CHAPTER 1

Basic Science

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Multiple Choice Questions

1. Which of the following syndromes does **not** have an autosomal dominant inheritance pattern?

a. Huntington disease.
b. Marfan syndrome.
c. Familial hypercholesterolemia.
d. Ehlers-Danlos syndrome.
e. Achondroplasia.

Answer: d

2. What does serum transferrin measure?

a. Iron stores.
b. Iron absorption.
c. Reticuloendothelial iron content.
d. Total body iron.
e. Total iron in the blood.

Answer: b
After iron is absorbed from the gastrointestinal (GI) tract, it is immediately bound by transferrin.

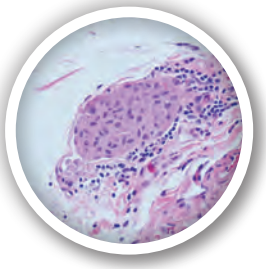
$$TSAT\ (transferrin\ saturation) = \frac{(serum\ iron) \times 100}{(total\ iron\ binding\ capacity)}$$

3. What type of organisms are *Nocardia* species?

a. Gram positive bacteria.
b. Gram negative bacteria.
c. Filamentous fungus.
d. Spore forming fungus.
e. Mycobacteria.

Answer: a
Nocardia species are gram positive bacteria that grow in bead-like chains. They cause skin, central nervous system (CNS), or respiratory infections in the immunocompromised patient.

4. Disseminated intravascular coagulation (DIC) depletes all factors **except**:



a. Platelet.
b. Fibrinogen.
c. Fibrin split products.
d. Antithrombin 3.
e. Factor 8.

Answer: c

5. Which inheritable genetic condition exhibits anticipation?

a. Huntington disease.
b. Down syndrome.
c. Gaucher disease.
d. Klinefelter syndrome.

Answer: a
Anticipation is the phenomenon of earlier and more severe disease onset associated with inheritable trinucleotide repeat diseases, such as Huntington disease.

6. *JAK2* mutations are associated with which condition?

a. Polycythemia vera.
b. Crohn disease.
c. Ulcerative colitis.
d. Burkitt lymphoma.

Answer: a
Up to 95% of all cases of polycythemia vera are associated with *JAK2* mutations.

7. Which protein is **anti**apoptotic?

a. *BAX*.
b. Caspase 9.
c. Caspase 3.
d. *BCL-2*.

Answer: d
BCL-2 is antiapoptotic and is upregulated in malignancies as compared to nonmalignant conditions (i.e., follicular lymphoma versus reactive lymph nodes).

8. Pediatric cardiac rhabdomyomas are associated with which condition?

a. Prader-Willi syndrome.
b. Tuberous sclerosis.
c. Niemann-Pick disease.
d. Down syndrome.

Answer: b
Cardiac rhabdomyomas are detected in > 50% of neonates with tuberous sclerosis.

9. Which is **not** a feature of apoptosis?

a. Nuclear shrinkage.
b. DNA laddering.
c. Associated inflammation.
d. Membrane blebbing.

Answer: c
Necrosis generally induces a surrounding inflammatory response, unlike apoptosis.

10. Which cellular process is associated with cytochrome c release from the mitochondria?

a. Intrinsic pathway apoptosis.
b. Necrosis.
c. Extrinsic pathway apoptosis.
d. Autophagy.

Answer: a
The intrinsic pathway of apoptosis is associated with mitochondrial release of cytochrome c into the cytoplasm of the cell.

11. Patau syndrome is associated with which trisomy?

a. 13.
b. 21.
c. 18.
d. 12.

Answer: a
Trisomy 13 is also known as Patau syndrome.

12. Which disease is associated with HLA-DR3?

a. Diabetes.
b. Celiac disease.
c. Ankylosing spondylitis.
d. Crohn disease.

Answer: a

13. Which disease is associated with a t(8;14) translocation?

a. Gastrointestinal stromal tumor.
b. Diffuse large B cell lymphoma.
c. Chronic myelogenous leukemia.
d. Burkitt lymphoma.

Answer: d
Burkitt lymphoma is associated with a c-Myc/IgH translocation; c-Myc is located on chromosome 8 and IgH is located on chromosome 14.

14. What is **not** a function of normal p53?

a. Apoptosis.
b. Glucose metabolism.
c. Cell cycle arrest.
d. Response to DNA damage.

Answer: b
Normal p53 plays a central role in inducing cell cycle arrest and apoptosis due to various stimuli. It also upregulates genes involved in DNA damage from radiation. It has no direct effect on glucose metabolism.

15. Which tumor is associated with *SYT-SSX* translocation protein?

a. Diffuse large B cell lymphoma.
b. Gastrointestinal stromal tumor.
c. Synovial sarcoma.
d. Ewing sarcoma.

Answer: c
Synovial sarcoma is associated with the t(X:18) translocation that produces the *SYT:SSX* fusion protein.

16. Kimmelstiel-Wilson (KW) bodies are seen in which disease?

a. Churg-Strauss syndrome.
b. Diabetic nephropathy.
c. Scleroderma.
d. Hashimoto thyroiditis.

Answer: b
KW bodies are pink hyaline nodules that form in glomerular capillaries in diabetic nephropathy.

17. Which kidney disease is associated with "full house" immunofluorescence staining?

a. Lupus nephritis.
b. Goodpasture syndrome.
c. IgA nephropathy.
d. Focal segmental glomerulosclerosis.

Answer: a
"Full house" staining refers to positive immunofluorescence for IgG, IgA, IgM, and complement proteins. It is commonly seen in lupus nephritis.

18. Which of the following proteins is a protooncogene?

a. p53.
b. p16.
c. Retinoblastoma.
d. K-ras.

Answer: d
K-ras is the only protooncogene. The other proteins are tumor suppressors.

19. What virus has been implicated in Kaposi sarcoma?

a. Coxsackie B.
b. Human T cell lymphotropic virus (HTLV).
c. Human herpesvirus 8 (HHV-8).
d. Herpes simplex virus 1 (HSV-1).

Answer: c
All forms of Kaposi sarcoma are associated with HHV-8 viral infection.

20. What condition is associated with abnormal genomic imprinting?

a. Down syndrome.
b. Huntington disease.
c. Prader-Willi syndrome.
d. Beckwith-Wiedemann syndrome.

Answer: c
Most cases of Prader-Willi syndrome are associated with 15q11–13 deletion in the paternally derived chromosome. Most cases of Angelman syndrome are associated with a 15q11–13 deletion in the maternally derived chromosome.

21. What is the most common type of epidermal growth factor receptor (*EGFR*) mutation that occurs in lung adenocarcinoma?

a. Point mutation.
b. Inversion.
c. Deletion.
d. Insertion.

Answer: c
Deletion of *EGFR* exon 19 is the most common mutation occurring in lung adenocarcinoma. Point mutation of L858 is the next most common alteration.

22. Which *EGFR* mutation does **not** confer sensitivity to tyrosine kinase inhibitors?

a. Exon 19 deletion.
b. L858R mutation.
c. T790M mutation.
d. Exon 19 insertion.

Answer: c
Threonine 790 to methionine (T790M) mutation confers resistance to most standard tyrosine kinase inhibitors in adenocarcinomas of the lung. All other mutations listed are sensitive to these therapeutic agents.

23. What is the most common type of mutation that occurs in melanoma?

a. Point mutation.
b. Inversion.
c. Deletion.
d. Insertion.

Answer: a
BRAF point mutations, resulting in the V600E transition, are the most common mutation in melanoma.

24. Calreticulin mutations are associated with which disease?

a. Chronic eosinophilic leukemia (CEL).
b. Primary myelofibrosis (PMF).
c. Chronic myelogenous leukemia (CML).
d. Polycythemia rubra vera (PV).

Answer: d
Calreticulin mutations are a newly discovered etiology of primary myelofibrosis. They occur independently of *JAK2* and account for approximately 50–60% of *JAK2* negative cases of PMF.

25. What type of mutation is seen in calreticulin in PMF?

a. Point mutation.
b. Inversion.
c. Deletion.
d. All of the above.

Answer: c

Out of frame deletions of calreticulin exon 9, specifically a 52bp deletion, are the most common mutation seen in PMF. A 5bp insertion can also be seen. These mutations have also been identified in most cases of *JAK2* negative essential thrombocytosis.

Short Answer Questions

26. Describe the effect of irradiating the whole human body.

LEVEL OF IRRADIATION	EFFECTS
2–10 Sv	• Bone marrow destruction (leucopenia, infections, nausea and vomiting, fatigue). • Skin burns/rash; some patients have desquamation of skin, mucosal sloughing, and ulceration (oral, head and neck, esophagus).
10–20 Sv	• Damage to small bowel: severe diarrhea, nausea and vomiting, fatigue.
> 50 Sv	• Damage to brain: coma and convulsions.

Notes
100 rads = 1 Sv = 1 Gy (gray)
rad = radiation absorbed dose
Sv = sieverts

27. What is the function of the Langerhans cell? What unique cytoplasmic structure can be seen on electron microscopy?

- Function: antigen presentation in the skin.
- Cytoplasmic structure: identified by the presence of Birbeck granules in the cytoplasm on electron microscopy.

28. What is the Lyon hypothesis (now the Lyon law)?

The Lyon law refers to the random and fixed inactivation (in the form of sex chromatin) of 1 X chromosome in mammalian cells at an early stage of embryogenesis, leading to mosaicism of paternal and maternal X chromosomes in females.

29. What are the functions of mast cells?

- Degranulation and release of preformed allergic mediators from granules in response to allergens (immediate hypersensitivity).
- Release of eicosanoids (thromboxane, PGD2, leukotrienes) for late phase allergic response.

30. Describe the types of prostaglandins and their function.

- PGI_2.
  - Promotes vasodilation.
  - Inhibits platelet aggregation.
- PGE_2.
  - Decreases gastric acid.
  - Increases gastric mucous.
  - Promotes vasodilatation and GI smooth muscle relaxation.
- PGD_2.
  - Promotes vasodilatation.
  - Increases vascular permeability.

31. Describe the structure of an IgG molecule.

- Y-shaped structure with variable domain regions at the top of the Y's arms and the Fc domain at the base.
- 2 heavy chains and 2 light chains with 4 disulfide bonds stabilizing the structure.

32. What is most likely the cause of fungus balls in the lung?

Aspergillosis, from the mold *Aspergillus fumigatus*.

33. What causes chronic granulomatosis disease (CGD)?

- Cause: a genetic defect in the phagocyte oxidase enzyme in neutrophils (NADPH oxidase) — the defect is either X-linked or autosomal recessive.
- Process: NADPH oxidase is required for the respiratory burst in neutrophils activated by bacteria. The generation of reactive oxygen species requires formation of a multiprotein complex (NADPH oxidase) that reduces oxygen to superoxide anion and forms H_20_2. H_20_2 is broken down by myeloperoxidase to generate hypochlorite and halides to kill bacteria. This occurs in intracellular phagocytic vacuoles. This deficiency makes CGD patients prone to bacterial infections.

34. Erythroblastosis fetalis is seen in what type of hypersensitivity reaction?

- Antibody mediated (type II) hypersensitivity reaction.
  - The IgM/IgG binds to red blood cells and triggers phagocytosis or lysis from formation of the complement membrane attack complex.

35. Describe common types of brown pigmentation in tissue.

PIGMENTATION	TISSUE
Bile — green to greenish brown.	• Commonly seen in bile canaliculi. • Intrahepatocellular bile (centrilobular) cannot be distinguished from lipofuscin or iron.
Copper — yellow brown.	• Seen in periportal hepatocytes. • Stain with rhodamine and orcein.
Iron — brown to golden brown.	• Seen in periportal hepatocytes, except in congestive liver. • In Kupffer cells, seen in hemolysis, iron overload, or recent hepatocyte necrosis. • In hepatocytes, seen in hemochromatosis. • Perls' Prussian blue stain shows positive.
Lipofuscin — brown.	• Seen in centrilobular hepatocytes (never periportal). • Can be seen in Kupffer cells in recent hepatocellular necrosis. • Acid fast stain (Fite) shows positive.
Melanin — black/brown.	• Seen in melanoma, nevi, melanocytes. • Not polarizable or refractile.

