Definition

- Pathological Anatomy is a Discipline which can be considered the binding bridge between Basic Sciences and Clinical medicine belonging to Laboratory medicine;
- It represents the fundamental step in the clinical diagnostic approach.

Programme - I

- Main histopathological activites: intraoperative histological diagnosis, surgical pathology, cytopathology, autopsy
- Necropsy assessment.
- Cardiac and cerebral death.
- General principles of biological material preparation: fixation, dehydration, embedding, sectioning and staining.

Programme - II

- Special methods and techniques. General aspects of histochemical staining . Immunocytochemistry, Flow Citometry. Electron Microscopy. Molecular Biology
- Tissue bank

Programme - III

- Tumor: definition, general features, classification.
- Benign and malignant tumors.
- Anaplasia; local invasion (expansive tumor growth and infiltrative); metastases.
- Grading and staging.
- Tumor epidemiology.

Programma – IV

- Lab Diagnostics of tumors.
  - Methods:
    - Hematochemistry
    - Citology
    - Histology
    - Ultrastructure
    - Immunohistochemistry
    - Flow cytometry
    - Molecular

- Recently many new methods and technologies are employed in diagnostics which are also became fundamental in prognosis definition for each patient
- Morphological data must be more and more integrated with clinical and functional data in a multidisciplinary approach to the patient

Histopathological activities

- Surgical Pathology
- Estemporanea
- Cytology (screener)
- Autopsy
- Diagnostic subspecialties
- Necroscopy
- Transplant and Tissue Bank

Surgical Pathology

- **Surgical pathology.** Surgical sample from operating room (very often they are constituted by small biopsies: liver, renal, etc).

Personal detail registration
Gross examination and dissection
Sampling
Slides

The mainstay of surgical pathology is the examination of the specimes following fixation in formalin, embedding in paraffin and staining with ematoxylin-eosin (H&E)

Activity (2)
Intraoperative histological diagnosis

- **Intraoperative histological diagnosis.** Pathologist is able to study the samples while the patient is still on the surgical table in order to indicate the surgeon about pathology features. Benign or malignant neoplastic lesion, extension. This operating way helps surgeon to avoid unnecessary tissue resection

Activity (3)
Autopsy

- **Autopsy.** This activity had mainly in the past a very important role in the anatomo-clinical correlation. However, it remains the last due of the pathologist to reach a diagnosis in very difficult and complexes cases and maintains an important role in the student learning

Activity (3)
Autopsy requirement

- Dead bodies without medical assistance
- Dead patients in public or private hospitals if diagnostic confirmation is needed and/or for scientific purposes)

Activity (3)

Forensic autopsy

- **Autopsy.** Forensic autopsy is usually done by the coroner when data must be collected as legal evidence in judicial processes

Activity (4)

Pathological Anatomy - Subspecialties

- **Subspecialties.** There are many branches of pathological Anatomy such as hematopathology, dermatopathology, Neuropathology due to the big progress of knowledges needing different and innovative methods and techniques to perform the final diagnosis.

Activity (5)

- Cytopathology
  - The cytopathology studies nuclear and cytoplasmic alterations in single cells which must be related to pathological processes of the tissues from which cell are derived .
  - It is a powerful technique: easy, cheap and quick technique mainly used in tumor screening, particularly in uterine cervix tumor (PAP Test)

Activity (5) - Cytopathology

- Sampling methods
  - Natural exfoliation. Cells from saliva and cervico-vaginal secretion
  - Brushing. Cells from bronchial, nasal, oesophageal, gastrointestinal, cervical brushing or brocho-alveolar lavage (BAL) . This samples are richer in cells.

Activity (5) - Cytopathology

- Cytocentrifugation o gradient centrifugation. Cells from physiological liquind are examined such as cephalorachid fluid , urine, pleural, pericardial or peritoneal exudate.

Attività (5) - Cytopathology

- FNAB / fine needle biopsy (20-27 gauge). Cells from supericial lesions are fine needle biopsied (breast, salivary glands, thyroid, lymph node) or, sound guided, from deep lesions (lung, kidney, liver, pancreas, etc.). With this technique it is possible to obtain small samples of tissue in order to evaluate both cytology and histoarchitecture of the lesion.

Methods.

a) Papanicolaou Test. Samples are smeared on the slide and fast fixed (within 10 min) in ethanol 95% or other commercial fixation solution.

Methods.

- Nuclei are stained in Blue with hematoxylin and counterstained with Orange G (cytoplasm orange if it is pleinful of cytocheratin) and with a eosin and light green mixture (cells with an acidophilic cytoplasm are stained with eosin , cell with basophilic cytoplasm are staind in light blue).

Main cytological features.

- Adequacy of the sample (cells in the samples are detectable)
- Cell population (cell number, cell debris, necrotic material, inflammatory cells)
- Nuclear and cytoplasmic characteristics

Main cytological features.

- Nucleus morphology (to distinguish benign from malignant cells) and cytoplasm morphology (to define cell differentiation)

Main cytological features.

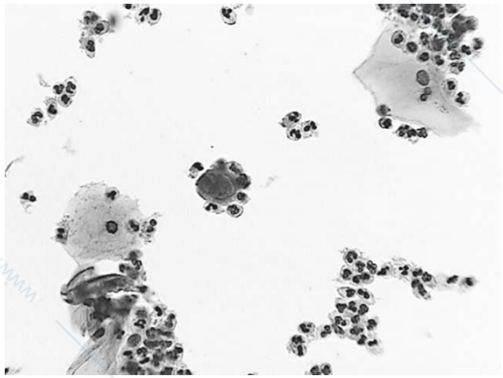
- normal;
- reactive;
- degenerating;
- dysplastic (cell in transition between the normal to neoplastic morphology which can be graded in low and high grade);

Main cytological features

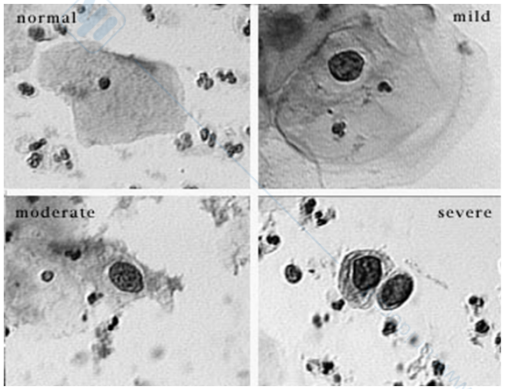
- neoplastic (cell are often aggregated in overlapping layers with a severe pleomorphism, nuclear atypia, mitoses, prominent and angulated , fragmented cytoplasm with anomalous differentiation features nucleoli).
- **IMPORTANT: It is not enough only one single parameter to define a cell as a neoplastic one's**

Normal Pattern

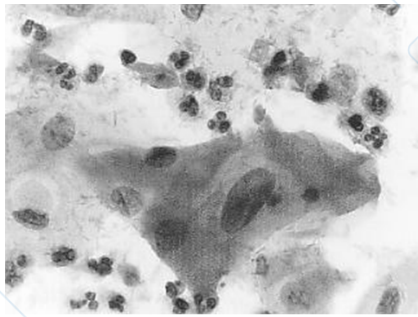
Reactive Pattern (herpes infection)

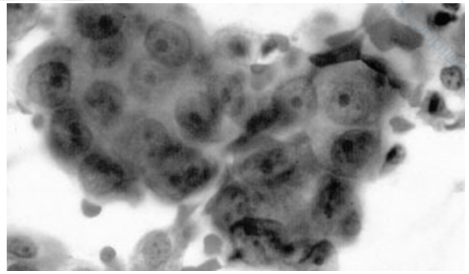


Dysplasia

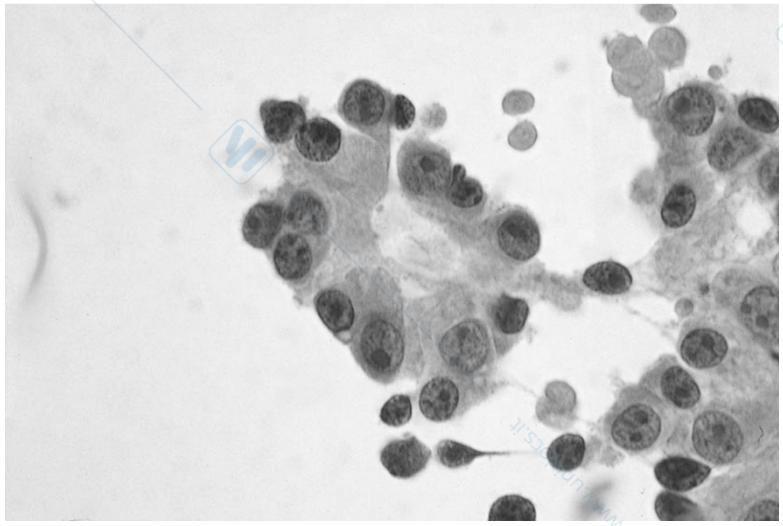


Neoplastic





FNAB



Activity (6)

- Necropsy assessment

in each death case the following steps needed:

- Ascertainment of death
- Necropsy assessment (hypostatic spots, rigor mortis)
- Osbervation period (24hrs or 48hrs for sudden death)

Activity(7)

- assessment of brain death

Transplant low

- **Cardiac death**
- **Brain death**.

medical committee

Cardiac death(1)

- Cardiac death. Clinical examination evidences listening silence of heart tones, absence of arterial pulses, the impossibility of measuring blood pressure, the immobility of the cardiac shadow to the scopia.
- Stopping breathing. Immobility of the chest, silence of heart tones and the immobility of the diaphragm to radioscopy.

Brain death(1)

- **Brain death**. Stop of cerebral activity and vegetative functions survival by resuscitation maneuvers. Brain death is due to massive necrosis of brain caused by primary lesions such as tumors, trauma and cerebral bleeding. After cerebral tissue death many events are following such as stop of spontaneous breathing and stop of cerebral electric activity: only cardiac activity spontaneously survives

Brain death(2)

- This kind of dissociate death is only possibly by resuscitation techniques with allow patient in a *coma dépassé* to maintain for an indefinite period of time vegetative functions (skin colorful, body temperature, matabolism, digestion and normal renal function)

Transplant low

- Medical board.

Collegio Medico

- Anaesthetist
- Neurologist and neurophysyology technician
- Pathologist /Forensic Pathologist /Doctor of Health Mangement

Regole tecniche

- a) coma depassè with muscle atony; tendinea areflexia of skeletal muscles depending from cranial nerves, plantar reflexe atony, paralytic mydriasis and absence of both corneal and pupillary reflex to light;
- b) absence of spontaneous breathing after stop of artificial resp, 2 m‘;
- c) absence of spontaneous or provoked electrical brain activity (pain, light, noise dolore, flashing light and convulsant chemical).

Condizioni (1)

- Le condizioni che garantiscono la realtà della morte cerebrale ed escludono i temporanei arresti dell'attività encefalo-elettrica sono le seguenti:
  - 1) carattere primitivo della lesione cerebrale, con esclusione dei casi di lesione cerebrale secondaria nei quali il legislatore ha ritenuto più difficile il controllo della irreversibilità del danno nervoso (coma da barbiturici, da CO ecc.);
  - 2) coesistenza dei segni clinici ed elettrici;

Condizioni (2)

- 3) persistenza ininterrotta di tali segni per un periodo di almeno 6 ore (adulto), durante il quale i segni neurologici e il silenzio respiratorio vanno controllati e rilevati ad intervalli di tempo non superiori a 1 ora, mentre l'EEG va praticato per periodi di mezz'ora ogni 3 ore;
- 4) assenza di effetti dovuti a somministrazione di farmaci depressivi del SNC e di ipotermia indotta artificialmente (temperatura corporea non inferiore a 35 gradi), pressione arteriosa non inferiore a 100 mm/Hg.

Histopathology

- **Main histological activities:** intraoperative histological diagnosis , **surgical pathology, cytopathology, autopsy,.**
- **Biological sample procedures: fixation, dehydration, embedding, sectioning, staining.**
- **Special techniques; Histochemical stains, Immunohistochemistry, Flux Cytometry, Electron microscopy, Molecula biology.**

Biological samples is ever associated with a request containing personal details, clinical history, clinical suspicion, site of origin and referring doctor and hospital ward.

Code number for each biological samples

Quality assurance procedures need that each step is recognizable (technician, pathologist, nurse, secretary)

Gross examination.

Pathologist and resident pathologist aided by technician described the specimen and select significant area putting them into small boxes contained in a fixative solution

Tissue fixation.

The principal aim of the fixation is to stop all metabolic vital mechanisms and to maintain the tissue structure that is during the complete processing mechanisms.

Fixation is the most critical step in biological material processing to avoid artifacts which could be limiting in the interpretation. Unappropriate fixation is able to impede structure reading

La formaldehyde is a coloroless gas water soluble. The product is available as a water solution 40% called Formalin . It is a slow fixative. In microscopy application formalin is used as a buffered solution.

Policard formalin is a 10% solution plus NaCl 0,9%.

After fixation tissue specimens must be washed vigorously to eliminate excess formalin which may cause staining artifacts.

The following step are dehydration, clarification and embedding.

dehydration is carried out by many steps in ascending graded ethanol from 25 to 100% (15min each depending on sample size)

Clearing

To cut sections, the tissue has to be embedded in paraffin wax, but wax is not soluble in water or alcohol. However, it is soluble in a paraffin solvent called 'xylene'. Therefore, the water in the tissue needs to be replaced with xylene. Finally, once the water has been replaced by 100% alcohol, the alcohol is replaced with xylene, which is miscible with alcohol. This final step is called 'clearing'.

The tissue is trimmed, and mounted on a cutting device called a microtome (shown in the picture). Thin sections are cut, 4-6 micron, which can be stained and mounted on a microscope slide.

https://youtu.be/qGyE4p9XanA

Inclusione

The tissue is placed in warm paraffin wax, and the melted wax fills the spaces that used to have water in them. After cooling, the tissue hardens, and can be used to cut slices (sectioned)..

Staining and Mounting

Unfortunately, most staining solutions are aqueous, so to stain the sections, the wax has to be dissolved and replaced with water (rehydration).
This is essentially step 2 in reverse. The sections are passed through xylene, and then decreasing strengths of alcohol (100% to 0%) and finally water. Once stained, the section is then dehydrated once again, and placed in xylene. It is then mounted on the microscope slide in mounting medium dissolved in xylene. A coverslip is placed on top, to protect the sample. Evaporation of xylene around the edges of the coverslip, dries the mounting medium and bonds the coverlips firmly to the slide.

https://youtu.be/2D0rj0m6dVs

What kinds of histological stains are there?

Most cells are colourless and transparent, and therefore histological sections have to be stained in some way to make the cells visible.

The techniques used can either be non-specific, staining most of the cells in much the same way, or specific, selectively staining particular chemical groupings or molecules within cells or tissues.

Staining usually works by using a dye, that stains some of the cells components a bright colour, together with a counterstain that stains the rest of the cell a different colour.

Basophilic and acidophilic staining

Acidic dyes react with cationic or **basic** components in cells. Proteins and other components in the cytoplasm are **basic**, and will bind to **acidic** dyes. Another way of saying this is that cytoplasmic proteins are **acidophilic**.

Basic dyes react with anionic or **acidic** components in cells. **Nucleic acids** are **acidic,** and therefore bind to **basic dyes.**

H&E staining

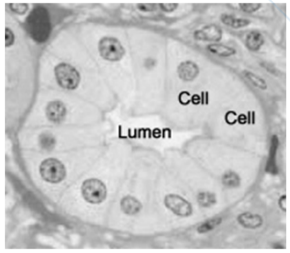
The most commonly used staining system is called **H&E** (Haemotoxylin and Eosin). **H&E** contains the two dyes haemotoxylin and eosin.
Eosin is an **acidic** dye: it is negatively charged (general formula for acidic dyes is: Na^+dye^-). It stains basic (or acidophilic) structures red or pink. This is also sometimes termed 'eosinophilic'. Thus the cytoplasm is stained pink in the picture below, by H&E staining.
Haematoxylin can be considered as a **basic** dye. **Haemotoxylin** is actually a dye called hematein (obtained from the log-wood tree) used in combination with aluminium ions (Al^{3+}). It is used to stain acidic (or basophilic) structures a purplish blue
Thus the nucleus is stained purple in the picture below, by H&E staining.
This means that the nucleus, and parts of the cytoplasm that contain RNA stain up in one colour (purple), and the rest of the cytoplasm stains up a different colour (pink).

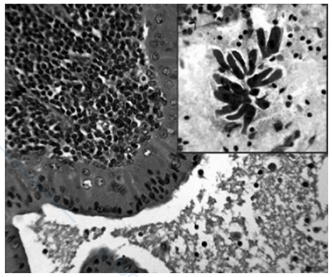
What structures are stained purple (basophilic)**?**
DNA (heterochromatin and the nucleolus) in the nucleus, and RNA in ribosomes and in the rough endoplasmic reticulum are both acidic, and so haemotoxylin binds to them and stains them purple. Some extracellular materials (i.e. carbohydrates in cartilage) are also basophilic.

What structures are stained pink (eosinophilic or acidophilic)**?**
Most proteins in the cytoplasm are basic, and so eosin binds to these proteins and stains them pink. This includes cytoplasmic filaments in muscle cells, intracellular membranes, and extracellular fibres.

