and as antagonists. They include raloxifene (60 mg/day), which acts on the bone by inhibiting osteoclast resorption, and bazedoxifene (20 mg/day).

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OBESITY

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7

DEFINITION AND EPIDEMIOLOGY

"Chronic disease characterized by excess body fat accumulation that can cause medical, psychological, social and economic problems."
(WHO, 2015)

The prevalence of obesity and overweight is steadily increasing worldwide: between 1980 and 2013 there was an increase of 27.5% in adults and 47.1% in children, for a total of 2.1 billion people. The prevalence tends to be higher in developed countries, where it usually affects men, while it is lower in developing countries, where it mainly affects women, and it is often associated with malnutrition.

CLASSIFICATION

Obesity is classically described by Body Mass Index (BMI), defined as the weight in kilograms divided by the height in square meters. According to the AACE/ACE 2016 guidelines, weight can be classified as follows:

- BMI<25 kg/m^2 – normal weight (<23 kg/m^2 in certain ethnic groups);
- BMI≥25 and <29.9 kg/m^2 – overweight;
- BMI≥30 and <35 kg/m^2 – class I obesity;
- BMI≥35 and <40 kg/m^2 – class II obesity;
- BMI≥40 kg/m^2 – class III obesity.

From an anatomical point of view, obesity is also described by the relationship between subcutaneous and visceral fat; this relationship is indirectly assessed by two anthropometric measurements:

- waist circumference, which has a cut-off of 94 cm in men and 80 cm in women according to the International Diabetes Federation (IDF) 2016 guidelines for Europid subjects, but the cut-off is 90 cm for South Asian, Chinese and Japanese men and remains 80 cm for women; the metabolic risk increases above these values;
- waist circumference/hip circumference ratio (waist-to-hip ratio), which has a cut-off of 0.90 in men and 0.85 in women according to the WHO; the risk of metabolic complications increases above these values.

DIAGNOSIS

The simplest and most popular method for diagnosing obesity is the BMI calculation; however, this index has the limit of considering only body weight and not the amount of body fat compared to lean mass. If we consider body composition, the gold standard is represented by dual energy X-ray densitometry (DEXA) which allows a rapid, accurate and reproducible analysis of body composition using a low dose of radiation.

Another method is bioelectrical impedance analysis (BIA), which evaluates body composition by measuring the body's resistance to the passage of a low-power, high-frequency (50 kHz) electric current; however, this method is less accurate when performed on people with a high BMI.

PATHOPHYSIOLOGY

Genetic predisposition and environmental influence

Obesity is a chronic, relapsing, progressive and multifactorial disease, linked to the coexistence of genetic factors (multiple and different mutations in various alleles) that predispose to susceptibility by up to 70% and environmental factors such as the availability of food and the level of physical activity performed. Obesity can also be secondary to rare pathological conditions, mostly endocrine (Cushing's Syndrome and growth hormone

deficiency), and to the intake of some drugs (steroids, some antidepressants, β-blockers, some antipsychotics and some antidiabetic drugs), which can influence the weight by up to 10%.

Energetic homoeostasis

Total energy expenditure (TEE) has two components:

- resting energy expenditure (REE), which represents up to 60-70% of the TEE (calculated by indirect calorimetry);
- activity energy expenditure (AEE), which represents 15-20% of TEE in sedentary subjects and 50% in athletes.

An energy intake in excess of the total energy expenditure results in an imbalance of energy homoeostasis, a condition that results in weight gain.

Endocrine weight control and regulation of the homoeostatic mechanisms

Food intake is regulated by complex integration of hormonal and neuronal signals between fat tissue, intestines and the central nervous system (CNS), where the hypothalamus is a key regulator with peripheral and cortical connections. The main peripheral hormones that influence weight regulation and appetite are leptin, peptide YY, glucagon-like peptide-1 (GLP-1), gastric inhibitory peptide (GIP), pancreatic polypeptide (PP) and ghrelin. The first five reduce appetite and increase energy expenditure (anorectic effect), while the sixth has the opposite effects (orectic effect).

Leptin is a hormone released from adipocytes in proportion to the amount of adipose tissue. Through their hypothalamic receptors, leptin and insulin stimulate specific hypothalamic neurons that induce the production of neuropeptides which act by reducing food intake and increasing energy expenditure. People with obesity have higher circulating leptin levels than people of normal weight. It is important to know that leptin must cross the blood-brain barrier via an insulin-independent saturated carrier in order to reach the hypothalamus. If the leptin plasma levels are excessive, as occurs in people with obesity due to excess adipose tissue, leptin cannot reach the hypothalamus and thus properly activate the satiety pathways. This phenomenon is the basis of leptin resistance and also

involves insulin, indirectly. Leptin resistance is therefore considered the main cause of obesity induction and maintenance.

Insulin is a hormone released by the pancreatic β-cells following the ingestion of nutrients and particularly of glucose. It has hypoglycæmic and anabolic effects, stimulating the preservation of nutrients in the form of glycogen, proteins and fats. Insulin resistance is a condition that can appear in subjects with obesity, favoring fat accumulation particularly in the abdominal area and the liver.

Glucagon-like peptide 1 (GLP-1) and gastric inhibitory polypeptide (GIP) both belong to the group of incretin hormones. These are released by the intestine after the ingestion of foods in order to stimulate the secretion of insulin from pancreatic β-cells. Both central and peripheral administration of GLP-1 agonists result in an increase in satiety and thermogenesis and a reduction of both food intake and body weight in rodents and humans. GIP, as well as GLP-1, can lead to a reduction in food intake. The GIP receptor is expressed in the arcuate nucleus and other hypothalamic regions involved in the regulation of food intake.

Peptide YY (PYY) is released by the L-cells of the intestine. Circulating levels of PYY are influenced by the composition of the meal and its caloric content. As with GIP and GLP-1, the peripheral administration of PYY inhibits food intake.

Pancreatic polypeptide (PP) is synthesized by the endocrine pancreas and to a lesser extent by the colon; its blood levels are low in fasting conditions and increase in proportion to calorie intake. Peripheral administration in rodents results in an increase in energy expenditure and a reduction in body weight; however, the metabolic pathways in humans have not yet been fully clarified.

Ghrelin is mainly produced in the stomach. It is the main gastrointestinal hormone with orexigenic action identified so far. Ghrelin binds to GHSR (the growth hormone secretagogue receptor), which is highly expressed in the hypothalamus and stimulates the appetite.

OBESITY-RELATED COMPLICATIONS

Obesity is associated with significant morbidity, mortality and disability. The obesity-related complications potentially affect all organs and systems,

including the gastrointestinal, endocrine, musculoskeletal, cardiovascular, respiratory and neurological systems. The underlying pathophysiological mechanisms that lead to the development of complications are mainly related to two conditions:

- insulin resistance induces metabolic complications, such as non-alcoholic fatty liver disease (NAFLD), type 2 diabetes (T2D), dyslipidæmia, some types of cancer, hypertension and infertility (Table 7.I);

TABLE 7.I. Comorbidity in obesity.

System	Comorbidity
Cardiovascular	Hypertension
	Myocardial infarction
	Heart failure
	Chronic venous insufficiency
Neurological	*Ictus cerebri*
	Idiopathic intracranial hypertension
Respiratory	Obstructive sleep apnea (OSAS)
	Pickwickian syndrome
	Asthma
	Higher incidence of complications during general anesthesia
Metabolic and endocrinological	Type 2 diabetes
	Dyslipidæmia
	Gestational diabetes, pre-eclampsia
	Metabolic syndrome
	Anovulation
	Precocious puberty
	Infertility
	Hyperandrogenism and polycystic ovary syndrome in women
	Male hypogonadotropic hypogonadism in men
Psychiatric	Anxiety/mood disorders
	Binge eating
	Sexual disorders
	Bulimia nervosa
Gastrointestinal	Gastroesophageal reflux diseases
	Non-alcoholic fatty liver disease (NAFLD)
	Cholelithiasis (gallstones)
	Intestinal hernias
	Colon cancer
Urinary	Urinary incontinence
	Chronic renal failure
Musculoskeletal	Osteoarthrosis (knee, hip and spine)
	Vertebral disc hernias
	Lumbar pain
Oncological	Colon
	Bladder
	Prostate
	Breast
	Pancreas
	Kidney

- mechanical and anatomical alterations due to weight gain lead, for example, to the development of arthritis and gastroesophageal reflux (Table 7.I).

Metabolic syndrome

To date, according to the IDF 2016 guidelines, metabolic syndrome (MS) is diagnosed when, in addition to the presence of central obesity defined as waist circumference ≥94 cm in men and ≥80 cm in women (the cut-off may vary according to ethnicity, see classification), at least any two of the following conditions are present:

- fasting blood glucose ≥100 mg/dL (or treatment with antidiabetic drugs);
- blood pressure ≥130/85 mm/Hg (or treatment with antihypertensive drugs);
- triglycerides ≥150 mg/dL (or treatment with blood triglyceride lowering drugs);
- HDL-C<40 mg/dL in men or <50 mg/dL in women (or treatment with drugs that increase HDL-C (high density lipoprotein cholesterol).

However, obesity is not always associated with the presence of MS. The absence of MS in some subjects with obesity has led to the development of the metabolically healthy obese condition. Conversely, MS does not always imply the presence of obesity: there are normal weight subjects, usually with increased waist circumference, presenting with a dysmetabolic state showing an increase in both cardiovascular and T2D risk. However, the condition of metabolically healthy obesity may be transitory and, in any case, less healthy than what observed in subjects with normal both body weight and waist circumference.

Insulin resistance and type 2 diabetes

The term "diabesity" was recently coined to describe the strong association between obesity and type 2 diabetes (T2D) in both genders and in all ethnic groups: in fact, about 50% of diabetic patients are obese. The risk of developing T2D increases by 20% for each BMI point when the BMI is >27.2 kg/m^2. The reason for this correlation lies in the fact that obesity is

a condition predisposing to the development of insulin resistance (IR), *i.e.*, a state in which greater insulin production is required in order to induce normal blood sugar levels. Visceral fat has a central role in IR development. IR is characterized by a chronic inflammatory state induced by the accumulation of adipose tissue in organs such as liver and muscle tissue. They can become the site of insulin resistance. The ectopic (deposited in the organs) adipose tissue and the visceral adipose tissue produce proinflammatory cytokines, such as interleukin (IL)-6, tumor necrosis factor alpha (TNFα), IL-1β, IL-17 and induce a decrease in the production of anti-inflammatory cytokines, such as adiponectin. IR leads to a chronic inflammatory state, confirmed by the fact that the adipose tissue of a subject with obesity is infiltrated by various proinflammatory cells such as monocytes, macrophages, natural killer cells and lymphocytes. This state of low and persistent inflammation and dysfunction of the adipocytes leads to the maintenance and progression of IR and the onset of T2D and cardiovascular complications.

Non-alcoholic liver disease

Non-alcoholic fatty liver disease (NAFLD) is a clinical condition that includes a broad spectrum of liver diseases, ranging from simple steatosis (fatty liver) to steatohepatitis with necroinflammation and fibrosis (non-alcoholic steatohepatitis, NASH), to cirrhosis, up to the possible development of hepatocellular carcinoma. The central link between NAFLD and obesity is IR, which is strongly associated with liver damage. The metabolic importance of NAFLD is demonstrated by the fact that this condition predicts all metabolic complications of obesity. Generally, the diagnosis of NAFLD is made in asymptomatic patients when, following a random identification of liver enzyme alterations, liver ultrasound tests are performed and the results indicate steatosis in the absence of other reasons for liver disease (such as alcohol abuse or infectious disease). Ultrasound has modest sensitivity and specificity, since it does not detect minor fat infiltrations (<33%) and does not distinguish steatosis from fibrosis. Liver biopsy, an invasive technique, is the only tool to date to diagnose NASH. Among non-invasive diagnostic tests, the biochemical markers of liver

function such as alanine transaminase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT) are often used. Unlike patients with alcoholic liver disease, NAFLD subjects usually exhibit an AST/ALT ratio <1, although this is not a specific NAFLD serum marker.

Dyslipidæmia

Obesity is associated with an altered lipid profile, which includes an increase in atherogenic cholesterol serum levels such as low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), triglycerides and apolipoprotein B and a reduction in high-density lipoprotein cholesterol (HDL-C). IR seems to be involved as it stimulates the liver synthesis of triglycerides and the increase of lipolysis in the fat tissue. Weight loss leads to LDL reduction and HDL increase but does not significantly influence cholesterol level in familial heterozygous hypercholesterolemia.

Cancer

The link between obesity and cancer has been known for decades. A 2007 meta-analysis emphasized that for every 5 BMI unit increase, there is an increased risk of esophageal, thyroid, colon, kidney and pancreatic cancer and that there is an inverse relation between BMI and cancer. The cause of this predisposition to cancer probably lies in the obesity-linked IR status: endogenous insulin, which is increased in these subjects, stimulates the hepatic production of insulin-like growth factor 1 (IGF-1) and causes changes in the IGF-binding protein (IGFBP) levels, increasing the available fraction of IGF-1, which is a cell proliferation stimulating hormone. In addition, obesity-related chronic inflammation may also influence cancer development.

Cardiovascular and cerebrovascular diseases

Hypertension is significantly related to obesity. It has been estimated that excess body fat is responsible for 26% of hypertension cases in men and 28% in women. A meta-analysis that analyzed 25 randomized controlled trials calculated that for every pound (about 0.45 kg) lost, systolic and diastolic blood pressure drops by about 1 mmHg. Weight loss must there-

fore be considered as a first-line approach in the high blood pressure therapeutic algorithm. The risk of ischemic heart disease increases by 9% for each unit increase in BMI; in addition, the common association of obesity with T2D significantly increases this risk. The risk of stroke is increased in obese women and men. A study conducted on 234,863 Korean men aged 40-64 years showed a 7% increase in the risk of ischemic stroke for each unit increase in BMI.

Obstructive sleep apnea

The main cause of obstructive sleep apnea (OSA) is obesity: in fact, 75% of OSA patients have an excess body weight of 120%. The increase in fat deposits around the upper airways has a key role. It increases their collapsibility and interferes with the function of the inspiratory and expiratory muscles. Fat increase predisposes to the development of pulmonary hypertension and chronic pulmonary heart disease. Weight loss improves night-time breathing.

Gastroesophageal reflux

Gastroesophageal reflux (GER) is a common disorder associated with weight gain. This association has been confirmed in many studies, while the correlation with esophageal erosions and esophageal adenocarcinoma, two GER-predisposing conditions, is less clear. However, a 2005 meta-analysis found a strong association between obesity, reflux and adenocarcinoma.

Hepatobiliary disease

In obese subjects there is a greater risk of symptomatic cholelithiasis (gallstones). However, people who lose weight rapidly, such as after bariatric surgery, tend to have a higher incidence of gallstones.

Psychiatric disorders

A positive association between high BMI and anxiety disorders, personality disorders and eating disorders has been reported. The relationship between obesity and psychiatric disorder appears to have an ambivalent

cause-effect relationship; in fact, on the one hand, obese patients show psychopathological patterns that predispose to anxiety and depression, such as progressive self-esteem reduction, relational isolation, decrease in social performance, self-image devaluation and self-loathing; on the other hand, it is known that some psychiatric disorders predispose to weight gain. Examples are major depression, which prevents any form of physical activity, and eating disorders, such as binge eating disorder; the latter is the expression of a psychological discomfort with food that results in a compulsive excess intake of food.

OBESITY, MORTALITY, AND DISABILITY

Not surprisingly, obesity and overweight are associated with a higher mortality rate and the risk increases with the obesity stage. Therefore, the increase in the prevalence of childhood obesity worldwide, which represents an increased risk of metabolic syndrome and complications in adulthood, is extremely alarming. The association between obesity and disability also needs a lot of attention: in fact, obesity is a chronic condition associated with various complications and therefore with different degrees of functional limitation.