36. List the classifications of burns and describe how to estimate their severity.

- Classifications:
  - First degree (partial thickness).
  - Second degree (superficial and deep).
  - Third degree (full thickness).
- Methods of estimating burn severity:
  - "Rule of Nines" — estimates body percentage as follows: each arm 9%, anterior torso 18%, posterior torso 18%, each leg 18%, head and neck 9%.
  - Severe burn (requires burn unit) — characterized by facial burns, perineal burns, circumferential extremity burns, burns that cross joints, inhalation injury, partial thickness burn with total body surface area (TBSA) > 10%, any full thickness (third degree) burn in any age group.

37. What is the role of adenosine-5′ triphosphate (ATP) in diffuse axonal injury?

- Diffuse axonal injury (DAI) is due to shear forces from rapid acceleration or deceleration (e.g., a motor vehicle accident) that lead to axonal disruption or separation in the brain stem, corpus callosum, cerebral hemispheres (white matter), cerebral cortex, cerebral peduncles, or basal ganglia.
  - Initially, the mechanism of DAI was thought to be rupture or tears in axons.
  - Evidence now suggests that biochemical cascades begin with the initial stimulus of disruption of the microtubule cytoskeleton. Axonal transport continues to the point of rupture where amyloid precursor protein is carried and accumulates via ATP dependent motor proteins (axonal swellings are seen histologically as a "retraction balls," the hallmark of DAI).
  - Other biochemical events include opening of sodium channels, loss of the calcium gradient, and subsequent activation of enzymes such as phospholipases and caspases.

38. What is Reye syndrome?

- A sudden, sometimes fatal disease of the brain (encephalopathy) with degeneration of the liver.
  - It occurs in children, most often 4–12 years of age.
  - It comes after chickenpox (varicella) or an influenza-type illness.
  - It is also associated with taking medications containing aspirin.
  - Children usually present with vomiting, lethargy, and progressive neurological dysfunction.
  - Pathology: acute noninflammatory encephalopathy and acute fatty liver changes.

STATISTICAL ANALYSIS

39. Define sensitivity and specificity.

- Sensitivity (SN): measurement of the rate of positive tests in patients who actually have a condition.

$$SN = \frac{true\ positive}{true\ positive\ +\ false\ negative}$$

  - Negative results in highly sensitive tests mean the condition is not present (SNOUT: sensitive tests rule out a condition when negative).
- Specificity (SP): the ability of a test to identify negative results in patients who do not actually have a condition.

$$SP = \frac{true\ negative}{true\ negative\ +\ false\ positive}$$

  - Positive results in highly specific tests mean the condition is present (SPIN: specificity rules in a condition when positive).

40. Define negative and positive predictive value.

- Positive predictive value (PPV): the ability of a test to identify true positives (condition present) among many positive tests.
- Negative predictive value (NPV): the ability of a test to identify true negatives (condition not present) among many negative tests.

41. Create a table to illustrate the meaning of sensitivity (SN), specificity (SP), positive predictive value (PPV), and negative predictive value (NPV).

	CONDITION + (PRESENT)	CONDITION – (ABSENT)	
Test +	A	B	PPV
Test –	C	D	NPV
	SN	SP	

$$SN = \frac{A\ or\ (true\ +)}{A\ +\ C\ (condition\ +)}$$

$$PPV = \frac{A\ or\ (true\ +)}{A\ +\ B\ (test\ +)}$$

$$SP = \frac{D\ or\ (true\ -)}{B\ +\ D\ (condition\ -)}$$

$$NPV = \frac{C\ or\ (true\ -)}{C\ +\ D\ (test\ -)}$$

ANGIOGENESIS

42. Define angiogenesis.

- Angiogenesis: the physiological process of developing new blood vessels from preexisting vessels that allows essential physiological processes to take place, including wound formation; revascularization after trauma, ischemia, or menstruation; and granulation tissue formation.
  - Pathological angiogenesis occurs with tumor growth, diabetic retinopathy, and chronic inflammation.
  - Vasculogenesis derives from circulating stem cells or angioblasts during embryonic development.

43. List 5 angiogenic growth factors.

- Vascular endothelial growth factor (*VEGF*).
- Angiopoietins 1 and 2.
- Platelet derived growth factor (*PDGF*).
- Transforming growth factor beta (*TGF-β*).
- Fibroblast growth factor (*FGF*).

44. How does vascular endothelial growth factor (*VEGF*) function?

- *VEGF* is the most important angiogenic factor.
  - Binds to *VEGF* receptors, which promotes the release of endothelial precursor cells from bone marrow.
  - Also stimulates endothelial cells to migrate and proliferate to new vessels from preexisting ones.
  - Is induced by: hypoxia; *TGF-β* released from fibroblasts (wounds); *PDGF* released from platelets (wound and injury); and *TGF-α* (inflammation).

HEALING AND REPAIR

45. At what point after injury does a wound reach 50% of its preinjury tensile strength?

- 1 month.
  - 1 week: 10% of original tensile strength.
  - 1 month: 50% of original tensile strength.
  - 3 months: 75–80% of maximal tensile strength.

46. Describe the cells and factors involved in the phases of wound healing.

- Phase 1: homeostasis phase (first 24 hours) — blot clot formation.
  - Cells: platelets, neutrophils.
  - Factors: fibrin, growth factors, chemokines, cytokines, clotting cascade.
- Phase 2: inflammatory phase (3–7 days post wound) — formation of granulation tissue; macrophages remove bacteria via phagocytosis.
  - Cells: fibroblasts, macrophages (48 hours), decreased neutrophils, endothelial stem cells.
  - Factors: *VEGF* (angiogenesis).
- Phase 3: proliferative phase (1–3 weeks).
  - Cells: fibroblasts (collagen deposits), myofibroblasts (wound contraction), epithelial cells (basement membrane formation and epithelization), angiogenesis.
  - Factors: extracellular matrix deposition, type III collagens, laminin, *FGF, TGFB*, chemokines.
- Phase 4: remodeling and maturation phase (3 weeks to months) — scar tissue formation (4 weeks), collagen cross linking/modifications, degradation of type III collagen (weaker), and deposition of type I collagen (stronger).
  - Cells: fibroblasts and macrophages.
  - Factors: matrix metalloproteinases (MMPs), tissue inhibitors of MMPs.

47. Describe the sequence of events in healing by primary intention.

- Occurs in small, clean cuts, where edges can approximate or line up.
- Occurs by the standard 4 phases of wound healing: homeostasis, inflammatory phase, proliferative phase, and maturation phase.
- Occurs in surgical wounds and small lacerations, and heals with minimal scarring.

48. Describe the sequence of events in healing by secondary intention.

- Larger wound heals with a larger amount of granulation tissue, scar, and wound remodeling and contraction.
- Broader scar formation; slow to heal if gets infected; requires wound care.

49. What are the basic features of repair by connective tissue deposition?

- Inflammation.
- Angiogenesis.
- Migration and proliferation of fibroblasts.
- Scar formation.
- Connective tissue remodeling.

50. List 4 local factors and 4 systemic factors that retard wound healing.

- Local factors:
  - Infection.
  - Mechanical factors, such as early motion of wounds.
  - Foreign bodies.
  - Size, location, and type of wound.
- Systemic factors:
  - Nutrition, such as protein deficiency and vitamin C deficiency.
  - Metabolic status, such as diabetes mellitus.
  - Circulatory status, such as inadequate blood supply caused by arteriosclerosis.
  - Hormones, such as glucocorticoids.

51. List 2 examples of abnormal wound healing.

- Excess scar formation or keloid.
- Wound dehiscence.

VITAMINS

52. Which types of vitamins are affected by pancreatic insufficiency?

Fat soluble vitamins (vitamins A, D, E, and K).

53. List fat soluble vitamins, their physiological function, and diseases that result from deficiency.

VITAMIN (FAT SOLUBLE)	FUNCTION	DISEASES FROM DEFICIENCY
Vitamin A (retinol)	• Vision. • Formation of teeth/bones. • Embryogenesis. • Epithelium formation. • Infection resistance. • Hematopoiesis.	• Night blindness (early). • Blindness (late). • Corneal dryness. • Infections. • Squamous metaplasia.
Vitamin D (calciferol)	• Calcium absorption from intestines. • Phosphate adsorption.	• Bone defects: rickets/osteomalacia, osteoporosis.
Vitamin E	• Antioxidant.	• Spinocerebellar degeneration/ataxia. • Muscle weakness and myopathies. • Retinopathies. • Immune response.
Vitamin K	• Cofactor for factors II, VII, IX and X in coagulation cascade.	• Bleeding.

54. List fat insoluble vitamins, their physiological function, and diseases that result from deficiency.

VITAMIN (FAT INSOLUBLE)	FUNCTION	DISEASES FROM DEFICIENCY
Vitamin B_1 (thiamine)	• Coenzyme in decarboxylation reactions.	• Beriberi. • Wernicke-Korsakoff syndrome.
Vitamin B_2 (riboflavin)	• Enzyme cofactor (think ribose).	• Angular cheilitis • Oral ulcers. • Stomatitis. • Corneal ulcers. • Sore throat. • Photophobia.
Vitamin B_3 (niacin)	• NADH and NADPH formation.	• Pellagra — dementia, dermatitis, diarrhea, necklace lesions of lower neck, hyperpigmentation, thick skin, delirium.
Vitamin B_6 (pyridoxine)	• Serotonin, dopamine, norepinephrine, epinephrine synthesis.	• Cheilosis. • Glossitis. • Dermatitis. • Peripheral neuropathy. • Depression, anxiety.
Vitamin B_{12} (cobalamin)	• DNA/folate synthesis.	• Megaloblastic pernicious anemia. • Posterior lateral spinal cord degeneration.
Vitamin C (ascorbic acid)	• Collagen hydroxylation. • Redox reactions.	• Scurvy — malaise, lethargy, skin spots, scorbutic gums, bleeding mucous membranes.
Folate	• Essential for DNA synthesis.	• Megaloblastic pernicious anemia. • Neural tube defects. • Cognitive decline. • Poor memory. • Pregnancy complications.
Pantothenic acid	• Required for the synthesis of coenzyme A.	• Very rare: numbness and painful burning and tingling in the feet.

55. List the pathological features of vitamin B_{12} deficiency.

- Autoimmune gastritis with pernicious anemia.
- Inadequate vitamin B_{12} intake (e.g., vegans, alcoholics).
- Terminal ileum damage such as resection.
- Crohn disease with loss of small bowel (ileum).
- Intestinal bacterial overgrowth.
- Fish tapeworm infestation.

56. Describe the pathological features of vitamin B_{12} deficiency.

- Peripheral neuropathy: degeneration of dorsal and lateral spinal columns (ataxia, weakness, paraplegia).
- Macrocytic anemia: leukopenia with macropolymorphonuclear neutrophils; giant platelets and precursors.
- Atrophy and intestinal metaplasia of stomach (pernicious anemia).

57. Describe the synthesis of vitamin D.

- 7-dehydrocholesterol interacts with UVB in the skin to form vitamin D_3.
- D_3 is processed in the liver by 25-hydroxylase to form 25-OH D_3.
- 25-OH D_3 is processed in the kidney by 1-alpha-hydroxylase to form 1,25 dihydroxy vitamin D.

58. Describe the biological effects of vitamin D.

- Increases calcium and phosphate absorption in the gut.
- Induces bone mineralization.
- Suppresses parathyroid hormone (PTH) secretion.