Haemotoxylin and Eosin

- **It is useful for quite all diagnosis**





Questions in oncology

- **What is tumor origin *(histogenetic diagnosis)***
- **What is tumor behaviour*(benign or malignant tumor)***
- **What is tumor extension? *(tumor staging)***

Special techniques

- Useful for:
  - diagnosis
  - etiology
  - histogenesis
  - pathogenesis

Special techniques

- Colorazioni speciali
- Histoenzymology
- Cell colture
- Morphometry
- Electron Microscopy
  - Xray microanalysis
- Immunoistochimica
- Citometria a flusso
- Cytogenetics
- Molecular Biology
  - Filter hybridization
  - in situhybridization
  - PCR

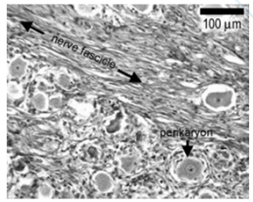
Special stains

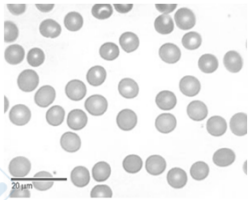
- PAS
- Bacteria, fungi, parasites
- Argentaffin and argyrophilicstains
- amyloid
- reticulin
- Tri-chrome
- PTAH
- Haemosiderin (Perls)
- Melanin (Masson-Fontana)
- Calcium (von Kossa)

This is often used to stain connective tissue. **Tri-chrome** - means the technique produces three colours.

- Nuclei and other basophilic (basic-liking) structures are stained **blue**,
- Cytoplasm, muscle, erythrocytes and keratin are stained bright-red
- Collagen is stained **green** or **blue**, depending on which variant of the technique is used.

Masson's trichrome.





Usually used for staining blood and bone-marrow smears. Nuclei are stained dark-blue to violet, cytoplasm pale blue, erythrocytes pale pink

Giemsa

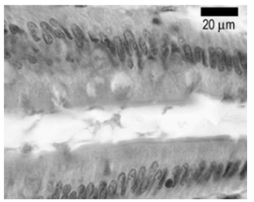
Special stains

- lipids
- Mucin
- Elastic fibers
- Myelin
- AgNOR

PAS

- glicogeno (diastasi)
- muco neutro
- membrane basali
- funghi e parassiti (molti)
- cristalli e inclusioni varie

The Schiff reagent is a bleached basic fuschin that reacts with aldehyde groups. This reaction results in a deep red colour in the section. It is the basis of the PAS stain.
PAS stains carbohydrates and carbohydrate rich macromolecules a deep red colour (magenta).
PAS will therefore stain up:
glycogen the intracellular storage form of carbohydrate in cells
Mucus in cells and tissues, Basement membranes, and Brush borders of kidney tubules and small and large intestines Reticular fibres (i.e. collagen) in connective tissue and Cartilage.
In the example shown above, The mucin produced by goblet cells is stained a purple colour by this stain.



Silver stains

- Argentaffin stain
  - Fontana-Masson
- Argyrophilic stain
  - Grimelius

Trichrome (Masson)

- nuclei, cytoplasm and connective tissue
- Collagen-specific

Special stains

- Perls
  - hemosiderin
- Von Kossa
  - calcium
- Congo Red
  - amyloid
- Oil Red O (ORO)
  - triglycerides

Special staining

- Specific stains for baceria, fungi azioni specifiche per batteri, funghi and parasites
  - Gram e Brown & Brenn (bacteria)
  - Ziehl-Neelsen (micobacteria)
  - argentica di Grocott (fungi and Pneumocystis)
  - PAS (fungi, Amoebae, Trichomonas)
  - Dieterle (Helicobacter, Legionella, syphilis, Lyme)

Tumor histogenesis

- **Immunohistochemistry**
- **Electron microscopy**

Immunohistochemistry is commonly used by clinicians to detect and diagnose abnormal cells found in disease states such as cancer. Such biomarkers are specific to the disease state and are characteristic of particular events such as cell death, apoptosis or proliferation, which give rise to the abnormality. Immunohistochemistry can also be used: 1. As a predictor for treatment, prognosis and outcome. For example Trastuzumab inhibits HER2 which is overexpressed in some but not all breast cancers. It is therefore not advisable to administer Trastuzumab to HER2 negative breast cancer patients. IHC can be used by clinicians to confirm overexpression of HER2 before administration of Trastuzumab. 2. During basic research, to evaluate the location and colocalization of proteins within a cell, for instance in the nucleus, cytoplasm or membrane.

www.researchgate.net

Immunohistochemistry (**IHC**) is a method for detecting antigens or haptens in cells of a tissue section by exploiting the **principle** of antibodies binding specifically to antigens in biological tissues. The antibody-antigen binding can be visualized in different manners.
IHC is widely used in many research and clinical laboratories because this technique makes it possible to visualize the distribution and localization of specific cellular components within cells and in proper tissue context. There are numerous IHC methods that can be used to localize antigens. The method selected should include consideration of parameters such as the specimen types and assay sensitivity.

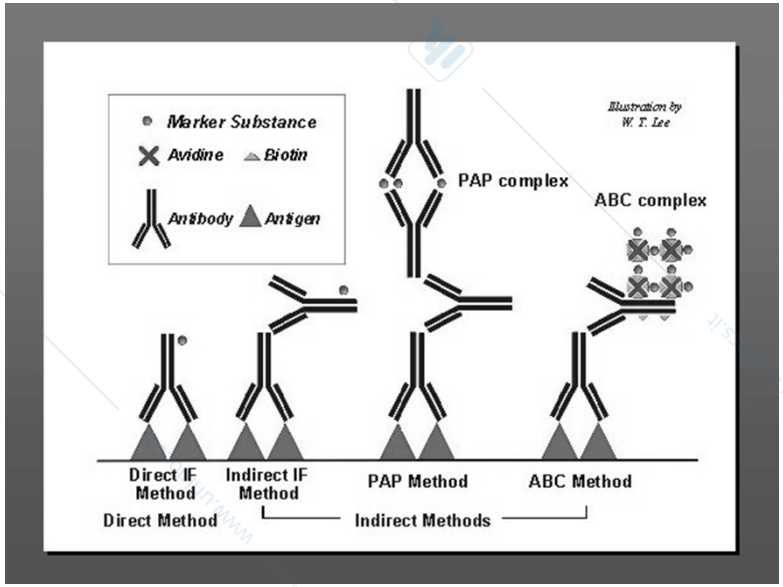
Immunocitochimica

- **L'immunocitochimica costituisce la tecnica di elezione per la localizzazione e quindi l'evidenziazione di sostanze e/o strutture intra- ed extracellulari in tessuti normali e patologici; tale tecnica si basa sull'utilizzazione di anticorpi altamente specifici verso antigeni che si desiderano rivelare e di opportuni marcatori e sonde che agiscono da rivelatori dell'avvenuta reazione tra antigene ed anticorpo**

FIXATION The principle of IHC tissue fixation is to maintain tissue structure and retain antigenicity. Users must be sure to preserve the readable tissue architecture and cell morphology, otherwise the localization of immune reactive products cannot be recognized. However, if the tissue is over-fixed, the antigenicity will be diminished or even completely extinguished thus resulting in false negative staining. There are a broad array of fixative methods commercially available. Researchers may choose appropriate fixatives based upon the tissue types and the requirement of individual experiments. However, we must iterate that there is no one universal fixative that is ideal for the staining for all antigens. In general, most antigens can be successfully identified in acetone-fixed tissue sections. Formalin-fixed tissue sections are also popularly used in IHC because formalin-fixed paraffin-embedded tissue can be stored for many years. It is very convenient to use archived paraffinembedded tissue to carry out IHC. In recent years the use of antigen retrieval techniques has increased the use of formalin fixation and paraffin-embedding as a more commonly used fixation method.

ANTIGEN RETRIEVAL After fixation, the epitopes may be cross-linked and covered making it difficult for antibody-binding. By pre-treatment with antigen retrieval reagents or procedures, investigators can re-open the cross-linked epitopes so that antibodies can easily bind to target antigens. Several approaches to antigen retrieval have been widely published, including heat and enzyme retrieval. Commercially available reagents are also available from multiple sources.

IHC METHODS Blocking Several endogenous substances may interfere with IHC results, such as endogenous peroxidase, fluorescence, antibody binding capability or biotin. Therefore, blocking the endogenous material prior to staining is crucial in IHC to avoid false positive staining. Visualization of the Antibody-Antigen Complex There are several potential options available to visualize the antibody-antigen complex in immunohistochemistry: 1. By directly conjugating an enzyme such as horseradish peroxidase (HRP) or alkaline phosphatase (AP), which can catalyze a color-producing reaction 2. By directly conjugating a fluorophore such as rhodamine or fluorescein 3. Indirectly through the use of a secondary antibody conjugated either to an enzyme or a fluorophore



Immunocytochemistry advantages

- High sensitivity and specificity
- Routine useful
- Morphology correlation (E-E)

Immunohistochemistry

- Actin (common, smooth muscle, sarcomeric)
- Intermediate filaments (Desmin, Neurofilaments, Cytokeratin, GFAP, Vimentin)
- hCG
- HMB-45
- PSA
- S-100
- Thyreoglobulin
- Calcitonin
- Chromogranin
- EMA
- Factor VIII

Immunohistochemistry

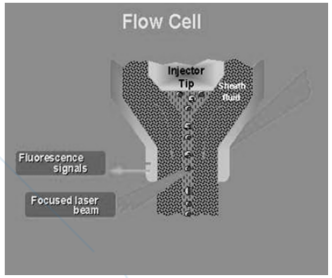
- Alpha-fetoproteina
- alpha-lactoalbumin
- B 72.3
- CD31
- CD34
- CEA
- type IV collagen
- Leu-M1
- Lysozyme
- Virus (selected)
- NSE
- PAP
- Peptide hormone
- PLAP
- Synaptophysin
- hematological (es. CD3/4/8/21)

ELECTRON MICROSCOPY

- Very useful depending on kind of pathology: selected tumor (undifferentuitated, small round cell, CNS tumor, pituitary tumors)
- Tumor hystogenesis
- Non neoplastic pathology: muscle, kidney, storage diseases
- TEM (> 90%)
- SEM (5%)
- Microanalisi a raggi X e altre tecniche analitiche (5%)

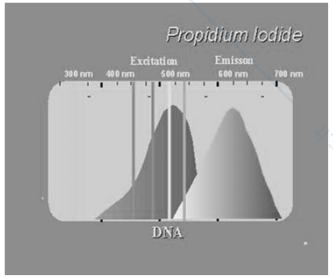
CITOMETRIA A FLUSSO

- **Misura diversi parametri citologici. Una sospensione cellulare viene fatta passare, in fila indiana, lungo una camera in corrispondenza della quale viene fatto convergere un fascio di luce laser. Le cellule emettono fotoni di luce di appropriata lunghezza d'onda che vengono rilevati da appositi rivelatori di segnale.**



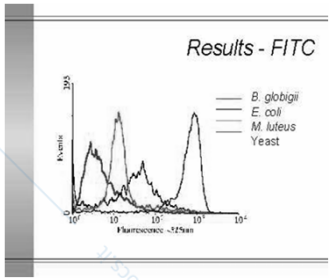
CITOMETRIA A FLUSSO

- 5000-10000 cellule/sec.
- Produce istogrammi relativi a:
  - dimensioni cellulari
  - granularità citoplasmatica
  - vitalità cellulare
  - lunghezza del ciclo (fase S)
  - contenuto in DNA (ploidia)
  - contenuto enzimatico
  - presenza di marker fenotipici di superficie



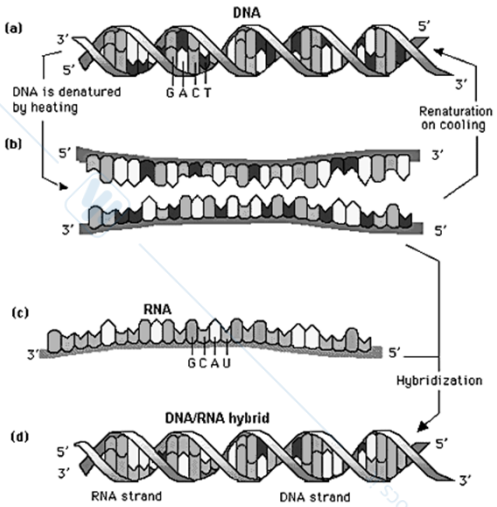
CITOMETRIA A FLUSSO

- Utile per leucemie e linfomi; tumori solidi ?
- Mediante lo studio della fase S del ciclo e della ploidia fornisce **<u>informazioni prognostiche</u>** indipendenti dalla staging e dal grading della neoplasia

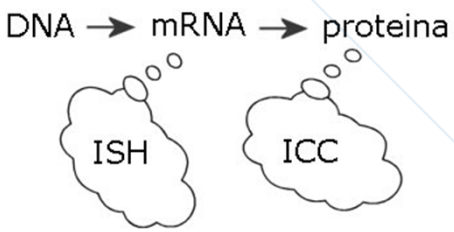


Ibridazione

- Reazioni di accoppiamento tra una molecola di DNA o di RNA marcate (sonda) e un acido nucleico complementare (bersaglio) a singola catena
- Il prodotto è una molecola duplex di DNA:DNA, DNA:RNA o RNA:RNA
- Le reazioni possono avvenire in soluzione, su filtro o ***in situ***
- Gli ibridi vengono visualizzati con metodo autoradiografico o immunocitochimico a livello di MO o ME



Nucleic Acid Hybridization

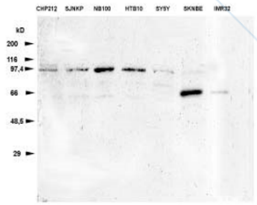


Ibridazione su filtro

- il DNA *estratto*, viene immobilizzato su filtri di nitrocellulosa (**dot blot**). Più comunemente, prima di procedere alla immobilizzazione, vengono selezionate le zone di DNA di interesse mediante uso di enzimi di restrizione o frazionamento dimensionale mediante elettroforesi su gel di poliacrilammide.
- dopo l'immobilizzazione, viene eseguita l'ibridazione e la visualizzazione, con metodo autoradiografico, dell'avvenuto accoppiamento su emulsione fotografica. Tale metodo è conosciuto con il nome di **Southern blot**

Ibridazione su filtro

- il **northern blot** e il **western blot** sono procedure analoghe al southern blot; in questo caso il frazionamento dimensionale riguarda RNA o proteine.



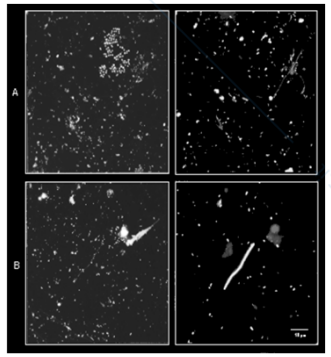
Necessità di materiale fresco

Ibridazione in situ

Consente di rilevare e localizzare specifiche sequenze di acidi nucleici (DNA/RNA) direttamente nelle cellule e nei tessuti, fornendo informazioni di tipo morfologico e molecolare. E' una tecnica altamente sensibile. Il più comune metodo di rilevamento è quello isotopico ma possono essere utilizzati metodi alternativi non-radiottivi (perossidasi, biotina, digossigenina).

Ibridazione in situ

- infezioni virali latenti (HPV, EBV, HIV, etc.)
- mRNA di vari ormoni peptidici (neoplasie endocrine), di catene leggere e pesanti delle IG (linfomi)
- espressione di proto-oncogeni (feto, tessuto normale, malattie infiammatorie, neoplasie)



PCR

- Polymerase chain reaction (PCR): rende possibile la replicazione e l'amplificazione di un particolare segmento anche da una singola molecola di DNA e l'estrazione di un gene, anche se in singola copia, da un insieme di sequenze genomiche.
- RT- PCR : mediante l'impiego dell'enzima retrovirale transcriptasi inversa, consente di amplificare l' RNA, permettendo l'analisi dell'espressione di singoli geni in piccole quantità di tessuto.

PCR

- identificazione di RNA virus (es. HIV)
- recidiva di LMC in trapiantati
- micrometastasi nel ca della prostata
- accertamento di proliferazioni clonali di B e T linfociti (Ig e TCR)

- identificazione di traslocazioni cromosomiche
- presenza di mutazioni puntiformi (*ras* attivato)
- alterazioni a carico dei geni onco-soppressori (Rb, p53)
- amplificazioni genetiche (*c-erb*-B-2 e N-*myc*)
- instabilità microsatellitare
- virus-tumore-associati (HPV, EBV)

Histopathology

Neoplasia

- Definition
  - Neoplasia means «new growth» and the new growth is a neoplasm. The term tumor was originally applied to the swelling caused by inflammation. Neoplasm also may induce swelling.
  - Cancer in the common term for all malignant tumors. It problably derives from latin crabs, cancer, because a cancer «adheres to any part that it seizes upon in an obstinate manner like a crab

- A neoplasm is an abnormal mass of tissue the growth of which exceeds and is uncoordinated with that of the normal tissues, and persists in the same excessive manner after cessation of the stimuli which evoked the change» (Willis, 1952
- The abnormal mass is purposeless, preys on the host, and it is virtually autonomous.
- The growth of the neoplatic tissues compees with normal cells and tissues for energy supplies and nutritional substrate.
- Later it becomes evident that such autonomy is not complete. All neoplass ultimately depend on the host for their nutrition and vascular supply

Epidemiology

- Study of cancer patterns in populations can contribute to knowledge about the origin of cancer.
- The major insights into the causes of cancer can be obtained by epidemiologic studies and relate particular environmental, racial (possibly hereditary) and cultural influences to the occurrence of malignant neoplasms.
- Certain diseases associated with an increased risk of developng cancer can provide insight into the pathogenesis of malignancy.

Main death causes in the western world

- cardiovascular diseases (31,4%)
- *neoplasia (23,3%)*
- cerebrovascular diseases (6,9%)
- lung non neoplastic diseases (5%)
- accidents (4%)
- others (29,4%)

Cancer Incidence

- An individual's likehood of developing a cancer is expressed by national incidence and mortality rates.
- USA (1 out of 5) **dati del 2000**
  - Male
    (Prostate 29%, lung 14%, colon 10%)
  - Female
    (breast 30%, lung 12%, colon 11%)

Cancer is the second leading cause of death globally, and is responsible for an estimated 9.6 million deaths in 2018.