OBESITY TREATMENT

Multidisciplinary approach

The peculiarity of the treatment of obesity is that it should be based on the collaboration of different professionals in order to obtain a multidimensional and integrated approach. The multidisciplinary team that manages the patient with obesity should consist of:

- general practitioner (or pediatrician, in case of childhood obesity) – this is the first contact with the patient with obesity at a local level; general practitioners acknowledge both therapeutic emergency and its predisposing factors, and refer patients to specialized centers, if appropriate. General Practitioners (GPs) will work together with the specialized centers once a therapeutic rehabilitation program has been launched;

- physicians with experience in the management of obesity (obesity doctor), usually an endocrinologist. They have the task of classifying the severity of obesity and excluding secondary causes, and assessing the patient from a clinical and metabolic point of view. They will identify possible complications and comorbidities, the social/family setting of the patient, and define weight loss goals. They will also define the appropriate diagnostic and therapeutic program, whether it is lifestyle modification (diet and physical activity), pharmacological or surgical, taking into account that the lifestyle modification approach should always be maintained if the pharmacological or the surgical approach have also been prescribed;
- dieticians – they should be able to give personalized dietary advice, adapting it according to the patient characteristics/habits and their needs;
- psychiatrists/psychologists – they work synergistically in the management of the patient with obesity; the psychiatrist must evaluate any primary psychiatric cause, manage the use of psychotropic drugs while trying to avoid those with harmful effects on energy balance and outline the psychopathological traits that contraindicate the use of bariatric surgery; the psychologist must interact with the patient through cognitive-behavioral therapy and motivational interviews, in order to increase the adherence to lifestyle changes;
- bariatric surgeons – they must perform the main types of surgery, if indicated, manage any postoperative surgical complications and follow the patient in a long-term follow-up, in collaboration with other clinical professionals (endocrinologist, dietician and psychologist);
- consultants: consultant physicians in cardiology, pneumology, otolaryngology, angiology, orthopedics, plastic surgery, obstetrics and gynecology, physical medicine/physiotherapy are crucial given the systemic complications of the disease.

Lifestyle changes and nutritional approach

Lifestyle intervention is the first approach in the treatment of obesity. According to the AACE/AHA 2016 guidelines, patients with obesity or

overweight without significant complications or with mild to moderate complications should undergo a lifestyle intervention consisting of:

- dietary therapy – there are different nutritional approaches that should be selected and adapted according to the patient's needs; according to the Italian Society for Obesity and the Italian Association of Dietetics and Clinical Nutrition - Onlus Foundation Italian Association of Dietetics and Clinical Nutrition (SIO-ADI) 2016-2017, a stable 10% reduction of the initial body weight, mainly by adipose tissue loss, improves and corrects the morbid obesity component. Any nutritional intervention should always include the patient's nutritional education and ensure a correct balance of macronutrients in the diet. Dietary restriction should be assessed on the base of the patient's energy expenditure, preferably measured by indirect calorimetry; an energy intake restriction of 500-1000 kcal less than the basal metabolic rate is generally recommended;
- physical activity – aerobic exercise for at least 150 minutes a week, reduction in the sedentary lifestyle;
- therapeutic education and lifestyle change, including empowerment of the patient, who must be informed about the risks and severity of their condition and then urged to practice self-monitoring of food intake and physical activity with the help of educational and group therapies.

Pharmacological therapy

To date, the drugs approved by the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA) that are commercially available in Italy are orlistat, liraglutide 3 mg and the naltrexone/bupropion combination, indicated for the treatment of adult obesity (in children under 12 years orlistat is approved only in the USA) with BMI≥30 or ≥27 with complications, in association with nutritional therapy.

Orlistat

This is a specific inhibitor of gastrointestinal lipase and therefore reduces the absorption of dietary triglycerides by 30%. The drug induces a slight weight reduction (about 2.89 kg after 12 months) compared to placebo and a reduction of T2D incidence in prediabetic subjects. It is generally

well tolerated, although it can have gastrointestinal effects such as abdominal pain, flatulence, fatty stools and fecal incontinence.

Liraglutide

Liraglutide 3 mg is a GLP-1 receptor agonist already used for the treatment of T2D; besides its role in blood glucose control, it has also shown a significant dose-dependent weight reduction. In association with diet and exercising, it guarantees an average body weight loss of 8%, a reduction in blood pressure and an improvement in the blood lipid and glucose profile. In addition, liraglutide 3 mg showed a reduction of T2D incidence in prediabetic subjects. The most common side effects are nausea, vomiting, diarrhea and hypoglycæmia. Among the side effects, pancreatitis and gallstones are the most serious effects to be considered.

Naltrexone/bupropion

This combination has two components: naltrexone, an μ-opiate receptor antagonist, and bupropion, a weak inhibitor of neuronal dopamine and norepinephrine reuptake. These components affect two main brain areas, namely the arcuate nucleus of the hypothalamus and the mesolimbic dopaminergic reward system. Preclinical data suggest that naltrexone and bupropion may have more than additive effects when administered together, such as to reduce food intake and stimulate energy expenditure.

Lorcaserin (withdrawn in 2019 for correlation with cancer)

Lorcaserin is a selective 5-hydroxytryptamine 2c (5-HT2c) receptor agonist. Activation of the brain 5-HT2c receptors, mainly in the hypothalamus, initiates a cascade that promotes the release of the stimulating hormone alpha-melanocortin, which acts on melanocortin-4 receptors to control the appetite.

Bariatric surgery/metabolic surgery

According to the international (ACC/AHA 2013) and national (SICOB 2016) guidelines, the indications for bariatric surgery as a therapeutic option are:

- age 18 to 65 years;
- obesity (BMI>40 kg/m^2 or BMI>35 kg/m^2 in the presence of complications that may improve with weight loss, such as metabolic, cardiopulmonary or joint disorders; or BMI>30 kg/m^2 in patients with T2D that is not well controlled using pharmacological treatments);
- documented failure of previous attempts to lose weight with non-surgical techniques.

The main contraindications are:

- no previous non-surgical treatment for weight loss;
- inability to undertake a follow-up protocol;
- severe mental illness;
- alcoholism and drug addiction;
- reduced life expectancy;
- inability to take care of themselves in the absence of adequate family support.

The surgical procedures currently used are all performed by laparoscopy for the best postoperative course, and they are:

- adjustable gastric band – an air chamber is positioned in the stomach subcardial region, thus creating a very small proximal gastric pouch. Weight loss ranging from 40 to 50% of the excess weight is reported, but long-term results depend on the patient's eating habits;
- vertical sleeve gastrectomy (VSG) – this involves creating a smaller stomach tube or sleeve that limits and reduces the amount of food ingested and causes premature satiety. Weight loss is estimated to be about 60% of the excess weight. To date, it is the most widely used bariatric surgery technique due to its relative safety and proven efficacy in weight reduction. This technique also showed metabolic improvement in patients with T2D. The mortality is 0.2%, and the main postoperative complications are stomach dilation and gastroesophageal reflux;
- gastric bypass – a proximal gastric pouch is created which connects directly to the jejunum, thus excluding the transit of food from the remaining portion of the stomach and duodenum. Weight loss is induced mainly by a change in incretin secretion and is about 55–60% of the excess weight. It is associated with a metabolic improvement in T2D and

dyslipidæmia and should be preferred over VSG when patients present with these complications. The intraoperative mortality is 0.5% and the postoperative complications are fistulas, stenosis, anastomotic ulcers and internal hernias;

- biliopancreatic diversion – this is a type of malabsorptive intervention and consists of reducing the stomach volume by means of a subtotal gastrectomy and creating an internal intestinal derivation. Weight loss is about 65–75% of the excess weight and it is very stable over time, as well as being associated with the metabolic improvement of T2D. The mortality is 1%. Postoperative complications include anastomotic peptic ulcer, anastomotic stenosis, occlusion and internal hernias. Nutritional complications are very frequent and include protein-calorie malnutrition, multifactorial anæmia, bone loss and fat-soluble vitamin deficiency; most of these can be prevented by an adequate nutritional intake;
- intragastric balloon – this is not actually a real surgical procedure, but consists of the insertion of a silicone prosthesis into the stomach for a limited period of time (about 6 months), to ensure a temporary artificial sensation of the stomach filling up. It is usually well tolerated, although it can cause dyspepsia, vomiting, gastric erosions, esophagitis, spontaneous rupture of the prosthesis with migration and risk of intestinal obstruction. Post-treatment recovery of lost weight is almost complete and is the main limitation of the technique. This technique can be used as a preparation for bariatric surgery or when the patient needs to lose a lot of weight before surgery (for example in orthopedics).

Bariatric surgery is also referred to as metabolic surgery for its role in inducing profound health benefits in a metabolic disorder such as T2D. Metabolic surgery exerts its antidiabetic effect not only via weight loss but also, and mainly, through the increased secretion of incretins after food ingestion. Vertical sleeve gastrectomy, gastric bypass and biliopancreatic diversion are considered metabolic surgery procedures while adjustable gastric banding not, because the latter induces weight loss only due to restrictive mechanisms and not through metabolic changes in incretins.

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AUTOIMMUNE POLYENDOCRININOPATHIES

8

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DEFINITION AND CLASSIFICATION

Autoimmune polyendocrine syndromes (APS) are heterogeneous clinical conditions characterized by the synchronous or metachronous development of two or more organ-specific endocrine diseases with autoimmune pathogenesis, often also associated with autoimmune disorders of non-endocrine areas (Table 8.I).

TABLE 8.I. APS classification according to Neufeld *et al.* (1980). Modified from Betterle (2008).

Type I APS	At least two of three conditions:
Autoimmune-poly-endocrine-candidiasis-ectodermal-dystrophy (APECED)	Chronic mucocutaneous candidiasis
Whitaker's syndrome	Chronic hypoparathyroidism
	Addison's disease
Type II APS	**Conditions:**
Schmidt's Syndrome	Addison's disease (always present) + autoimmune thyroid disease and/or type 1 diabetes
Type III APS	Autoimmune thyroid disease (always present) + any autoimmune disease (see Table 8.II), excluding Addison's disease
Type IV APS	Any other association between autoimmune diseases which is not included in those above

EPIDEMIOLOGY

Prevalence and incidence vary greatly depending on the APS type and the geographical area considered. Type III APS is the most common, while the rarest is type I APS.

PATHOGENESIS

With the exception of type I APS, caused by the *AIRE* gene mutation on chromosome 21 and inherited as an autosomal recessive trait, the other syndromes have multifactorial pathogenesis such as environmental and immunological factors and a different genetic [human leukocyte antigen (HLA)-related)] susceptibility (Table 8.II).

TABLE 8.II. Type III APS subtypes based on clinical manifestations.

Type III APS	
Autoimmune thyroid disease +	
3A	3B
Other autoimmune endocrine diseases	Gastrointestinal autoimmune diseases
Type 1 diabetes	Atrophic chronic gastritis
Hirata disease	Pernicious anæmia
Autoimmune hypophysitis	Celiac disease
Premature ovarian failure/ insufficiency (POF)	Intestinal chronic inflammatory disease
Autoimmune central diabetes insipidus	Autoimmune hepatitis
	Primary biliary cirrhosis
3C	3D
Autoimmune diseases with cutaneous/neuromuscular/hematopoietic system manifestations	Autoimmune rheumatic diseases
Vitiligo	Systemic/discoid lupus erythematosus
Alopecia	Mixed connective tissue disease
Myasthenia gravis	Rheumatoid arthritis
Stiff-person syndrome	Seronegative arthritis
Multiple sclerosis	Systemic sclerosis
	Sjogren's Syndrome
	Antiphospholipid antibody syndrome

THERAPY

Therapy is symptomatic and aimed at correcting the existing hormonal deficit. The only exception is Graves-Basedow's disease for which hyperthyroidism treatment is indicated (antithyroid therapy). It is essential to always have in mind certain associations of autoimmune diseases since their combined treatment requires special attention. For instance, in the case of hypothyroidism, concomitant low cortisol levels should be excluded before initiating replacement therapy, to avoid the precipitation of an adrenal crisis secondary to the administration of levothyrox-

ine (LT4), which accelerates cortisol clearance. Conversely, the appearance of hyperthyroidism in a patient with Addison's disease will require an increased dosage of steroid therapy until the thyroid clinical picture is solved.

TYPE 1 APS

Mucocutaneous candidiasis (90%), hypoparathyroidism (82%), Addison's disease (67%), generally occurring in this chronological order. Other associated diseases: premature ovarian failure (POF) (60%), alopecia (32%), malabsorption due to autoimmune enteropathy (22%), autoimmune hepatitis (20%), thyroid disease (15%), type 1 diabetes (14%), chronic atrophic gastritis and pernicious anæmia (13%), vitiligo (9%).

Epidemiology

The syndrome occurs early during childhood and is the rarest among the APS. The highest prevalence in Italy is recorded in Sardinia (1:14,000) and in the area of Vicenza (1:4400).

Clinical presentation

Chronic candidiasis (CC) is generally the first disease to appear (mean age at onset: 5 years) and is caused by an immunological deficiency of *T-cells*. It is manifested by nail, oral, esophageal, or genital infections and can have a chronic or recurrent course. Major complications include secondary esophageal stenosis, squamous cell carcinoma and systemic infections.

Chronic hypoparathyroidism (CH) is the second disease in order of occurrence (average age at onset: 10 years). Clinical manifestations are those typical of hypocalcemia (paranesthesia, tetany). Diagnosis is based on hypocalcemia, hyperphosphatemia, and low parathyroid hormone values. Specific antibodies are not yet used for routine diagnostics. Addison's disease (AD) occurs later (at around 15 years of age) and is secondary to autoimmune adrenalitis with adrenal gland atrophy. Symptoms include asthenia, low blood pressure, mucocutaneous hyperpigmentation (due to a compensatory increase in adrenocorticotropic hormone [ACTH] levels), weight loss, nausea and craving for salty foods. Diagnosis is based on elevated renin and

ACTH levels and low cortisol levels or on the failure of the cortisol response to the ACTH stimulation test. The most frequently responsible autoantibodies are those directed against the adrenal cortex or against the 21-hydroxylase enzyme, present in 90% of the affected individuals.

Diagnostic criteria

Diagnostic criteria include:

- presence of two of the three "major" pathologies (CC, CH and AD);
- only one major pathology, if a sibling is affected by type I APS;
- diagnosed homozygous or combined heterozygous mutation of the *AIRE* gene.

Screening

Screening for type I APS is recommended in the following cases:

- chronic candidiasis;
- paranesthesia, tetany or other manifestations simulating an epileptic seizure;
- first-degree relative affected by type I APS.

Level I tests

Level I tests are:

- calcemia, phosphatemia;
- ACTH, basal cortisol levels.

Level II tests

Level II tests are:

- ACTH stimulation test for adrenal insufficiency;
- detection of antiadrenocortical and anti-21-hydroxylase autoantibodies;
- detection of *AIRE* gene mutations.

TYPE 2 APS

Addison's disease (100%), thyroid disease (69%), type 1 diabetes (52%) in variable chronological order. Other associated diseases: atrophic gastritis

and pernicious anæmia (13%), hypogonadism (5%), vitiligo (5%), coeliac disease (2-3%), myasthenia gravis (1-5%), hypophysitis.

Epidemiology

Prevalence is 1.4-2 cases x 100,000 inhabitants and more frequent in women (F/M ratio = 3/1). Peak incidence is in the third and the fourth decade of life, but very rare in childhood.

Clinical presentation

Addison's disease is always present: it represents the clinical picture at onset in patients with type II APS in 50% of cases. Chronic autoimmune thyroiditis (CAT) is the most common manifestation of autoimmune thyroid disease. Antithyroid peroxidase antibodies (TPOAb) are present in 90-100% and antithyroglobulin antibodies (TGAb) in 60-70% of CAT patients.

Diagnosis

Diagnosis is based on the clinical picture and the detection of organ-specific antibodies.

TYPE 3 APS

Autoimmune thyroid disease (100%) associated with one or more autoimmune diseases, not necessarily endocrine, except for Addison's disease. Depending on the organs involved, it is possible to distinguish type III APS into four subgroups (Table 8.III).

Epidemiology

Type 3 APS is the most frequent type of APS. Prevalence is much higher in women (F/M ratio = 10-15/1). Peak incidence is in adulthood, as for type II APS.

Clinical presentation

Type 1 diabetes is the disorder most frequently associated with autoimmune thyroid disease.

TABLE 8.III. Type I and type II APS characteristics.

	Type I APS	Type II APS
Epidemiology	Monogenic	Polygenic
	APECED/*AIRE* (*autoimmune regulator gene*) gene mutation (21q22.3)	HLA DR3-DQ2*; DR4-DQ8*
	Autosomal recessive	
	No relationship with HLA	
	Onset during childhood	Onset during adulthood
	M/F ratio = 1:1	Prevalent in women
Antibodies (Ab)	Addison's disease	Addison's disease (see APS I)
	Anti-21-hydroxylase Ab	Autoimmune thyroid disease:
	Anti-adrenal cortex Ab	Antithyroglobulin Ab (TGAb)
	Anti-17-hydroxylase Ab	Antithyroid peroxidase Ab (TPOAb)
	Antisteroid-producing cell Ab	Anti-TSH receptor Ab (TRAb)
	Hypoparathyroidism	Type 1 diabetes
	Antiparathyroid antibodies	Antiglutamic acid decarboxylase Ab (GADA)
	Low sensitivity and specificity	Anti-insulin Ab
		Antityrosine phosphatase Ab (ICA512 or IA-2)
		Antipancreatic islet cell Ab (ICA)

*HLA DR3-DQ2 and DR4-DQ8 are expressed by 90% of patients with TID.