CELL DAMAGE

59. Which part of the complement system is responsible for lysing of red blood cells?

- The lysis of red blood cells requires the formation of the membrane attack complex — made of complement proteins — from the classical pathway.
  - Classical pathway: IgM or IgG bind antigen and C1 protein; C3 binds and activates C3-convertase, generating C3b and C3a. C3a forms C5-convertase, and splits C5 into C5a. This results in the formation of the membrane attack complex, composed of C6 and C9 proteins, which lyse red blood cells.
  - Alternate pathway: triggered by microbial molecules (lipopolysaccharide), recruits complement.
  - Lectin pathway: binds sugars on bacteria, recruits complement.

60. Oxygen toxicity causes what type of damage to cells?

- Oxidative damage.
  - Excess oxygen (like reperfusion injury) causes the formation of free radicals and reactive oxygen species, which can lead to direct damage to cell membranes, nuclear DNA, and cellular organelles.

61. Ultraviolet (UV) radiation causes what type of DNA damage?

- UV-B: cross-links between adjacent cytosine and thymidine forming pyrimidine dimers (repaired by nucleotide excision repair pathway, enzymes of which are deficient in patients with xeroderma pigmentosum.
- UV-A: free radical formation causes indirect DNA damage or breaks.

62. List 5 types of DNA damage.

- Oxidation of base pairs and DNA strand breaks from reactive oxygen species.
- Alkylation of bases (methylation).
- Hydrolysis, including deamination, depurination, depyrimidination.
- Adduct formation, bulky modification with benzo[*a*]pyrene (e.g., smokers).
- DNA mismatch, errors in DNA replication.

63. List at least 3 biochemical mechanisms responsible for cell membrane damage.

- ATP depletion, which results in the loss of function of the sodium-potassium pump.
- pH drop from lactic acid accumulation and anaerobic metabolism, which causes denaturation of cellular enzymes.
- Entry of calcium ions (Ca^{2+}) into cells with activation of proteases, phospholipases, and endonucleases.
- Osmotic changes, which can lead to cell rupture.
- Detachment of ribosomes from endoplasmic reticulum with decreased protein synthesis.

APOPTOSIS

64. Define apoptosis.

Apoptosis: the programmed cell death pathway, regulated by an intricate enzyme cascade, which ultimately leads to DNA fragmentation, apoptotic bodies, and cytoplasm degradation.

65. Describe the pathogenesis of apoptosis.

- Intrinsic pathway: release of mitochondrial proteins (cytochrome c) to activate caspase 9.
  - Proapoptosis: *BAX, BAK*.
  - Antiapoptosis: *BCL-2, BCL-X, MCL-1*.
- Extrinsic pathway: plasma membrane receptors containing FADD/death domains can bind their appropriate ligands and activate caspases, resulting in apoptosis (i.e., FAS ligand binding to the FAS receptor).
- The common downstream effect of both pathways is the activation of executioner caspases (3, 6), leading to activation of DNases to cleave DNA. This results in DNA fragmentation into "DNA ladders" of approximately 200 base pairs.

66. List at least 3 morphological differences between apoptosis and cell necrosis.

MORPHOLOGICAL CATEGORY	APOPTOSIS	CELL NECROSIS
Surrounding tissue	• No inflammation.	• Inflammatory reaction frequent.
Histology	• Isolated cells affected in healthy tissue.	• Cells die together, cause structure disintegration.
Cytology	• Pyknotic nuclei, condensed chromatin (pyknosis), chromatin fragmentation (karyorrhexis), minor changes in cytoplasmic organelles, and overall cell shrinkage, blebbing of the plasma membrane, and formation of apoptotic bodies that contain nuclear or cytoplasmic material.	• Karyorrhexis, edema, fading of chromatin.
Staining	• Cell membrane not permeable to staining agents.	• Cell membrane permeable to staining agents.
Electron microscopy	• Dense nuclear crescents and apoptotic bodies, intact plasma membrane.	• Swollen mitochondria, vacuoles in the cytoplasm, fragmented organelles, ruptured plasma membrane and nuclear membrane.

67. List 4 physiological situations where apoptosis occurs.

- Programmed destruction of cells during embryogenesis.
- Involution of hormone dependent tissues upon hormone withdrawal, such as endometrial atrophy after menopause.
- Cell loss in proliferating cell populations, such as immature lymphocytes in bone marrow and thymus that fail to express useful antigen receptors.
- Elimination of potentially harmful self-reactive lymphocytes.
- Death of host cells that have served their useful purpose, such as neutrophils in an acute inflammatory response.

68. List 4 pathological situations where apoptosis occurs.

- DNA damage, which can result — directly or indirectly via the production of free radicals — from radiation, cytotoxic anticancer drugs, or hypoxia.
- Accumulation of misfolded proteins, as seen in neurodegenerative disease and α_1-antitrypsin deficiency.
- Cell death in certain infections, particularly viral infections such as acute hepatitis.
- Pathologic atrophy in parenchymal organs after duct obstruction, such as occurs in the pancreas, parotid gland, and kidney.

69. What is the key gene involved in regulating apoptosis?

BCL-2.

p53 GENE

70. List the functions of p53 in the normal cell cycle.

- p53 thwarts neoplastic transformation by 3 interlocking mechanisms:
  - Activation of temporary cell cycle arrest (quiescence) and induction of DNA repair.
  - Induction of permanent cell cycle arrest (senescence).
  - Triggering of programmed cell death (apoptosis).

71. Describe the role of p53 in human carcinogenesis.

- It acts as the "guardian of the genome." It functions at the G1/S cell checkpoint.
- It senses DNA damage and induces DNA repair; p53 mutation leads to accumulation of DNA damage.
- It induces cell senescence or apoptosis; p53 mutation leads to immortalization of the cell via defective apoptosis.
- p53 mutation leads to aneuploidy via centrosome destabilization and multipolar mitotic spindle.

72. What are the clinical implications of testing p53 mutations?

- Confirming cancer diagnosis: lung, colon, breast.
- Identifying cancer subtypes, such as triple negative breast cancer and high grade serous carcinoma of the mullerian tract.
- Suggesting the mechanism of carcinogenesis: Li-Fraumeni syndrome equates with sarcomas, brain tumors, leukemias, osteosarcomas.
- Predicting the response to chemotherapy and radiotherapy.

73. What methods detect p53 mutations in cells and tissues?

- Polymerase chain reaction (PCR) assay.
- Direct sequencing.
- Immunohistochemistry.

74. Describe the function of miR-34 with respect to p53.

- p53 activates the transcription of miR-34.
- miR-34 is a microRNA. MicroRNA are small (22 bp) RNA molecules that target mRNA for destruction. MiR-34 targets cyclins, *BCL*-2, myc, cyclin-dependent kinases, and notch for degradation, which leads to apoptosis, quiescence, or senescence.

CELL NECROSIS

75. Define cell necrosis.

- Cell necrosis: the morphological changes indicative of cell death caused by progressive enzymatic degradation.
  - It may affect groups of cells, parts of a structure, or an entire organ.

76. Describe some pathological processes involved in cell death.

- Factors external to the cell, such as infections or hypoxia, which lead to irreversible cell injury.
- ATP depletion (ion pump failures), pH changes, osmotic forces, which lead to denaturation of intracellular proteins and loss of plasma membrane integrity.
- Release of enzymes from lysosomes, which leads to degradation.

77. List at least 3 histologic types of cell necrosis and 1 example of each.

- Coagulative necrosis: myocardial infarct, splenic infarct.
- Liquefactive necrosis: abscesses, stroke.
- Gangrenous necrosis: diabetic foot ulcer.
- Caseous necrosis: TB, atypical mycobacterium, histoplasmosis, cryptococcosis, coccidiomycosis.
- Fat necrosis: acute pancreatitis.
- Fibrinoid necrosis: vasculitis (leukocytoclastic vasculitis, Churg-Strauss syndrome, Wegener granulomatosis).

78. Name 2 histologic features of reversible cell damage.

- Cellular swelling.
- Fatty change.

INFLAMMATION

79. List 4 vascular changes in acute inflammation.

- Vasodilation.
- Endothelial permeability.
- Neutrophil recruitment.
- Stasis of blood flow.

80. List 4 endothelial leukocyte adhesion molecules.

- L-selectin (*CD34*).
- VLA-4 (B1) integrins (*VCAM-1*).
- Sialyl-Lewis X–modified proteins (P- and L-selectins).
- B2 integrins (*CD18*), which bind *ICAM*, fibrinogen, and fibronectin.

81. List at least 5 inflammatory mediators.

- Histamine.
- Serotonin.
- Cytokines.
- Tumor necrosis factor (*TNF*).
- Interleukins.
- Chemokines.
- Leukotrienes.
- Complement factors.
- Nitric oxide.
- Reactive oxygen species.

DIABETES MELLITUS

82. List 5 gross morphological features of diabetes mellitus.

- Blood vessels (macrovascular): atherosclerosis of aorta (large and medium-sized blood vessels), peripheral vascular disease (with ischemic ulcers, gangrenous necrosis of lower extremities), hypertension (hyaline arteriolosclerosis), coronary artery atherosclerosis (with myocardial infarction), renal artery stenosis, stroke.
- Kidneys: nephrosclerosis of kidneys (due to glomerulosclerosis, arteriosclerosis, pyelonephritis).
- Eyes: retinopathy, glaucoma, cataracts.
- Skin: cellulitis, infections, ulcers due to peripheral neuropathy.
- Bladder: dysfunctional bladder with incontinence due to autonomic neuropathy.

83. List 5 clinical features in untreated diabetes.

- Diabetic retinopathy.
- Diabetic peripheral neuropathy.
- Diabetic foot necessitating amputation.
- Diabetic nephropathy.
- Atherosclerotic cardiovascular disease.

84. List 4 common causes of death in diabetes patients.

- Stroke.
- Myocardial infarction.
- Infection/sepsis.
- Renal failure.

85. What invasive fungal nasal infection can occur in a diabetic?

- Mucormycosis or zygomycosis — the fungus has large, non-septa-wide hyphae (5–60 um), 90-degree-angle branching, and nonparallel walls. It is angioinvasive, and can cause tissue necrosis and hemorrhage.
- Inhaled spores cause infection in the nasal sinuses, lung, and brain.

COAGULATION

86. What are the 3 components of the Virchow triad?

- The Virchow triad describes the 3 broad categories of factors that are thought to contribute to thrombosis:
  - Hypercoagulability.
  - Hemodynamic changes (stasis, turbulence).
  - Endothelial injury/dysfunction.

87. List 2 types of infarcts.

- Red infarct (venous occlusion).
- White infarct (arterial occlusion).

88. List 6 types of emboli.

- Fat emboli.
- Air emboli.
- Amniotic emboli.
- Tumor emboli.
- Bacteria emboli.
- Thrombotic emboli.

AMYLOIDOSIS

89. Define amyloid and amyloidosis.

- Amyloid: a pathologic proteinaceous substance composed of a heterogeneous group of fibrillar proteins that share the ability to aggregate into an insoluble, cross beta pleated sheet tertiary conformation. It can be deposited in the extracellular space of various tissues and organs of the body in a wide variety of clinical settings.
  - With electron microscopy (EM), seen as a 7.5–10 nm diameter fiber. (See electron microscopy section in this chapter.)
  - With X-ray crystallography, seen as a beta pleated sheet.
- Amyloidosis: abnormal accumulation of amyloid proteins, which are resistant to degradation and get deposited in various organs, causing systemic disease.