- Globally, about 1 in 6 deaths is due to cancer.
- Approximately 70% of deaths from cancer occur in low- and middle-income countries.
- Around 1/3 of deaths from cancer are due to the 5 leading behavioral and dietary risks: high body mass index, low fruit and vegetable intake, lack of physical activity, tobacco use, and alcohol use.
- Tobacco use is the most important risk factor for cancer and is responsible for approximately 22% of cancer deaths

Cancer is a leading cause of death worldwide, accounting for an estimated 9.6 million deaths in 2018. The most common cancers are:
•Lung (2.09 million cases)
•Breast (2.09 million cases)
•Colorectal (1.80 million cases)
•Prostate (1.28 million cases)
•Skin cancer (non-melanoma) (1.04 million cases)
•Stomach (1.03 million cases)

The most common causes of cancer death are cancers of:
•Lung (1.76 million deaths)
•Colorectal (862 000 deaths)
•Stomach (783 000 deaths)
•Liver (782 000 deaths)
•Breast (627 000 deaths)

•Cancer causing infections, such as hepatitis and human papilloma virus (HPV), are responsible for up to 25% of cancer cases in low- and middle-income countries

•Late-stage presentation and inaccessible diagnosis and treatment are common. More than 90% of high-income countries reported treatment services are available compared to less than 30% of low-income countries.

•The economic impact of cancer is significant and is increasing. The total annual economic cost of cancer in 2010 was estimated at approximately US$ 1.16 trillion.

•Only 1 in 5 low- and middle-income countries have the necessary data to drive cancer policy

Mortality rate

- A **mortality rate** is a measure of the frequency of occurrence of death in a defined population during a specified interval
- USA
  - Male (lung 31%, Prostate 11%, Colon 10%)
  - Female (lung 25%, breast 15%, Colon 11%)

Geographic and environmental risk factor

- Gastric Ca
  (⇑ Japane ⇓ USA)
- Melanoma
  (⇑ New Zealand⇓ Island)
- UV
- Asbestos
- Eating habits

Geographic and environmental risk factor

- Alcohol abuse (liver cirrhosis ⇒ hepatocarcinoma)
- Smoking (Ca. mouth, pharynx, larynx,, laringe, esophagus, pancreas, bladder and lung)
- Cervical Ca (age at first intercourse and the number of sex partners: this association point to a possile causal role fr venereal transmission of cervical viral infections)

Cancer arises from the transformation of normal cells into tumour cells in a multistage process that generally progresses from a pre-cancerous lesion to a malignant tumour.

These changes are the result of the interaction between a person's genetic factors and 3 categories of external agents, including:

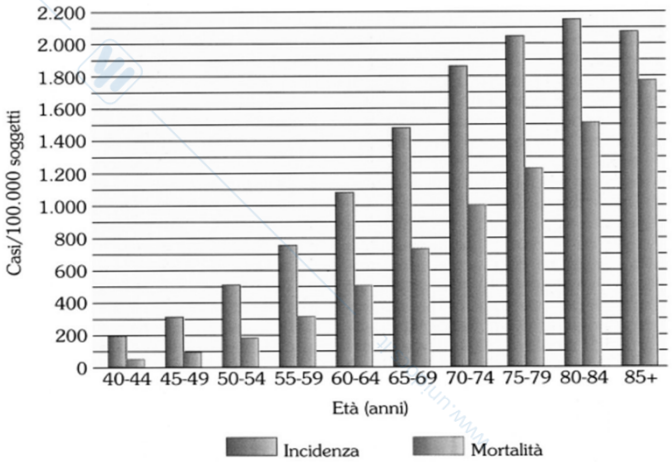
•physical carcinogens (ultraviolet and ionizing radiation);

•chemical carcinogens, such as asbestos, components of tobacco smoke, aflatoxin, and arsenic;

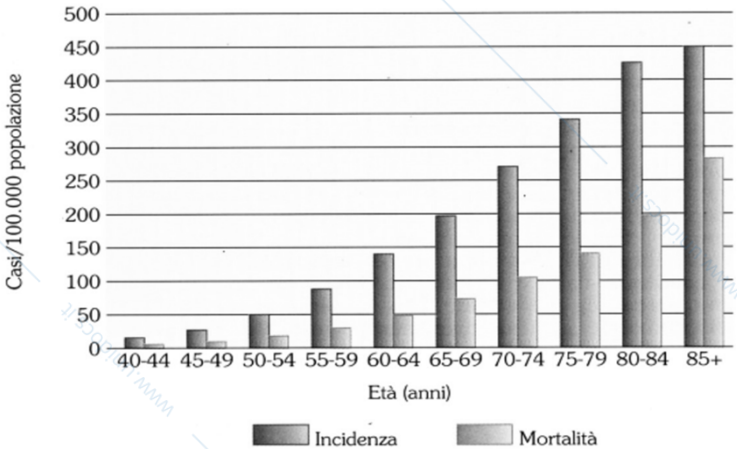
•biological carcinogens, (infections from certain viruses, bacteria, or parasites.

Age

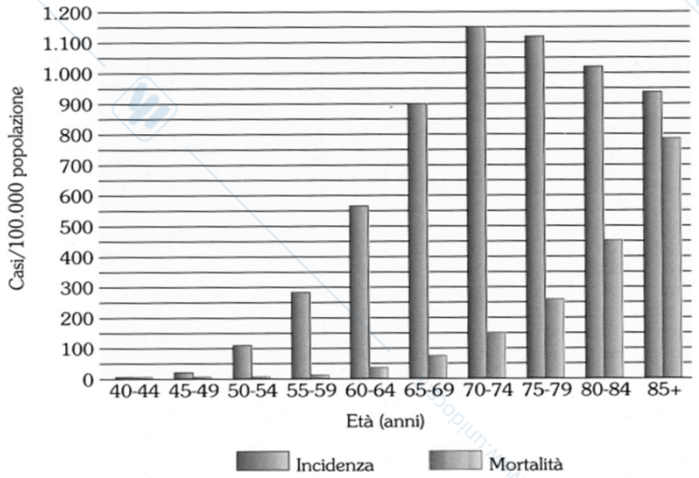
- Age is an important influence on the likehood of being afflicted with cancer!! (tendency for cellular repair mechanisms to be less effective) Most ca. occur in the later years of life (> 55)
- Children (acute leukemia , CNS neoplasms del SNC, neuroblastoma, Wilms tumor, retinoblastoma, rhabdomyosarcomas)



Incidence and mortality colon Ca. Age related (1993-1997)



Incidence and mortality prosate Ca. Age related (1993-1997)



Incidence / Age /Sex

- 0-39 yrs → 1:62 (M) e 1:52 (F)
- 40-59 yrs → 1:12 (M) e 1:11 (F)
- 60-79 yrs → 1:3 (M) e 1:4 (F)

Early detection

Cancer mortality can be reduced if cases are detected and treated early. There are 2 components of early detection:

Early diagnosis

When identified early, cancer is more likely to respond to effective treatment and can result in a greater probability of surviving, less morbidity, and less expensive treatment. Significant improvements can be made in the lives of cancer patients by detecting cancer early and avoiding delays in care.

Early diagnosis consists of 3 steps that must be integrated and provided in a timely manner:

•awareness and accessing care
•clinical evaluation, diagnosis and staging
•access to treatment.

Early diagnosis is relevant in all settings and the majority of cancers. In absence of early diagnosis, patients are diagnosed at late stages when curative treatment may no longer be an option. Programmes can be designed to reduce delays in, and barriers to, care, allowing patients to access treatment in a timely manner

Screening

Screening aims to identify individuals with abnormalities suggestive of a specific cancer or pre-cancer who have not developed any symptoms and refer them promptly for diagnosis and treatment.

Screening programmes can be effective for select cancer types when appropriate tests are used, implemented effectively, linked to other steps in the screening process and when quality is assured. In general, a screening programme is a far more complex public health intervention compared to early diagnosis.

Examples of screening methods are:

•visual inspection with acetic acid (VIA) for cervical cancer in low-income settings;
•HPV testing for cervical cancer;
•PAP cytology test for cervical cancer in middle- and high-income settings; and
•mammography screening for breast cancer in settings with strong or relatively strong health systems

Heredity (5-10%)

hereditary forms can be divided in three categories:

- Inherited cancer syndromes (autosomal dominant)
  - Poliposi adenomatosa familiare (FAP)
  - MEN
  - Neurofibromatosis type 1 and 2
  - Von Hippel-Lindau S
- Familial cancers but role of inherited predisposition my not be clear in an individual case
  - Colon, ovarian , breast
- Autosomal recessive syndromes of defective DNA repair → BRCA1 e BRCA2

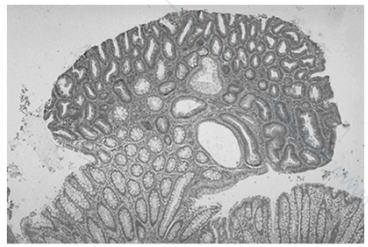
Certain forms of benign neoplasia also constitute precancerous conditins. (villous adenoma of colon as it increases in size develops cancerous changes in up to 50% of cases)

NEOPLASIA

- Parenchyma:
  neoplastic cells
- Supportive Stroma :
  connective tissue and blood vessels from host

NEOPLASIA

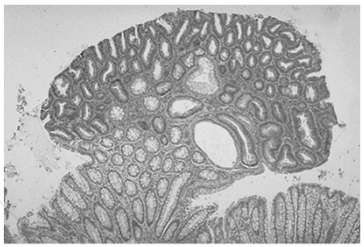
- Beningn
  - **epithelial**: classifying gross morphology and microscopic architecture
  - **mesenchymal**: attaching the suffix -oma to the cell of origin are classified depending on the origin cell



NEOPLASIA

- Benign epithelial tumors:

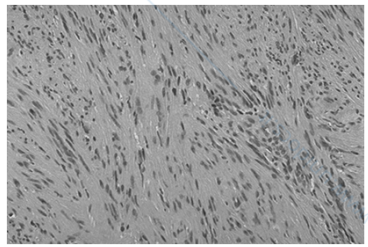
adenoma
papilloma
cystodenoma
papillary cystoadenoma
polyp



NEOPLASIA

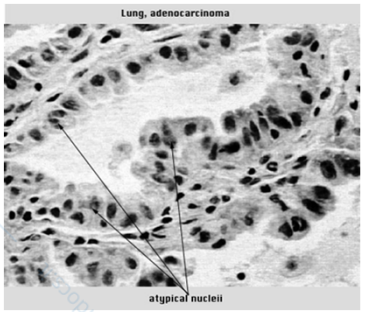
- Mesenchymal benign tumors:

leomyoma
lipoma
rhabdomyoma
schwannoma
fibroma



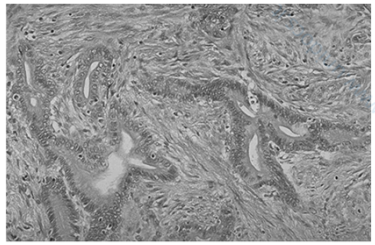
NEOPLASIA

- Malignant tumors
  - Carcinoma: malignant epithelial cell origin, derived from any of the three germ (ectodermal /mesodermal/ endodermal)
  - Sarcoma: malignant tumors arising in mesenchymal tissue because they have little connective tissue troma and so are fleshy



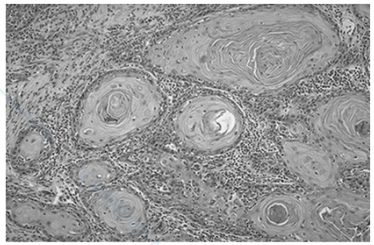
NEOPLASIA

- Epithelial malignant tumors
  - Adenocarcinoma glandular growth pattern is microscopically recognizable



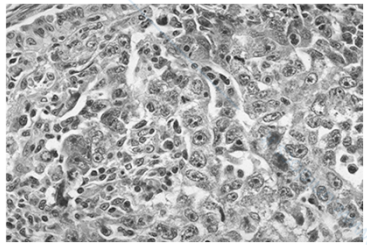
NEOPLASIA

- Malignant epithelial tumors
  - Squamous carcinoma
    squamous cell deriving from any multilayered flat epithelium



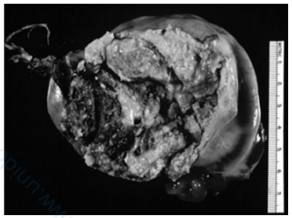
NEOPLASIA

- Undifferentiated malignant tumors
- It is not possible to identify an origin cell
- Sometimes it is impossible to make the differential diagnosis between carcinoma and sarcoma



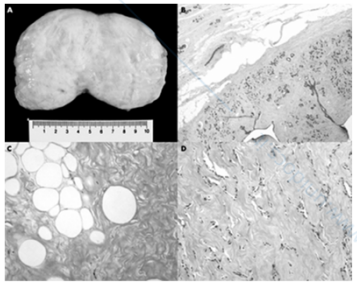
NEOPLASIA

- **Mixed tumor**: divergent differentiation of a single line of parenchymal cells creates what are called mixed tumors
- **Teratoma**: a variety of parenchymal cell types representative of more than one germ layer, totipotential cell, usually all three(principally encountered in the gonads



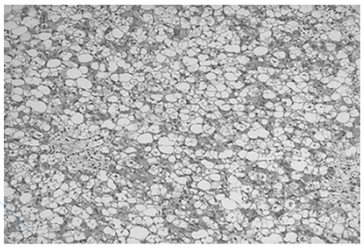
NEOPLASIA

- **Choristoma**: rest of normal tissue
- **Hamartoma**: aberrant differentiation of dysorganized mature cells (lung hamartoma contains islands of cartiage, blood vessels, bronchial-type structures and lymphoid tissue)



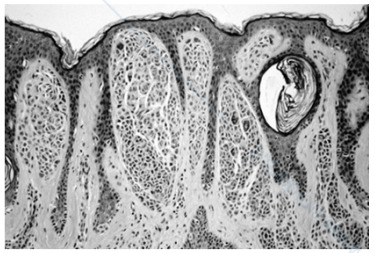
NEOPLASIA

- Differences between benign and malignant tumor
  - *Differentiation and anaplasia*
  - *Rate of growth*
  - *Local invasion*
  - *metastasis*



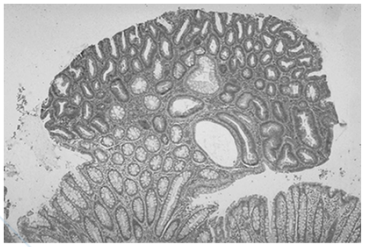
NEOPLASIA

- <u>Differentiation</u>
- Refers to the extent to which parenchymal cells resemble comèarable normal cells, both morphologically and functionally.



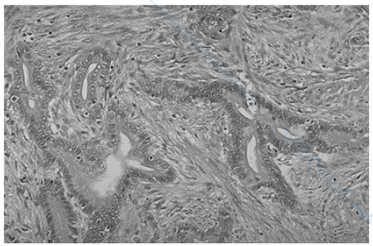
NEOPLASIA

- Benign tumors are well differentiated



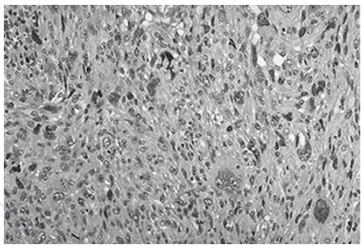
NEOPLASIA

- Malignant tumors range from well differentiated to undifferentiated



NEOPLASIA

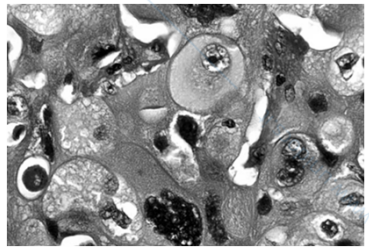
- Malignant neoplasms composed of undifferentiated cells are said to be anaplastic.
- Anaplasia = lack of differentiation.
- Anaplasia is considered a hallmark of malignant transformation.