Chronic atrophic gastritis (42%) is another condition frequently encountered in patients with type III APS.

Anæmia, generally microcytic, should alert the physician. A suitable diagnostic procedure should be started, including a differential diagnosis with coeliac disease, present in 7-8% of patients with type III APS (with or without atrophic gastritis).

Pernicious anæmia is instead rarer, as it is found only in 15-20% of patients with atrophic gastritis. Antiphospholipid antibody syndrome (8.5%) should always be suspected in patients with autoimmune thyroid disease and a history of recurrent miscarriage.

Diagnosis

Diagnosis is clinically driven and based on the detection of organ-specific antibodies (Table 8.IV). In patients diagnosed with type 3 APS, the detection of autoantibodies should not be carried out indiscriminately or across the board but always based on clinical suspicion, familiarity or in any case limited to the screening of the most frequently associated pathologies (such as the TPOAb assay in patients with type 1 diabetes).

TABLE 8.IV. Epidemiological, clinical and laboratory characteristics of type III APS.

	Type III APS
Epidemiology	Polygenic
	HLA DQA1 (0301 and 0303)
	HLA DQB1 (0401 and 0405)
	Onset during adulthood
	F/M ratio = 10-15:1
Non-endocrine autoimmune diseases with increased frequency and related antibodies (Ab)	Atrophic gastritis (42%): anti-H^+/K^+ ATP-ase pump Ab
	Vitiligo (18%): anti-SOX9, anti-SOX10, anti-tyrosinase Ab
	Pernicious anæmia (15%): anti-intrinsic factor Ab
	Antiphospholipid antibody syndrome (8.5%): anticardiolipin Ab, lupus anticoagulant Ab, anti-β2-glycoprotein 1 Ab
	Celiac disease (7.5%): antitransglutaminase, antigliadin, antiendomysium Ab
	Sjogren's Syndrome (6.5%): anti-SSa/Ro, anti-SSb/La Ab

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9 MULTIPLE ENDOCRINE NEOPLASIA

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Multiple endocrine neoplasia, also known as MEN, is defined as a rare, usually hereditary syndrome with autosomal dominant transmission. It is characterized by the presence of neoplasms localized in two or more endocrine tissues. It may occur as single case (sporadic form) or in a family (familial form) with hormone-secreting or hormone-producing neoplasia in many tissue types. MEN are classified into four subgroups: MEN1, MEN2A, MEN2B and MEN4.

GENETICS

MEN1 is a complex genetic syndrome resulting from mutation of the tumor suppressor gene located on chromosome 11q13. The gene is organized as 10 exons and encodes a protein, called "menin," which is involved in cell division, genome stability and transcription regulation. It is not yet known how the mutations in this gene determine the development of syndrome-associated tumors. By contrast, MEN2A and MEN2B exhibit the inherited *RET* mutation that involves an aminoacid switch. MEN4 results from *CDKN1B*/p27 gene mutation, which must therefore be considered a new susceptibility gene for the development of multiple endocrine tumors.

MEN1

MEN1 syndrome results in a combination of >20 different types of endocrine and non-endocrine tumors. The familial form is much more frequent in comparison with the sporadic or the simple form.

Disorders involved in MEN1

The endocrine disorders involved in MEN1 (Table 9.I) are:

- hyperparathyroidism – it is the most common endocrine disease associated with the syndrome and represents the first clinical manifestation in about 90% of patients. Generally, all the parathyroid glands are involved in an asymmetrical and asynchronous mode;
- pituitary gland tumors – they may represent the first clinical manifestation of the syndrome in 25-30% of MEN1 sporadic cases, but do not exceed 10% in familial forms. Most MEN1 pituitary tumors are macroadenomas;
- gastroenteropancreatic (GEP) tract neuroendocrine tumors – their prevalence varies from 30% to 75% according to case studies. Symptoms are mainly those related to hormone production (even with negative imaging):
  - gastrinomas and Zollinger-Ellison Syndrome – in MEN1, these are prevalent in the duodenum. Gastrin hypersecretion and consequent increase in stomach acid secretion can lead to multiple ulcerations of the duodenal mucosa;
  - insulinomas – multiple or solitary, they may spread throughout the pancreas. They are present in about 10% of MEN1 patients. The clinical picture is typical of hyperinsulinism (hypoglycæmia);
  - glucagonomas – they represent 5% of the pancreas endocrine tumors, most of which originate in the tail of the pancreas. Clinical signs

TABLE 9.I. MEN1 syndrome.

MEN1 lesion	Clinical manifestation	Treatment
Hyperparathyroidism	↑ PTH,↑ Ca^{++}	Parathyroidectomy
Pituitary gland adenomas: prolactinoma	↑ Prolactin	Pharmacological and surgical therapy in certain cases
Insulinoma	↑ Insulin, ↓ glycæmia, hypoglycæmic symptoms	Surgical treatment
Gastrinoma	↑ Gastrin, gastritis, ulcer, gastric hyperacidity	Pharmacological +/- surgical therapy
Thymic carcinoids	-	Preventive thymectomy

and symptoms include: necrolytic migratory erythema (perineum and lower limbs), glossitis, cheilitis, onycholysis, urethritis, hyperglycæmia, normocytic anæmia, venous thrombosis, and weight loss. Glucagon levels above 150 pg/mL are suggestive for diagnosis;
  - VIPomas – clinically characterized by Verner-Morrison Syndrome (watery diarrhea, hypokalemia, achlorhydria). The specific markers are insulin, glucagon, gastrin, somatostatin, vasoactive intestinal peptide (VIP);
- carcinoids – MEN1-associated carcinoid tumors are generally non-hormone-secreting. They may manifest as a large mass after 50 years of age. Thymic, bronchial, and type II gastric enterochromaffin cell-like (ECL) carcinoids occur in 10% of people with MEN1 syndrome. They rarely produce adrenocorticotropic hormone (ACTH), calcitonin, growth hormone-releasing hormone (GHRH), serotonin or histamine and equally rarely cause carcinoid syndrome.

Non-endocrine tumors included in MEN1 are facial angiofibromas, collagenomas, lipomas, meningiomas, ependymomas and meiomyomas.

Diagnosis

MEN1 may be diagnosed through:

- genetic testing;
- clinical assessment for associated tumors;
- blood tests: calcium, parathyroid hormone (PTH), gastrin, prolactin, insulin and C-peptide plasma levels; the "gold standard" for insulinoma diagnostic confirmation remains the prolonged fasting test for 72 hours;
- tumor localization with magnetic resonance imaging (MRI), computed tomography (CT) or ultrasonography.

MEN1 screening may be considered in patients diagnosed with hyperparathyroidism before the age of 30. If a MEN1 patient is diagnosed in a family, first-degree relatives should undergo genetic screening tests.

Treatment

MEN1 treatment includes:

- surgery if possible;

- drug treatment to control hormonal excess;
- hyperparathyroidism – first-line treatment is parathyroidectomy. Cinacalcet may be used to control recurrent or persistent hypercalcemia after surgery;
- prolactinoma – treatment with dopamine agonists;
- gastrinomas – surgery where possible. Frequently, proton pump inhibitors can manage the long-term symptoms of gastric ulcer;
- insulinomas – surgical treatment. If no formation can be detected, distal subtotal pancreatectomy with enucleation of any palpable mass of the pancreas head is recommended. Somatostatin analogues (octreotide) may be effective in the treatment of hypoglycæmia. Other anticancer drugs may reduce symptoms by reducing the lesion. Somatostatin analogues may block hormone secretion also caused by other pancreatic tumors that do not produce gastrin.

Follow-up

Blood tests

Blood tests that must be carried out yearly are: calcium (corrected for albumin), ionized calcium, intact PTH level, phosphorus, 25-hydroxy-vitamin D, bone alkaline phosphatase, serum creatinine, urinalysis, 24-hour urine calcium and phosphate, prolactin, insulin-like growth factor-1 (IGF-1), thyroid-stimulating hormone (TSH), ACTH, glycæmia, insulinemia, chromogranin A, pancreatic polypeptide, glucagon, VIP, gastrin, serum cortisol, dehydroepiandrosterone sulphate (DHEAS), urine aldosterone, 24-hour urine metanephrine and normetanephrine, aspartate aminotransferase (AST), alanine transaminase (ALT), gamma-glutamyl transferase (GGT), total bilirubin, direct bilirubin, total cholesterol, high- and low-density lipoprotein (HDL/LDL), blood urea nitrogen (BUN), serum uric acid.

X-ray

X-ray tests that must be carried out yearly include neck ultrasound and parathyroid scintigraphy, and complete abdominal ultrasound (search for microlithiasis/kidney stones).

X-ray tests that must be carried out every 2 years are: MRI of the brain – *sella turcica* every 2 years in the presence of pituitary micro-adenoma, every 12 months in the presence of pituitary macroadenoma; abdominal CT/MRI; EGD (esophagogastroduodenoscopy) + pancreas endoscopic ultrasound; SRS (somatostatin receptor scintigraphy) in the presence of uptake lesions.

MEN2A

MEN2A is an autosomal dominant syndrome involving predominantly four tissues: the C- or parafollicular cells of the thyroid, the gastrointestinal autonomic nervous system, the medullary part of the adrenal gland and the parathyroid glands. It is characterized by medullary thyroid carcinoma in 90% of adults carrying the gene, unilateral or bilateral pheochromocytoma in 50% of cases and multiglandular parathyroid tumors in 20–30% of cases. All MEN2A variants are caused by an activating point mutation (gain of function) in the *RET* gene (Table 9.II).

Disorders included in MEN2A

Medullary thyroid cancer

Medullary thyroid carcinoma (discussed in another chapter) is a multicentric C-cell thyroid cancer. Lesions are multicentric and bilateral. From a histopathological point of view, there are two variants: cellular and fibrous. If the carcinoma passes the capsule, it results in laterocervical and mediastinal lymph node, lung, liver, bone, and brain metastasis. The treatment of choice is total thyroidectomy associated with lymphadenectomy of the central and/or laterocervical compartment depending on the size of

TABLE 9.II. MEN2A syndrome.

MEN2A lesion	Clinical manifestation	Treatment
Medullary thyroid carcinoma	↑ Calcitonin, ↑ CEA	Total thyroidectomy and lymphadenectomy of the central compartment
Pheochromocytoma	↑ Adrenaline, ↑ noradrenaline, hypertensive crisis	Adrenalectomy
Hyperparathyroidism	↑ PTH, ↑ Ca^{++}	Parathyroidectomy

the tumor. For metastatic medullary thyroid carcinoma there are experimental treatments that have shown partial responses.

Pheochromocytoma

Pheochromocytoma is evident in about 50% of MEN2A or MEN2B patients; it may be multiple and bilateral, due to chromaffin tissue hyperplasia. Many patients have no signs or symptoms of pheochromocytoma concomitantly with the diagnosis of medullary thyroid carcinoma. Specific symptoms are hypertensive crisis, tachycardia, headache episodes, sweating and tremors. Pheochromocytoma surgery must be performed before surgery for medullary thyroid cancer.

Hyperparathyroidism

Hyperparathyroidism is the least evident manifestation in MEN2A. It results from hyperplasia and subsequently adenomatosis of the parathyroid glands. Hypercalcemia is mild and about 85% of patients are asymptomatic.

Diagnosis

Diagnosis of medullary thyroid carcinoma reveals high calcitonin levels, carcinoembryonic antigen (CEA), genetic analysis for *RET* mutation. Diagnosis of pheochromocytoma presents hypersecretion of adrenaline and noradrenaline. These must be measured in the presence of symptoms such as: hypertensive crisis, tachycardia, sweating, headache, and tremors. Diagnosis of hyperparathyroidism includes high or inappropriately high PTH levels associated with hypercalcemia.

Treatment

The therapy of choice is surgical removal in the case of medullary thyroid carcinoma, pheochromocytoma and hyperparathyroidism.

Follow-up

For medullary thyroid cancer the following tests are recommended: calcitonin and CEA levels, neck CT, liver MRI, Octreoscan (Curium, Petten, the Netherlands) and positron emission tomography (PET).

MEN2B

MEN2B syndrome, also called mucosal neuroma syndrome, has a sporadic and therefore rather unknown incidence. Hyperparathyroidism is rarely observed in MEN2B compared with MEN1 or MEN2A. MEN2B-characterizing mucosal neuromas consist of small swellings (hypertrophy), in the lips, tongue and oral mucous membrane with frequent involvement of eyelids, conjunctiva and cornea as well. Skeletal anomalies such as marfanoid habitus, spine anomalies (lordosis, kyphosis, scoliosis), cavus foot and talipes equinovarus foot (congenital clubfoot) are common. Gastrointestinal disorders such as diarrhea or constipation may be associated. In addition to mucosal neuromas, medullary thyroid carcinoma (in this case particularly aggressive) and pheochromocytomas which tend to be bilateral and multicentric may also frequently be found in this type of MEN syndrome. (Table 9.III).

MEN4

MEN4 syndrome is a new MEN syndrome identified in humans, characterized by *CDKN1B*/p27 gene mutations. Therefore, this must be considered a new susceptibility gene for the development of multiple endocrine tumors. Protein P27 is a cyclin-dependent kinase inhibitor, involved in processes of cell cycle regulation and cell proliferation. Considering the small number of cases of MEN4 patients described to date, a specific clinical picture is not currently defined, neither are there guidelines for

TABLE 9.III. MEN2B syndrome.

MEN2B lesion	Clinical manifestation	Treatment
Medullary thyroid carcinoma	↑ Calcitonin, ↑ CEA	Total thyroidectomy and lymphadenectomy
Pheochromocytoma	↑ Adrenaline, ↑ noradrenaline, hypertensive crisis	Adrenalectomy
Ganglioneuromas	Mucous membranes and nerve ganglia tumors	
Marfanoid habitus	Lordosis, kyphosis, scoliosis, cavus foot, and talipes equinovarus foot (congenital clubfoot)	

biochemical and instrumental screening. Consequently, these patients are followed as MEN1 and MEN2A-like patients.

OTHER GENETIC SYNDROMES ASSOCIATED WITH ENDOCRINE TUMORS

McCune-Albright Syndrome

McCune-Albright Syndrome is a syndrome characterized by the mutation (missense) of a gene located in the q13.2 position on chromosome 20, which causes the formation of an abnormal Gs α protein (Guanine nucleotide-binding protein -G alpha subunit). This abnormal protein may cause autonomous and excessive cellular activation and hormonal hypersecretion affecting multiple glands (gonads, pituitary glands, thyroid, and adrenal glands). The following lesions may be associated with:

- melanocytes resulting from an excessive proliferation of hyperpigmented cells in some skin areas;
- bone fibrous dysplasia;
- growth hormone (GH)/prolactin (PRL) secreting pituitary adenomas/hyperplasia; adrenal hyperplasia/adenoma with Cushing's Syndrome; precocious puberty; autonomous thyroid nodules; hypophosphatemic rickets.

McCune-Albright Syndrome is diagnosed by means of functional and instrumental tests, such as:

- instrumental tests – X-ray, bone scan, MRI, genital/pelvic ultrasound, thyroid ultrasound, adrenal ultrasound;
- dynamic tests – luteinizing hormone (LH), follicle stimulating hormone (FSH), ACTH, TSH, GH, PRL.

Neurofibromatosis type 1 (von Recklinghausen disease)

Neurofibromatosis type 1 (von Recklinghausen disease) is a genetic disorder, with autosomal dominant inheritance characterized by the mutation/deletion of the gene located on the long arm of chromosome 17, encoding a protein called "neurofibromin." A neurofibromin dysfunction determines an uncontrolled activation of this pathway, with an increased

susceptibility to the development of benign and malignant tumors. Associated lesions are *café-au-lait* spots (macular, uniformly pigmented, and well-marked lesions); freckles in the axillary and inguinal areas; Lisch nodules; skin or plexiform neurofibromas; specific bone lesions (scoliosis, sphenoid or tibial dysplasia); visual defects (symptoms suggestive of optic nerve glioma); early/late puberty; pheochromocytoma; neuroendocrine tumors of the pancreas.