90. List the types of amyloid and their associated clinical conditions.

TYPE OF AMYLOID	ASSOCIATED DISEASE/CONDITION
SYSTEMIC AMYLOIDOSIS	
• Primary amyloidosis - Amyloid light chain (AL).	• All types of systemic primary amyloidosis are associated with: - Multiple myeloma and other monoclonal plasma cell proliferations. - Chronic inflammatory conditions.
• Secondary amyloidosis - Amyloid associated protein (AA). - Transthyretin (ATTR). - β2-microglobulin (A-β2-m).	• All types of systemic secondary amyloidosis are associated with: - Systemic senile amyloidosis. - Hemodialysis (for chronic renal failure).
HEREDITARY AMYLOIDOSIS	
• Amyloid associated protein (AA). • Transthyretin (ATTR).	• All types of hereditary amyloidosis are associated with: - Familial Mediterranean fever. - Familial amyloidotic neuropathies.
LOCALIZED AMYLOIDOSIS	
• Amyloid precursor protein (Aβ). • Calcitonin (A Cal). • Islet amyloid peptide (AIAPP). • Atrial natriuretic factor (AANF).	• All types of localized amyloidosis are associated with: - Alzheimer disease. - Medullary carcinoma of thyroid. - Type 2 diabetes.

91. List the microscopic features of amyloidosis and the special stains for detecting amyloidosis.

- Microscopic features:
  - Eosinophilic, pink waxy accumulation in the extracellular space.
- Special stains:
  - Congo red stain shows apple green birefringence.
  - Thioflavin T shows yellow color.
  - Methyl/crystal violet shows a red-purple stain for amyloid on blue normal background.

92. How is amyloidosis diagnosed clinically?

- Abdominal fat pad aspiration.
- Biopsies: rectum, tongue, gingiva.
- Serum and urine electrophoresis.
- Imaging/scintigraphy.

ANTINEUTROPHIL CYTOPLASMIC ANTIBODY (ANCA)

93. What is the pathogenesis of ANCA?

- Molecular mimicry of components of bacteria leading to ANCA formation.
- Neutrophils with a defective apoptosis mechanism releasing cellular components.
- NETosis (**n**eutrophil **e**xtracellular **t**rap formation): death/lysis of neutrophils, releasing chromatin/DNA, MPO, PR3, and elastase in response to microorganisms.

94. List types of ANCA and associated diseases.

- c-ANCA (antibody against proteinase 3): Wegener granulomatosis.
- p-ANCA (antibody against myeloperoxidase): Churg-Strauss syndrome, microscopic polyangiitis, ulcerative colitis, primary sclerosing cholangitis, rheumatoid arthritis, and polyarteritis nodosa.

CALCIFICATION

95. Name the type of calcium visible on X-rays that might be invisible on slides.

Calcium oxalate.

96. List types of calcium crystals.

- Calcium oxalate — polarize as flat rhomboids, refractile pale yellow or clear, positive mammogram. Found in apocrine cysts, giant cell reactions, but not carcinomas.
- Calcium phosphate — purple granular material histologically; fails to polarize; most common source of positive mammogram. Found in chronic inflammation, heart valves, breast cysts, sclerosing adenosis, hyalinized fibroadenomas, ductal carcinoma in situ (DCIS), and invasive breast carcinoma.
- Calcium pyrophosphate — blue to purple rhomboidal crystals found in joints with pseudogout.

97. Describe how to proceed in the case of a suspicious mammogram with calcifications where Ca^{2+} is not found on histology.

- Polarize.
- Cut deeper.
- Perform radiography on the tissue blocks (flat and on edge).
- Check fixation time: calcium can dissolve if left in formalin for over 24 hours.
- Rule out specimen mix-up.

SARCOIDOSIS

98. What causes hypercalcemia in sarcoidosis?

- Serum angiotensin converting enzyme is elevated in up to 75% of untreated sarcoidosis patients.
  - This leads to elevation of serum calcium levels due to extrarenal production of calcitriol by activated macrophages in the kidney (independent of PTH).
  - This leads to increased intestinal absorption of calcium with serum hypercalcemia and hypercalciuria.
- The same mechanism can also lead to hypercalcemia in other granulomatous disorders including tuberculosis.

99. What methods can you use to diagnose sarcoidosis?

- Pulmonary imaging: chest X-ray, computed tomography (CT) scan, positron emission tomography (PET) scan, radionuclide scan.
- Skin biopsy (naked granulomas).
- Nasal mucosal biopsy.
- Tonsil and lymph node biopsy.
- Biopsies of other involved organs (liver, spleen, bone marrow, muscle).

GRAFT-VERSUS-HOST DISEASE (GVHD)

100. Describe the pathophysiology of GVHD.

T cells derived from a bone marrow transplant recognize the recipient (host) as foreign tissue and mount an immune response, typically targeting the skin, mucosa, liver, or gastrointestinal tract.

101. Describe the characteristic histomorphology of GVHD.

TISSUE	ACUTE GVHD (10–50 DAYS AFTER TRANSPLANT)	CHRONIC GVHD (> 100 DAYS AFTER TRANSPLANT)
Skin	• Grade 0: no changes. • Grade 1: vacuolar alteration of the epidermal/dermal junction. • Grade 2: dyskeratotic keratinocytes. • Grade 3: partial separation of the epidermis from dermis. • Grade 4: complete separation of the epidermis from dermis.	• Lichenoid GVHD or sclerodermoid GVHD.
Colon	Always rule out CMV infection with immunohistochemistry. • Grade 1: increased epithelial cell apoptosis (mainly crypts). • Grade 2: with crypt abscess. • Grade 3: necrosis of individual crypts. • Grade 4: complete destruction of mucosal lining.	• Ischemic changes from fibrosis. • Crypt loss.
Liver	• Severe presents as portal/periportal inflammation with extension into hepatic parenchyma and hepatocyte necrosis. • Resembles chronic hepatitis.	• Bile duct damage and loss. • Portal tract inflammation. • Fibrosis. • Endothelialitis with lifting of endothelial cells from the portal veins or hepatic veins.
Esophagus	• Similar to skin, desquamation. • Submucosal fibrosis.	
Stomach	• Glandular destruction. • Apoptotic debris.	

CORTICOSTEROID THERAPY

102. Describe the effects of corticosteroids and their mechanisms.

- Effects:
  - Corticosteroids, produced in the adrenal cortex, affect stress response, immune response, carbohydrate metabolism, and lipid and protein metabolism.
- Mechanisms:
  - Inflammation: bind to glucocorticoid receptor and enter nucleus; bind DNA and upregulate genes involved in antiinflammation (transactivation), and downregulate genes involved with inflammation (transrepression).
  - Metabolism: metabolize glucose via stimulation of gluconeogenesis in liver, mobilization of amino acids from other tissues, inhibition of glucose uptake by adipose tissue, and stimulation of fatty acid release and fat breakdown.
  - CNS: cross the blood-brain barrier and bind to receptors in the central nervous system to regulate blood pressure, salt excretion, and sympathetic activation (fight or flight).
  - Embryogenesis: promote surfactant synthesis (used in premature deliveries).

103. List the complications of long-term steroid use.

- Immunodeficiency — decreased function and number of neutrophils, lymphocytes, and macrophages. This predisposes patients to infection.
- Adrenal insufficiency crisis — from sudden withdrawal of long-term steroids.
- Cushing syndrome — bilateral adrenal cortical atrophy from exogenous steroids, decreased corticotropin.
- Hyperglycemia, diabetes mellitus, insulin resistance.
- Osteoporosis — reduced bore density.
- Cataracts.
- Hypertension.
- Hypothyroidism.
- Growth failure/pubertal delay.
- Glaucoma — increased intracranial pressure.
- Slow wound healing.

LEAD POISONING

104. List 4 occupations that have increased risk of lead poisoning.

- Lead miners.
- Plumbers.
- Welders.
- Battery recycling workers.

105. List the organ systems affected by lead poisoning and their clinical presentations.

- CNS: decreased IQ, peripheral neuropathy, behavioral changes, coma.
- Bone: lead lines in the epiphysis.
- Hematopoietic system: microcytic anemia, basophilic stippling sideroblasts.
- Kidney: proximal tubular damage (Fanconi syndrome).
- Gastrointestinal tract: diffuse abdominal pain, gingiva lead line.

MOLECULAR DIAGNOSTICS AND CYTOMETRY

106. Name 3 methods to measure cell proliferation, stating advantages and disadvantages of each method.

METHOD	ADVANTAGES	DISADVANTAGES
Ki 67 (MIB-1)	• Easy application in paraffin embedded tissue. • Established prognostic marker for some tumors. • Captures all proliferating cells irrespective of the stage in the cell cycle.	• Formalin fixation only. • Not a true measure of mitosis.
Mitotic cell count	• Specific for mitotic cells. • Can identify aberrant mitosis/aneuploidy. • Recognized in H&E sections, no need for special stains.	• Tumor heterogeneity. • Unable to measure proliferating cells outside mitotic phase.
Flow cytometry/ FACS analysis	• Can count cells in each phase of cell cycle.	• Equipment cost. • Needs fresh tissue.
	• Can determine ploidy status. • Used as an ancillary technique in cytology.	• Requires technical expertise to perform the test and interpret the result. • 2–3 day turnaround time.

107. Describe the principles of the polymerase chain reaction (PCR) technique.

- PCR is a scientific technique in molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.
- It essentially involves amplification of a target sequence of DNA using 3′ and 5′ DNA primers. Each amplification cycle is made up of 3 steps:
  - Step 1: heat denaturation (sequences come apart, dsDNA to ssDNA).
  - Step 2: addition of primers. Cooling to reanneal primers to ssDNA.
  - Step 3: DNA synthesis by DNA polymerase.
- After 25 cycles, 10^7 copies of target DNA sequence are made.

108. List 3 applications of PCR as a diagnostic tool in the laboratory.

- Direct DNA sequencing — forensics, cancer gene/translocations, paternity assays.
- Clonality assays — T cell receptor genes in lymphomas, B cell lymphomas.
- DNA mismatch repair — MSI (microsatellite sequence identification).
- Identification of infectious agents (tuberculosis, *H. pylori*, syphilis, human papillomavirus, hepatitis C, Epstein-Barr virus, toxoplasmosis).

109. Describe the principles of Southern blot analysis.

- Is routinely used in molecular biology for detection of a specific DNA sequence in DNA samples.
- Combines transfer of electrophoresis separated DNA fragments to a filter membrane, and subsequent fragment detection by probe hybridization.
- Uses radioactive or fluorescent dye labels to identify the DNA fragments of interest.

110. List the steps involved in Southern blot analysis.

- Use restriction endonucleases to digest DNA.
- Use electrophoresis to separate the DNA.
- Denature the DNA to ssDNA with alkaline buffer.
- Transfer the digested DNA to a nitrocellulose membrane.
- Hybridize a complementary DNA probe to the membrane to detect the specific gene of interest (radioactive or fluorescent labeling).

111. Give at least 3 examples of clinical applications of Southern blot analysis.

- Syndrome detection (trinucleotide repeat such as fragile X syndrome and Huntington disease).
- DNA fingerprinting in forensics.
- Detection of restriction fragment length polymorphisms (noncoding repeats or satellite DNA sequence variation between individuals of a species — method used to detect sickle cell disease).

112. Describe the principles of Northern blot analysis.

- It identifies specific sequences of RNA.
- RNA molecules are separated by electrophoresis, transferred to nitrocellulose, and identified with a suitable probe.

113. List the steps involved in Northern blot analysis.

- Denature total cellular RNA with chemicals (formaldehyde).
- Note that RNA unfolds and is linear.
- Run an agarose gel (size separation).
- Transfer to nitrocellulose.
- Add radiolabeled DNA or RNA probes to detect specific messenger RNA (mRNA) levels or expression.

114. Give examples of clinical applications of Northern blot analysis.

- Comparison of expression of tumors versus normal tissue to identify potential oncogenes.
- Detection of mRNA.
- Prognostic information via expression profiling.
- Pathogenesis studies, specific mRNA levels in disease tissue.

115. Describe the principles of fluorescence in situ hybridization (FISH).

- Interphase nuclei of interest are typically placed on a microscope slide, either by touch prep or by blood drop. Tagged fluorescent probes bind specific DNA sequences on intact chromosomes prepared from interphase nuclei.
  - Fusion FISH: 2 probes come together to give specific color (translocations).
  - Break apart FISH: 2 probes are together in wild type/normal cells (color A); when translocation occurs they separate (color B).
- The labeled probes are then visualized as colored signals on the prepared slides using fluorescent microscopy.