«to form backward»

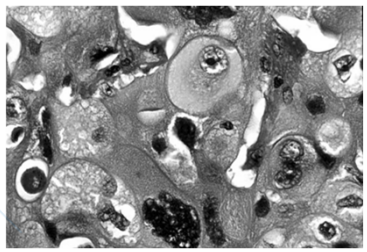
NEOPLASIA

- Anaplasia is marked by a number pf morphological and functional changes.
- cytology
  - **pleomorphism** (variation inn size and shap)



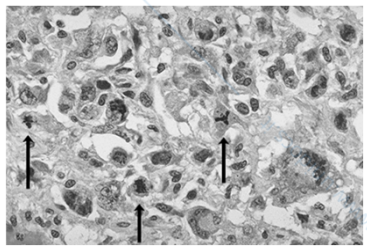
NEOPLASIA

- **Hyperchromatic nuclei**
- **high N/C**
- **Prominent nucleoli**



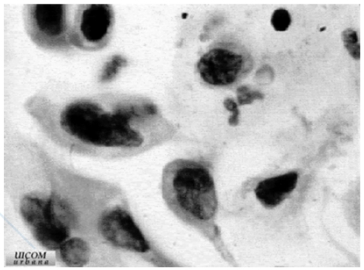
NEOPLASIA

- **Large number of mitoses**
- **Atypical and bizarre mitoses**
- **Tumor giant cells**



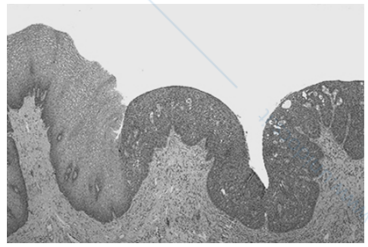
NEOPLASIA

- Histology
  - **Disturbed orientation**
  - **Loss of adhesiveness**
  - **necrosis**
  - **Dystrophic calcification**



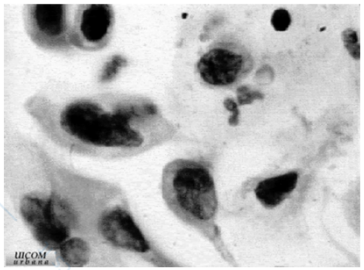
NEOPLASIA

- Dysplasia: epithelia disordered growth a loss in the uniformity of the idividual cells as well as a loss in their architectural orientation



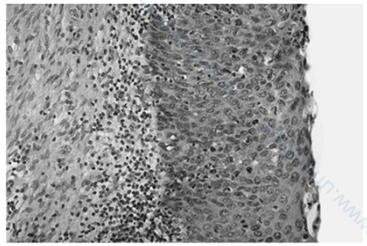
NEOPLASIA

- Displasia: the cells exhibit considerable pleomorphism and deeply stained nuclei; mitotic figures are more abundant although almost invariably they conform to normal pattern: sometimes they appear in abnormal location within the epithelium



NEOPLASIA

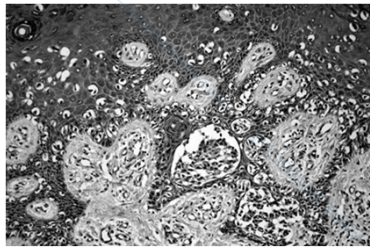
- Dysplasia: when dysplastic changes are marked and involve the entire thickness of the epithelium the lesion is considered a pre invasion neoplasm and it is referred to as *carcinoma in situ.*



NEOPLASIA

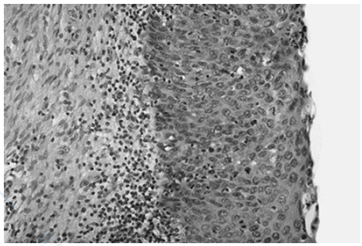
- Most benign tumors grow slowly over a period of years, whereas most cancers groe rapidly, sometimes at an erratic pace and eventually spread and kill their hosts.
- In general, the growth rate of tumors correlates with their level of differentiation and thus most malinant tumors grow rapidly than do enign lesions

- Nearly all benign tumors grow as cohesive expansile masses that remain localizaed to their site of origin and do not have the capacity to infiltrate, invade or metastatize to distant site as do malignant tumors; they usually develop a rim o of compressed connective tissue sometimes called a fibrous capsule
- The growth of cancers is accompaniec by infiltration, invasion, and destruction of the surrounding tissues.



NEOPLASIA

- Next to the development of metastases invasiveness is the most reliable feature that differentiates malignant from benign tumors
- **N.B.** In situ carcinoma dysplay the cytologic features of malignacy without invasion of the basement membrane

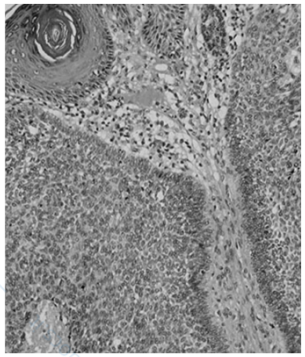


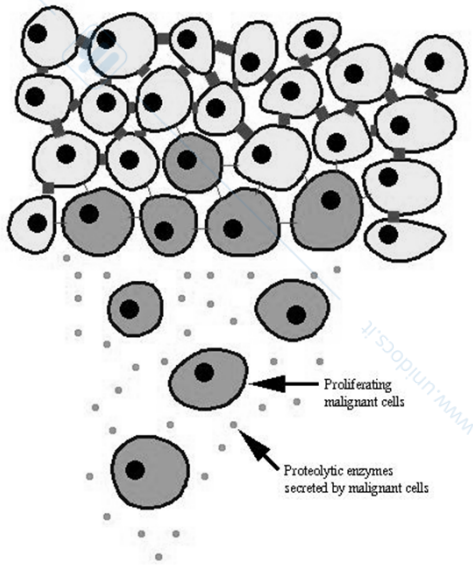
NEOPLASIA

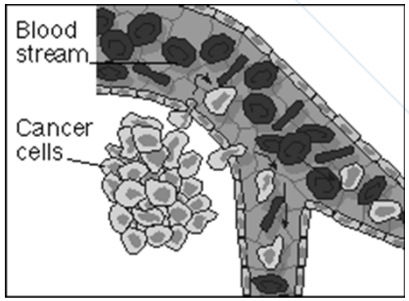
- Metastases are tumor implants discontnuous with the primary tumor.
- Metastasi unequivocally marks a tumor as a malignant because benign neoplasms do not metastatize.

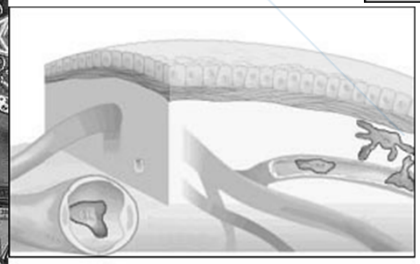
NEOPLASIA

- With few exceptions, all cancers can metastatize
  - **Gliomas**
  - **Basal cell ca. of the skin (rodent ulcer because of their invasive destructiveness)**

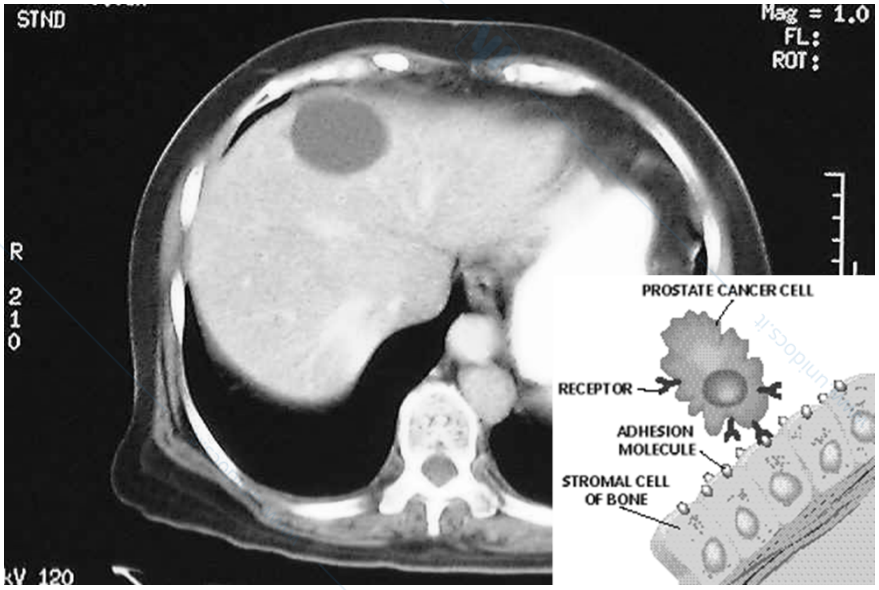








Distant Metastases

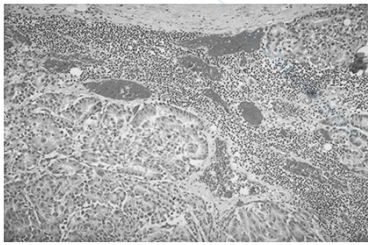


NEOPLASIA

- Seedind of body cavities and surfaces (pleural, pericardial, subarachnoid and joint space)
- Lymphatic spread
- Hematogenous spread

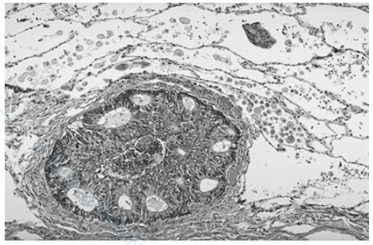
NEOPLASIA

- Lymphatica spread: is the most common pathway for the initial dissemination of carcinomas.
- The pattern of lymphnode involvement follows the natural routes of drainage.
- LN reactive hyperplasia (drainage of tumor cell debris or tumor antigens or both)!!



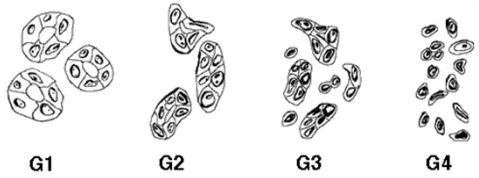
NEOPLASIA

- Hematogenous spread is typical of sarcomas.
- Veins (thin walls) are more easily penetrated than arteries
- Neoplastic emboli follow the venous flow draining the site of neoplasm seguono il flusso ematico (vena cava-lung; portal vein-liver; paravertebral plexus-vertebral metastases).



NEOPLASIA

- Grading (level of differentiation) and staging (extent of spread of a cancer within the patient): are parameters of the clinical gravity of the disease

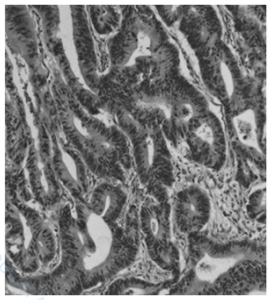


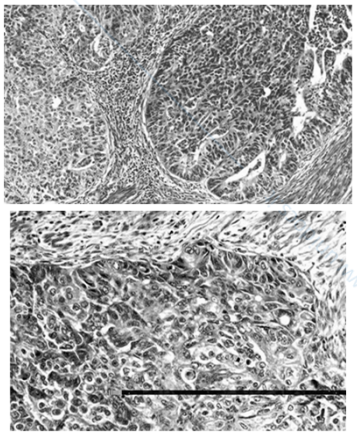
Cancers are classified as Grade I to IV with increasing anaplasia

NEOPLASIA

- **Grading** of a cancer is based on the degree of differentiation of the tumor cells and the number of motoses within the tumor as preseumed correlates of the neoplasm aggressiveness.
- The correlation between histological appearance and biologic behavior is less than perfect: in general grading of cancers has proved of less clinical value than has staging

NEOPLASIA

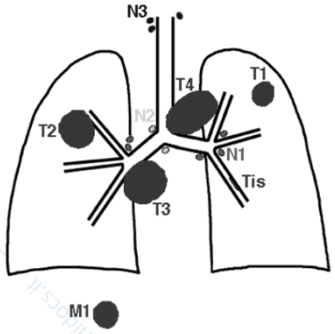




- **Uterine adenoCa**:
  grade I-II-III

NEOPLASIA

- Staging: is based on the size of the primary lesion, its extent of spread to regional lymph node, and the presence or absence of blood borne metastases
- TNM (UICC)
- 0-IV (AJC)



Malignant neoplasms of the thyroid

Neoplasms of the thyroid gland

Benign, in the majority of cases

- **Malignant, rare cases**
- **Genetic predisposition**
- **Radiation**
- **Diagnosis made by means of fine needle aspiration.**

Molecular

RET proto-oncogene at 10q11.2, which encodes a tyrosine kinase receptor not normally expressed by thyroid follicular cells; RET may be rearranged and put under control of a gene constitutively expressed in the thyroid (PTC/RET oncogene)

II. Malignant Tumors
- A. Differentiated
  - 1. Papillary adenocarcinoma
    - a. Pure papillary adenocarcinoma
    - b.Mixed papillary and follicular carcinoma (variants including tall cell, follicular, oxyphyl, solid)
  - 2. Follicular adenocarcinomas (variants: "malignant adenoma", Hurthle cell carcinoma or oxyphil carcinoma, clear-cell carcinoma, insular carcinoma)
- B. Medullary carcinoma
- C. Undifferentiated
  - 1. Small cell (to be differentiated from lymphoma)
  - 2. Giant cell
  - 3. Carcinosarcoma
- D. Miscellaneous
  - 1. Lymphoma, sarcoma
  - 2. Squamous cell epidermoid carcinoma
  - 3. Fibrosarcoma
  - 4. Mucoepithelial carcinoma
  - 5. Metastatic tumor

Papillary carcinoma

• Conventional

• Variants:

Papillary and follicular microcarcinoma and tall cell, columnar cell, sclerosing

Follicular carcinoma

Hurthle cell carcinoma

Medullary Carcinoma

Poorly differentiated carcinoma, insular Ca.

Undifferentiated carcinoma, anaplastic papillary Ca

Papillary Carcinoma

80-90% Ca thyroid

A rare neoplasm (1%)

Favorable prognosis in spite of a high incidence of lymph node metastasis

Associated with radiation exposure

Papillary Carcinoma

Women (70%) in reproductive age;
Occult tumors in 6% of autopsies (from 1 to 10mm), 46% multicentric, 14% with lymph node metastasis

Papillary Carcinoma

Risk factors:

Environmental (diet rich in iodine)

Radiation (therapeutic or accidental)

Autoimmune disease

Genetic syndromes (Gardner syndrome or Cowden syndrome)

Gardner's syndrome: autosomal dominant, multiple colorectal adenomas with carcinoma at age 35-40, multiple osteomas, fibromatosis, pigmented ocular lesions

Cowden's syndrome: autosomal dominant, multiple hamartomas or tumors of the skin, oral mucosa, breast, thyroid, bowel, multiple trichilemmomas of the skin

Papillary Carcinoma

MACRO:

- **Localization**
- **Size, 2-3cm**
- **Invasive, firm to hard consistency, white-tan in color**
- **With or without a capsule**
- **Calcifications**
- **Necrosis, rare (high grade forms)**

Papillary Carcinoma

MICRO:

Papillary proliferation of the epithelium

Nuclei, clear, "ground glass", large with numerous pseudoinclusions

Prominent nucleoli

Scarce mitosis

Psammomatous calcifications (lamellated)

Fibrosis

Lymph node metastasis (50% of cases) do not change prognosis

Papillary carcinoma

IHC:

Thyroglobulin

CAM5.2

AE1/3 (follicular lesions absent and papillary hyperplasia)

Unfavorable prognostic factors:

- age 40+ or older,
- Male sex
- Locally invasive
- Distant metastasis (bone > lung)
- Tumor size
- Tall cell/columnar or diffuse sclerosing
- Exposure to radiation
- 5-20% local recurrences, 10-15% distant metastasis (lung, bone, CNS)

Papillary carcinoma

VARIANTS:

- Follicular
- Dd carcinoma follicolare
- Follicular appearance associated with nuclear alterations
- Micro and macrofollicular patterns
- Multicentric

Papillary carcinoma

VARIANTS:

Papillary microcarcinoma

- <1 cm (<5mm, minute, or between 5 and 10mm, tiny)
- Silent
- Often have a follicular structure with papillary carcinoma-type nuclei
- A high incidence of distant and lymph node metastasis

Papillary carcinoma

VARIANTS:

Tall-cell

- 10% of papillary carcinomas
- > aggressive, mortality rate of 25%
- More advanced age
- MACRO: >6cm, vascular invasion
- MICRO: papillae with very high cells, twice as high as normal; convoluted nuclei and eosinophilic cytoplasm; mitotic figures; involves surrounding structures and lymphatic invasion; can be associated with lymphocitic thyroiditis

Papillary carcinoma

VARIANTS:

- Columnar-cell
- <10%
- Particularly aggressive
- Male sex

MACRO

- Involves surrounding structures

MICRO: prominent nuclear stratification; papillary and microfollicular pattern; no convoluted nuclei

Papillary carcinoma

VARIANTS:

- Diffusely sclerosing
- Rare
- Female sex
- Cervical lymph nodes
- Pulmonary metastasis
- >aggressive

Papillary proliferation with solid areas; squamous metaplasia appearance; dense sclerosis; psammomatous bodies; convoluted nuclei

Follicular carcinoma

- 5% of malignant neoplasms
- Increased incidence in geographical areas of endemic goitre
- Rarely occult
- <female sex
- Age > papillary Ca
- Risk factors: iodine deficiency, advanced age, exposure to radiation
- 2 subtypes: minimally invasive (capsulated) and widely invasive.