The diagnosis of type 1 neurofibromatosis is exclusively clinical and therefore requires the presence of at least two clinical signs among those established by the NIH (1988):

- six or more *café-au-lait* (CAL) spots >5 mm in size during childhood and >15 mm in post-pubescent subjects;
- axillary and/or inguinal freckles;
- two or more Lisch nodules on the iris surface;
- two or more cutaneous/subcutaneous neurofibromas or at least one plexiform neurofibroma;
- optic nerve and/or optic chiasm glioma;
- typical bone lesions (sphenoid dysplasia or cortical thinning of long bones with or without pseudarthrosis);
- first-degree relative with type 1 neurofibromatosis (according to the above criteria).

von Hippel-Lindau Syndrome

Von Hippel-Lindau (VHL) syndrome has an autosomal dominant inheritance pattern. The VHL tumor suppressor gene located on the short arm of chromosome 3 is responsible for the disease. Onset frequently occurs in adulthood; retinal hemangioma is often diagnosed (the average age of diagnosis is 25-30 years). Associated injuries include central nervous system tumors; retinal hemangioblastomas; renal cell carcinomas; renal and pancreatic cysts; endolymphatic sac tumors; spinal cord hemangiomas; pheochromocytomas.

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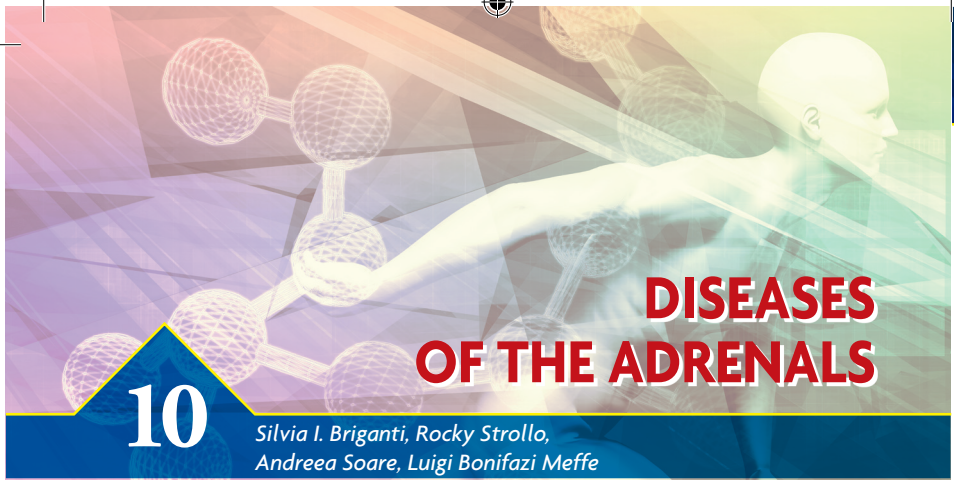

10 DISEASES OF THE ADRENALS

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HYPERCORTICOADRENALISM (CUSHING'S SYNDROME)

Cushing's Syndrome is an endocrine disease secondary to an excess of glucocorticoid. The syndrome presents itself in two forms. The first is exogenous, secondary to chronic corticosteroid therapy. The second is the endogenous form, secondary to glucocorticoid-secreting neoplasms of the adrenal glands (primary hypercortisolism) and to adrenocorticotropic hormone (ACTH)-secreting neoplasms of pituitary or ectopic localization (secondary hypercortisolism).

Epidemiology

Cushing's Syndrome has an incidence of 2 to 3 cases x 1,000,000 people *per* year with a male to female ratio of about 1:3. In the absence of early diagnosis, mortality is estimated at about 50%, while patients treated promptly have a mortality comparable to that of general population.

Clinical manifestations and diagnosis

The clinical presentation of Cushing's Syndrome is complex. High blood pressure and visceral obesity accompanied by characteristic abdominal *strie rubrae* are specific characteristics. Possible psychiatric manifestations include depression, anxiety, and psychosis. Women may have hirsutism and period irregularities, while men frequently have decreased libido. In

more advanced cases, signs and symptoms of complications may appear, such as osteoporosis with fragility fractures, and diabetes mellitus. In the forms with pituitary or ectopic origin, visual disturbances, headaches, and straightforward hypopituitarism may be present.

Hypercortisolism can be confirmed by performing the low-dose dexamethasone suppression test. It involves the administration of 1 mg dexamethasone at 11:00 p.m., followed by a blood sample for the determination of plasma cortisol the following morning at 8:00 a.m. The test is considered negative for levels below 1.8 µg/dL and diagnostic for levels over 5 µg/mL. In cases of incomplete suppression, it is possible to carry out the Liddle test as a confirmation test: it consists of the administration of 0.5 mg dexamethasone every 6 hours for 48 hours with plasma cortisol determination at 8:00 a.m. on the third day. Even in this case, the test is considered negative for cortisol levels below 1.8 µg/dL.

ACTH measurement allows the clinician to distinguish primary from secondary hypercortisolism. In ACTH-dependent Cushing's Syndrome, the ACTH levels are generally over 500 pg/mL in ectopic forms and over 200 pg/mL in pituitary forms. ACTH measurement should be followed by dynamic tests to distinguish the pituitary or ectopic origin of the tumor lesion. The corticotropin-releasing hormone (CRH) stimulation test involves i.v. administration of CRH with subsequent measurement of ACTH blood levels through serial sampling. Pituitary tumors respond to the CRH-mediated stimulation with increased ACTH levels, which are not observed in ectopic tumors. Selective catheterization of petrosal sinuses is an invasive investigation that makes it possible to measure and compare ACTH levels in central and peripheral veins after CRH stimulation. In case of neoplasm with pituitary localization, the test is positive when there is a central ACTH/peripheral ACTH ratio ≥3. Finally, the high-dose dexamethasone suppression test consists of administering 2 mg dexamethasone every 6 hours for 48 hours with plasma cortisol measurement at 8:00 a.m. on the morning of the third day or 8 mg dexamethasone at 11:00 p.m., with plasma cortisol measurement at 8:00 a.m. The test is negative if the cortisol level is below 1.8 µg/dL.

Imaging methods allow the localization of tumor lesions. Magnetic resonance imaging (MRI) with gadolinium identifies pituitary expansive le-

sions with good accuracy. High-resolution chest and abdomen computed tomography (CT) imaging with contrast agents can often identify secreting lesions with ectopic localization.

Treatment

First-line treatment is surgery. If Cushing's Syndrome is caused by pituitary ACTH-secreting lesions, the patient will undergo neurosurgical removal via the transsphenoidal route. The neurosurgical route is limited to removing the neoplastic lesion to avoid complications such as hypopituitarism and brain hemorrhage. About 25 to 30% of patients undergoing neurosurgery to remove ACTH-secreting adenoma manifest disease recurrence regardless of postsurgical cortisol levels. Patients with ACTH-secreting ectopic neoplasms may undergo surgery if the disease is not in a too advanced stage; however, the prognosis is generally unfavorable with a poor 5-year survival rate.

In Cushing's Syndrome of adrenal origin, adrenalectomy is the treatment of choice. Bilateral adrenalectomy is a surgical option to be considered in case of ACTH-dependent Cushing's Syndrome due to occult neoplasia or in case of postsurgical ACTH-dependent relapsed Cushing's Syndrome.

Radiotherapy is the treatment of choice in patients unsuitable for surgery or with relapsed disease. It is possible to schedule fractionated treatments or single sessions of radiosurgery. In both cases, the relapse rate is considerable, even after 10 years. The most relevant side effect is hypopituitarism, which is developed by about 70% of patients and which requires lifelong hormone replacement therapy.

TABLE 10.I. Classification of drugs by target and action.

	Neuromodulators	GABA-agonists/serotonin antagonists
Hypothalamic-pituitary target	Active on neoplastic cells	Dopamine analogues: bromocriptine, cabergoline
		Somatostatin analogues: octreotide, pasireotide
Adrenal target	Steroidogenesis inhibitors (cytotoxic)	Mitotane
	Steroidogenesis inhibitors (cytostatics)	Metyrapone, ketoconazole
Receptor target	Glucocorticoid antagonists	Mifepristone

Medical therapy is the treatment of choice in all patients who cannot undergo surgery or radiotherapy. Potentially useful drug classes and mechanism of action are summarized in Table 10.I.

Ketoconazole is an antifungal drug indicated in all patients over 12 years of age in a daily starting dose of 400 to 600 mg/day, to be increased up to a maximum of 800 to 1200 mg/day, divided into two to three doses. The main side effect is hepatotoxicity; therefore, the liver cytolysis enzymes must always be requested prior to starting the treatment and then periodically after starting administration. Treatment should be stopped in case of elevation of transaminase levels >3 times the reference value. Metyrapone is indicated in the treatment of endogenous Cushing's Syndrome with a starting dose of 250 mg, which can be increased up to 1000 mg/day.

Pasireotide is a drug that works by binding somatostatin receptors in the pituitary gland. It is indicated in patients who cannot be treated surgically or who have experienced postsurgery disease recurrence or persistence. The daily dose varies from 0.3 to 0.9 mg in double daily injections. In case of poor response after 2 months of treatment, it is advisable to stop the drug. Major side effects include QT prolongation and cholelithiasis, potential hypopituitarism due to interference with the secretory function of healthy pituitary cells, and diabetes mellitus.

ADRENAL CORTICAL INSUFFICIENCY

Adrenal cortical insufficiency is a clinical condition characterized by reduced hormonal secretion from the adrenal cortex. It can be distinguished into primary and secondary forms. The causes of adrenal cortical insufficiency are reported in Table 10.II, 10.III. Addison's disease or autoimmune hypocortisolism is the most frequent disorder in the context of primary adrenal insufficiency, with an incidence of about 4 to 5 cases *x* 1,000,000 people *per* year.

Clinical manifestations and diagnosis

Acute adrenal insufficiency is confirmed in the presence of at least two of the following clinical signs in association with an improvement in gen-

eral condition after the administration of glucocorticoids:

- asthenia;
- drowsiness;
- nausea and vomiting;
- hypotension (systolic blood pressure less than 100 mmHg);
- fever;
- hyponatremia ± hypokalemia;
- hypoglycæmia.

In chronic adrenal insufficiency the clinical picture is vaguer. Asthenia, fatigue, weight loss, nausea and vomiting, desire to eat mainly salty foods, abdominal pain and polymyalgia are often present. Androgen deficiency can lead to depression and decreased libido in women. In primary forms, skin hyperpigmentation on palmar creases and scars can be observed. Blood pressure tends to be reduced in the primary forms and normal in the secondary forms.

TABLE 10.II. Causes of primary adrenal cortical insufficiency.

Autoimmune (90% of all cases of primary adrenal cortical insufficiency)	Addison's disease 40% of isolated cases (prevalence M >F); 60% of APS-associated cases (prevalence F>M)
Infectious	Tuberculosis, HIV, cytomegalovirus
Neoplastic	Adrenal carcinoma (rare)
	Metastasis (mainly pulmonary)
Infiltrative	Hemochromatosis, amyloidosis, sarcoidosis, histiocytosis
Hemorrhagic	Anticoagulant therapy, trauma
	Waterhouse-Friderichsen Syndrome (during *Neisseria meningitidis*)
Thrombotic	SLE, panarteritis nodosa, antiphospholipid antibody syndrome
Congenital	Adrenoleukodystrophy, congenital adrenal hypoplasia, congenital adrenal hyperplasia
Drugs-related	Mitotane, mifepristone, ketoconazole
Iatrogenic	Bilateral adrenalectomy

M: male; F: female; APS: autoimmune polyendocrine syndrome; HIV: human immunodeficiency virus; SLE: systemic lupus erythematosus.

In acute adrenal insufficiency, blood samples for cortisol, ACTH, blood count, sodium, potassium, calcium, blood glucose and creatinine must be taken; blood gas analysis must be performed promptly. In chronic adrenal insufficiency, cortisol, ACTH, aldosterone, and plasma renin activity (PRA) must always be determined. Cortisol levels lower than 3 µg/dL are diagnostic for adrenal insufficiency, while levels higher than 18 µg/dL rule it out. The grey area between 3 µg/dL and 18 µg/dL requires the ACTH test for assessing plasma cortisol levels, 30 and 60 minutes, respectively, after the administration of 250 µg ACTH. In the secondary forms, ACTH

TABLE 10.III. Causes of secondary adrenal cortical insufficiency.

Congenital	Hereditary
	Malformative (empty sella, Rathke's pouch cyst)
Neoplastic	Pituitary adenomas
	Craniopharyngioma
	Metastasis (rare)
Vascular (pituitary apoplexy)	Ischemia further to *post-partum* hemorrhagic shock (Sheehan Syndrome)
	Anticoagulant/antiplatelet therapy
	Cerebral or subarachnoid hemorrhage
Inflammatory	Autoimmune hypophysitis (rare)
	Drug-induced hypophysitis
	Vasculitis
Iatrogenic	Radiotherapy
	Neurosurgery
Traumatic	Cranial traumas
Infectious	Meningitis, encephalitis, cerebral abscesses
Functional	Exogenous hypercortisolism (chronic corticosteroid therapy)

levels are low or non-measurable, while in the primary forms they are increased and tend to exceed 100 pg/mL. In pituitary adenomas, the pituitary reserve must be estimated. In primary forms, aldosterone is reduced with a compensatory increase in PRA due to the loss of mineralocorticoid function.

Treatment

The treatment of adrenal insufficiency is pharmacological and consists of the restoration of the hormone to physiological ranges. Replacement therapy must be aimed at reproducing cortisol rhythmicity as much as possible to guarantee an adequate quality of life to the patient. The pharmacological options available are varied and they are summarized in Table 10.IV. Dosage adjustment of corticosteroid replacement therapy is essential when the patient undergoes stressful events, as reported in Table 10.V.

TABLE 10.IV. Corticosteroid replacement therapy.

	Anti-inflammatory action	Mineralocorticoid action	Posology equivalence (mg)	Half-life (h)
Cortisone acetate	0.8	0.8	25	8-12
Hydrocortisone	1	1	20	8-12
Prednisone	4	0.8	5	12-36
Dexamethasone	25	0	0.75	36-72

TABLE 10.V. Stressful events and related therapeutic management.

Stressful event	Therapeutic management
Febrile states (infections)	Double standard drug dose
Minor surgery (tooth extractions, endoscopy)	100 mg hydrocortisone i.m. before the procedure and double standard drug dose for 24 hours
Major surgery in ordinary hospitalization	100 mg of hydrocortisone i.m. before the procedure, then 100 mg hydrocortisone i.m. or i.v. every 6 hours for 24-48 hours and double standard drug dose for 24-48 hours
Multiple traumas, transplantation, emergency surgery	100 mg of i.v. hydrocortisone every 6 hours until the patient is re-fed, then double standard drug dose for at least 48 hours
Septic shock	100 mg of i.v. hydrocortisone every 6 hours until resolution or at least for 72 hours

The adrenal crisis represents a real medical emergency with high mortality (mortality rate 0.5/100 patients). If diagnosed early and treated appropriately, it is generally resolved within 24 hours. Medical management involves the immediate administration of hydrocortisone 100 mg i.m. or i.v. together with the infusion of 0.9% NaCl solution at 1 L *per* hour, followed by the administration of hydrocortisone in boluses of 50 to 100 mg i.m. or i.v. every 6 hours for at least 24 hours and the infusion of another 3 L of NaCl solution at a rate of 500 mL/hour.

Replacement therapy for mineralocorticoids should only be used in patients with primary adrenal insufficiency and marked low blood pressure. The drug of choice is fludrocortisone in daily single doses of 50 to 100 mg, taken in the morning.

Androgen deficiency is not treated pharmacologically in all patients with adrenal insufficiency, but only in female patients with symptoms of depression and/or decreased libido. In these cases, the drug of choice is dehydroepiandrosterone (DHEA) in galenic formulation, in daily doses of 25 to 50 mg/day.

ENDOCRINE HYPERTENSION

Arterial hypertension affects average 33% of men and 31% of women in the adult population and, in most cases, it is primary (essential or idiopath-

ic). However, a subgroup of about 15% has secondary hypertension. More than 50% of children with hypertension have a secondary cause. In young adults (<40 years), the prevalence of secondary hypertension is about 30%. Secondary causes of hypertension include renal causes (*e.g.*, renal parenchymal disease) and endocrine causes. Endocrine hypertension is divided into primary, due to alterations in the hormones that primarily regulate blood pressure, and secondary, due to endocrine disorders that can also lead to an increase in blood pressure. Hypertension can be the initial clinical manifestation for at least 15 endocrine disorders (**Table 10.VI**). An accurate diagnostic framework offers the opportunity to obtain the best clinical response to specific pharmacological therapy or surgical therapy.

TABLE 10.VI. Endocrine causes of hypertension.