116. Give examples of clinical applications of FISH.

- Detection of:
  - Trisomy 13, 18, and 21 in prenatal testing (FISH is used for chromosome counting).
  - DiGeorge syndrome.
  - Chromosome aneuploidy in cancers.
  - Deletions or amplifications in cancers.
  - Cancer specific translocations (UroVysion for urothelial carcinoma).
  - *ERBB2* (formerly *HER2/neu*) (breast cancer).
  - Gliomas (1p/19q).
  - Lymphomas and sarcomas (see specific translocations at end of this chapter).

117. Describe the principles of comparative genomic array–based microarrays (CGH-based microarrays).

- CGH-based microarrays compare DNA content from 2 differentially labeled genomes.
- The 2 genomes, a test (or patient), and a reference (or control) — each labeled with a different dye — are cohybridized onto a solid support (usually a glass microscope slide), on which cloned or synthesized DNA fragments have been immobilized.
- Arrays have been built with a variety of DNA substrates that may include oligonucleotides, cDNAs, or bacterial artificial chromosomes (BACs).

118. List the steps involved in CGH-based microarrays.

- Label genomic DNA from diseased tissue and control tissue with a different dye.
- Affix microarray DNA gene probes to slide (microarray).
- Hybridize genomic DNA on the slide.
- Compare levels of fluorescence with specific genes.

119. Give examples of clinical applications of CGH-based microarrays.

- Tumor molecular classification (e.g., reclassification of breast cancer based on microarray gene expression, lymphoma classification).
- Definition of origin of metastatic tumor.
- Prediction of disease behavior or response to therapy.
- Prognosis (i.e., defining different prognostic groups).
- Study of pathogenesis.

120. What is a major limitation of CGH-based microarrays?

CGH arrays cannot detect balanced translocations or inversions because these techniques are hybridization techniques. If the DNA content does not change, the signal remains the same and the translocation is therefore undetectable.

121. Describe the technical processes for electron microscopy.

- Fixation and processing.
  - Fix tissue in glutaraldehyde.
  - Apply osmium tetroxide postfix.
  - Dehydrate tissue in a graded series of ethanols.
  - Infiltrate tissue with EM epoxy resin.
- Embedding.
  - Embed tissue in a mold.
- Sectioning.
  - Heat resin-infiltrated tissue and harden into blocks.
  - Cut sections with a microtome (60–90 µm thick).
  - Collect sections onto specimen grids.
- Microscopy.
  - Image specimens under electron microscope.
  - Obtain digital images.

122. List examples of clinical applications of electron microscopy.

- Pediatric pathology — storage diseases, cilia (primary ciliary dyskinesia), small blue cell tumors, others.
- Renal pathology — basement membrane change, identifying deposits.
- Adult tumors — carcinomas, sarcomas, neuroendocrine tumors, melanoma.
- Infections — viral (severe acute respiratory syndrome), parasites, bacteria (Whipple disease), others.
- Neuropathology — tauopathies, cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopath (CADASIL: deposits in blood vessels), metabolic storage diseases.

123. Describe the principles of flow cytometry.

- Label cells with specific dyes.
- Note that dyes can be attached to antibodies that detect specific proteins (such as cell lineage markers) or they can bind directly to cellular components (such as DNA or lipids).
- Measure the fluorescence of the dyes after exposure to an argon laser — fluorescence occurs as the cells, in a liquid medium, "flow" past the laser.
- Note that cell size and number can also be determined.

124. Describe the value of flow cytometry in diagnosis.

- Cell marker/cell type analysis, as in detection of leukemia/lymphoma (CD antigens).
- Cell size analysis, as in defining lymphoma types.
- DNA ploidy analysis, as in molar pregnancy.
- Cell cycle analysis (G1, S, G2, M phases of cell cycle).

125. Describe the principles of DNA hybridization.

- The DNA strand complementary to a gene of interest is labeled (e.g., with a radioactive or fluorescent tag) to create a probe.
- The specimen DNA is denatured in situ, or isolated, and transferred to a membrane/slide.
- The specimen DNA is incubated with the labeled probe at a specific hybridization temperature (determined by the type of probe).
- Excess probe is washed off and an image is produced using an appropriate detection method.

126. List 3 applications of DNA hybridization in laboratory diagnostics.

- Southern blot DNA mutational analysis.
- Comparative genomic hybridization.
- FISH for detection of gene amplification, rearrangements, or translocations.

127. In addition to FISH and conventional cytogenetics, what is the best test to detect the translocation of *SYT* given a stable chimeric transcript?

Polymerase chain reaction (PCR).

128. Describe how to make a tissue microarray, and its clinical and research applications.

- Procedure:
  - Selected specimens are taken from a donor block of selected tissue with a punch.
  - An array block is made by transferring the core to a blank wax block. Immunohistochemistry stains using selected antibodies can be performed on these tissue arrays.
- Applications:
  - Comparing protein expression across multiple tissues for expression profiling, or to determine protein expression across multiple tumor types.

129. List 2 advantages of chromogenic in situ hybridization (CISH) over FISH.

- No loss of signal over time.
- No need for a fluorescent microscope.

130. List 2 strategies used to detect certain translocations.

- RT-PCR if there is a stable fusion product.
- DNA hybridization (in situ or Southern blot).

131. What component of the Epstein-Barr virus (EBV) is tested by CISH?

EBER-1/EBER-2 (EBV encoded snRNA).

IMMUNOHISTOCHEMISTRY AND SPECIAL STAINS

132. Describe the principles of the unlabeled antibody peroxidase-antiperoxidase (PAP) method of immunohistochemical testing.

- Primary antibody recognizes epitope of interest (e.g., rabbit IgG).
- Linking antibody recognizes primary Ab (anti-rabbit IgG).
- An antiperoxidase antibody from the same species as the primary antibody (e.g., rabbit antiperoxidase), complexed with peroxidase, is incubated to allow a colorimetric detection.
- Peroxidase polymerizes diaminobenzidine into a pigment that stains tissue.

133. What is the histochemical stain for acid mucopolysaccharides? Describe the biochemistry for how it stains.

Alcian blue: copper based dye that binds electrostatically to negatively charged (acidic) mucopolysaccharides to impart a blue color.

134. Describe the biochemical mechanism of silver stains (for argyrophilic and argentaffin cells) and their applications in histopathology.

- Silver stains tissues at sites of reduction of silver ions.
  - Argyrophilic cells: the reducing agent is light or an external substance. Example: Grimelius stain for neuroendocrine cells.
  - Argentaffin cells: the reducing substance is in the tissue that is stained. Example: Masson-Fontana stain for melanin.

135. Describe the biochemical mechanism of the periodic acid-Schiff (PAS) stain and its applications in histopathology.

- Principle: periodic acid and Schiff reagent interact with diols on carbohydrates (glycogen, glycoproteins, proteoglycans), resulting in a red stain.
- Application: identifying glycogen producing tumors, and staining of basement membranes, mucin, and fungal walls.

136. List 5 tissue antigen markers that can be demonstrated by immunoperoxidase staining of formalin fixed paraffin sections. Indicate the diagnostic value of each marker.

- CD45: hematopoietic lineage marker.
- S100: neurological and neural crest cell marker.
- Synaptophysin: neuroendocrine cell marker.
- Desmin: muscle marker.
- Inhibin: germ cell line marker.

137. List 5 histochemical methods (stains) and indicate the diagnostic value of each method.

- Gram stain: used to identify bacteria; gram positive bacteria stain purple/blue and gram negative bacteria stain red.
- Grocott stain: silver stain used to detect fungi.
- Ziehl–Neelsen: used to identify mycobacteria, such as *M. tuberculosis.*
- PAS/Alcian blue: detects acidic and neutral mucin.
- Congo red: detects amyloid protein.

138. What is S100 and what is its use in immunohistochemistry?

- S100: a calcium binding protein. Its name derives from solubility in 100% saturated ammonium sulfate.
- Immunohistochemistry: S100 is typically found on cells derived from neural crest cells.

139. What is HMB45?

Human melanoma black protein — a common marker for melanoma and other tumors with melanocytic differentiation. It stains immature melanosomes.

GENETIC SYNDROMES

140. What causes hereditary angioedema?

- Deficiency of the C1 protease inhibitor of the complement cascade.
  - C1 protease inhibitor deficiency causes accumulation of factor XII and bradykinins.
  - This leads to episodes of edema of the skin, larynx, and GI tract.

141. What genetic syndrome produces degenerative arthropathy and black urine?

- Alkaptonuria (ochronosis) — an autosomal recessive disorder caused by deficiency in homogentisic oxidase.
  - It is characterized by deposits of phenols (homogentisic acid) into cartilage and connective tissue, with bluish black pigmentation of joints.
  - It looks like solar elastosis in the skin.
  - Hardened connective tissue can induce a foreign body–type reaction.
  - It can cause excess pigment excretion into ducts (breast, prostate, sweat glands).

142. A person with renal cysts, angiomyolipoma, and cerebral hemangioblastoma has what syndrome?

- Von Hippel-Lindau disease — a rare, autosomal dominant genetic condition.
  - It results from a mutation in the von Hippel-Lindau tumor suppressor gene on chromosome 3p25.3.
  - It is associated with several pathologies, including hemangioblastomas in the cerebellum; spinal cord, kidney, and retina angioma; renal cell carcinoma (clear cell variety); angiomyolipomas and pheochromocytoma; pancreatic cysts, and also renal cysts (75%).

143. A person with renal cysts and berry aneurysm has what syndrome?

- Adult form autosomal dominant polycystic kidney disease or syndrome (ADPKD).
  - ADPKD is 1 of 2 forms of polycystic kidney disease (PKD), a genetic disorder of the kidneys.
  - The other form of PKD is autosomal recessive polycystic kidney disease (ARPKD), which is less common and occurs in childhood.

TYPE OF PKD	INHERITANCE	PATHOLOGIC FEATURES	CLINICAL FEATURES OR COMPLICATIONS	TYPICAL OUTCOME
Adult form polycystic kidney disease (ADPKD)	• Autosomal dominant. • Mutations at chromosome 16p13.3 (*PKD1*) and 4q21 (*PKD2*). • Encodes polycystin-1 and polycystin-2.	• Large multicystic kidneys. • Liver cysts. • Berry aneurysms.	• Hematuria. • Flank pain. • Urinary tract infection. • Renal stones. • Hypertension.	• Chronic renal failure beginning at age 40–60 years.
Childhood form polycystic kidney disease (ARPKD)	• Autosomal recessive. • Mutations of the *PKHD1* gene at chromosome 6p21–p23.	• Enlarged, cystic kidneys at birth.	• Hepatic fibrosis.	• Variable: death in infancy or childhood.

144. Define Gaucher disease.

Gaucher disease: a rare hereditary autosome recessive disorder that causes glucocerebroside to build up in the spleen, liver, lungs, bones, and sometimes the brain. The buildup prevents these organs from working properly. Glucocerebrosides also accumulate within phagocytic cells throughout the body.

145. Describe the genetic and clinical variants of Gaucher disease.

- Type 1:
  - Is the most common form.
  - Causes liver and spleen enlargement, bone pain, broken bones, and, sometimes, lung and kidney problems.
  - Has no brain involvement.
  - Can occur at any age.
- Type 2:
  - Causes severe brain damage.
  - Appears in infants — most children who have it die by age 2.
- Type 3:
  - Is intermediate between type 1 and type 2.
  - May involve liver and spleen enlargement.
  - Shows gradual signs of brain involvement.

146. Describe the morphological characteristics of Gaucher disease.

- Gaucher cells (distended phagocytic cells) can be identified in spleen, liver, bone marrow, and lymphoid tissue.
- The phagocytic cells exhibit abundant "tissue paper" cytoplasm.
- Gaucher cells show positive with PAS, and sometimes also with Perls' Prussian blue stain (iron).
- Electron microscopy findings: cytoplasm contains numerous elongated and tapering single membrane bound lysosomes.