Minimally invasive follicular carcinoma

- Thick capsule
- Similar to adenoma foll
- > cells
- > mitosis
- Capsular invasion (difficult diagnosis with FNAB); angioinvasion
- IHC: similar to adenoma (vim, low-weight ck, thyroglobulin)
- PROGNOSIS: metastasis via bloodstream (lung, bone, snc, liver); good prognosis

Minimally invasive follicular carcinoma

Negative prognostic factors:

- Age >50 years
- Distant metastasis
- Size >4 cm
- Angioinvasion
- Extracapsular extension
- Scarcely differentiated areas

HURTHLE CELL LESIONS

BENIGN AND MALIGNANT

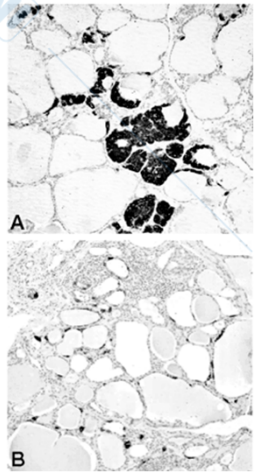
- From 1 to varying cm, mahogany brown in color
- haemorragic areas, necrosis
- Hurthle cells
- Follicular pattern
- Diagnosis of malignancy: invasion of the capsule and/or angioinvasion
- Metastasis via lymphatic system or bloodstream
- Thyroglobulin +

Medullary Carcinoma

- Malignant tumor derived from C-cells, parafollicular
- <10%
- Highly aggressive
- Associated with hereditary syndromes: MEN 2A e 2B
- Preceded by C-cell hyperplasia

Medullary Carcinoma

C-cell hyperplasia



Sporadic: 80%, ages 40-60, solitary, usually have paraneoplastic syndromes (diarrhea from VIP, Cushing's syndrome), dysphagia and hoarseness from tumor bulk
Familial: 20%, due to MEN 2A, 2B syndromes (has >90% penetrance), familial medullary thyroid carcinoma syndrome, von Hippel-Lindau disease or neurofibromatosis; usually bilateral, multicentric and younger patients (mean age 35), usually discovered by screening serum calcitonin or peripheral blood RET oncogene mutational analysis
MEN 2A: due to germ line mutations in RET proto-oncogene; fewer distant metastases
RET: normally expressed in C cells; carcinomas may have germline point mutations causing constitutive activation of receptor

Medullary Carcinoma

MACRO:

- **Variable in size**
- **Soft, not capsulated**
- **Calcifications and fibrosis**
- **Bilateral, above all in familial syndromes**

Medullary Carcinoma

MICRO:

- **Varied histology**
- **Solid, trabecular pattern, invades surrounding tissue**
- **Follicular or papillary pattern**
- **Round or fused cells with "salt-and-pepper" nucleus**
- **Mucin vacuoles**
- **Necrosis and hemorrhage in the larger lesions**

Medullary Carcinoma

MICRO:

- **Amyloid (80% of cases)**
- **Diffusion via lymphatic system and bloodstream**

IHC:

- **Calcitonin, CEA**
- **ACTH, HCG, substance P, VIP, glucagon, gastrin,....**
- **NSE, CHR, SYN**

Medullary Carcinoma

Favorable prognostic factors:

- Young age,
- Female sex,
- Hereditary forms,
- Microcarcinoma

Unfavorable prognostic factors:

- Advanced age
- Male sex,
- Sporadic forms,
- Elevatad mitotic activity,
- Small cells

Anaplastic Carcinoma (undifferentiated ca)

·MICRO:

- Giant or fused cells; clear cells; atypical

·Invasion of surrounding tissue, palisade necrosis, angioinvasion

- fused cell lesions associated with giant "osteoclast-like" elements (stromal or histiocytic origin)

·IHC: low and high molecular weight cytokeratin; thyroglobulin +/-

Anaplastic Carcinoma (undifferentiated ca)

·Highly aggressive (life expectancy of three months from diagnosis)

- A pre-exisiting low-grade lesion is hypothesized
- Women >60 years of age
- Rapidly growing mass (dispnea and dysfagia)
- Lymph node and distant metastasis
- MACRO: invasive mass with necrosis and hemorrhage

2016 WHO CLASSIFICATION of CNS TUMORS

1. **Neuroepithelial T**
2. **Peripheral nerve T**
3. **Meningeal T**
4. **Haematopoietic T and lymphoma**
5. **Germ cell T**
6. **Sellar T**
7. **Metastatic T**

The classification of brain tumors has been based largely on concepts of histogenesis that tumors can be classified according to their microscopic similarities with different putative cells of origin and their presumed levels of differentiation.

The characterization of such histological similarities has been primarily dependent on:

- light microscopic features in hematoxylin and eosin-stained sections,
- immunohistochemical expression of lineage-associated proteins
- ultrastructural characterization.

CNS Tumors

- Intracranial (intraparenchymal and extraparenchymal)
- Extracranial
- Intraparenchymal and extraparenchymal (primary and metastatic)

1. Cytotype
2. Mixed cell
3. Mixed differentiation
4. Localization (supratentorial,adult; subtentorial,children)

NEUROEPITHELIAL TUMORS

- **ASTROCYTIC**
- **OLIGODENDRIAL**
- **OLIGOASTROCYTIC**
- **EPENDYMAL**
- **CHOROID PLEXUS**
- **OTHER GLIOMA**
- **NEURONAL AND NEURONAL-GLIAL MIXED T**
- **PINEAL PARENCHYMA**
- **EMBRYONAL T(MB, CNS PNET, ATRT)**

CNS Tumors

• CNS primary T are 9% out of all the tumors

• T account for 20% of all cancers of childhood;

➢ Clinical features (localization and growth pattern)

•Headache (high intracranial pressure)

•Epilepsia (infiltrative T)

•False focal sign (III e VI nerve palsy) cerebral herniation and pressure due to per erniazioni e presione

•Functional failures with a slow progression

•Oedema, hemorrhage, hydrocephalus

Biological features

•Biological malignancy/histological malignancy

•infiltrating

•Intraneural dissemination

•Extraneural metastases (uncommon)

Differential diagnosis

T. with histological similar features

- Different histogenesis
- Different prognosis

Classificazione WHO 2007

The current update (2016 CNS WHO) thus breaks with the century-old principle of diagnosis based entirely on microscopy by incorporating molecular parameters into the classification of CNS tumor entities

The use of "integrated" phenotypic and genotypic parameters for CNS tumor classification adds a level of objectivity that has been missing from some aspects of the diagnostic process in the past.

It is hoped that this additional objectivity will yield more biologically homogeneous and narrowly defined diagnostic entities than in prior classifications, which in turn should lead to greater diagnostic accuracy as well as improved patient management and more accurate determinations of prognosis and treatment response.

It will, however, also create potentially larger groups of tumors that do not fit into these more narrowly defined entities (e.g., the not otherwise specified/NOS designations, see below)—groups that themselves will be more amenable to subsequent study and improved classification.

A compelling example of this refinement relates to the diagnosis of oligoastrocytoma—a diagnostic category that has always been difficult to define.

Using both genotype (i.e., IDH mutation and 1p/19q codeletion status) and phenotype to diagnose these tumors results in nearly all of them being compatible with either an astrocytoma or oligodendroglioma, with only rare reports of molecularly "true" oligoastrocytomas consisting of histologically and genetically distinct astrocytic (IDH-mutant, *ATRX-mutant, 1p/19q-intact)* and oligodendroglial (IDH-mutant, *ATRX-wildtype and* 1p/19q-codeleted) tumor populations

As detailed below, CNS tumor diagnoses should consist of a histopathological name followed by the genetic features, with the genetic features following a comma and as adjectives, as in:

•*Diffuse astrocytoma, IDH-mutant*
• *Medulloblastoma, WNT-activated.*

The diagnostic use of both histology and molecular genetic features also raises the possibility of discordant results, e.g., a diffuse glioma that histologically appears astrocytic but proves to have IDH mutation and 1p/19q codeletion, or a tumor that resembles oligodendroglioma by light microscopy but has IDH, *ATRX and TP53 mutations* in the setting of intact 1p and 19q. Notably, in each of these situations, the genotype trumps the histological phenotype, necessitating a diagnosis of o*ligodendroglioma, IDH-mutant and 1p/19q-codeleted in the first instance and diffuse astrocytoma, IDH-mutant in the second.*

WHO classification of tumours of the central nervous system

Diffuse astrocytic and oligodendroglial tumours	
Diffuse astrocytoma, IDH-mutant	9400/3
Gemistocytic astrocytoma, IDH-mutant	9411/3
Diffuse astrocytoma, IDH-wildtype	*9400/3*
Diffuse astrocytoma, NOS	9400/3
Anaplastic astrocytoma, IDH-mutant	9401/3
Anaplastic astrocytoma, IDH-wildtype	*9401/3*
Anaplastic astrocytoma, NOS	9401/3
Glioblastoma, IDH-wildtype	9440/3
Giant cell glioblastoma	9441/3
Gliosarcoma	9442/3
Epithelioid glioblastoma	*9440/3*
Glioblastoma, IDH-mutant	9445/3*
Glioblastoma, NOS	9440/3
Diffuse midline glioma, H3 K27M-mutant	9385/3*
Oligodendroglioma, IDH-mutant and 1p/19q-codeleted	9450/3
Oligodendroglioma, NOS	9450/3
Anaplastic oligodendroglioma, IDH-mutant and 1p/19q-codeleted	9451/3
Anaplastic oligodendroglioma, NOS	*9451/3*
Oligoastrocytoma, NOS	*9382/3*
Anaplastic oligoastrocytoma, NOS	*9382/3*
Other astrocytic tumours	
Pilocytic astrocytoma	9421/1
Pilomyxoid astrocytoma	9425/3
Subependymal giant cell astrocytoma	9384/1
Pleomorphic xanthoastrocytoma	9424/3
Anaplastic pleomorphic xanthoastrocytoma	9424/3
Ependymal tumours	
Subependymoma	9383/1
Myxopapillary ependymoma	9394/1
Ependymoma	9391/3
Papillary ependymoma	9393/3
Clear cell ependymoma	9391/3
Tanycytic ependymoma	9391/3
Ependymoma, *RELA* fusion-positive	9396/3*
Anaplastic ependymoma	9392/3
Other gliomas	
Choroid glioma of the third ventricle	9444/1
Angiocentric glioma	9431/1
Astroblastoma	9430/3
Choroid plexus tumours	
Choroid plexus papilloma	9390/0
Atypical choroid plexus papilloma	9390/1
Choroid plexus carcinoma	9390/3
Neuronal and mixed neuronal-glial tumours	
Dysembryoplastic neuroepithelial tumour	9413/0
Gangliocytoma	9492/0
Ganglioglioma	9505/1
Anaplastic ganglioglioma	9505/3
Dysplastic cerebellar gangliocytoma (Lhermitte-Duclos disease)	9493/0
Desmoplastic infantile astrocytoma and ganglioglioma	9412/1
Papillary glioneuronal tumour	9509/1
Rosette-forming glioneuronal tumour	9509/1
Diffuse leptomeningeal glioneuronal tumour	
Central neurocytoma	9506/1
Extraventricular neurocytoma	9506/1
Cerebellar liponeurocytoma	9506/1
Paraganglioma	8693/1
Tumours of the pineal region	
Pineocytoma	9361/1
Pineal parenchymal tumour of intermediate differentiation	9362/3
Pineoblastoma	9362/3
Papillary tumour of the pineal region	9395/3
Embryonal tumours	
Medulloblastomas, genetically defined	
Medulloblastoma, WNT-activated	9475/3*
Medulloblastoma, SHH-activated and *TP53*-mutant	9476/3*
Medulloblastoma, SHH-activated and *TP53*-wildtype	9471/3
Medulloblastoma, non-WNT/non-SHH	9477/3*
Medulloblastoma, group 3	
Medulloblastoma, group 4	
Medulloblastomas, histologically defined	
Medulloblastoma, classic	9470/3
Medulloblastoma, desmoplastic/nodular	9471/3
Medulloblastoma with extensive nodularity	9471/3
Medulloblastoma, large cell / anaplastic	9474/3
Medulloblastoma, NOS	9470/3
Embryonal tumour with multilayered rosettes, C19MC-altered	9478/3*
Embryonal tumour with multilayered rosettes, NOS	*9478/3*
Medulloepithelioma	9501/3
CNS neuroblastoma	9500/3
CNS ganglioneuroblastoma	9490/3
CNS embryonal tumour, NOS	9473/3
Atypical teratoid/rhabdoid tumour	9508/3
CNS embryonal tumour with rhabdoid features	*9508/3*
Tumours of the cranial and paraspinal nerves	
Schwannoma	9560/0
Cellular schwannoma	9560/0
Plexiform schwannoma	9560/0

Melanotic schwannoma	9560/1
Neurofibroma	9540/0
Atypical neurofibroma	9540/0
Plexiform neurofibroma	9550/0
Perineurioma	9571/0
Hybrid nerve sheath tumours	
Malignant peripheral nerve sheath tumour	9540/3
Epithelioid MPNST	9540/3
MPNST with perineurial differentiation	9540/3
Meningiomas	
Meningioma	9530/0
Meningothelial meningioma	9531/0
Fibrous meningioma	9532/0
Transitional meningioma	9537/0
Psammomatous meningioma	9533/0
Angiomatous meningioma	9534/0
Microcystic meningioma	9530/0
Secretory meningioma	9530/0
Lymphoplasmacyte-rich meningioma	9530/0
Metaplastic meningioma	9530/0
Chordoid meningioma	9538/1
Clear cell meningioma	9538/1
Atypical meningioma	9539/1
Papillary meningioma	9538/3
Rhabdoid meningioma	9538/3
Anaplastic (malignant) meningioma	9530/3
Mesenchymal, non-meningothelial tumours	
Solitary fibrous tumour / haemangiopericytoma**	
Grade 1	8815/0
Grade 2	8815/1
Grade 3	8815/3
Haemangioblastoma	9161/1
Haemangioma	9120/0
Epithelioid haemangioendothelioma	9133/3
Angiosarcoma	9120/3
Kaposi sarcoma	9140/3
Ewing sarcoma / PNET	9364/3
Lipoma	8850/0
Angiolipoma	8861/0
Hibernoma	8880/0
Liposarcoma	8850/3
Desmoid-type fibromatosis	8821/1
Myofibroblastoma	8825/0
Inflammatory myofibroblastic tumour	8825/1
Benign fibrous histiocytoma	8830/0
Fibrosarcoma	8810/3
Undifferentiated pleomorphic sarcoma / malignant fibrous histiocytoma	8802/3
Leiomyoma	8890/0
Leiomyosarcoma	8890/3
Rhabdomyoma	8900/0
Rhabdomyosarcoma	8900/3
Chondroma	9210/0
Chondrosarcoma	9220/3
Osteoma	9180/0
Osteochondroma	9210/0
Osteosarcoma	9180/3
Melanocytic tumours	
Meningeal melanocytosis	8728/0
Meningeal melanocytoma	8728/1
Meningeal melanoma	8720/3
Meningeal melanomatosis	8728/3
Lymphomas	
Diffuse large B-cell lymphoma of the CNS	9680/3
Immunodeficiency-associated CNS lymphomas	
AIDS-related diffuse large B-cell lymphoma	
EBV-positive diffuse large B-cell lymphoma, NOS	
Lymphomatoid granulomatosis	9766/1
Intravascular large B-cell lymphoma	9712/3
Low-grade B-cell lymphomas of the CNS	
T-cell and NK/T-cell lymphomas of the CNS	
Anaplastic large cell lymphoma, ALK-positive	9714/3
Anaplastic large cell lymphoma, ALK-negative	9702/3
MALT lymphoma of the dura	9699/3
Histiocytic tumours	
Langerhans cell histiocytosis	9751/3
Erdheim–Chester disease	9750/1
Rosai–Dorfman disease	
Juvenile xanthogranuloma	
Histiocytic sarcoma	9755/3
Germ cell tumours	
Germinoma	9064/3
Embryonal carcinoma	9070/3
Yolk sac tumour	9071/3
Choriocarcinoma	9100/3
Teratoma	9080/1
Mature teratoma	9080/0
Immature teratoma	9080/3
Teratoma with malignant transformation	9084/3
Mixed germ cell tumour	9085/3
Tumours of the sellar region	
Craniopharyngioma	9350/1
Adamantinomatous craniopharyngioma	9351/1
Papillary craniopharyngioma	9352/1
Granular cell tumour of the sellar region	9582/0
Pituicytoma	9432/1
Spindle cell oncocytoma	8290/0
Metastatic tumours	

The morphology codes are from the International Classification of Diseases for Oncology (ICD-O) [142A]. Behaviour is coded */0* for benign tumours; */1* for unspecified, borderline, or uncertain behaviour; */2* for carcinoma in situ and grade III intraepithelial neoplasia; and */3* for malignant tumours. The classification is modified from the previous WHO classification, taking into account changes in our understanding of these lesions.
*These new codes were approved by the IARC/WHO Committee for ICD-O.
Italics: Provisional tumour entities. **Grading according to the 2013 *WHO Classification of Tumours of Soft Tissue and Bone*

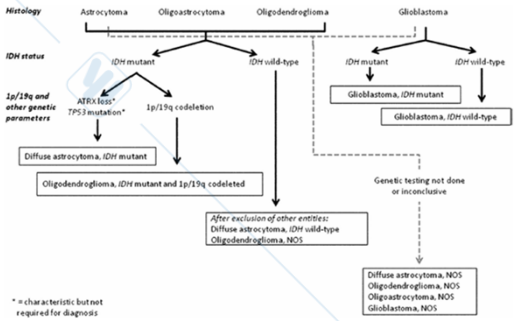


Fig. 1 A simplified algorithm for classification of the diffuse gliomas based on histological and genetic features (see text and 2016 CNS WHO for details). A caveat to this diagram is that the diagnostic "flow" does not necessarily always proceed from histology first to molecular genetic features next, since molecular signatures can sometimes outweigh histological characteristics in achieving an "integrated" diagnosis. A similar algorithm can be followed for anaplastic-level diffuse gliomas; * Characteristic but not required for diagnosis. Reprinted from [27], with permission from the WHO

Table 3 Grading of selected CNS tumors according to the 2016 CNS WHO

WHO grades of select CNS tumours	
Diffuse astrocytic and oligodendroglial tumours	
Diffuse astrocytoma, IDH-mutant	II
Anaplastic astrocytoma, IDH-mutant	III
Glioblastoma, IDH-wildtype	IV
Glioblastoma, IDH-mutant	IV
Diffuse midline glioma, H3 K27M-mutant	IV
Oligodendroglioma, IDH-mutant and 1p/19q-codeleted	II
Anaplastic oligodendroglioma, IDH-mutant and 1p/19q-codeleted	III
Other astrocytic tumours	
Pilocytic astrocytoma	I
Subependymal giant cell astrocytoma	I
Pleomorphic xanthoastrocytoma	II
Anaplastic pleomorphic xanthoastrocytoma	III
Ependymal tumours	
Subependymoma	I
Myxopapillary ependymoma	I
Ependymoma	II
Ependymoma, *RELA* fusion–positive	II or III
Anaplastic ependymoma	III
Other gliomas	
Angiocentric glioma	I
Chordoid glioma of third ventricle	II
Choroid plexus tumours	
Choroid plexus papilloma	I
Atypical choroid plexus papilloma	II
Choroid plexus carcinoma	III
Neuronal and mixed neuronal-glial tumours	
Dysembryoplastic neuroepithelial tumour	I
Gangliocytoma	I
Ganglioglioma	I
Anaplastic ganglioglioma	III
Dysplastic gangliocytoma of cerebellum (Lhermitte–Duclos)	I
Desmoplastic infantile astrocytoma and ganglioglioma	I
Papillary glioneuronal tumour	I
Rosette-forming glioneuronal tumour	I
Central neurocytoma	II
Extraventricular neurocytoma	II
Cerebellar liponeurocytoma	II
Tumours of the pineal region	
Pineocytoma	I
Pineal parenchymal tumour of intermediate differentiation	II or III
Pineoblastoma	IV
Papillary tumour of the pineal region	II or III
Embryonal tumours	
Medulloblastoma (all subtypes)	IV
Embryonal tumour with multilayered rosettes, C19MC-altered	IV
Medulloepithelioma	IV
CNS embryonal tumour, NOS	IV
Atypical teratoid/rhabdoid tumour	IV
CNS embryonal tumour with rhabdoid features	IV
Tumours of the cranial and paraspinal nerves	
Schwannoma	I
Neurofibroma	I
Perineurioma	I
Malignant peripheral nerve sheath tumour (MPNST)	II, III or IV
Meningiomas	
Meningioma	I
Atypical meningioma	II
Anaplastic (malignant) meningioma	III
Mesenchymal, non-meningothelial tumours	
Solitary fibrous tumour / haemangiopericytoma	I, II or III
Haemangioblastoma	I
Tumours of the sellar region	
Craniopharyngioma	I
Granular cell tumour	I
Pituicytoma	I
Spindle cell oncocytoma	I

Reprinted from [27], with permission from the WHO

Table 4 Key characteristics of IDH-wildtype and IDH-mutant glioblastomas

	IDH-wildtype glioblastoma	IDH-mutant glioblastoma	References
Synonym	Primary glioblastoma, IDH-wildtype	Secondary glioblastoma, IDH-mutant	{1830}
Precursor lesion	Not identifiable; develops de novo	Diffuse astrocytoma Anaplastic astrocytoma	{1827}
Proportion of glioblastomas	~90%	~10%	{1797}
Median age at diagnosis	~62 years	~44 years	{214,1078,1797, 2103}
Male-to-female ratio	1.42:1	1.05:1	{214,1417,1797}
Mean length of clinical history	4 months	15 months	{1797}
Median overall survival			
Surgery + radiotherapy	9.9 months	24 months	{1797}
Surgery + radiotherapy + chemotherapy	15 months	31 months	{2810}
Location	Supratentorial	Preferentially frontal	{1417}
Necrosis	Extensive	Limited	{1417}
TERT promoter mutations	72%	26%	{1801,1830}
TP53 mutations	27%	81%	{1797}
ATRX mutations	Exceptional	71%	{1519}
EGFR amplification	35%	Exceptional	{1797}
PTEN mutations	24%	Exceptional	{1797}

Data from [29, 30]. Reprinted from [27], with permission from the WHO

WHO grade determinations are still made on the basis of histologic criteria.