Due to adrenal gland
Pheochromocytoma and sympathetic paraganglioma
Primary aldosteronism
Hyperdeoxycorticosteronism
Congenital adrenal hyperplasia
Deficiency of 11β-hydroxylase
Deficiency of 17α-hydroxylase
Deoxycorticosterone-secreting tumors
Primary cortisol resistance
Cushing's Syndrome
Apparent excess of mineralocorticoids/11β-hydroxysteroid dehydrogenase deficiency
Genetic
Acquired
Liquorice or carbenoxolone intake
Cushing's Syndrome
Due to parathyroid glands
Hyperparathyroidism
Pituitary causes
Acromegaly
Cushing's Syndrome
Secondary hyperaldosteronism
Renovascular hypertension
Due to thyroid
Hypothyroidism
Hyperthyroidism
Complex effects
Obstructive sleep apnea

Pheochromocytoma and paraganglioma

Causes and prevalence

These are rare neuroendocrine tumors derived from the adrenal medulla (chromaffin cells) and characterized by the ability to synthesize and secrete catecholamines and their precursors, often in enormous quantities. Hypertension is due to the excess of catecholamines. In 80 to 85% of cases the tumor is localized in the adrenal medulla, while in 15 to 20% it is found in extra-adrenal sites where chromaffin cells are present, for example in the paravertebral lymph nodes of

the sympathetic chain. They are mostly benign tumors and are malignant only in 5 to 10% of cases. Usually, the paragangliomas located in the neck region at the base of the skull are hormonally inactive; however, some of them secrete hormones such as dopamine. The prevalence of pheochromocytomas and paragangliomas (PPGLs) in the general population is extremely low (1.5 to 1.6 *x* 10,000 people), but higher in patients with hypertension (20 to 60 *x* 10,000 patients).

There are two PPGL biochemical phenotypes: adrenergic and noradrenergic tumors.

Adrenergic tumours originate in the adrenal medulla and produce epinephrine, metanephrine (the main metabolite of epinephrine) and variable amounts of noradrenaline.

Noradrenergic tumours originate from the adrenal medulla or from extra-adrenal sites and produce norepinephrine (predominantly or exclusively) and normetanephrine (the main metabolite of norepinephrine). The lack of adrenaline secretion in these tumors is due to the absence of the phenylethanolamine N-methyltransferase enzyme in extra-adrenal tumors; it is essential in converting norepinephrine to adrenaline.

The biochemical phenotype is important for several reasons. Firstly, it can predict the type of germline mutation (*e.g.*, the noradrenergic tumors are more likely to be associated with mutations of the hypoxia signaling pathway [cluster 1: von Hippel Lindau and succinate dehydrogenase subunit mutations], adrenergic tumors are more likely to be associated with mutations of the protein kinase signaling pathway [cluster 2: multiple endocrine neoplasia type 2 and neurofibromatosis type 1]). Secondly, patients with adrenergic tumors may have more paroxysmal symptoms than those with noradrenergic tumors.

Clinical presentation

The symptomatology of patients with PPGL varies widely from patient to patient: from asymptomatic subjects to those with life-threatening symptoms and clinical conditions. Generally, 1 in 10 patients is completely asymptomatic. Symptoms are much more frequent in those patients with a casual finding of an adrenal mass (incidentaloma). The symptomatic pic-

ture is characterized by paroxysmal or persistent high blood pressure, very often associated with symptoms and signs specific to increased sympathetic activity such as headache, increased heartbeat, sweating, pallor, anxiety, tremors, dizziness, adynamia, polyuria and nocturia. In adrenaline-producing tumors, high blood pressure sometimes alternates with normal blood pressure or even orthostatic hypotension. Sometimes the typical triad – headache, increased heartbeat, sweating – may be present and can last from a few minutes to an hour. Normally there is a complete resolution of the symptoms between episodes. The frequency of the episodes can vary from several times daily to few times monthly. Crises can be triggered by various causes: exercise, stress, urination, defecation, purgatives, anesthesia induction, smoking, palpation of the abdomen, pressure exerted by an enlarged uterus during pregnancy, trauma, pain, ingestion of some foods or drinks containing high amounts of tyramine (*e.g.*, cheese, beer, wine). High blood pressure variability is present in patients with paroxysmal hypertension (about 35% of cases) as well as in patients who may have high blood pressure peaks that sometimes overlap, leading to sustained high blood pressure with the potential to progress to hypertensive crisis. These blood pressure peaks with underlying catecholamine release from the tumor are responsible for the high prevalence of cardiovascular emergencies, such as myocardial infarction, stroke, and heart failure in this group of patients.

Screening tests

Routine biochemical screening is recommended in all patients, regardless of blood pressure levels, who have signs or symptoms of excessive catecholamine secretion (Table 10.VII).

TABLE 10.VII. Conditions requiring pheochromocytoma/paraganglioma screening.

Conditions
Paroxysmal signs or symptoms suggesting excess catecholamines
Paroxysmal hypertension to drugs, surgery, or anesthesia
Resistant hypertension
Adrenal incidentaloma (with or without hypertension)
Previous diagnosis of pheochromocytoma or paraganglioma (as part of the annual follow-up for any relapses)
Hereditary predisposition for pheochromocytoma or paraganglioma
Syndromic characteristic indicating a hereditary syndrome related to pheochromocytoma

Biochemical tests should generally precede imaging procedures because only an excessive production of catecholamines can justify more expensive imaging procedures. Fractionated free plasma metanephrines (metanephrine, normetanephrine) and fractionated urinary metanephrines (metanephrine, normetanephrine/24 h) measurements performed on two different occasions have very high specificity and allow an accurate diagnosis. Before performing biochemical tests, the presence of coexisting conditions associated with increased sympathetic activity, such as known or unknown heart failure, renal failure, and hypoglycæmia, which can induce false positives, must be taken into consideration. As secondary tests, plasma chromogranin A assay can be used to rule out falsely elevated plasma metanephrine levels. A more laborious test is the clonidine suppression test which differentiates the actual tumor production of normetanephrine from false positives such as sympathetic activation.

Primary hyperaldosteronism

Causes and prevalence

Primary hyperaldosteronism is a condition characterized by an inappropriately high, autonomous, and non-suppressible production of aldosterone. The two main forms are bilateral adrenal hyperplasia and aldosterone-secreting adenoma (Conn's disease). In addition to being sporadic, primary hyperaldosteronism can be familiar and manifest in at least three forms: type I (or suppressible glucocorticoid hyperaldosteronism), type II and type III.

Primary hyperaldosteronism is the most frequent cause of secondary hypertension, with a prevalence estimated to be around 5 to 12% in unselected hypertensive subjects. In the group of patients with resistant hypertension, the prevalence is about 20%. In most patients, primary hyperaldosteronism is diagnosed within the third to the sixth decade of life. Excessive unregulated aldosterone production leads to increased sodium reabsorption by its normal regulator, the renin-angiotensin II system, through amiloride-sensitive epithelial channels within the distal nephron, with consequent hypertension and suppression of the renin–angiotensin II system. The resulting urinary loss of potassium and hydrogen ions in

exchange with sodium within the distal nephron can cause hypokalemia and metabolic alkalosis.

Clinical presentation

Hypertension occurs in most patients with primary hyperaldosteronism: it can be moderate or severe but in rare cases it can be malignant. In familial type I hyperaldosteronism, hypertension is often a late sign, especially in female patients, but it can have an early and severe onset leading to early death, usually following a hemorrhagic stroke. Family screening in familial type I hyperaldosteronism and familial type II hyperaldosteronism identifies very different phenotypes, some patients having normal blood pressure, consistent with the presence of the preclinical phase in primary hyperaldosteronism. Less than a quarter of patients diagnosed with primary hyperaldosteronism and less than half of those with aldosterone-producing adenoma have hypokalemia. In these patients, in order to differentiate primary hyperaldosteronism from essential hypertension, plasma renin and aldosterone levels must be measured. When it is manifested, hypokalemia can be associated with nocturia, polyuria, muscle weakness, cramps, paresthesias and/or palpitations. Furthermore, the prevalence of obstructive sleep apnea is increased in these patients: it improves with specific hyperaldosteronism therapy. The severity of hypertension and symptoms can improve or worsen with pregnancy. The improvement appears to be due to the antimineralocorticoid action produced by high circulating levels of placental progesterone, which antagonize the action of aldosterone at the receptor level. Furthermore, hyperaldosteronism has a direct harmful effect on cardiovascular and renal tissues (*e.g.*, inflammation, remodeling, and fibrosis) with adverse metabolic effects partly independent of its effect on blood pressure. Consequently, the rates of cardiovascular events (*e.g.*, arrhythmias, myocardial infarction, stroke, and cardiovascular mortality) are higher in patients with primary hyperaldosteronism compared to those with essential hypertension matched for blood pressure level. It is important to emphasize that the excess of cardiovascular morbidity is reversed after treatment, providing convincing support for early diagnosis of hyperaldosteronism in subjects at risk.

Screening tests

Screening is recommended in most patients with hypertension. This is partially due to the fact that hypertension in hyperaldosteronism responds well to specific treatment against excess aldosterone. Unilateral laparoscopic adrenalectomy in patients with unilateral forms of primary hyperaldosteronism leads to resolution of hypertension in 50 to 60% of cases and to a significant improvement in the remaining hypertensive patients. For inoperable patients, pharmacological therapy with aldosterone antagonists (*e.g.*, spironolactone, eplerenone and amiloride) leads to a substantial improvement in the control of hypertension. Since only a minority (approximately 20%) of patients have low potassium levels, plasma levels cannot be used as screening test. However, when hypokalemia is present (especially when it is not induced using diuretics), plasma potassium levels can lead to suspicion of primary hyperaldosteronism. Among the available screening tests, the aldosterone/renin ratio (ARR) is the most reliable; it is more specific than the renin measurement (whose levels are almost always suppressed in patients with primary hyperaldosteronism) and more sensitive than the plasma potassium or aldosterone measurement. The ratio becomes elevated before plasma aldosterone or potassium levels leave their normal ranges. Diagnosis is based on the aldosterone/renin ratio as screening test, confirmation tests and CT or MRI imaging. Venous catheterization of the adrenal veins is the 'gold standard' for the diagnosis of subtype and surgically correctable forms. Genetic tests are used to rule out familial forms.

Other forms of mineralcorticoid excess

Table 10.VI lists the clinical conditions associated with mineralocorticoid excess derived from deoxycorticosterone (DOC) or cortisol, to be taken into consideration in hypertensive patients with hypokalemia and reduced plasma aldosterone and renin levels.

Congenital adrenal hyperplasia

Congenital adrenal hyperplasia (CAH) is the term commonly used, together with adrenogenital syndrome, in order to describe a group of auto-

somal recessive disorders caused by the lack of one of the five enzymes involved in cortisol biosynthesis in the adrenal cortex. In the adrenal gland, enzymatic deficits lead to insufficient cortisol production resulting in compensatory adrenal cortex hyperplasia due to hyperstimulation by the adrenocorticotropic hormone (ACTH). The most frequent enzyme deficiency is that involving the 21-hydroxylase enzyme and it causes over 90 to 95% of congenital adrenal hyperplasia, but it does not cause hypertension. The mutation results in a reduced production of glucocorticoid and mineralocorticoid hormones, with a subsequent increase in androgens. Some adrenal steroidogenesis deficiencies cause an excess of aldosterone precursors with mineralocorticoid activity such as deoxycorticosterone (DOC). They include 11-β-hydroxylase deficiency (*CYP11B1*, P450c11) and 17 α-hydroxylase deficiency (*CYP17*, P450c17) or Biglieri Syndrome. Clinical manifestations are hypogonadism and hypertension with hypokalemia. Even if they are typically diagnosed during childhood, it has been demonstrated that they can also cause hypertension in the adult population.

Deoxycorticosterone secreting tumors

Deoxycorticosterone (DOC) is a synthetic steroid, but it is also present in the adrenal glands, causing water and sodium retention with loss of potassium. DOC-secreting adrenergic tumors are very rare and usually they are large sized and malignant. Some secrete both androgen and estrogen as well as DOC, which can cause virilization in women or feminization in men. The typical clinical presentation is represented by the relatively rapid onset of marked hypertension associated with hypokalemia and low blood aldosterone and renin levels. In patients manifesting hypertension, spontaneous hypokalemia, and low aldosterone and renin levels, the diagnosis is confirmed by high plasma DOC or urinary tetrahydrodeoxycorticosterone levels with a large adrenal tumor evidenced on CT. Aldosterone secretion in these patients is generally suppressed.

Cortisol-resistance syndrome

Selective resistance to cortisol (glucocorticoid) causes, in severe forms, arterial hypertension, hypokalemia and metabolic alkalosis secondary to the

excess of mineralocorticoids. It is due to deficiency of the glucocorticoid receptor, which has a reduced affinity for the hormone with a consequent increase in ACTH secretion. All patients (especially children) with hypertension, spontaneous hypokalemia, and low aldosterone and renin levels should be screened. Initial screening tests include blood levels of cortisol, DOC, 11-deoxycortisol, androstenedione, testosterone, and dehydroepiandrosterone sulfate (DHEA-S) levels, which are usually above the upper limit of their respective reference ranges (**Table 10.VIII**). In addition, it also increases the 24-hour urinary cortisol excretion and plasma ACTH is not suppressed. Germinal mutation tests are available as confirmatory tests.

Apparent mineralocorticoid excess syndrome

Apparent mineralocorticoid excess (AME) is a rare syndrome characterized by hypertension, hypokalemia, and reduced renin and aldosterone levels.

TABLE 10.VIII. Screening tests to evaluate the excess/effect of non-aldosterone mediated mineralocorticoids. Modified from Young *et al.* (2017)

Disorder	24-h UFC	Urinary cortisol: cortisone ratio	DOC	11-deoxycortisol	Androstenedione	DHEA-S
11-β-hydroxylase deficiency	↓	#	↑↑↑	↑↑↑	↑↑↑	↑↑↑
17-α-hydroxylase deficiency	↓	#	↑	↑	↓	↓
Deoxycorticosterone-secreting tumors	#	#	↑↑↑	#	#	#
Cortisol-resistance syndrome	↑↑	#	↑	↑	↑	↑
Apparent mineralocorticoid excess (AME) syndrome	↑↑↑↑*	↑↑↑↑	↑	#	#	#

All these patients have low or undetectable aldosterone and renin levels.
UFC: urinary free cortisol; DOC: deoxycorticosterone; DHEAS: dehydroepiandrosterone sulphate.
#No changes; *24-h UFC is markedly increased in apparent mineralocorticoid excess syndrome due to severe Cushing's Syndrome (e.g., ectopic ACTH syndrome).

There are two forms, congenital and iatrogenic, where the apparent mineralocorticoid excess is due to impaired activity of the microsomal enzyme 11-β-hydroxysteroid dehydrogenase type II, encoded by the *HSD11B2* gene, which normally inactivates cortisol in the kidney by converting it into cortisone. The congenital form (Ulick Syndrome) is a very rare form of juvenile hypertension due to deficiency of 11-β-hydroxysteroid dehydrogenase type II: reduced conversion of cortisol into cortisone results in an increased plasma half-life of cortisol, which can bind to the aldosterone receptor causing hypertension. The secondary form (caused by liquorice and drug ingestion) is due to inhibition of 11-β-hydroxysteroid dehydrogenase type II activity by glycyrrhizic acid, contained in liquorice and carbenoxolone. The diagnosis is confirmed by the increased ratio of the urinary metabolites of cortisol and cortisone, which reflects the decrease in 11-β-hydroxysteroid dehydrogenase type II enzyme activity and is typically 10 times higher than normal.

Liddle Syndrome

First described in 1963 by Grant Liddle, this syndrome is extremely rare, with less than 30 families reported worldwide. It is an inherited syndrome with autosomal dominant inheritance, characterized by high blood pressure, hypokalemia, and suppressed renin and aldosterone levels that do not respond to spironolactone therapy. It is caused by a mutation in the β or γ subunit of the amiloride-sensitive sodium channel present on the epithelium of the distal convoluted tubule and the renal collecting duct, resulting in increased sodium reabsorption and potassium excretion. Once the other causes of hypertension with hypokalemia (**Table 10.VIII**) have been excluded, a family history of hypokalemia-associated hypertension makes Liddle Syndrome more likely. Furthermore, clinical genetic tests are available.

Secondary aldosteronism and renovascular hypertension

Causes and prevalence

Secondary hyperaldosteronism reflects abnormally elevated aldosterone levels due to activation of the renin–angiotensin axis; renovascular hyper-

tension (RVH) is one of these causes. Other situations include renal infarction, blood volume depletion with or without the administration of diuretic drugs, renal hypoperfusion related to heart or liver failure or, rarely, primary renin overproduction by a juxtaglomerular cell tumor. The actual prevalence of RVH is substantially lower than that of renal artery stenosis; 1% to 5% of hypertensive subjects may have an RVH component. Most cases of renal artery stenosis are caused by atherosclerosis (≈85%) or by some form of fibromuscular dysplasia (≈15%). The prevalence of atherosclerotic renal artery stenosis increases with age and with other atherosclerotic manifestations.