147. Define Niemann-Pick disease.

Niemann-Pick disease: a hereditary autosome recessive biochemical disorder, caused by intracellular accumulation of sphingomyelin, resulting in progressive hepatosplenomegaly, lymphadenopathy, anemia, and mental and physical deterioration.

148. Describe the clinical variants of Niemann-Pick disease.

- At least 5 forms of Niemann-Pick disease have been distinguished:
  - Classical infantile form (type A).
  - Visceral (organ) form (type B).
  - Subacute or juvenile form (type C).
  - Nova Scotian variant (type D).
  - Adult form (type E).

149. List 3 diseases caused by sex chromosome imbalance.

- XO: Turner syndrome.
- XXY: Klinefelter syndrome.
- XYY: unclear if associated with a genetic syndrome.

150. List 3 diseases caused by autosomal chromosome imbalance.

- Trisomy 21: Down syndrome.
- Trisomy 13: Patau syndrome.
- Trisomy 18: Edward syndrome.

151. List 4 specific HLA subtypes and their associated diseases.

- HLA-DR3: diabetes (type 1), systemic lupus erythematosus, autoimmune hepatitis, Sjogren syndrome, rheumatoid arthritis.
- HLA-B27: ankylosing spondylitis.
- HLA-DQ2/8: celiac disease.
- HLA-DR15: multiple sclerosis.

152. Define Down syndrome.

- Down syndrome: a common chromosome disorder due to an extra chromosome number 21 (trisomy 21).
- It causes:
  - Mental retardation.
  - Characteristic facial features — slight flattening of the face, minimal squaring off of the top of the ear, a low bridge of the nose (lower than the usually flat nasal bridge of the normal newborn), an epicanthic fold (fold of skin over top of the inner corner of the eye, which can also be seen less frequently in normal babies), a ring of tiny harmless white spots around the iris, and a minor narrowing of the palate.
  - Congenital heart defects — endocardial cushion defect and ventricular septal defects.
  - Other malformations — duodenal atresia; a minor but still significant risk of acute leukemia.

153. Define phenyl ketonuria.

- Phenyl ketonuria: an autosomal recessive inheritable mutation in phenylalanine hydroxylase.
  - Patients cannot metabolize phenylalanine, which results in toxic accumulation causing permanent developmental delay, seizures, and retardation.
  - Restriction of phenylalanine in the diet is an effective treatment.

154. Describe the genetic abnormality and neoplasms associated with von Hippel-Lindau disease.

- Genetic abnormalities: mutation of the von Hippel-Lindau protein (pVHL), resulting in constitutive hypoxia signaling.
- Associated pathological conditions: renal cell carcinoma, pheochromocytoma, pancreatic neoplasms, retinal and cerebellar hemangiomas, hemangioblastomas, endolymphatic sac tumors, and papillary cystadenoma of the epididymis/broad ligament.

155. Describe the genetic abnormality and findings associated with neurofibromatosis type 1 (NF1).

- Genetic abnormalities: mutation in the *NF1* gene that encodes neurofibromin protein.
- Associated pathological conditions: neurofibromas (plexiform type), café au lait spots, Lisch nodules in the iris, malignant peripheral nerve sheath tumors, scoliosis, somatostatinoma, gangliocytic paraganglioma, gastrointestinal stromal tumor (GIST), pheochromocytoma, juvenile xanthogranuloma, and optic glioma.

TUMOR BIOLOGY AND GENETICS

156. List at least 4 neoplasms associated with the Epstein-Barr virus (EBV).

- Burkitt lymphoma.
- B cell lymphoma.
- Nasopharyngeal carcinoma.
- Posttransplant lymphoproliferative disorder.
- Lymphomatoid granulomatosis.
- Plasmablastic lymphoma.
- Peripheral T cell lymphoma.
- Classic Hodgkin lymphoma.
- NK T cell lymphoma.
- Angioimmunoblastic lymphoma.
- Primary effusion lymphoma (coinfection with HHV-8).
- Smooth muscle tumors in immunosuppressed patients.

157. List at least 4 methods for detecting EBV in neoplasms.

- Quantitative or qualitative PCR — viral load (number of viral particles) or absence/presence of virus.
- EBER — small nuclear RNAs, imitates latent stage of infection.
- Peripheral smear — atypical lymphocytes (lymphocytosis).
- Monospot test — positive.
- Immunohistochemical staining — latent membrane protein 1.

158. Describe the role of EBV in associated neoplasms.

- Infection with EBV leads to expression of *EBNA1*, *EBNA2*, and *LMP-1* (oncogene).
- *EBNA2* increases transcription of cell cycle proteins (cyclin D).
- *LMP1* activates *NFKB* and *JAK/STAT* pathways.
- *NFKB* enters nucleus leading to transcription of cell cycle proteins (proliferation).
- *LMP1* prevents apoptosis by increased expression of *BCL-2* (immortalization).
- Translocation (8,14) leads to c-Myc translocation in Burkitt lymphoma.

159. What nonneoplastic disorders are associated with EBV?

- Mononucleosis — sore throat, fever, lymphadenitis.
- Fever of unknown origin.
- Generalized rash.
- Splenomegaly.
- EBV hepatitis.
- Meningitis.
- Pneumonia.
- Encephalitis.
- Oral hairy leukoplakia.

160. Define oncogene.

Oncogene: a gene found in chromosomes of cancer cells which, when expressed, may directly or indirectly contribute to tumor cell growth, immortalization, dedifferentiation, and metastatic potential.

161. What is the function of oncogenes in normal cells?

- The normal gene counterpart in a cell is called a proto-oncogene.
- When mutated or abnormally expressed, the proto-oncogene contributes to cancer growth and is called an oncogene.

162. What are some of the roles of oncogenes in carcinogenesis?

- Can be antiapoptotic.
- Can be defective DNA repair proteins that cause accumulation of mutated or damaged DNA.
- Can result in increased cell motility and metastasis.
- Can cause growth factor receptors to become constitutively active without need for growth factors.
- Can cause continuous cell cycling or prevent cell cycle arrest.
- Can cause dedifferentiation.
- Can cause abnormal mitosis, resulting in chromosomal imbalance.
- Can induce telomerase expression and overcome cellular senescence.

163. What methods are used to identify oncogenes?

- Microarrays — test that compares expression of proteins in cancer versus control tissue.
- Clonality assays — PCR based assay to detect gene rearrangements.
- RNA interference — gene knockdown (partial) or knockout (complete) to study gene function.
- Transfection assays — introduction of genes (under control of an inducible promoter) into cells in culture to study the effects of overexpression.
- Laser microdissection — cutting out an area of interest on a slide with a laser in order to use other assays (i.e., PCR) to study which genes are expressed.
- Cytogenetics — study of chromosome translocations in cancer cells and identification of genes involved.
- Mutational analysis/chemical carcinogen analysis — introduction of a mutagen into cultured cells and isolation of mutated genes.
- Cell motility assays — study of cell motility through a matrix or gel to study role of genes in metastasis.
- Epigenetics — study of DNA or histone modifications such as methylation or acetylation in differential gene expression.
- Cell cycle analysis — study of the role of the oncogene in cell cycle distribution via FACS analysis.
- Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assays — used to detect apoptotic cells by detecting caspase induced DNA cleavage products (200bp).

164. List 5 common oncogenic viruses.

- EBV — Burkitt lymphoma, Hodgkin lymphoma, nasopharyngeal carcinoma.
- HHV-8 — Kaposi sarcoma.
- HPV (16, 18, 6, 11) — squamous cell carcinoma and adenocarcinoma of the cervix.
- Human T cell leukemia virus (HTLV) — T cell leukemia/lymphoma.
- Hepatitis B and C virus — hepatocellular carcinoma.

165. List at least 5 neoplasms encountered in patients with AIDS.

- Kaposi sarcoma (HHV-8).
- CNS lymphoma (HIV associated lymphomas, EBV driven).
- Systemic lymphomas (diffuse large B cell lymphoma and Burkitt).
- Plasmablastic lymphoma.
- Primary effusion lymphoma, body cavity.
- Squamous cell carcinoma of anus (males).
- Cervical cancer (females).
- Hepatocellular carcinoma (hepatitis B and hepatitis C positive).
- Lung cancer.
- Melanoma.
- Hodgkin lymphoma.
- Carcinomas associated with the head and neck.

166. Describe the relationship between malignancy and thrombosis.

- Malignancies can cause a hypercoagulable state. The most commonly accepted theory for this is that CD142 (factor III or tissue factor) expressed on tumor cells induces thrombin formation.
- Pancreatic and bronchogenic carcinoma can cause the Trousseau phenomenon with secretion of mediators that promote thrombosis (also called migratory thrombophlebitis).

167. List 3 examples of chromosome changes in cancer.

- Translocation — *BCR-ABL* in CML.
- Deletion — 3p in lung cancer, 1p/19q in oligodendroglioma.
- Amplification — 3q in lung cancer, *ERBB2* in breast cancer, N-myc in neuroblastoma.

168. What are oncofetal antigens and what is their clinical significance?

- Oncofetal antigens refer to tissue antigens that are expressed during early development but are lost in adulthood.
- Clinical significance: commonly reexpressed in malignancies (e.g., α-fetoprotein in hepatocellular carcinoma or carcinoembryonic antigen in colon adenocarcinoma).

169. Indicate the genetic abnormality associated with each sarcoma listed below.

- Alveolar soft part sarcoma: t(X;17)(p11;q25).
- Synovial sarcoma: t(X;18)(p11;q11).
- Myxoid liposarcoma: t(12;16)(q13;p11).

170. List 4 familial cancer syndromes and name 1 associated tumor for each.

- Peutz-Jeghers syndrome: colon adenocarcinoma.
- MEN1: gastrointestinal carcinoid.
- Familial atypical melanoma syndrome: melanoma.
- Fanconi anemia: acute myeloid leukemia.

171. Indicate the genes that are altered in each of the cancers listed below.

- Mantle cell lymphoma: IgH-cyclin D1.
- Follicular lymphoma: IgH/*BCL-2*.
- Chronic myeloid leukemia: *BCR-ABL* fusion gene.
- Gastrointestinal stromal tumor: *c-KIT* gene (CD117), *PDGFRA*.

172. List 3 syndromes due to abnormality of DNA repair and give an example of an associated neoplasm.

- Fanconi anemia: colorectal cancer.
- Ataxia telangiectasias: lymphomas and leukemias.
- Xeroderma pigmentosum: melanoma and squamous cell carcinomas.

173. List 3 tumors treated with tyrosine kinase receptor inhibitors.

- Gastrointestinal stromal tumor — treated with imatinib mesylate.
- Chronic myeloid leukemia — treated with imatinib.
- *EGFR* positive nonsmall-cell lung cancer — treated with gefitinib and erlotinib.
- *ERBB2* positive breast carcinoma — treated with herceptin.

174. List at least 2 genetic mutations that predict poor response to *EGFR* inhibitor therapy.

- *KRAS* mutation.
- *BRAF* mutation.
- *ALK* translocation.

175. List at least 3 tumors associated with *BRAF* V600E mutations.

- Melanoma.
- Papillary thyroid cancer (all variants).
- Anaplastic thyroid cancer.
- Ameloblastoma.
- Hairy cell leukemia.
- Subset of GIST.

176. List at least five reportable lung biomarkers.

- *EGFR*.
- *ALK*.
- *KRAS*.
- *RET*.
- *ROS1*.
- *MET*.
- *ERBB2*.
- *BRAF*.

177. List the components of the *RAS* kinase pathway in order.

Receptor tyrosine kinase > *RAS* > *RAF* > *MEK* > transcription factor (e.g., *FOS/JUN*).

178. What is the major feature that separates standard DNA sequencing from next generation sequencing (NGS)?

Traditional DNA sequencing (i.e., Sanger sequencing) sequences only 1 specific DNA target at a time. NGS is massively parallel sequencing that sequences multiple DNA targets/locations simultaneously.