DIFFUSE ASTROCYTOMA

- Macroscopy: because of their infiltrative nature these T usually show blurring of the grossanatomical boundaries

Diffuse astrocytoma, IDH mutant

•CNS (supratentorial, preferentiallyfrontal and temporal lobe)

•60% out of all primary CNS T

•diffusely infiltrating

•Young adults

• component resembling oligodendroglioma is compatible with this diagnosis in the absence of 1p/19q codeletion

Histopathological features: grading (St.Anne/Mayo)

WHO		StAnne/Mayo	
I	**Pylocytic astrocytoma**		
II	**Diffuse astrocytoma**	**Astro grade 2**	**nuclear atypia**
III	**Anaplastic astrocytoma**	**Astro Grade 3**	**+ mitoses**
IV	**glioblastoma**	**Astro Grade 4**	**+angiog + necrosis**

Histopathology

- Well differentiated fibrillary astrocytes
- Cellularity is moderately increased compared with that of normal brain
- Nuclear atypia; vesicular nucleus with intermediate masses of chromatin and often with a distinct nucleolus
- Mitotic activity generally absent (single mitosis does not justify the diagnosis of anaplastic astrocytoma

Genetic alterations:

- IDH1/2 mutations with a more favorable course from a IDH-wild type
- 7q gain and 10q loss negative factors

Genetic susceptibility

- Diffuse astro can occur in patients with inherited TP53 germilne mutation (Li-Fraumeni syndrome, although affected family members more frequently develop anaplastic astro

Prognosis and predictive factors

•Median survival time:6-8 yrs

Negative prognostic factors:

• age>40yrs

• limited surgical resection

•Astrocytoma histology

•Size T>6cm

•Tumor crossing the midline

•Neurological deficits prior to surgery

Inferior outcome

Histopathological factors: gemistocytic astrocytoma: early progression

ANAPLASTIC ASTROCYTOMA

A diffusely infiltrating astrocytoma with focal or dispersed anaplasia, signoficant proliferative activity and mutation in either the IDH1/IDH2 gene

Can arise from low-grade diffuse astro, but are commonnly diagnosed without a less-malignant precursor lesion

Intrinsic tendency for malignant progression to IDH mutant glioblastoma

•age: mean age 38yrs 41

•Sex: male > female

•Grading: III, high grade

•Localization: any region of the CNS ,but most frequently in the cerebrum

•Macro: infiltrating without causing frank tissue destruction; tumor mass

•Histopath: high cellularity, nuclear atypia and multinucleated cells., mitotic activity, microvascular proliferation and necrosis are abstent

•Immunephenotype:

•Genetic profile IDH1/2 and TP53 and ATRX in the majority of tumors; 9p and 19q loss higher frequence:

Survival: 3-5yrs

Prognostic factors: age, genetic mutations IDH1/2, extent of surgical resection

GLIOBLASTOMA IDH-wild type or Primary GBM IDH wild type

- a high grade glioma with astrocytic differentiation; nuclear atypia, cellular pleomorphism, mittic activity, diffuse growth pattern microvascular proliferation and/or necrosis lacking mutation of IDH1/2 genes, tipicaly affectind adults (62yrs)
- the most common and the most malignant astrocytic glioma accounting for 90% of all glioma
- Age: any age; preferentially affects older adults 55-85
- Incidence: 12-15% all intracranial CNS T
- Sex distribution: male to female 1.60:1
- Grade: IV
- Localization: subcortical white matter and deeper grey matter of cerebral emispheres (temporal>parietal>frontal>occipital lobe) IDH-mutant GBM have a striking predilection for the frontal lobe

•Clinical features: develops rapidly. Symptoms depend largely on T location, primarily manifesting as focal neurologcal deficits and tumor-associated oedema with increased intracranial pressure. Inaugural seizures

•Macro: poorly delineated greysh mass, colour variability with a central areas of yellowish necrosis from myelin breakdown. Red and brown foci of recent and remote haemorrhage. (butterfly pattern)

•Diffusion: often surprisingly large at time of diagnosis occupying much of a lobe. Usually unilateral but those in brain stem and corpus callosum can be bilaterally simmetrical (butterfly pattern)

• Few metastases via liquor, very rare haematogenic (iatrogenic)

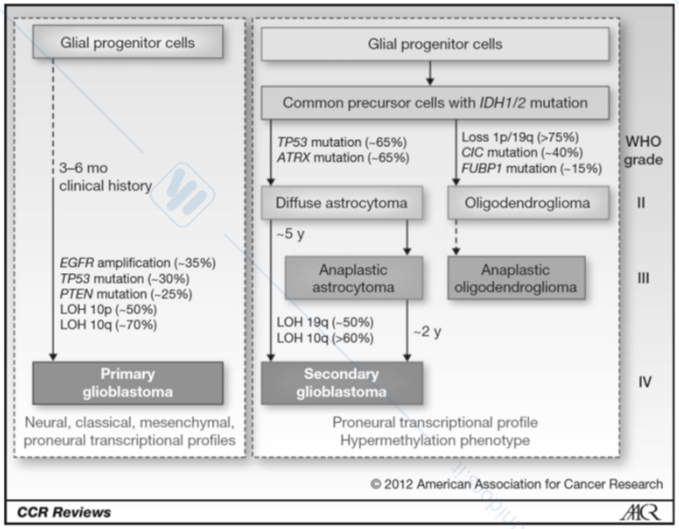
•Histopathology: poorly differentiated astrocytes., nuclear atypia and brisk mitotic activity, prominent microvascular proliferation (glomeruloid appearance), necrosis (palisading), gland-like structures, multinucleated giant cells, lipidized cells, granular cells, GFAP+

•EM: morphological variability, degenerating mitochondria, intermediate filaments (glial) depending from differentiation

•Genetics: mutazione TP53, gain ofLOH 17p,(progressione da grado III); LOH 10p, amplificazione EGFR (GBM de novo)

•Prognosis: almost invariably fatal<15/18 months from diagnosis indipendente da chemio- e radioterapia

•Prognostic factors: younger age (< 50 yrs), greater extent of necrosis -, complete surgical resection, MGMT promoter methylation and/or IDH mutation (closely associated with secondary GBM), , EGFR -/+, loss 10q -, PTEN ?.



Key characteristics of IDH-wildtype and IDH-mutant GBM in adults

	IDH wild type	IDH-mutated
Precursor lesion	De novo	Diffuse astro
Clinical history	4 mo	15 mo
Median survival • surgery +radioth • Surg+radioth+chemoth	 9,9 mo 15 mo	 24 mo 31mo
necrosis	extensive	limited
ATRX mut	exceptional	71%
TP53 mut	27%	81%
EGFR amplif	35%	exceptional

ASTROCITOMA PILOCITICO

•circumscribed, slow growing, biphasic pattern (bipolar cells with Rosenthal fibers + multipolar cells + microcysts and occasional granular bodies)

•Age: 10-20 yrs

•Clinical features: focal neurological deficits or non-localizing signs such as macrocephal, headache, endocrinopathy, increased intracranial pressure due to mass effect, visual loss, seizures uncommon

•Sex: no prevalence

•Grade: I

•Localization: neuraxis; in paediatric population are infratentorial; optic nerve, optic chiasm/hypothalamus, brain stem, cerebellum

•Macro: soft, grey , relatively discrete

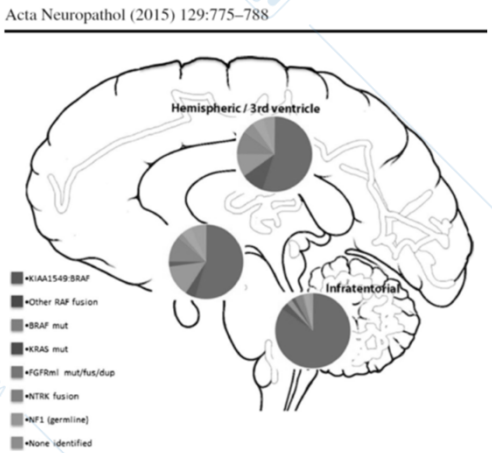
•Histopathology: biphasic pattern, scarsa cellularità, hyperchromatic nuclei, hair-like cell processes, rare mitoses, glomeruloid vascular proliferation, regressive changes (calcifications, infarct-like necrosis, lymphocytic infiltrate), GFAP +, Rosenthal fibers (hyaline masses intracytoplasmic, amorphous elements surrounded by intermediate-glial filaments,αβcristallina), EGB, globular aggregates within the astrocytic processes (α1chymotrypsin and α1trypsina)

•Immunophenotype: GFAP,S100, OLIG2, P53 neg

•Genetics: see next slide

•Genetic susceptibility: NF1 association (15% of indivuduals with NF1 develop pilocytic astro particularly ofthe optic nerve (optic patway glioma); Noonan syndrome (neuro.cardiac, facial-cutaneous syn) caused by mutationsMAPK pathway gene

•Prognosis : benign; slow growing, low-grade tumorwith a favorable prognosis. (>95%) ater surgical intervention alone; very occasional cases progress with more anaplastic features; patient age and extent of resection are key prognosticfactors



Oligodendroglial tumors

Oligodendroglioma

Anaplastic Oligodendroglioma

Mixed glioma

Oligoastrocytoma

Anaplastic Oligoastrocytoma

OLIGODENDROGLIOMA

•Oligodendroglial cells are well differentiated; diffusely infiltrating and slow growing glioma

•Age: 50-60 yrs, rare in children; Incidence: 4.2% CNS

•Sex: male:female = 1.3:1

•Grade: II

•Localization: the white matter and cortex of the frontal lobe is the most common location followed by the temporal, parietal and occipital

•Clinical features: seizures, headache and focal neurological deficits

•Macro: relatively well defined, soft, greyish-pink mass; occasionally densely calcified areas and cystic degeneration.

•Istopat: moderate cellularity, rounded in shape with clear cytoplasm (fried egg appearance); micrcalcifications and a dense network of delicate branching capillaries, no specific markers (S-100, Leu7, GFAP, Vim

•EM: round cells, electronlucent cytoplasm, regular nucleus, perinuclear microtubules and short microvilli and mitochondrial aggregates

•Molecular genetics : LOH 1p/19q; IHD1 or IDH2 mutated. Lacking ATRX mutation but virtually always carry activating mutation in the TERT promoter region. Strong expression of EGFRmRNA.

•Prognosis: Median survival 11.6yrs. Typically slow-growing tumor associated with a relatively long overall survival. Generally recurlocally

•Prognostic fators: young age, frontal localization, surgical resection

Ependymal tumors

Ependymoma

•Cellular

•Papillary

•Clear cells

•Tanicytic

Anaplastic Ependymoma

Mixopapillary Ependymoma

Subependymoma

EPENDYMOMA *RELA fusion-positive*

•Well differentiated ependymal cells; low growth children and young adults; ventricular walls

•Incidence: 3-9% of all neuroepithelial tumors

•Age: children, subtentorial

•30-40 yrs, spinal chord

•Sex: no prevalence

•Grade: II

•Localization: posterior fossa and spinal chord; III, IV and lateral ventricles

•Clinical features: depends on the localaization dalla sede (subtentorial, hydrocephalus; posterior fossa, cerebellar ataxia; supratentorial, neurological deficits, seizures)

•Macro: well circumscribed, soft and spongy, tan colored, occasionally calcium deposits and cysts, foci of necrosis or hemorragic

•Histopatology: monomorphic cells; round to oval nuclei perivascular pseudorosettes and true ependymal rosettes, few or rare mitosis, occasional necrosis; GFAP, Vim., S-100, EMA, focal CK

•Papillary,with well formed papillae

Clear cell, characterized by an oligodenrocyte-like appearance;

DD: Oligodendroglioma, neurocytoma; EM diagnostic role

Tanicytic, characterized by and arrangement of tumor cells in fascicles of variable width and by elongated cells

EM: fusiform cells, glial filaments, zipper-like junctions, microrosettes with microvilli and cilia

MIXOPAPILLARY EPENDYMOMA

Young adults, filum terminale of the cauda equina, papillary pattern

•Incidence: 13% of ependymomas; intraspinal very common

•Age: averages 36 yrs

•Sex: female > male

•Grade: I

•Localization: cauda, rarely hemisphere

•Clinical features: back pain often of long duration

•Macro: soft, lobulated and grey or tan in color; often encapsulated

•Histology: papillary pattern with cuboidal to elongated cells, mixoid matrix, few ir rare mitosis; ICC: GFAP, S-100, Vim

•EM: zipper-like junctions, microrosettes

•Genetics: correlated with NF2

•Genetic profile: broad range of cytogenetic aberrations (gains of 1q, 5, 7, 9 etc. and losses of 1p, 3, 6q, etc.; LOH 17p). Rela fusion-positive ependymoma accounts for approximately 70% of all childhood supratentorial tumors exhibiting a range of histopathological features. Rela fusion-positive ependymoma have the wirst outcome of the supratentorial group.

•Prognosis and predictive factors: children have a worse prognosis than adults; <1 yr 42% have a 5yr overall survival rate and 76% among patients 10-14 yrs .

• extent of surgical resection is a rliable indicator of outcome; gross total resection is associated with siginficantky improved survival.

•Localization (spinal > sovratentorial > fossa).

- Prognosis: favorable with a 5 year survval rate of 98,4% after total or partial resection. Late recurrence and distant metastases can occur after incomplete resections in both adults and children

Neuronal tumors and mixed glio-neuronal tumors

Gangliocytoma

Desmoplastic Infantile Astrocytoma, DIG / Ganglioglioma

DNT, Dysembryoplastic neuroepithelial tumors

Ganglioglioma

Anaplastic Ganglioglioma

Central Neurocytoma

Cerebellar Liponeurocytoma

Paraganglioma

Central neurocytoma

•Uncommon intraventricular neoplasm composed by uniform, round cells with a neuronal immunophemotype and low proliferation rate; region of Foramen of Monro; favorable prognosis

•Incidence: 0.25-0.5% SNC

•Age: young adults, mean patient age, 28,5 yrs

•Sex: both sexes equally affected

•Grade: II

•Localization: supratentoriallly in the lateral ventricle and/or III ventricle;

•Clinical features: increased intracranial pressure, occasionally visual and cognitive deficits

•Macro: greyish and friable mass; calcifications and haemorrhages can occur,

•Microscopy: uniform round cells, oligo-like; ultrastructuiral features of neuronal differentiation; fibrillar areas mimicking neuropil; low proliferation rate; arborizing microvasculature pattern; ICC : Synaptophysin (false + or -), NeuN, MAP2 or class III beta-tubulin

• DD oligodendroglioma, clear cell ependymoma, pineocytoma, DNT

•EM: round cell, uniform nuclei, microtubules , neuroendocrines granules, prolungamenti neuritici cytoplasmic projections with microtubules, synaptic-like structures

•Genetic profile: DNA copy number alterations (MYCN gain, overexpression of WNT pattern genes, calcium function and maintenance of neural progenitors

•Prognosis and predictive factors: usually benign with the extent of resection being the most important prognostic factor; craniospinal dissemination is exceptional; atypical histological features is more controversial.