Clinical presentation

Renovascular occlusive disease leading to activation of the renin–angiotensin–aldosterone system can produce a spectrum of manifestations including RVH, accelerated/malignant phase hypertension, impaired heart function, circulatory congestion ("flash" pulmonary oedema) and, eventually, renal parenchyma lesions with irreversible loss of renal function. Table 10.IX lists the clinical manifestations that justify RVH screening.

TABLE 10.IX. Clinical manifestations associated with renovascular disease.

Clinical pictures
Hypertension onset before 30 years of age
Accelerated, resistant, malignant hypertension
Deterioration of renal function (increase in creatinine of up to 30% over pretreatment levels) in response to ACE inhibitors or angiotensin receptor blockers
New hypertension onset after 50 years of age (suggestive of atherosclerotic renal artery stenosis)
Renal asymmetry with a size difference of more than 1.5 cm and an otherwise unexplained loss of renal function
Sudden and unexplained pulmonary oedema ("flash" pulmonary œdema)

ACE: angiotensin-converting-enzyme.

Screening tests

Image diagnostics

Echo-color-Doppler of the renal arteries has sensitivity of over 85% and specificity of 92% for atherosclerotic disease with more than 60% of lumen occlusion; it is relatively cheap and easily available; it provides information about renal resistive index, kidney size and perfusion; false negative results are operator-dependent.

CT angiography has sensitivity of over 90% and specificity of 97%; it provides detailed images of the renal cortex and medulla and high resolution of the vascular part. However, it is relatively expensive, and the potential toxicity of the iodine contrast must be considered.

MR angiography provides detailed, relatively expensive imaging with potential gadolinium toxicity.

Radionuclide scintigraphy, with or without angiotensin converting enzyme (ACE) inhibition (nephrogram or renography), rarely produces false negative results; it provides limited information regarding great vessel disease.

Hormonal assays

These may be employed to define secondary aldosterone excess: peripheral activity of plasma renin and plasma aldosterone levels. In clinical practice, renin measurement in the renal vein is commonly used for diagnostic tests related to surgical revascularization.

Specific hormonal and diagnostic procedures

Plasma cortisol measurement

Plasma cortisol measurement is conducted at 8.00 a.m. Levels below 3 µg/dL are considered diagnostic for adrenal insufficiency, while levels over 18 µg/dL basically exclude it. The grey zone from 3 µg/dL to 18 µg/dL requires closer consideration of diagnosis by performing a dynamic test which assesses plasma cortisol levels in response to stimulation after the administration of 250 µg ACTH. If cortisol does not reach values of at least 18 to 22 µg/dL by 30 to 60 minutes after ACTH administration, the test is considered positive.

24-hour urinary free cortisol measurement

24-hour urinary free cortisol measurement consists of 24-hour urine collection. It is therefore essential to evaluate daily diuresis and to measure urine creatinine to ascertain the reliability of the sample collected, together with cortisol levels. At least two measurements are taken. Instructing the patient about the correct procedure (inclusion/exclusion of the first morning urine, storage in the refrigerator) is essential in order to avoid delays

and diagnostic errors. Patients suffering from chronic renal insufficiency cannot be classified for hypercortisolism by urinary free cortisol measurement, while this assay seems to be the first choice in pregnant women. Urinary free cortisol levels over 50 mg/24 hours are considered diagnostic.

Night-time salivary cortisol measurement

Night-time salivary cortisol measurement involves the patient collecting a sample of saliva before going to bed, after having chewed the detecting swab for about 5 minutes. Furthermore, at least two measurements are taken. The patient must not smoke, eat, or drink for at least two hours before collecting saliva. Salivary cortisol levels are resistant to degradation when the sample is stored in the refrigerator; therefore, the patient can deliver the sample to the laboratory the morning after collecting it. The cut-off considered diagnostic of Cushing's Syndrome for night-time salivary cortisol is set at 4.3 nmol/L.

Low-dose dexamethasone suppression test (Nugent test)

Low-dose dexamethasone suppression test (Nugent test) is probably the most frequently used test in clinical practice. The patient should take 1 mg dexamethasone at 11.00 pm before going to bed; a blood sample for plasma cortisol is taken the following morning at 8.00 am. The test is considered as negative if the cortisol level is below 1.8 μg/dL. In doubtful cases of incomplete suppression with the low-dose dexamethasone test (such as in patients suspected of pseudo-Cushing's), the Liddle test can be performed as an additional confirmatory test.

Liddle test

Liddle test involves the administration of 0.5 mg of dexamethasone every 6 hours for 48 hours with plasma cortisol measurement at 8:00 a.m. on the third day. The test is considered as negative if the cortisol level is below 1.8 μg/dL.

ACTH test

ACTH test is a dynamic test evaluating poststimulation plasma cortisol levels after the administration of 250 μg ACTH; if cortisol does not reach values of

at least 18 to 22 µg/dL by 30 to 60 minutes after ACTH administration, the test is considered positive. ACTH level measurement is useful for differential diagnosis between primary and secondary forms; however, the results are often poorly reliable due to the high degradability of ACTH at room temperature. In the secondary forms the ACTH levels are low or non-measurable, while in the primary forms they are increased and tend to exceed 100 pg/mL.

CRH stimulation test

CRH stimulation test is a dynamic test that evaluates the possible pituitary or ectopic origin of an ACTH-secreting tumor. It consists of intravenous administration of CRH with subsequent measurement of the blood ACTH response levels by serial sampling. Benign and well-differentiated pituitary tumors respond to CRH-mediated stimulation with increased blood ACTH levels.

Selective catheterization of the lower petrosal sinuses

Selective catheterization of the lower petrosal sinuses is an invasive procedure that allows measurement and comparison of ACTH levels in central and peripheral veins after CRH stimulation. The test is positive when the central ACTH/peripheral ACTH ratio is ≥3.

High-dose dexamethasone suppression test

High-dose dexamethasone suppression test is a dynamic test that evaluates the possible pituitary or ectopic origin of an ACTH-secreting tumor. It is performed in two ways: administering 2 mg dexamethasone every 6 hours for 48 hours with plasma cortisol measurement at 8:00 a.m. on the morning of the third day; or dexamethasone 8 mg at 11:00 p.m., with plasma cortisol measurement at 8:00 a.m. the following day. The test is negative if the cortisol level is below 1.8 µg/dL.

Chest/abdomen CT imaging with contrast agents

Chest/abdomen CT imaging with contrast agents can identify secreting lesions suggestive of ectopic ACTH secretion and ACTH-independent forms of adrenal origin.

Basal aldosterone measurement and plasma renin activity

Basal aldosterone measurement and plasma renin activity (PRA) should be carried out:

- after compensating any possible hypokalemia;
- having ensured a normal dietary sodium intake in the previous days, which can be evaluated if the urine sodium is >150 mEq/24 h;
- after at least 3-6 weeks of wash-out from interfering drug therapy (diuretics, ACE inhibitors, β-blockers, clonidine, aldosterone antagonists, sartans);
- with the use of α-blockers and calcium channel blockers to control blood pressure.

Metanephrine and 24-h urine creatinine (2 collections)

The correct mode of 24-h urine collection is the following: in the 3 days prior to collection, avoid intense physical activity and eating bananas, coffee, tea, chewing gum, vanilla, peanuts, aubergines, chocolate, and cereals. Also, no anticoughing tablets must be ingested before testing. Collection must be performed from 8:00 a.m. (eliminating 5 a.m. urine) to 8:00 a.m. the next morning in a container previously brought to the laboratory for the addition of hydrochloric acid. The container must be kept in the refrigerator during the entire collection period and must be shielded with aluminium foil. Urine metanephrine levels over 3 to 4 times the maximum normal limit are diagnostic in almost 100% of cases.

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11 DISEASES OF THE MALE GONADS

Giuseppe Defeudis, Silvia Pieralice, Luigi Bonifazi Meffe

PHYSIOLOGY NOTES

The male gonads have two main functions:

- hormonal – synthesis and release of androgens, namely testosterone, androstenedione and dehydroepiandrosterone (DHEA), *via* the interstitial cells of Leydig, under the stimulus of luteinizing hormone (LH);
- spermatogenic – maturation of male germ cells within the seminiferous tubules through the support of Sertoli cells, under the stimulus of follicle-stimulating hormone (FSH) and testosterone.

TABLE 11.I. Causes of primary hypogonadism.

Congenital forms	Acquired forms
Gonadal dysgenesis	Post-traumatic
Klinefelter Syndrome	Radiation-induced
Down Syndrome	Induced by drugs that inhibit testosterone synthesis or receptor activity
Noonan syndrome	Postorchitis
Bilateral anorchia	Bilateral torsion
Germ-cell aplasia (Del Castillo Syndrome)	Retroperitoneal fibrosis
Cryptorchidism	Iatrogenic
Myotonic dystrophy	Immune
Leydig cell aplasia	Senile (late-onset hypogonadism LOH)
Sexual differentiation abnormality	
Real hermaphroditism	
Pseudohermaphro ditism (anti-Müllerian hormone deficiency; Morris Syndrome; 5α-reductase deficiency)	

Male gonads are controlled by the hypothalamic-pituitary axis. Gonadotropin-releasing hormone (GnRH), released with a pulsatile pattern by the hypothalamus, stimulates the gonadotropin cells of the anterior pituitary gland to release LH and FSH.

LH stimulates testosterone secretion by the interstitial cells of Leydig; conversely, testosterone has a negative feedback effect on the hypothalamic-pituitary axis.

FSH targets the Sertoli cells, which in turn release androgen-binding protein, which promotes spermatogenesis, and inhibin. The latter acts by a negative feedback mechanism on the pituitary gland, selectively inhibiting FSH secretion.

HYPOGONADISM

Definition

Male hypogonadism is a clinical, congenital, or acquired condition, characterized by a reduction in testicular function that can manifest itself through a biochemical and symptomatic picture of androgen deficiency and/or infertility.

Classification

The classification of hypogonadism is based on the

TABLE 11.II. Causes of secondary hypogonadism.

Congenital forms	Acquired forms
Pituitary causes (LH and/or FSH deficiency)	Pituitary causes (LH and/or FSH deficiency)
Mutation of the LH β subunit gene (fertile eunuch syndrome)	Autoimmune hypophysitis
GnRH receptor mutation	Pituitary adenomas (both due to the mass effect and the release of hormones capable of interfering with the hypothalamic-pituitary-gonadal axis, such as in the case of prolactin and ACTH)
PROP1 or *HESX1* gene mutation	
Hypothalamic causes (GnRH deficit)	Hypothalamic causes (GnRH deficit) Structural lesions: tumors (craniopharyngioma, glioma, meningioma), infiltrative disorders (hemochromatosis, histiocytosis X, TB, sarcoidosis), trauma, radiotherapy.
Kallmann Syndrome	Functional forms: stress, weight loss, intense exercise, opioids, anabolic steroids.
Normosmic idiopathic hypogonadotropic hypogonadism	
Prader-Willi Syndrome	
Laurence-Moon-Biedl Syndrome	

main affected site: primary if at testicular level (Table 11.I) or secondary if at central (hypothalamic pituitary) level (Table 11.II).

Primary or testicular hypogonadism

The characteristics of primary or testicular hypogonadism are described in Table 11.I.

Secondary or central hypogonadism

The characteristics of secondary or central hypogonadism are described in Table 11.II.

Clinical presentation

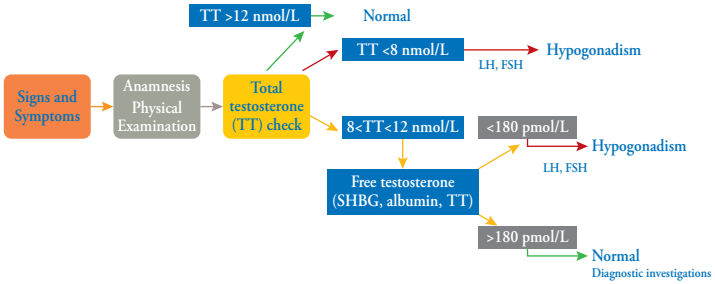
Clinical presentation is detailed in Table 11.III.

TABLE 11.III. Main signs and symptoms that may occur during hypogonadism. Modified from: Hackett G, *et al.* (2017), and Defeudis (2017).

Decreased libido	Oligo- or azoospermia	Scant pubic and axillary hair*
Erectile dysfunction	Reduced testicular volume*	Hypercholesterolemia
Reduced muscle force	Reduced bone mineral density	Increased BMI or obesity
Mood disorders	Reduced penis length*	Insulin resistance or diabetes

BMI: Body Mass Index.
*Frequent in prepubertal hypogonadism.

FIGURE 11.1. Diagnostic features.



Diagnosis

Diagnostic features can be found in (Figure 11.1).

Therapy notes

Treatment of hypogonadism is essentially based on the actual type of hypogonadism:

- primary hypogonadism is treated with testosterone replacement therapy, subject to exclusion of risk factors (previous breast or prostate cancer, prostate-specific antigen [PSA] >4 ng/mL, hematocrit levels >50%, decompensated chronic obstructive pulmonary disease [COPD], heart failure not controlled with common therapies, International Prostate Symptom Score [IPSS] questionnaire score >19);
- secondary or central hypogonadism is treated with testosterone replacement therapy, gonadotropins, antiestrogens, or aromatase inhibitors.

MALE INFERTILITY

Definition

Male infertility is defined as the inability to conceive naturally after 12 months of unprotected sexual intercourse.

Epidemiology

The prevalence of infertile couples in Western countries is about 10-15%. Risk factors are related to smoking, alcohol, drug abuse, anabolic drugs, stress, etc.

Classification

The etiopathogenetic classification is the most used, as shown in Table 11.IV, 11.V). In 30% of patients, diagnosis is impossible, and the cause remains unknown.

Diagnosis

Anamnesis

Anamnesis pays particular attention to lifestyle, recreational habits and the use of drugs that can alter spermatogenesis.

TABLE 11.IV. Pretesticular and testicular causes of male infertility. Modified from: Quaderni del Ministero della Salute N. 28 (2017).

Pretesticular causes (hypogonadotropic hypogonadism)	Testicular causes (hypogonadotropic hypogonadism)
Congenital forms	Congenital forms
Hypothalamic (Kallmann Syndrome, idiopathic normosmic hypogonadotropic hypogonadism, Prader-Willi Syndrome, Laurence-Moon–Biedl Syndrome)	Gonadal dysgenesis
Pituitary (LH β subunit gene mutation, GnRH receptor mutation, *PROP1* or *HESX1* gene mutation)	Sexual differentiation abnormality
Acquired forms	Acquired forms
Hypothalamic (tumors, trauma, infiltrative pathologies, radiation therapy)	Exposure to toxic agents
Pituitary (autoimmune hypophysitis, pituitary adenomas)	Traumas
	Tumors
	Varicocele
	Hydrocele
	Iatrogenic cause
	Immune causes
	Adult hypogonadism (late-onset hypogonadism [LOH])

TABLE 11.V. Post-testicular causes of male infertility. Modified from: Quaderni del Ministero della Salute N. 28 (2017).

Genital tract disorders
Congenital
Vas deferens aplasia, isolated or associated with cystic fibrosis
Acquired
Vasectomy, iatrogenic damage during surgery
Infections
Gonorrhea, syphilis, *Chlamydia trachomatis* and mycoplasma infections, non-specific urethritis, TB, mumps orchitis
Sperm function disorders
Kartagener Syndrome
Antisperm antibodies
Penile malformations and hypospadias
Sexual dysfunctions and ejaculation disorders
Premature ejaculation
Delayed ejaculation
Retrograde ejaculation
Anejaculation
Erectile dysfunction

Physical examination

Physical examinations search for alterations in primary and secondary sexual characteristics, syndromic phenotypic characteristics; identification of any varicocele (enlargement of the veins in the scrotum). It also includes semen examination (according to WHO guidelines).

Hormone assessment

Hormone assessment is based on the measurement of gonadotropins (LH, FSH) and total testosterone must be requested in all cases of infertility.

Albumin and sex hormone binding globulin (SHBG) measurement can be additionally requested in case of total testosterone within the low range limits to assess free testosterone levels.

Infectious disease screening

Infectious disease screening must be requested when infection-related causes are suspected (blood sampling, sperm culture and urethral swab).

Genetic investigations

Genetic investigations (in case of oligospermia or azoospermia) usually include: karyotype study, search for Y chromosome microdeletions, and search for mutations in the cystic fibrosis gene (*CFTR* gene).

Other investigations

Other investigations are represented by transmission electron microscopy, seminal biochemistry, spermatozoon biological and functional tests, and immunological tests for the detection of antispermatozoon antibodies (agglutination test).