179. What are some of the clinical utilities of NGS sequencing?

- Tumor mutational profiling in a single patient specimen (i.e., simultaneous detection of K-ras mutations and *EGFR* insertions, deletions, and point mutations in lung cancer; mutational profiling of acute myelogenous leukemia).
- Identification of diagnostic mutations in difficult-to-diagnose disease states (i.e., mutational analysis of MDS and MPN).
- Whole genome sequencing for the identification of constitutional mutations in pediatric populations (i.e., inherited diseases).
- Identification of mutations in multiple genes (e.g., *BRAF*, K-ras) simultaneously in multiple patient specimens in 1 run. This costs less than individual testing of each gene in each separate patient specimen. (One NGS test is less expensive than three separate PCRs).

Checklists

GENETIC ALTERATIONS CHECKLIST

HEMATOPOIETIC ALTERATIONS

Acute lymphoblastic leukemia/lymphoma (B cell)

Good:
- Hyperdiploidy.
- t(12;21).

Bad:
- t(9;22).
- Translocations involving 11q23 (*MLL* gene).

Acute myelogenous leukemia

Good:
- t(8;21).
- inv(16).

Intermediate (APML):
- t(15;17) 15-*PML* (promyelocytic leukemia).
- 17-*RAR*-alpha.

Bad:
- t(11q23;v).

Anaplastic large cell lymphoma

t(2;5) 2-*ALK*, 5-*NPM*.

Burkitt lymphoma

t(8;14) 8-*c-MYC*, 14-IgH.

t(2;8) 2-kappa, 8-*c-MYC*.

t(8;22) 8-*c-MYC*, 22-lambda.

Chronic myelogenous leukemia

t(9;22) 9-*ABL*, 22-*BCR* (fusion protein with tyrosine kinase activity).

Chronic neutrophilic leukemia

Colony stimulating factor 3R (*CSF3R*).

Diffuse large B cell lymphoma

30% have *BCL-6* (chromosome 3) translocation.

10–20% have t(14;18) involving *BCL-2*.

Essential thrombocytosis

JAK2 V617F mutations.

CALR mutations.

Follicular lymphoma

t(14;18) 14-IgH, 18-*BCL-2*.

Mantle cell lymphoma

t(11;14) 11-cyclin D1, 14-IgH.

Marginal zone lymphoma

t(11;18).

Mastocytosis

c-KIT mutations (D816V common).

Polycythemia rubra vera

JAK2 V617F mutation.

JAK2 exon 12 insertions and deletions.

Primary myelofibrosis

JAK2 V617F mutations.

CALR mutations.

GENETIC SYNDROMES

Angelman syndrome

15q11–13 deletion in the **maternally** derived chromosome.

Note the relation to Prader-Willi.

Autosomal dominant (adult) polycystic kidney disease

Polycystin-1 (16p), polycystin-2 (4q).

Cystic fibrosis

Cystic fibrosis transmembrane conductance receptor (7q31.2); chloride channel.

Most common mutation: deltaF508.

Ehlers-Danlos syndrome

Defects in collagen synthesis.

Fragile X syndrome

Xq27.3 (FMR-1 protein) — CGG repeats.

Gaucher disease

Glucocerebrosidase deficiency.

Hemochromatosis

HFE (chromosome 6) C282Y mutation.

Huntington disease

4p16.3 (Huntington protein) — CAG repeats.

Hyper-IgM syndrome

CD40L (on T cells, Xq26) or CD40 (on B cells) mutation; can't class switch.

Marfan syndrome

Fibrillin-1: glycoprotein scaffolding for elastin deposition (15q21.1) — dominant negative mutation.

Mucopolysaccharidoses (e.g., Hurler, Hunter)

Deficiency of enzymes that degrade mucopolysaccharides.

Niemann-Pick disease

Sphingomyelinase deficiency.

Prader-Willi syndrome

15q11–13 deletion in the **paternally** derived chromosome.

Tay-Sachs disease

Alpha-subunit of hexosaminidase enzyme complex mutation.
- Accumulation of GM2-gangliosides.

X-linked agammaglobulinemia of Bruton

BTK (B cell tyrosine kinase) mutation.

TUMORS

Alveolar soft part sarcoma

t(X;17) *TFE3-ASPL*.

Breast carcinoma

BRCA1 (17q21).

BRCA2 (13q12.3).

Low grade ductal and lobular carcinoma: 16q LOH.

ER/PR — positive if > 1% of cells have nuclear staining.

ERBB2 (criteria may vary) — see table below.

POSITIVE	NEGATIVE
IHC3+: uniform, intense membranous staining > 10% tumor cells. FISH ≥ 6 *ERBB2* gene copies/nucleus. FISH ratio (*ERBB2* gene signals: chromosome 17 signals) ≥ 2.0.	IHC 0 or 1+. FISH < 4 gene copies/nucleus. **– and –** FISH ratio < 2.0.

> Note: the lab should have > 95% concordance with another validated test for positive and negative values.

Clear cell sarcoma (malignant melanoma of soft parts)

t(12;22) *EWS-ATF1*.

Colorectal carcinogenesis

Mucosa at risk: *APC* **(5q21)**, loss of DNA methyl groups, beta-catenin, *MSH2*.

Adenomas: ***KRAS* (12p12)**, *SMADs*, **p53 (17p13)**, LOH at 18q21.

Invasive carcinoma: **telomerase**.

> Note: the bold terms highlight the most important changes in the adenoma/carcinoma progression pathway.

Congenital fibrosarcoma

t(12;15) *ETV6-NTRK3*.

Desmoplastic small round cell tumor

t(11;22) *EWS-WT1*.

DFSP

t(17;22) *COLA1-PDGFB*.

Endometrial adenocarcinoma

Type 1: MSI, *PTEN*, K-ras, beta-catenin.

Type 2: p53 overexpression.

Endometrial stromal sarcoma

t(7;17) *JAZF1-JJAZ1*.

t(10;17) *YWHAE-FAM22*.

Ewing sarcoma/primitive neuroectodermal tumor

t(11;22)(q24;q12) 11-*FLI1*, 22-*EWS*.

t(21;22) 21-*ERG*, 22-*EWS*.

t(7;22) 7-*ETV1*, 22-*EWS*.

Extraskeletal myxoid chondrosarcoma

t(9;22) *CHN-EWS*.

Gastrointestinal stromal tumor

Most: *c-KIT* mutation.

Some: platelet derived growth factor receptor-alpha mutation.

Germ cell tumors

i(12p).

Liposarcoma (myxoid/round cell type)

t(12;16) *CHOP/TLS*.

Melanoma (dysplastic nevus syndrome/heritable melanoma syndrome)

p16INK4A/CDNK2 (9p21).

BRAF V600E,K,R,D.

NRAS Q61, G12, G13.

KIT mutations.

Neuroblastoma

Good (< 12 months):
- Schwannian stroma.
- TrkA expression.

Bad (> 5 years):
- Diploidy, near-diploidy, near-tetraploidy.
- N-myc amplification.
- 17q gain, 1p loss.
- Telomerase overexpression.

Oligodendroglioma

Loss of 1p/19q.

IDH1/2 mutations.

Pancreatic carcinoma

Very common: *KRAS* (12p), *p16/CDKN2A* (9p).

Common: *TP53* (17p), *SMAD4* (18q).

Parathyroid adenoma

PRAD1 (cyclin D1) overexpression.

MEN1 inactivation.

Renal cell carcinoma (papillary type)

MET mutation (familial), PRCC (sporadic).

Rhabdomyosarcoma (alveolar type)

t(2;13) *PAX3-FKHR*.

t(1;13) *PAX7-FKHR*.

Synovial sarcoma

t(X;18) *SYT/SSX*.

Thyroid carcinoma

Follicular:
- RAS family of oncogenes mutation.
- t(2;3) fusion of *PAX*-8 and *PPAR* gamma1.

Papillary:
- *RET* tyrosine kinase receptor (10q) rearrangement.
- *BRAF* V600E mutation.

Medullary:
- *RET* protooncogene mutation (MEN2).

Anaplastic:
- p53 mutation.
- *BRAF* V600E mutation.

UNCLASSIFIED ALTERATIONS

Alkaptonuria (ochronosis)

Lack homogentisic oxidase.

- Accumulation of homogentisic acid.
- Black urine, black pigmentation of ears/nose/cheeks, arthropathy.

Celiac

HLA B8, DQ2.

DM type 1

HLA DR3 or HLA DR4.

Familial hypercholesterolemia

Low density lipoprotein receptor mutation.

Hirschsprung disease

RET mutation.

Rheumatoid arthritis

HLA DR4 or HLA DR1.

Seronegative spondyloarthropathies

HLA B27.

SYNDROMES CHECKLIST

Ataxia-telangiectasis (AT: DNA repair after radiation injury — 11q22.3)

Cerebellar dysfunction.

Recurrent infection (no thymus).

Lymphoid malignancy.

Telangiectasis (especially conjunctival).

Hypoplastic gonads.

Autoimmune polyendocrinopathy syndrome type 1 (APS1: autoimmune regulator — 21q22)

Mucocutaneous candidiasis.

Ectodermal dystrophy.

Pernicious anemia.

Hypoparathyroid.

Primary adrenal insufficiency.

Idiopathic hypogonadism.

Autoimmune polyendocrinopathy syndrome type 2 (Schmidt) (not monogenic)

Adrenal insufficiency.

Autoimmune thyroiditis.

Diabetes mellitus, type 1.

Beckwith-Wiedemann syndrome (Wilms tumor 2 locus — 11p)

Organomegaly.

Hemihypertrophy.

Adrenal cytomegaly.

Renal medullary cysts.

Wilms and other primitive tumors.

BRCA1 (DNA repair, 17q21)

Breast carcinoma (medullary, poorly differentiated), ER/PR–, ERBB2–.

Ovarian carcinoma.

Also prostate, colon, and pancreas carcinomas.

BRCA2 (DNA repair, 13q12.3)

Breast carcinoma, including male breast (but histology similar to sporadics).

Ovarian carcinoma.

Also prostate, pancreas, stomach, melanoma, and colon carcinomas.

Carney syndrome

Spotty pigmentation.

Psammomatous melanotic schwannoma.

Endocrine overactivity (primary pigmented nodular adrenal cortical hyperplasia, Cushing disease, GH pituitary adenoma).

Cardiac myxoma, extracardiac myxoma.

Myxoid breast fibroadenomas.

Large cell calcifying Sertoli cell testicular tumor.

Carney triad

Pulmonary hamartoma.

GIST.

Extra-adrenal paraganglioma.

Chromosome 22q11.2 deletion syndrome (DiGeorge)

Developmental delay.
Palate abnormalities.
Facial dysmorphism.
Variable T cell deficiency.
Hypoparathyroidism.
Congenital heart defects.

Cowden syndrome (*PTEN* — 10q23)

Multiple trichilemmomas.
Acral keratoses.
Oral mucosal papillomas.
Breast carcinoma.
Colorectal polyps.

Denys-Drash syndrome (Wilms tumor 1 — 11p)

Gonadal dysgenesis.
Nephropathy resulting in renal failure.
Wilms tumor.

Down syndrome (trisomy 21)

Mental retardation.
Epicanthic folds, flat facial profile.
Abundant neck skin.
Simian crease.
Congenital heart defects (septal defects).
Intestinal stenosis.
Umbilical hernia.
Hirschsprung.
Hypotonia.
Gap between first and second toes.
Predisposition to leukemia.
Infections due to abnormal immune responses.
Premature Alzheimer disease.

Edwards syndrome (trisomy 18)

Prominent occiput.
Mental retardation.
Micrognathia.
Low set ears, short neck.
Overlapping fingers.
Congenital heart defects.
Renal malformations (horseshoe).
Limited hip abduction.
Rocker bottom feet.