GANGLIOGLIOMA

•Combination of neuronal and glial cell elements, with mrked heterogeneity; dysplastic neurons, multipolari + glial component showing variability, but constitutes the the proliferative cell population of tumor; no Necrosis Incidenza: 0.4% SNC

•Age: range from 2 months to 70 yrs; mean age 8,5-25yrs

•Sex: male:female=1.1:1

•Grado: I

•Localization: throughout the CNS, including cerebrum, brain stem, cerebellum, spinal cord, optic nerve, pituitary, pineal gland

•Clinical features: according with tumor size and site

•Macro: well defined solid or cystic lesion; calcification may be observed; haemorrhage and necrosis are rare

MEDULLOBLASTOMA

•Embryonal neuroepithelial tumor, cerebellum or dorsal brain stem, mainly in childhood;densely packed small round undifferentiated cells with mild to moderate nuclear pleomorphism abd a high mitotic count

•Incidence: annual overall incidence 1,8 cases x 1.000.000; the annual childhood is 6 cases x 1.000.000

•Age and sex distribution: median 9 yrs anni; adulthood, 21-40 yrs; male-to-female ratio is 2:1

•Grading: irrespective of histological or genetic characterization, IV

•Localization: fourth ventricle, cerebellar parenchyma, (vermis); some laterally located in a hemisphere

•Clinical features: MB growing in the fourth ventricle causes increased intracranial pressure by exerting mass effect and blocking cerebrospinal fuid pathways; nausea upon waking, bouts of vomiting; cerebellar ataxia

•Spread: MB like other embryonal tumors has propensity to spread through cerebrospinal fluid pathways to seed neuraxis with metastatic tumor deposits; rarely outside the CNS

•Macroscopy: pink or grey often friable masses that fill the IV ventricle; in the cerebellar emisphere tend to be firm and more circumscribed generally corresponding to to desmoplastic/nodular variant with SHH pathway; necrosis

•Histopathology: several morphological variants

Classic MB, syncytial arrangement of densely packed undifferentiated embryonal cells; mitotic figures; Homer-Wright rosettes che

Desmoplastic/nodular MB nodular, reticulin-free zones (pale islands) surrounded by densely-packed, undifferentiated proliferative cells with hyperchromatic , pleomorphic nuclei

MB with extensive nodularity, an expanded lobular architecture due to reticulin-free zones becomes unusually enlarged and rich in meuropile-like tissue containing a population of small cells with round nuclei with neurocytic differentiation and exhibit a streaming pattern; mitotic activity low or absent in these neurocytic areas

Large cells/anaplastic MB marked nuclear pleomorphism accompanied by particularly high mitotic and apoptotic counts; nuclear moulding and «cell wrapping»; large cell variant lack variability in cell size and shape characterizing the anaplastic variant (nearly all contain regions with anaplastic phenotype)

ICC: Syn, class III beta tubulin, NeuN, GFAP (10% of MB), NFPs is rare

EM: neuroblastic differentiation with neurites; neuroendrocrine granules; synaptic-like structures; glial differentiation areas

•Genetic susceptibility: associated with several inherited cancer syndromes: nevoid basal Ca (Gorlin S); Li-Fraumeni S.; mismatch repair cancer S. (Turcot S.)

Table 5 Summary of the most common integrated medulloblastoma diagnoses, with clinical correlates

Genetic profile	Histology	Prognosis
Medulloblastoma, WNT-activated	Classic	Low-risk tumour; classic morphology found in almost all WNT-activated tumours
	Large cell / anaplastic (very rare)	Tumour of uncertain clinicopathological significance
Medulloblastoma, SHH-activated, *TP53*-mutant	Classic	Uncommon high-risk tumour
	Large cell / anaplastic	High-risk tumour; prevalent in children aged 7–17 years
	Desmoplastic / nodular (very rare)	Tumour of uncertain clinicopathological significance
Medulloblastoma, SHH-activated, *TP53*-wildtype	Classic	Standard-risk tumour
	Large cell / anaplastic	Tumour of uncertain clinicopathological significance
	Desmoplastic / nodular	Low-risk tumour in infants; prevalent in infants and adults
	Extensive nodularity	Low-risk tumour of infancy
Medulloblastoma, non-WNT/non-SHH, group 3	Classic	Standard-risk tumour
	Large cell / anaplastic	High-risk tumour
Medulloblastoma, non-WNT/non-SHH, group 4	Classic	Standard-risk tumour; classic morphology found in almost all group 4 tumours
	Large cell / anaplastic (rare)	Tumour of uncertain clinicopathological significance

Reprinted from [27], with permission from the WHO

LC/A large cell/anaplastic, *DN* desmoplastic/nodular, *MBEN* medulloblastoma with extensive nodularity

•Molecular genetics: MYC; LOH 17p, 1q, 10q,5q (APC)

•Prognosis and predictive factors: 50-70% 5 yrs survival

Negative factors: age<3 yrs, metastases at the onset, partial surgical removal, Anaplastic/large cell MB, glial differentiation, LOH 17p, MYC amplification

Positive factors: Desmoplastic/nodular MB, MB with extensive nodularity

MENINGIOMA

•Slow growing , mostly benign tumour deriving from meningothelial cells of arachnoid layer

•Incidence: 36% of all CNS T ; 90% are solitary

•Age and sex distribution: median age 65 yrs; females are at greater risk than males (annual incidence 10,5 cases x 100.000)

•Grading: I, II (Chordoid M; Atypical M), III (Papillary M, Rhabdoid M, Anaplastic M)

•Localization: intracranial, intraspinal or orbital locations (cerebral convexities in association with the falx, olfactory grooves, optic nerve sheath and posterior fossa),; metastases most often involve lung, pleura, bone, liver

•Clinical features: neurological signs and symptoms due to compression of adjacent structures; headache and seizures are common

•Macro: rubbery or firm, well demarcated,sometimes lobulated

•Histopathology: a wide range of histological appearances

Meningothelial or sinciziale, whorls e corpi psammomatosi

Fibrous, cellule fusate in fasci separati da abbondante collagene

Transitional, intermediate between meningothelial and fibrous

Atypical Meningioma, mitoses plus 3 or more following oarameters (cellularity, high N/C, prominent nucleolus, sheet-likenecrosis),

Anaplastic Meningioma, sarcoma-like, >20 mitoses x 10HPF

Variants: Psammomatous, microcystic, secretory, chordoid (II), a clear cells (II) , papillary (III), rhabdoid (III)

•ICC: EMA, VIM, S-100, CEA (secretory)

•EM: desmosomes, interdiditated cytoplasmic projections, intermediate filaments, nuclei with pseudoinclusions.

•Genetics:

Meningioma, grade I – LOH 22q, NF2 mutation

Meningioma atypical, grado II – LOH 1p, 6q, 10q, 18q; gain 1q, 9q, 12q, 15q, 17q, 20q.

Anaplastic meningioma, grade III – LOH 6q, 9p, 10 e 14 q, amplification 17q, rare TP53, PTEN mutation, rare deletions CDKN2A

•PROGNOSIS: recurrences are difficult to prognose and survival (grado III); extent of resection, localization, age, grading, proliferation index (MIB1), progesteron receptor.

Ischemic Heart Disease, IHD

•IHD and its various forms is the leading cause of death for both men and women in the Usa and other industrialized nations.

•80% of all deaths due to cardiovascular diseases

=

imbalance between supply (perfusion) and demand of the heart for oxygenated blood

In more than 90% of cases the cause of myocardial ischemia is the reduction in coronary blood flow due to atherosclerotic coronary arterial obstructions

IHD is termed coronary artery disease

ATHEROSCLEROSIS

UNPREDICTABLE AND ABRUPT CONVERSION OF A STABLE ATHEROSCLEROTIC PLAQUE TO AN UNSTABLE POTENTIALLY LIFE-THREATENING ATHEROTHROMBOTIC LESION THROUGH SUPERFICIAL EROSION, ULCERATION, FISSURING RUPTURE OR DEEP HEMORRAGE, USUALLY WITH SUPERIMPOSED THROMBOSIS

Permanent coronary stenosis (75% of arterial lumen)

Secondary effects

•Platelet aggregation + vasospasm

•Vasospasm (endothelial dysfunction with a reduces secretion of vasodilatative factors ;smoke induced vasoconstriction);

Clinical manifestation of IHD

•Angina pectoris

•Sudden heart death

•Myocardial infarct

•Chronc ischemic heart disease

Angina pectoris

Is characterized by paroxysmal and usually recurrent attacks of substernal or precordial chest discomfort caused by transient (15 sec to 15 min) myocardial ischemia inducing cellular necrosis that defines infarcion

•Owing to fixed stenosing plaques
•Thrombosis
•Platelet aggregation
•embolization

Myocardium infarction

•Defined myocardium area of necrosis caused by focal ischemia (20-30 min necrosis post-ischemia; 3-6 hrs full extent of ischemic area)

•Size and localization depend on coronary circle anatomy (R or L dominant) and on collateral circle

Sudden cardiac death

Unexpected death from cardiac causes early (usually within 1 hour) after or without the onset of symptoms

It is a complication and often the first clinical manifestation of IHD
•Atherosclerotic causes
•Non atherosclerotic causes:

➢Congenital structural or coronary arterial abnormalities
➢Aortic valve stenosis
➢Mitral valve stenosis
➢Myocarditis
➢Dilated or hyperthrophic cardiomyopathy
➢Pulmonary hyoertension

EPIDEMIOLOGY

Prevention

Therapeutic advances (coronary care unit, trombolysis, angioplasty, stents, coronary by-pass)

Risk factors

•hypertension
•smoking
•Elevated blood cholesterol.

Transmural versus subendocardial infarction

- Most of the MI are Transmural in which the ischemic necrosis involves the full or full thickness of the ventricular wall in the distribution of a singlle coronary artery (chronic coronay atherosclerosis, acute plaqua change, obstructive thrombosis)

- Subendocardial infarcts constitutes an area of ischemic necrosis limited to the inner one third or at most one half of the ventricular wall

- The subendocardial area is normally the least well perfused region and the most vulnerable zone to any reduction in coronary flow.
- Diffuse stenosing coronary atherosclerosis and reduction of coronary flow.

In approximately 10% of cases transmural MI is not associated with Atheroscleotic plaque thrombosis. Other mechanisms as follows:

- Vasospasm associated with platelet aggregation;

- Emboli from left atrium in association with atrial fibrillation, a left-side mural thrombus or vegetative endocarditis

- Unexplained: one third of such cases the coronary arteries are free of obstruction by angiography (unusual diseases of small intramural coronary vessels, hematologic abnormalities such as hemoglobinopathies or other disorders.

Sequence of events

- Sudden change in the morphology of an atheromatous plaque (disruption)
- Exposed to subendothelial collagen andnecrotic plaque contents, platelets undergo adhesion, aggregation, activation and rlease of potent aggregators
- Activation of the extrinsic pathway of coagulation and adding to the bulk of the thrombus
- the thrombus evolves to occlude completely the lumen of the culprit coronary vessel
- Therapeutic thrombolysis and primary angioplasty

MI etiology

. 85% occlusione trombotica

• 10% plaque thromboembolic damage

• 5% platelet aggregation/vasospasm

MI localization

•Left descending coronary (anterior wall, ventricle apex and adjacent septum setto adiacente) – 40/50%

•Right coronary (posterior free wall and and ventricle basis)- 30/40%

•Circumflex coronary (lateral wall of Left ventricle)

MI Extensions

•Subendocardial (1/3 of the wall)

•Transmural

Differential diagnosis between subendocardial and transmural infarct

multifocal	focal
spotty	uniform
circumferential	In the area depending on coronary flow
Coronay thrombosis very rare	Coronary thrombosis common
hypotension and shock	shock
No Pericarditis	Pericarditis
No aneurysms or heart rupture	Aneurysms and heart rupture

Early stages:

. 10 sec, cyanotic and dilates in sys instead of contracting : if the cause is removed the contraction restarts even if it remains dpressed for many hours (Stunned myocardium)

•20-30 min of ischemia ⟶ myocyte necrosis

• for up to 12 hrs the infarct is not grossly identifiable
•Sarcolemma damage
⟶ enzyme loss (myoglobin, LDH. CK, Troponine I and T)

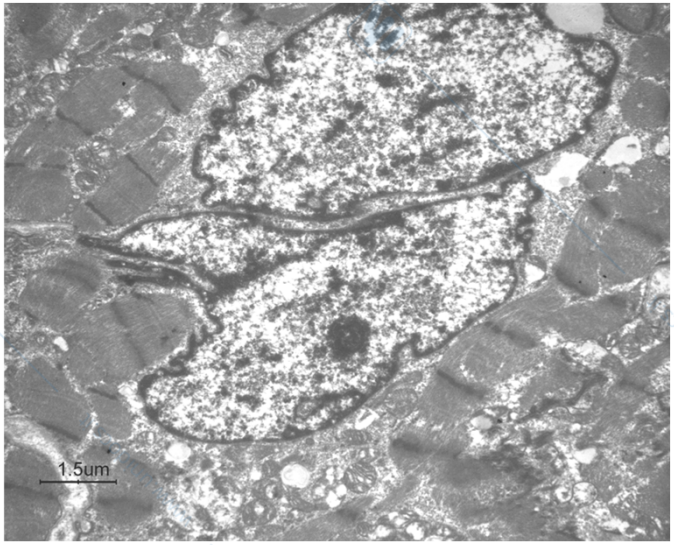
Morphological lesion of ischemic damage

•Coagulative necrosis (eosinophilic , piknotic nuclei)

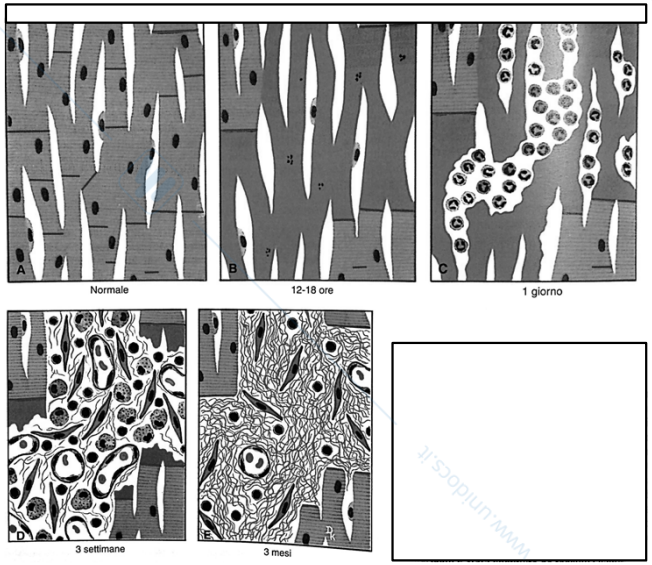
•Contraction band necrosis (Ca influx into degenerated cells or due to early reperfusion)

• Vacuolization (myocytolysis) characterizes subendocardial myocytes

Inflammation, healing



Time from onset	Gross morphological findings
18-24 hrs	Pallor of the myocardium
24-72 hrs	Pallor with some hyperemia
3-7 days	Hyperemic border with central yellowing
10-21 days	Maximally yellow and soft with vascular margins
7 weeks	White fibrosis



TIME FROM ONSET	MICROSCOPIC MORPHOLOGICAL FINDINGS
1-3 hrs	Wavy myocardial fibers
2-3 hrs	Staining defect with tetrazolium or basic fuchsin dye,coagulation necrosis with loss of cross striations, contraction bands, edema, hemorrage, and early neutrophilic infiltrate
18-24 hrs	Continuing coagulation necrosis, pyknosis of nuclei, and marginal contraction bands
24-72 hrs	Total loss of nuclei and striations along with heavy neutrophilic infiltrate
3-7 days	Macrophage and mononuclear infiltration begin, fibrovascular response begins
10-21 days	Fibrovascular response with prominent granulation tissue
7 weeks	fibrosis

Infarct consequences and complications

- **Arrythmias: electrolitic imbalance, drugs, high muscle pressure ⟹ sinus bradycardia, ventricular tachycardia, fibrillation or asystole**

- **CARDIOGENIC SHOCK, or pump failure : occurs in 10-15% of patients inducating a large infarct (often greater than 40% of the left ventricle).**
- **PROGRESSIVE LATE HEART FAILURE: compensatory hypertrophy and regeneration mechanism induces less cardiac compliance and increases lung pressure.**

MI complications

- **MYOCARDIAL RUPTURE: 4-7 days after onset results from the mechanical weakeniing that ocirs in necrotic and susequently inlfamed myocardium and includes rupture of the ventricular free wall or rupture of the ventricular septum, papillary muscle rupture.**
- **ACUTE PERICARDIS FIBRINOUS OR FIBROHAEMORRHAGIC pericarditis usually develops about the 2° or 3° day after a transmural infarct; it is the epicardial manifestation of the underlying myocardial inflammation**
- **INFARCT EXTENSION OR EXPANSION: new necrosis adjacent to an existing infarct or disproportionate stretching, thinning and dilation of the infarct region**

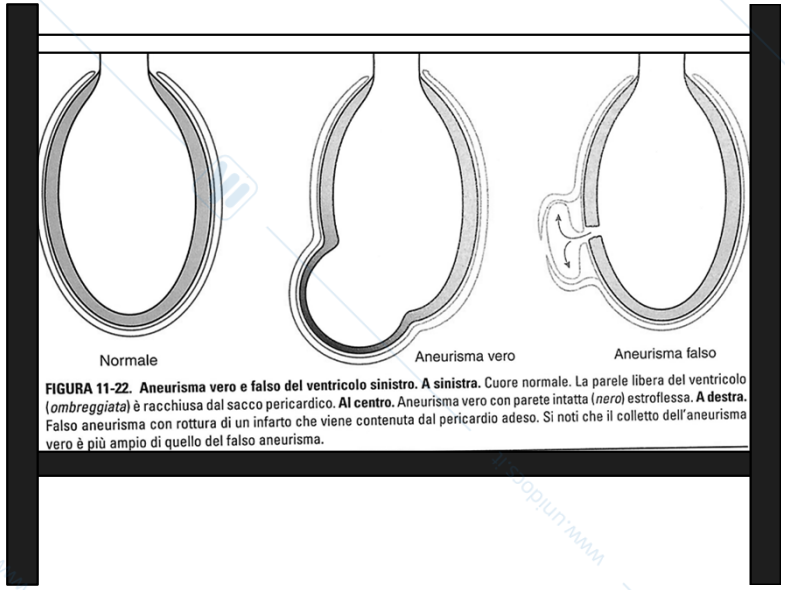


FIGURA 11-22. Aneurisma vero e falso del ventricolo sinistro. A sinistra. Cuore normale. La parele libera del ventricolo (*ombreggiata*) è racchiusa dal sacco pericardico. **Al centro.** Aneurisma vero con parete intatta (*nero*) estroflessa. **A destra.** Falso aneurisma con rottura di un infarto che viene contenuta dal pericardio adeso. Si noti che il colletto dell'aneurisma vero è più ampio di quello del falso aneurisma.