Instrumental tests

Instrumental tests include:

- scrotum color-Doppler ultrasonography, to investigate the possible presence of varicocele and/or alterations of testicle and/or ejaculatory ducts;
- prostate vesicular ultrasound;
- other (level II and III) investigations, such as testicular fine-needle aspiration (TESA) and testicular biopsy (TESE), used for diagnostic and therapeutic purposes (cryopreservation and assisted reproductive techniques [ART]), magnetic resonance imaging (MRI) of the pituitary region if there is suspicion of hypothalamic-pituitary lesions.

Therapy notes

Hormonal treatment of male infertility is only possible in hypogonadotropic (secondary) hypogonadism using gonadotropins, antiestrogens, or aromatase inhibitors. Medical therapies are particularly effective in patients

with reproductive system infections and are based on the use of targeted antibiotics and anti-inflammatory drugs, while the use of nutraceuticals (including antioxidants, arginine, etc.) is associated with the improvement of certain less severe infertility conditions. Surgical therapy in varicocele can determine an improvement in seminal parameters.

TESTICULAR TUMORS

Epidemiology

Testicular tumors represent 1-2% of male cancers (the most frequent malignant tumor in men aged 15 to 40 years). Incidence in Western countries is about 3-6 new cases *per* year in 100,000 men. Prognosis is of 5-year survival rate on 90%.

Risk factors

Risk factors are detailed in (Table 11.VI).

Anatomopathological classification

Germ-cell tumors: further divided into seminomatous (more frequent) and non-seminomatous forms. The first group includes typical seminoma (the most common), spermatocyte seminoma, seminoma with syncytiotrophoblast cells and anaplastic seminoma, which is more aggressive. The second group includes instead trophoblastic tumors comprising choriocarcinoma (aggressive), yolk sac tumor, embryonal carcinoma and teratoma. There are also mixed type germ-cell tumors, characterized by the coexistence of multiple histological types, expressed in different percentages (Table 11.VII).

TABLE 11.VI. Main risk factors for testicular cancer.

Cryptorchidism (the most important)	Testicular hypotrophy
Genetic syndromes (Klinefelter Syndrome)	Endocrine disruptors
Testicular dysgenesis syndrome	Infertility
Family history of testicular cancer	Testicle exposure to high temperatures
Contralateral testicle cancer	Occupational activity (exposure to pesticides)

Clinical presentation

Generally, the diagnostic approach starts by finding an often-painless testicular swelling (nodule), which can be variably associated with a feeling of weight or changes in the testicle volume. Sometimes the onset of bilateral gynecomastia can be a sign of β human chorionic gonadotropin (β-HCG) overproduction.

TABLE 11.VII. Anatomopathological classification of testicular cancer.

Germ-cell tumors (90%)*	Non-germ-cell tumors (10%) (stromal tumors and sex cord stromal tumors)
Intratubular germ-cell neoplasia undifferentiated (IGCNU)	Leydig cell tumors
Seminoma	Sertoli cell tumors
Non-seminomatous tumors	Granulosa cell tumors
Embryonal carcinoma	Gonadoblastoma
Yolk sac (or endodermal sinus) tumor	Non-classified type tumors
Teratoma	
Choriocarcinoma	
Mixed tumors	

*Benign teratoma and yolk sac tumor prevail in prepubertal children, seminoma prevails in adolescents and young adults.

Diagnosis

Diagnosis includes:

- testicular self-examination, anamnesis, and physical examination;
- testicular color-Doppler ultrasonography (possibly associated with elastography or contrast media);
- measurements of tumor markers (α-fetoprotein, β-HCG, lactate dehydrogenase [LDH], placental alkaline phosphatase [PLAP], neuron-specific enolase [NSE]), which are particularly important for the follow-up;
- other exams, such as TNM classification, chest computed tomography (CT) and abdominal or pelvic CT (or MRI), that are required. Other useful diagnostic procedures are testicular MRI and testicular biopsy, which are required only in special cases. Measurement of gonadotropins and sex hormones is generally required; however, it is not essential for diagnosis and often their values are not altered.

Staging

The TNM classification is the most used for staging of germ-cell tumors. In case of metastatic disease, the IGCCCG classification is used instead. It includes the second and third stages of the TNM classification and considers histology, tumor markers, and primary and secondary sites.

Therapy notes

Treatment includes:

- orchiectomy (or funicular orchiectomy) – gold standard treatment;
- chemotherapy – carboplatin (for seminomatous tumors), cisplatin, etoposide, bleomycin (for non-seminomatous tumors);
- radiotherapy – seminomas are more radiosensitive.

ERECTILE DYSFUNCTION

Definition and epidemiology

Erectile dysfunction consists of persistent or recurrent inability to obtain and/or to maintain an effective penile erection to allow penetration into the vagina and thus satisfactory sexual intercourse. The prevalence is variable, from 49% among subjects aged 40 to 65 years up to about 80-90% in subjects with comorbidities such as diabetes or hypertension.

Pathogenesis and classification

Organic causes include:

- vascular disorders (atherosclerosis, diabetes, high blood pressure, etc.);
- neurological disorders (diabetes, multiple sclerosis, spinal trauma, etc.);
- endocrine-metabolic disorders (hypogonadism, hyperprolactinemia, thyroid diseases, etc.);
- systemic disorders (diabetes, heart failure, renal failure, etc.);
- iatrogenic (antihypertensive drugs, diuretics, narcotics, etc.).

Non-organic (psychological) causes: depressive syndrome, anxiety, personal experience, etc.

Therapy notes

Treatment includes:

- research into, and, if possible, elimination of the causes and risk factors;
- medical therapy, including type 5 phosphodiesterase enzyme inhibitors (PDE5i; sildenafil, tadalafil, vardenafil, avanafil). Contraindications may be concomitant treatment with nitrates, retinitis pigmentosa, recent episode of myocardial infarction, stroke, or heart failure;

- intracavernous or topical administration of prostaglandin (alprostadil) (in patients unresponsive to PDE5i);
- other treatments, such as hormonal treatment (*i.e.*, hypogonadism), shockwaves therapy, vacuum devices, psycosexual approach);
- surgical therapy (*e.g.*, penile prosthesis).

PREMATURE EJACULATION

Definition

Male sexual dysfunction is characterized by: 1) ejaculation that always or almost always occurs within 1 minute following vaginal penetration, or after a latency time ≤3 minutes; or 2) inability to delay ejaculation in all or almost all vaginal penetrations.

Epidemiology

Premature ejaculation is one of the most frequent disorders in adolescents (about 20%). Causes: organic (prostatitis, hyperthyroidism, etc.) or non-organic (psychological).

Classification

Classification is made considering:

- onset time – primary or secondary (acquired);
- frequency – absolute (independent of partner or intercurrent events) or relative (dependent on the partner or intercurrent events);
- moment of occurrence (*ante portam*, *i.e.*, before penetration, or *intra moenia*, *i.e.*, during coitus);
- simple (in the absence of concomitant sexual dysfunction) or complicated (in the presence of concomitant sexual dysfunction).

Therapy notes

Treatment can be both pharmacological and psychosexual, or mixed, and depends on the patient's clinical characteristics. Pharmacological treatment can be based on the use of low-dose psychotropic drugs or topical anesthetic creams.

HORMONAL AND INSTRUMENTAL INVESTIGATIONS SPECIFIC FOR THE STUDY OF MALE GONADAL FUNCTION

Semen analysis

Semen analysis is the gold standard for the male infertility evaluation. It should be performed after 2-7 days of sexual abstinence and, for a correct diagnosis, it should be repeated at least twice. The exam assesses seminal volume, pH and viscosity, sperm concentration, morphology and motility; it also searches for leukocytes, spermatogenic cells and agglutination areas; the latter are detectable in case of autoantibodies or infections in progress. Seminal alterations mainly include (Table 11.VIII):

- reduced number of sperm in the ejaculate;
- absence of sperm in the ejaculate, defined as azoospermia;
- reduced sperm motility;
- altered sperm morphology.

TABLE 11.VIII. Level I, II and III panel tests in andrology.

Level I tests	Level II tests	Level III tests
Anamnesis	Main blood chemistry tests: total testosterone, LH, FSH, total PSA, inhibin B	Testicular needle examination
	Secondary blood chemistry tests: TSH, blood glucose, prolactin	
Physical examination	Seminal fluid examination	Testicular biopsy
Identification of causes and risk factors	Semen culture, urethral swab, prostate secretion culture	TESA
		TESE
		PESE
		MESA
Psychometric tests (IIEF 5 or 15, Androtest, etc.)	Testicular ultrasonography (with elastography, with contrast media), prostatic, penile ultrasound, vascular color-Doppler ultrasonography	Sperm cryopreservation
	Genetic tests (Y chromosome microdeletions, CFTR, karyotype examination)	Genetic tests
	Psychosexual counselling and investigations	

IIEF: International Index of Erectile Function; MESA: microepididymal sperm aspiration; PESA: percutaneous epididymal sperm aspiration; TESA: testicular sperm aspiration; TESE: testicular sperm extraction; TSH: thyroid-stimulating hormone.

However, the last WHO edition of laboratory manual for the examination and processing of human semen (2021) suggests evaluating the percentiles and no more the use of cut offs; anyway, these percentiles do not represent distinct limits between fertile and subfertile men.

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12 DISEASES OF THE FEMALE GONADS

Daria Maggi, Giovanni Rossini, Silvia Egiddi

AMENORRHEA

Definition

Amenorrhea can be defined as the absence of menstrual bleeding. It can be differentiated into physiological amenorrhea (prepubertal age, pregnancy, and postmenopause) and pathological amenorrhea, and is present in about 3 to 4% of women.

Primary amenorrhea

Primary amenorrhea occurs when menarche does not appear by 15 years of age (in the presence of secondary sexual characteristics) or by 13 years of age (in the absence of sexual characteristics). Primary amenorrhea is a rather rare condition that affects less than 1% of the female population.

Classification

It can be classified according to different criteria:

- etiological (ovarian insufficiency, hypothalamus-pituitary-ovary axis alterations, congenital anomalies of the uterus and vagina, abnormalities of sexual differentiation, constitutional puberty delay);
- gonadotropin levels (hypogonadotropic, normogonadotropic, hypergonadotropic);
- clinical classification (absence or presence of sexual maturation signs, abnormalities of sexual differentiation).

TABLE 12.I. Classification of primary amenorrhea.

Absence of sexual maturation			Normal sexual maturation
Ovarian insufficiency (hypergonadotropic hypogonadism)	Hypothalamic-pituitary insufficiency (hypogonadotropic hypogonadism)	Constitutional puberty delay	Anatomical uterine and vaginal abnormalities (Rokitansky Syndrome; imperforate hymen; vaginal septa)
Gonadal dysgenesis	Hypothalamic causes		Abnormalities of sexual differentiation
Steroidogenesis deficiency	Organic (tumors, radiotherapy, traumas)		Real hermaphroditism
Resistant ovary syndrome	Functional (anorexia, weight loss, exercise)		Male pseudohermaphroditism
	Kallman Syndrome		Female pseudohermaphroditism
	Pituitary causes		
	Tumors, radiotherapy, surgery, hypopituitarism		

The latter subdivision appears to be the most useful for clinical purposes in a process-oriented diagnosis. The classification and the main causes of primary amenorrhea are listed in Table 12.I.

Diagnostic procedure in primary amenorrhea

The etiological diagnosis of amenorrhea can be quite complex and requires a careful medical history with physical examination and certain blood chemistry and instrumental tests. Diagnostic tests are usually performed in all female patients with amenorrhea by 14 years or even earlier in the absence of normal pubertal development or short stature. The request for diagnostic tests is performed by considering the distinction between amenorrhea in the absence of secondary sexual characteristics and amenorrhea with normal development of secondary sexual characteristics (Table 12.II).

Secondary amenorrhea

Secondary amenorrhea is defined as no menstrual bleeding after menarche for over 3 months in women with previous regular menstrual cycles or over 6 months in women with irregular menstrual cycles. Secondary amenorrhea affects 2-3% of the female population. The causes of secondary amenorrhea are divided into three main groups: chronic anovulation,

TABLE 12.II. Diagnostic tests.

Amenorrhea in the absence of secondary sexual characteristics	
Level I tests	**Level II tests**
FSH, LH (elevated in case of ovarian insufficiency; reduced in case of hypothalamic–pituitary insufficiency)	Pituitary tropins (GH, ACTH, TSH, FT3, FT4, cortisol): to exclude panhypopituitarism or partial hypopituitarism
17 β-estradiol (reduced)	Carpal radiology for bone age determination: this shows delayed bone age in case of delayed constitutional puberty
Karyotype (to be performed only in case of high FSH and LH levels. It is altered in Turner's Syndrome; normal in pure gonadal dysgenesis, in steroidogenesis deficiency and in resistant ovary syndrome)	
Pelvic ultrasound (shows fibrous streak ovaries in Turner's Syndrome)	
GnRH test (useful for distinguishing constitutional delay from hypothalamic–pituitary insufficiency: it shows a prepubertal response with LH levels <2 mU/mL in both cases. A LH to FSH peak ratio >1 indicates pubertal activation of the hypothalamic–pituitary axis)	
Pituitary magnetic resonance imaging (MRI): indicated in patients with gonadotropin deficiency; used to distinguish organic from functional causes	
Amenorrhea in the presence of secondary sexual characteristics	
Gynecological examination with pelvic ultrasound: evaluation of vaginal and uterine pathologies (malformations, agenesis, hypoplasia)	
Karyotype	

ACTH: adrenocorticotropic hormone; FSH: follicle-stimulation hormone; FT3: free T3; FT4: free T4; GH: growth hormone; GnRH: gonadotropin-releasing hormone; LH: luteinizing hormone; TSH: thyroid-stimulating hormone.

ovarian failure, and uterine disorders. Secondary amenorrhea has been further classified according to gonadotropin levels as hypo-, hyper- and normogonadotropic (**Table 12.III**). The most frequent cause of secondary amenorrhea is polycystic ovary syndrome.

Diagnostic procedure in secondary amenorrhea

The occurrence of menstrual flow requires an intact hypothalamic-pituitary-gonadal axis; therefore, to diagnose secondary amenorrhea, it is necessary to identify the level where the abnormality occurs.

Anamnesis

Anamnesis is carried out:

- ruling out the possibility of pregnancy;
- evaluating time of menarche and regularity/irregularity of the menstrual cycle;
- ruling out psychological causes, major emotional stress, weight changes and exercise associated with low calorie diets;
- assessing pelvic surgery, previous miscarriages, radio- and chemotherapy, use of drugs that can interfere with ovarian function.

Signs and symptoms

Signs and symptoms evaluate the assessment of the occurrence of flushing associated with night sweats, headache, visual disturbances, galactorrhea, hirsutism, and acne.

Diagnostic tests

Level I

Level I diagnostic tests consider:

- plasma β-hCG (β human chorionic gonadotropin);
- prolactin;

TABLE 12.III. Classification of secondary amenorrhea.

Chronic anovulatory cycles
With reduced estrogen levels (hypogonadotropic hypogonadism)
Hypothalamic
Organic causes: tumors (craniopharyngioma, gliomas of the optic nerve and hypothalamus, hamartomas)
Infectious and infiltrative diseases (encephalitis, meningitis, tuberculosis, sarcoidosis)
Functional causes: psychophysical stress, weight loss, eating disorders
Idiopathic
Pituitary
Tumors: prolactinomas, other secreting tumors (ACTH, TSH, GH), non-secreting tumors, empty sella syndrome
Necrosis: panhypopituitarism, Sheehan's Syndrome
Infiltrative disorders
Iatrogenic damage: surgery, radiotherapy
With normal or increased estrogen levels
Ovarian diseases: polycystic ovary syndrome, granulosa cell tumors
Adrenal diseases: Cushing's Syndrome, Addison's disease, late onset steroidogenesis deficit, androgen-secreting tumors
Thyroid disorders: hypothyroidism, hyperthyroidism
Ovarian insufficiency (hypergonadotropic hypogonadism)
Genetic disorder
Variants of gonadal dysgenesis
Early menopause
Idiopathic, autoimmune, iatrogenic, associated with impairment of gonadotropin action
Uterine disorders
Uterine synechiae (Asherman's Syndrome)
Chronic endometritis

- TSH;
- MAP test, which consists of administering 10 mg of medroxyprogesterone acetate orally for 5 days or 100 mg of progesterone i.m. for 1 to 2 days. The occurrence of bleeding within 7 to 10 days from suspension indicates the presence of an anovulatory cycle with preserved estrogen levels (*e.g.,* polycystic ovary syndrome), while the absence of bleeding could be an expression of outflow tract imperviousness or of an inadequately estrogenized endometrium;
- estrogen-progestin challenge test for 21 consecutive days to rule out suspected anatomical anomalies of the genital tract (it should be performed in case of a negative MAP test). The occurrence of withdrawal bleeding will suggest that the outflow tract and the endometrium, appropriately stimulated, function normally and hence that there is chronic anovulation with low estrogen levels;
- 17 β-estradiol;
- FSH and LH-FSH levels, in which >40 mU/mL are indicative of hypergonadotropic hypogonadism (ovarian failure); normal or low levels lead to differential diagnosis between the amenorrhea caused by hypothalamic-pituitary insufficiency and the amenorrhea caused by genital tract anatomical lesions;
- GnRH test, which is necessary to identify the site of the lesion and the cause (organic or functional) of anovulation. The response is reduced or absent in case of pituitary insufficiency. In the event of a reduced response, the test should be repeated after GnRH administration for several days: if the response is normal, there may be a hypothalamic deficiency, even functional;
- pelvic ultrasonography, that may evidence micropolycystic ovaries, thin endometrium in cases of hypestrogenism or increased endometrial thickness in chronic anovulation with normal estrogen production.