Familial adenomatous polyposis (*APC* — 5q21)

Stomach and small bowel polyps (fundic gland polyps and lymphoid hyperplasia).
Colorectal carcinoma.
Gallbladder, pancreas carcinoma.
Thyroid, adrenal carcinoma.

Gardner syndrome

Familial adenomatous polyposis with additional findings such as:
Abnormal dentition (impacted teeth).
Epidermal cysts.
Fibromatosis (desmoid tumors).
Osteomas (mandible, skull, long bone).
Duodenal and thyroid carcinomas.

Hereditary hemorrhagic telangiectasia (Osler-Weber-Rendu syndrome)

Multiple small aneurysmal telangiectasis on skin and mucosal membranes.
- Epistaxis, hemoptysis, GI/GU bleeding.

Hereditary nonpolyposis colon cancer (mismatch repair: *MSH2*, *MLH1*, *MSH6*)

Colorectal carcinoma.
Gastric carcinoma.
Duodenal/jejunal carcinoma.
Endometrial carcinoma.
Ovarian carcinoma.
TCC.

IgG4 associated diseases

Idiopathic fibrosing inflammatory conditions (not really a syndrome).
Inflammatory pseudotumor of the orbit.
Riedel thyroiditis.
Chronic sclerosing sialadenitis.
Sclerosing cholangitis.
Autoimmune pancreatitis.
Fibrosing mediastinitis.
Retroperitoneal fibrosis.

Juvenile polyposis syndrome (*SMAD4*, *BMPRIA*)

Juvenile polyps.
Carcinomas of stomach, small intestine, colon, pancreas.

Klinefelter syndrome (47,XXY in 80%, remainder mostly mosaics — e.g., 46,XY/47,XXY)

Eunuchoid body habitus.
Failure of male secondary sexual characteristics, atrophic testes.
Gynecomastia, increased risk of breast carcinoma and autoimmune diseases.
Male infertility.
High FSH and estrogen levels; low testosterone levels.

Li-Fraumeni syndrome (p53, *CHEK2*)

Soft tissue sarcoma.
Osteosarcoma.
Breast carcinoma.
Carcinoma of colon, pancreas, adrenal cortex.
Leukemia.
Melanoma.
Glioma.

Maffucci syndrome

Somatic mutations of *IDH1/2*.
Multiple hemangiomas.
Enchondroma.
Chondrosarcoma (20% of cases).

McCune-Albright syndrome

Irregular skin pigmentation.
Polyostotic fibrous dysplasia.
Precocious sexual development.
Mosaic loss of *GNAS1* (nonmosaic loss is embryonic lethal).

MEN1 (Wermer) (Menin — 11q11–13)

Pituitary adenomas.
Pancreatic endocrine tumors (especially pancreatic polypeptide secreting).
Parathyroid hyperplasia/adenoma.
Also duodenal gastrinomas, carcinoids, thyroid/adrenocortical adenomas.

MEN2A (Sipple) (*RET* activating mutation — 10)

Medullary thyroid carcinoma.
Parathyroid hyperplasia.
Pheochromocytoma.

MEN2B (*RET* mutation different from MEN2A)

Includes all of MEN2A plus:

- Neuromas/gangliomas in GIT, etc.
- Marfanoid habitus.
- **No** parathyroid hyperplasia.

Muir-Torre syndrome (*MLH1* or *MSH2*)

Benign/malignant sebaceous tumors.
Internal adenocarcinoma.

Muscular dystrophy (Xp21 — dystrophin)

Duchenne.
Becker.

Neurofibromatosis 1 (von Recklinghausen syndrome) (neurofibromin: downregulates p21 — 17)

CNS tumors (schwannomas, meningiomas, optic gliomas).
Reduced intelligence.
Lisch nodules.
Neurofibromas, MPNST.
Café au lait spots.
Skeletal abnormalities (bone cysts, scoliosis, erosions).
Gynecomastia.
Pheochromocytoma.
Gastrointestinal stromal tumors.
Small intestinal adenocarcinomas.

Neurofibromatosis 2 (merlin: tumor suppressor — 22)

Bilateral acoustic schwannomas.
Other CNS tumors: meningioma, ependymoma (spinal).
Schwannosis (Schwann cells grow into spinal cord).
Meningioangiomatosis (meningeal cells and blood vessels grow into brain).
Café au lait spots.
No Lisch nodules.

Nevoid basal cell carcinoma syndrome (*PTCH*: development — 9q22.3) (Gorlin)

Multiple BCCs < 20 years old.
Pits of palms and soles.
Medulloblastoma.
Developmental abnormalities.
Odontogenic keratocysts.
Ovarian fibroma.

Patau syndrome (trisomy 13)

Microcephaly, mental retardation.
Microphthalmia.
Cleft lip and palate.
Polydactyly.
Cardiac defects.
Umbilical hernia.
Renal defects.
Rocker bottom feet.
PEComa family of tumors
Renal angiomyolipoma.
Lymphangiomyomatosis of lung and lymph nodes.
Clear cell/sugar tumor of lung.

Peutz-Jeghers syndrome (*STK11*)

Nasal polyps.
Melanotic pigmentation of skin and mucosa.
Small and large intestine hamartomatous polyps.
Adenoma malignum of cervix.
Sertoli cell proliferation.
Carcinomas in pancreas, breast, lung, ovary (sex cord tumor with annular tubules, mucinous tumors), uterus.

POEMS syndrome

Polyneuropathy.
Organomegaly.
Endocrinopathy.
Monoclonal gammopathy.
Skin changes.

Retinoblastoma (*RB* — 13q14)

Retinoblastoma.
Pinealoblastoma.
Osteosarcoma.

Sturge-Weber syndrome (etiology unclear)

Leptomeningeal angiomatous masses.
Mental retardation.
Seizures.
Hemiplegia.
Skull radiopacities.
Glaucoma.
Facial port wine nevi.
Pheochromocytoma.

Tuberous sclerosis (*TSC1* hamartin — 9q34, *TSC2* tuberin — 16p13.3)

Seizures.
Mental retardation.
Cortical tubers.
Subependymal giant astrocytoma (hamartomatous).
Shagreen patches, "ash leaf" patches.
Subungual fibroma.
Cardiac rhabdomyoma (hamartomatous).
GI inflammatory polyps.
Hepatic/renal/pancreatic cysts.
Renal angiomyolipomas.

Turcot syndrome

2/3 FAP, medulloblastoma.
1/3 HNPCC, glioblastoma.

Turner syndrome (45,X)

Short stature.
Lymphedema of neck, hands, feet.
Webbing of neck.
Congenital heart disease (aortic coarctation).
Broad chest, widely spaced nipples.
Failure of breast development.
Infantile external genitalia.
Ovaries atrophic and fibrous (streak ovaries).
Primary amenorrhea.

Von Hippel-Lindau (*VHL* — 3p)

Cavernous hemangiomas in cerebellum, brainstem, eye.
Cerebellar/retinal hemangioblastomas.
Angiomatous/cystic neoplasms in pancreas and liver.
Pheochromocytoma, paragangliomas.
Renal cell carcinoma, clear cell type.

WAGR syndrome (Wilms tumor 1 and *PAX6* — 11p13)

Wilms tumor.
Aniridia.
Genital anomalies.
Mental **r**etardation.

Xeroderma pigmentosum

Melanoma.
Nonmelanoma skin carcinomas.

ELECTRON MICROSCOPY CHECKLIST

Active protein synthesis (1161)

Abundant granular ER (ribosomes).

Adenocarcinomas

Lumens, intracellular lumens.
Microvilli.
Mucigen granules.
Glycogen.
Prominent Golgi.
Intermediate filaments (juxta nuclear).
Interdigitating cell membranes, cell junctions, rare cilia, basal lamina.

Alveolar soft part sarcoma

Rhomboid crystals.

Amyloid

Nonbranching fibrils, indefinite length, diameter approximately 7.5–10 nm.

Carcinoid tumor

Dense core bodies, neurosecretory granules.

Clear cell carcinomas (e.g., kidney, vagina)

Abundant glycogen and/or lipid (both in RCC).
Lumen, microvilli, junctional complex.

Ewing sarcoma

Prominent pools of cytoplasmic glycogen.

Glomerulonephritides

TYPE	CHARACTERISTICS
Poststreptococcal	Subepithelial humps.
Goodpasture syndrome	GBM disruptions, fibrin, no deposits.
RPGN	GBM wrinkling, disruptions.No deposits (unless immune complex type).
Membranous	Subepithelial deposits.
Minimal change	Loss of foot processes, no deposits.
FSGS	Loss of foot processes, epithelial denudation.
MPGN type I	Subendothelial deposits.
MPGN type 2	Dense deposits.
IgA nephropathy	Mesangial and paramesangial dense deposits.
Alport	Split of GBM, thin GBM, "bread crumb" degeneration.
Diabetic glomerulosclerosis	Massive increase in mesangial matrix, thickened GBM (greater than 300–350 nm) and Bowman capsule.
Crescentic GN	+/– deposits, crescents, disrupted GBM.
Lupus	Mesangial deposits (type I, II). Mesangial and subendothelial deposits (type III, IV).
Chromophobe RCC	Microvesicles.

Glucagonoma (1191)

Granules with closely apportioned membranes, dense round center.

Granular cell tumor

Lysosomes.

IHC of basal/myoepithelial cells

Prostate basal cells: K903+, p63+, CK5/6+, S100–, SMA–.

Breast myoepithelial cells: SMA+, p63+, S100+.

Salivary gland myoepithelial cells: SMA+, S100+.

Insulinoma (1191)

Membrane bound granules.

Dense, paracrystalline, often rectangular core.

Distinct halo separates core from membrane.

Irreversible cell injury

Mitochondria markedly swollen, contain amorphous densities.

Cell membranes disrupted.

Dense pyknotic nucleus.

Kaposi sarcoma

Vascular channels.

Langerhans cell histiocytosis

Birbeck granules with characteristic periodicity and dilated terminal end.

Leiomyosarcoma

Myofilaments.

Smooth muscle derivation.

Medullary thyroid carcinoma (1182)

Membrane bound, electron dense granules.

Melanoma

Premelanosomes, melanosomes.

Neuroendocrine tumors (APUDomas) (e.g., carcinoid, islet cell tumors, medullary thyroid carcinoma, pituitary adenomas)

Neurosecretory type granules.

Microfilaments, cell junctions, basal lamina.

Oncocytic neoplasms (e.g., Hürthle cell tumor, oncocytoma, Warthin tumor)

Lots of mitochondria with stacked lamelliform cristae that lack matrix granules.

Intercellular junctions, lumens (microvilli).

Pheochromocytoma (1220)

Membrane bound secretory granules, distinct halo.

Pulmonary adenocarcinoma versus mesothelioma

Adenocarcinoma: short, plump microvilli.

Mesothelioma: numerous, long, slender microvilli in gaps between cells (3 long:1 wide).

Reversible cell injury

Microvilli lost.

Blebs, extrude into lumen.

Mitochondria slightly dilated.

Rhabdomyosarcoma/rhabdomyoma

Actin (6 nm) filaments, myosin (15 nm) fibrils in parallel arrays.

Sarcomeres, glycogen, primitive cell junctions, external lamina.

Schwannoma

Complexly entangled long cell processes.

Intermediate filaments, microtubules, long spacing collagen.

Smooth muscle tumors

Actin microfilaments, interspersed fusiform dense bodies.

Plasmalemmal attachment plaques, pinocytic vesicles.

Squamous cell carcinoma/squamous metaplasia

Cytokeratin filaments (tonofilaments), filopodia (fingerlike cell processes).

Well-developed desmosomes, primitive cell junctions.

Steroid producing tumors

Prominent smooth endoplasmic reticulum.

Mitochondria with tubulovesicular cristae.

Vascular tumors (EC origin)

Weibel-Palade bodies.

Bundles of intermediate filaments, tight and primitive cell junctions.

Pinocytotic vesicles.

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