Level II

Level II diagnostic tests include:

- clomiphene test, which is performed in patients with a positive MAP test. The response to the test is considered normal if there is a 50% increase in FSH and LH levels compared to baseline and ovulation occurs.

Based on this result, severe hypothalamic disorders can be excluded. The test is also useful for the induction of ovulation;

- androgens (testosterone, dehydroepiandrosterone sulphate [DHEAS], δ-4-androstenedione, 17-hydroxyprogesterone), that can be elevated in patients with signs of hyperandrogenism;
- Pituitary MRI, useful to identify sellar region expansive lesions;
- antiovarian antibodies, it is useful to investigate other associated autoimmune endocrine disorders in case of positivity (*e.g.,* hypothyroidism, hypocortisolism, hypoparathyroidism);
- karyotype, that must be carried out especially in patients under 30 years of age, in order to exclude gonadal dysgenesis;
- hysteroscopy with endometrial biopsy, which (if positive) shows the presence of synechiae in the uterine cavity. The endometrial biopsy shows the loss of the basal layer.

HYPERANDROGENISM

Hyperandrogenism occurs in 5 to 10% of the female population. The most common cause of hirsutism is polycystic ovary syndrome (about 70 to 80% of cases). Idiopathic hirsutism, on the other hand, accounts for 10 to 15% of all causes of hirsutism: it can be caused by increased skin activity of 5-α-reductase, which activates the testosterone in the hair follicles, or by increased sensitivity of androgen receptors. It can sometimes be associated with increased levels of circulating androgens, in women with regular cycles and normal appearance of the ovaries on ultrasound (Table 12.IV).

TABLE 12.IV. Causes of hyperandrogenism.

Causes	Pathology
Ovarian	Polycystic ovary syndrome
	Ovarian tumors
	Hyperthecosis
Idiopathic	
Adrenal	Adrenal hyperplasia
	Steroidogenesis
	Cushing's Syndrome
	Androgen-secreting tumors
Endocrine disorders	Thyroid disorders
	Cushing's disease
	Acromegaly
Iatrogenic	Exogenous androgens
	Corticosteroids
	Antirejection drugs
	Antiepileptic drugs
	Antidepressants
	Diazoxide

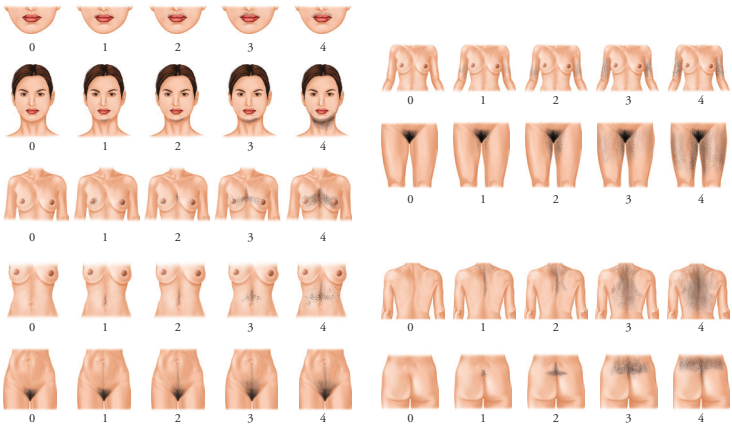
Diagnostic procedures in hyperandrogenism

Clinical manifestations

Clinical manifestations of hyperandrogenism are represented by:

- hirsutism (the presence of terminal hair on skin areas with typical male distribution);
- hypertrichosis (excessive hair on the entire body surface);
- skin manifestations:
  - acne vulgaris;
  - seborrhea (excess sebum production by the sebaceous glands);
  - androgenetic alopecia;
  - period irregularities (frequently related to polycystic ovary syndrome);
  - virilization – loss of female sexual characteristics with clitoris hypertrophy, increased muscle mass, android distribution of body fat, breast atrophy, lowering in the voice pitch and amenorrhea (rare manifestation, important to exclude androgen-secreting neoplasm).

FIGURE 12.1. Modified Ferriman-Gallwey Scale. From: Kopera *et al.* (2010).



Clinical assessment of hirsutism

Figure 12.1 shows the modified Ferriman-Gallwey Scale, which assesses hair growth in different areas of the body, with a scale ranging from 0 to 4 for each area:

- score <8 – normal;
- score range 8 to 15 – mild hirsutism;
- score range 16 to 25 – moderate hirsutism;
- score >25 – severe hirsutism.

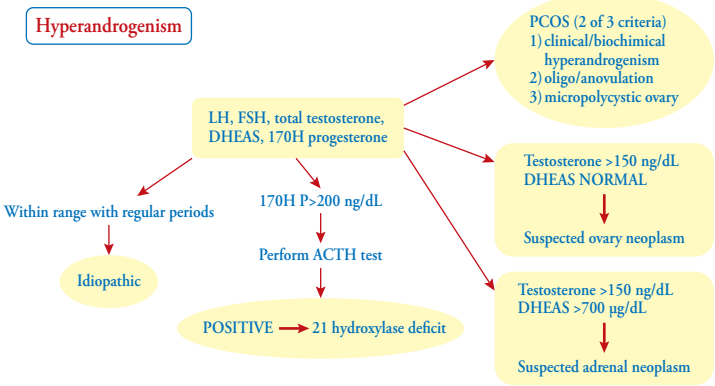
Anamnesis

Anamnesis considers hirsutism modality and time of onset: occurrence during the prepubertal period suggests a non-classical form of congenital adrenal hyperplasia; a slow and progressive postpubertal onset of hirsutism frequently associated with period irregularity is typical of the idiopathic form; worsening postmenopausal hirsutism is characteristic of an androgen-secreting neoplasm.

Diagnostic tests

Figure 12.2 summarizes the diagnostic key points of hyperandrogenism.

FIGURE 12.2. Hyperandrogenism flow chart.



Level I

Level I diagnostic tests consider:

- total testosterone and sex hormone binding globulin (SHBG) should be performed in the early follicular stage together with androstenedione and DHEAS. Significantly increased testosterone and androstenedione levels indicate an androgen-secreting neoplasm of ovarian origin, while testosterone and DHEAS levels two times higher indicate an androgen-secreting neoplasm of adrenal origin;
- progesterone in luteal stage in order to check that ovulation occurs in patients with regular cycles;
- FSH and LH in patients with amenorrhea;
- 17-hydroxyprogesterone (to be performed in the early follicular phase) in order to rule out "late onset" adrenogenital syndrome;
- TSH;
- prolactin.

Level II

Level II diagnostic tests analyze:

- low-dose dexamethasone suppression test in order to rule out Cushing's Syndrome;
- ACTH challenge test to confirm a possible suspicion of adrenogenital syndrome;
- blood glucose/insulin response curve and evaluation of the lipid panel should be performed in patients with clinical signs of insulin resistance or in obese patients.

Diagnostic procedures

Diagnostic procedures include:

- pelvic ultrasound, possibly transvaginal, in the follicular phase with follicle count and ovarian volume assessment;
- MRI or abdominal CT (computed tomography) in suspected androgen-secreting neoplasms;
- karyotype and gonadal biopsy for changes in sexual differentiation.

POLYCYSTIC OVARY SYNDROME

Definition

Polycystic ovary syndrome (PCOS) is a condition characterized by different combinations of hyperandrogenism, oligo-anovulation and polycystic ovary morphology. These criteria are part of the Rotterdam consensus (2003), which includes the presence of at least two of the following PCOS diagnostic criteria:

- oligo-anovulation;
- hyperandrogenism (biochemical and/or clinical);
- polycystic ovaries on ultrasound (presence of at least 12 follicles in one of the two ovaries 2 to 9 mm in diameter and/or increased ovary volume >10 mL).

Previous criteria require the exclusion of other causes of hyperandrogenism and anovulation. In the adolescent female population these criteria are not easily applicable, mainly due to the immaturity of the reproductive system and the subsequent irregularity of menstrual cycles. Therefore, for PCOS diagnosis in adolescence, the following criteria must be fulfilled:

- irregular and oligo-anovulatory periods (abnormal for chronological and gynecological age and persistent for at least 1-2 years);
- biochemical and/or clinical hyperandrogenism.

Epidemiology

Polycystic ovary syndrome is the most frequent endocrine disorder in women. Prevalence depends on the criteria adopted for diagnosis: it ranges from 6 to 10%. Some conditions are associated with a higher PCOS prevalence, *i.e.,* obesity, diabetes mellitus, history of premature adrenarche, first-degree family members with PCOS, ethnicity (Mexican, Australian) and the use of certain drugs (*e.g.*, valproate).

Clinical characteristics

Alterations of reproductive function

Alterations of reproductive function present as:

- menstrual dysfunction – oligo-amenorrhea caused by oligo-anovulation. Period alterations usually begin in the peripubertal period. The presence

of normal estrogen levels and low progesterone levels associated with oligo-anovulation is associated with an increased risk of endometrial hyperplasia and carcinoma;
- infertility – PCOS accounts for more than two thirds of female infertility;
- pregnancy-related complications – the miscarriage rate in women with PCOS is 20 to 40% higher than in the normal population. There is also an increased risk of gestational diabetes, pre-eclampsia, and premature births.

Hyperandrogenism

Hyperandrogenism presents with following features:
- the most frequent clinical features are acne (25 to 30%), hirsutism (about 50%) and male pattern alopecia. In rare cases, an increased muscle mass and changes in voice tone can be observed (more often consequences of virilization secondary to hyperthecosis or ovarian or adrenal tumors);
- other manifestations of hyperandrogenism are seborrhea, hyperhidrosis, and hidradenitis suppurativa.

Metabolic alterations

Metabolic alterations occur as:
- prevalence of obesity (about 50%);
- non-alcoholic fatty liver disease (NAFLD, about 50%), including non-alcoholic steatohepatitis (NASH);
- metabolic syndrome;
- type 2 diabetes (7% to 10%) and gestational diabetes;
- impaired carbohydrate tolerance (35%);
- obstructive sleep apnea syndrome (OSAS);
- dyslipidæmia with high-density lipoprotein (HDL) reduction and triglyceride increase.

Other associated diseases

PCOS appears to be associated with:
- an increased cardiovascular risk when other comorbidities such as diabetes, obesity and dyslipidæmia are present. The increased C-reactive

protein (CRP) levels and endothelial dysfunction seem to contribute, at least partially, to an increased risk;
- venous thromboembolism (for which PCOS is a risk factor), although currently the influence of obesity and other comorbidities on the increased risk cannot be determined;
- an increase in mood and eating behaviour disorders. In fact, it has been observed in women with PCOS, associated with a reduced quality of sleep and life.

Diagnostic procedures in PCOS

Lab tests

Level I

Level I lab test consider:
- total testosterone – the upper limit of total testosterone levels is around 45 to 60 ng/dL. In women with PCOS, total testosterone values range from 29 to 150 ng/dL. If the levels exceed 150 ng/dL, more serious causes of hyperandrogenism (ovarian and adrenal tumors) should be excluded. Free testosterone measurement is not recommended due to limitations of the test method;
- sex hormone binding globulin (SHBG) – can provide an indirect estimate of free testosterone levels by using specific formulas;
- LH and FSH – an increase in LH/FSH ratio is typical in PCOS;
- β-hCG – to exclude an ongoing pregnancy;
- panel for chronic diseases – complete blood count, erythrocyte sedimentation rate, CRP and metabolic panel to exclude chronic diseases that cause chronic anovulation.

Level II

Level II lab test are useful to exclude secondary causes of hyperandrogenism and alterations in the menstrual cycle. They analyze:
- DHEAS – levels >700 μg/dL are usually associated with adrenal carcinomas and severe virilization. Slightly increased levels are found in 25 to 50% of women with PCOS;
- TSH – both hyper- and hypothyroidism can cause period alterations;

- 17-hydroxyprogesterone – should be measured between the second and fifth day after the last period. It is necessary to exclude non-classical congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Levels >200 ng/dL are suggestive of congenital adrenal hyperplasia and require the performance of an ACTH challenge test;
- prolactin – hyperprolactinemia causes hypogonadism and is accompanied by period dysfunction. In 40% of cases, it is associated with hyperandrogenism;
- IGF-1 – should be requested in patients with suspected acromegaly or gigantism;
- 1 mg dexamethasone test – to rule out the presence of Cushing's Syndrome.

Diagnostic procedures

Diagnostic procedures include:

- pelvic and abdominal ultrasound – ovarian ultrasound allows assessment of the presence of polycystic ovary morphology, according to the Rotterdam criteria. It can also be useful in order to exclude ovarian tumors and evaluate endometrial thickness;
- MRI or abdominal CT in suspected androgen-secreting neoplasms.

Therapy notes

Obesity/overweight

Weight loss is the first-line treatment for PCOS. Even modest weight losses (5 to 10% of body weight) can re-establish ovulatory cycles, increase the pregnancy rate and improve the metabolic profile. Bariatric surgery can represent an excellent therapeutic strategy in women with PCOS and severe obesity.

Irregular periods and endometrial protection

Chronic anovulation in PCOS is associated with an increased risk of endometrial hyperplasia and carcinoma.

First-line treatment is the estrogen-progestogen pill. It leads to various benefits, including endometrial protection through daily exposure to pro-

gestogen, and skin benefits in hirsutism through the suppression of androgen production.

The use of estroprogestins increases the risk of thromboembolism, especially in women >40 years, smokers, with obesity and polymorphisms of clotting factors.

In women who are unable or unwilling to take estroprogestins, alternative therapies are intermittent or long-term use of progestogens alone, or the use of intrauterine devices that release progesterone. However, this treatment is not effective in hirsutism.

Metformin is also a potential alternative therapy for restoring ovulatory cycles and is effective in 30% to 50% of women. However, its effectiveness for endometrial protection is unclear, and therefore it represents a second-line therapy for this purpose; further, metformin is not an effective treatment for hirsutism. Metformin can improve insulin sensitivity and blood glucose metabolism and it seems to have a good effect in preventing diabetes in subjects at risk. The recommended daily doses are 1000 to 2000 mg. The combination with the estrogen-progestogen pill does not provide further advantages for hirsutism, but it can be useful in controlling the negative effects of estrogen-progestogen on glucose metabolism and in controlling body weight.

Hirsutism and hyperandrogenism

Medical therapy can act on various levels: suppression of ovarian androgen production, reduction of free androgens, increased SHBG production, and androgen action blockage at the receptor level.

The first-line treatment is the estrogen-progestogen pill, which acts on the first two issues. The initial estrogen dose is 20 µg of ethinylestradiol, although some women require higher doses in order to achieve a good ovarian androgen suppression. It is preferable to use progestogen combinations with antiandrogenic activity.

If after 6 months of treatment the cosmetic result is not satisfactory, it is possible to add an antiandrogen drug while continuing treatment with the estrogen-progestogen pill. It should be noted that antiandrogen drugs have important teratogenic effects. The most used antiandrogen drugs are

spironolactone in doses of 50 to 100 mg once or twice daily, finasteride 2.5 to 5 mg/day, cyproterone acetate 50 to 100 mg/day or 3 mg in combination with estrogen in predefined formulations, or flutamide 250 to 750 mg/day.

Other less commonly used therapies are GnRH agonists and glucocorticoids, mainly in hyperandrogenism of adrenal origin and when there are no benefits from first-line estrogen-progestogen and antiandrogen treatment.

Topical treatments with eflornithine cream (for facial hirsutism) or cosmetic treatments such as photoepilation, electrolysis, hair removal creams or waxing are effective treatments for hirsutism, especially when used in combination.

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