- VENTRICUALR ANEURISM: late complication results from a large transmural infarct that heals into a large region of thin scar tissue paradoxically bulging during systol.
- MURAL THROMBUS: combination of local abnormality in contractility (causing stasis) with endocardial damage (causing a thrombogenis surface) can foster mural thrombosis and poterntially thromboembolism.
- PAPILLARY MUSCLE DYSFUNCTION: post-infarct mitral regurgitation is most commonly due to early ischemic dysfuntcion of a papillary muscle and underlying myocardium without rupture and later to papillary muscle fibrosis.

Chronic ischemic disease

•The disease is caused by severe atherosclerosis involving all the largest coronary branches. This results in inadequate blood supply with myocyte loss and associated reactive fibrosis with interstitial collagen deposition.

• ⟹less cardiac compliance, cardiac dilation and reactive hypertrophy (survival myocyte overloading).

•Compensatory hypertrophy leads to progressive cardiac failure.

ANGINA – ACUTE INFARCT – DILATED CARDIOMYOPATHY

Ischaemic cardiomyopathy: impaired left ventricular function resulting from coronary artery disease causing myocardial injury and ventricular remodelling.

Stem cells (including bone marrow (BM)-derived stem cells, endothelial progenitor cells (EPCs) and resident cardiac stem cells), are involved in the natural response to ischaemic tissue injury and have become a promising target of clinical research over the last decade.

It has been proposed that **paracrine factors** may mediate the favourable effects of stem cell engraftment.

The **chemokine stromal cell-derived factor 1α** (SDF-1α/CXCL12) and its corresponding receptor CXCR4 have been identified as key regulator

Vascular endothelial growth factor (VEGF) is a major regulator of blood vessel formation through promoting endothelial cells (ECs) proliferation, migration and survival1.
The majority of ECs remain quiescent during adulthood and proliferate only after angiogenic activation mostly by stimulation of VEGF.

VEGF-mediated angiogenesis is integral for tissue restoration in cases of injury, **ischemia** and wound healing.

The administration of recombinant VEGF protein or VEGF gene into ischemic myocardium has been shown to enhance collateral vessel flow and improve cardiac function

The type I (about 80%) and type III (about 10%) collagen are the main components of cardiac ECM .

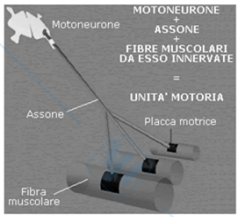
The production of type I collagen increases in ischemic area after MI.
Type I collagen may be used as the target for certain growth factors to improve cardiac function.

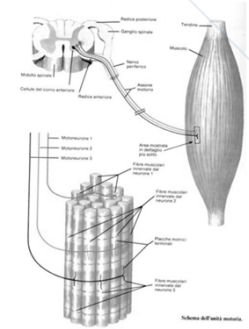
Fusion proteins of VEGF or SDF-1α combined with a polypeptide TKKTLRT named collagen-binding domain (CBD) have been demonstrated to significantly improve cardiac function after MI

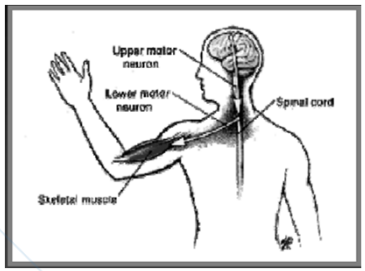
Neuromuscular disorders

Myopathies are muscle diseases characterized by symptoms and sign due to biochemical, electrophysological or pathological alterations of one of motor unit components:

1. Inferior motoneuron
2. Neuromuscular plaque
3. Muscle fiber (or interstitial tissue)







Functional muscle integrity is largely dependent on central and peripheral nervous system

Structural Aspects

Muscle fiber

- **Morphological syncitium**
- **Diameter up to 100 micron**
- **Lenght between 1 and 35 cm**
- **SARCOMER = morpho-functional unit**
- **ENDOMYSIUM**
- **PERIMYSIUM**
- **EPIMYSIUM**

Type 1 fibers (slow fiber, RED)

High content of MYOGLOBIN

MITOCHONDRIA (AEROBIC Metabolism, REDOX)

ATP-ase 4.2

Tonic contractions and posture maintainance

Type 2 fibers (fast fibers, WHITE)

Glycolitic enzymes (anaerobic metabolism)

ATP-ase 9.4

Fast contractions

S: ***slow and fatigue resistant (MHC1)***
FR: ***fast and fatigue resistant esprimenti (MHC 2A (fiber type 2A)***
FF: ***fast and NOT fatigue resistant (MHC 2X (fiber type 2X).***

Each Muscle contain two muscle fiber types

Many factors can influence fiber typing such as:

- Innervation
- Hormones
- Exercise
- Disuse
- Drugs
- Age
- Neuromuscular disorders

Myopathies Classification

1. **myogenic**
2. **neurogenic**

Myopathies Classification

2. **neurogenic**

- **Myasthenia**
- **Degenerative atrophies**

Myopathic alterations

Several myopathies (trauma, vascular, toxic)

- vacuolization
- NECROSIS
  - Segmental necrosis (drugs)
  - Hyalin necrosis (Duchenne Dystrophy)

- eosinophilia
- "ghost fibre"
- Macrophage infiltrate
- TEM (RS, mitochondria, myofilaments)

Myopathic alterations

- REGENERATIVE

1. continuous
   - Nuclei migration
2. dyscontinuous
   - Stem cells (satellite) activation

Diameter <normal

Few myofibrils (ATPase scarsely reactive)

Lipid vacuoles

Basophilic (RER prominent)

Myopathic alterations

- Increased diameter variability
- hypertrophy
- atrophy (neurogenic, partial regeneration, splitting)
- Central nuclei (Myotonic Dystrophy)
- Selective atrophy type 2 fibres (disuse atrophy)
- Type 1 fibre predominance (Duchenne dystrophy)
- splittng
- Regeneration (distrofie, miopatie neurogene croniche)

Myopathic alterations

- Endomysial fibrosis and adipose substitution

- WHORLED, MOTHE-EATEN, LOBULATED

Inflammatory myopathies and LGMD (mitochondrial pattern alterations)

NEUROGENIC ALTERATIONS

ACUTE AND CHRONIC

Denervation ⟹ Atrophy

- **Single cells and/or o cluster**
- **Small groups or large groups**

Angulated and polygonal shape

Target fibre (clear core, dark stained and peripheral normal staining)

Central, rounded, piknotic nuclei

Loss of ATPase staining

NEUROGENIC ALTERATIONS

Reinnervation

- ❖ **Acute**
- **Degenerated axon regeneration**
- ❖ **Progressive diseases**
- **Sprouting from survival neurons**
- **reinnervated fibres change fibre type**
- **Reinnervated fbres change the histochemical type**

1. **Fibre-type grouping**
2. **Fibre-type predominance**

Fibrosis impairs reinnervation

ALTERAZIONI NEUROGENE

Secondary myopathic alterations

- **central nuclei**
- **splitting**
- **hypertrophy**
- **degeneration**
- **diameter variability**
- **fibrosis**

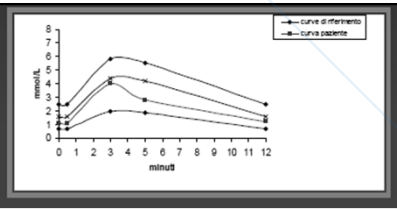
Myopathies – DIAGNOSIS

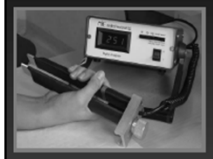
1. Clinical features

- anamestic data
- strength deficit
- muscle fatigue
- muscle mass variation
  Hypo-atrophy
  Pseudo hypertrophy
- mialgia
- muscle contractures
- cramps
- stiffness

Myopathies – DIAGNOSIS

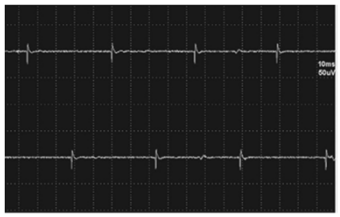
2. Lab diagnostics
- ➢ Enzyme assay
- • CPK
- • LDH
- • Aldolase
- ➢ Lactic acide assay
- • Ischemic
- • aerobic

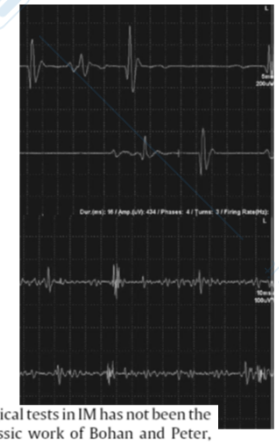




Myopathies – DIAGNOSIS

3. Electromyography





•Sensitivity (abnormal vs normal):
- •depends on type of myopathy:
  - •Duchenne, myositis…….. 90-99%
  - •Metabolic myopathy …….may be very low

•Specificity (classification):
- •EMG usually not specific in separating subgroups

The sensitivity of neurophysiological tests in IM has not been the subject of many studies. In the classic work of Bohan and Peter, the sensitivity of these was 89%.[6] Therefore, it must be remem-

4. Muscle Biopsy

Histology → Ematoxylin/Eosin, Gomori, Oil Red O, PAS

Histochemistry

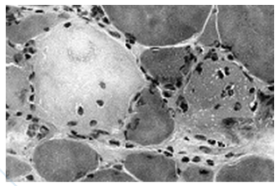
Histoenzymology

Immunohistochemistry → Antigen-antibody reaction (protein expression on muscle biopsy

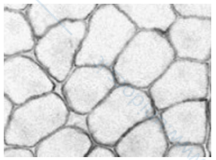
Morphometry

Ultrastructure

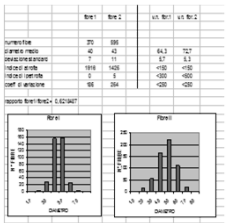
Muscle biopsies take place after clinical, biological and electrophysiological studies



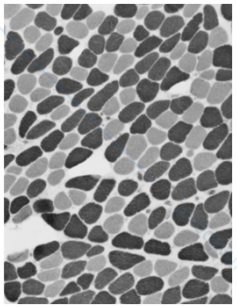
Histology



Immunohistochemistry



Quantification by computerized system



Enzyme histochemistry

20

MUSCLE BIOPSY

Enzymatic pattern

Frozen Tissue

Muscle Biopsy

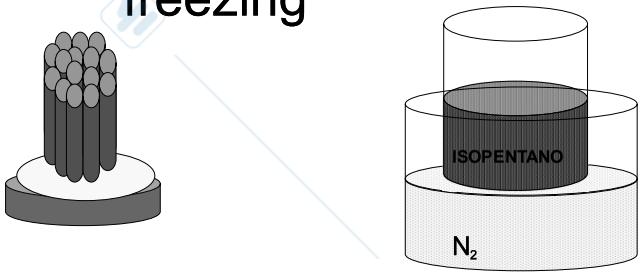
Histoenzymatic analysis

structure

metabolism

Biopsia muscolare

freezing



pre-cooled Isopentane

in Liquid Nitrogen

Muscle Biopsy

sectioning

(8-10µ)

cryostate

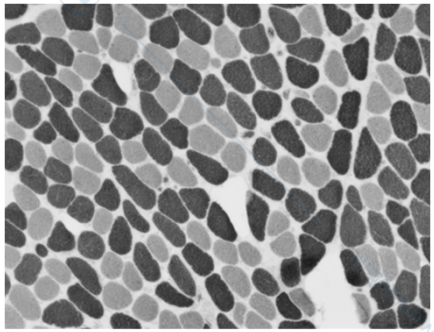
coverslip

storing at –20°C

staining

Enzymatic Pathway

Each muscle is constituted by both type of fibres: type I and type II



Histological stains

- Hematoxylin and Eosin
- Gomori Trichrome

Basic stains	➡ nuclei, membranes, organelles
Acidic stains	➡ myofibrils and connective tissue

Histochemical stains

- Oil red O (lipids)
- PAS (glycogen)

Histoenzymatic stains

- NADH
- SDH
- Cox
- ALkALINE phosphatase
- ACIDA phosphatase
- ATPase 4.35; 4.63; 10.4

Histoenzymatic analysis

Size and shape alteration

- Atrophy
- Hypertrophy
- Diameter variability

Histoenzymatic analysis

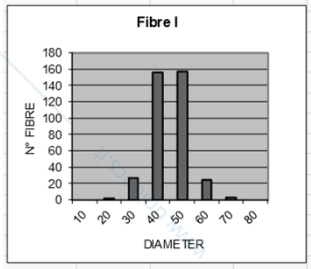
Arhitectural alterations

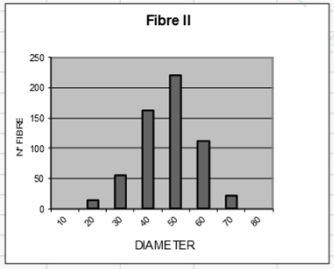
- Central core - Minicore
- Target
- Moth-eaten
- Ragged red
- Ring fibre
- Coiled fibre –whorled
- Rod

Morphometric analysis

- Atrophy index
- Hypertrophy index
- Variation coefficient
- Type I fibre :type II fibre Ratio (1:2)

		fibre 1	fibre 2	v.n. fibr.1	v.n. fibr.2
fibre number		370	595		
medium diameter		40	43	64,3	72,7
standard deviation		7	11	5,7	5,3
atrophy index		1916	1425	<150	<150
hypetrophy index		0	5	<300	<500
variation coeff		186	254	<250	<250
ratio fibre1/fibre2 =	0,6218487				





Myopathies classification

- **Hereditary and /or congenital**
  - Muscle dystrophy
  - Congenital myopathoes
  - Metabolic myipathies
  - Canalopathies and myotonic disorders
  - Mitochondrial myopathies

- **Acquired myopathies**
  - Inflammatory myopathies
  - Toxic myopathies
  - Systemic disorders associated

Inflammatory Myopathies, IM

AutoImmune Myositis, AIM

Idiopathic Inflammatory Myopathy, IIM

- the largest group of potentially treatable myopathies in children and adults.
- a heterogenous group of systemic autoimmune conditions with dominant effects on skeletal muscle, though a range of extra-muscular features
- difficult to treat (high-dose glucocorticoids and immunosuppressive agents such as methotrexate, azathioprine, cyclosporine or mycophenolate mofetil) .
- a significant proportion of patients have an incomplete response,

quick diagnosis to install appropriate treatment

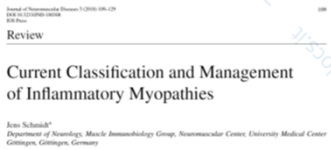

EDITORIAL

A New Classification of Adult Autoimmune Myositis

Epidemiology

All forms of myositis are considered rare diseases (Euromyositis registry)

- OM is the most frequent subgroup, accounting for 50%
- DM was the most common disorder with 31%.
- IMNM is thought to be the second largest group with 20%
- PM is controversial, ranging from the 27% down to the rarest condition, 5%


Review

Current Classification and Management of Inflammatory Myopathies

Jens Schmidt*

Department of Neurology, Muscle Immunobiology Group, Neuromuscular Center, University Medical Center Göttingen, Göttingen, Germany

DIAGNOSIS

- Clinical: muscle weakness
- EMG: myopathic triad (small polyphasic motor unit potentials, fibrillation potentials even at rest and bizarre, high-frequency repetitive discharges)
- Imaging (muscle MRI): muscle oedema on T2/STIR sequences are all suggestive of IIM
- Laboratory: elevated levels of muscle enzymes (creatine kinase—CK, LDH, aspartate aminotransferase, alanine aminotransferase and aldolase). Autoantibodies identified, including both myositis-specific antibodies (MSA) and myositis-associated antibodies (MAA)

Immune Mediated Necrotizing Myopathy

IMNM

distinct clinicopathologic entity that occurs more frequently than polymyositis

3 subgroups according to positive autoantibodies:

1. anti-3-hydroxy-3-methyglutaryl coenzyme A reductase (HMGCR)
2. antisignal recognition particle (SRP),
3. seronegative IMNM (frequent occurrence of associated connective tissue disorders and significantly higher rates of extramuscular disease activity

The diseases tend to be more severe in younger patients and can be chronically progressed mimicking muscular dystrophy

MUSCLE BIOPSY

- scattered myofiber necrotic
- regenerating process,
- no perifascicular atrophy
- sparse inflammatory cell infiltration (predominantly macrophages),
- mild-to-moderate expression of major histocompatibility complex class I (MHC class I, HLA-ABC) on nonnecrotic/nonregenerating fibers,
- MAC deposition on the sarcolemma of scattered nonnecrotic myofibers

Seronegative cases: confirmation of the necrotizing muscle disease is essential

Polymyositis

Polymyositis is rare (5% of all cases) as a stand-alone entity and is often misdiagnosed

Polymyositis remains a diagnosis of exclusion because its non specific phenotype (subacute proximal myopathy without overlap features or autoantibodies)

Muscle Biopsy

- ✓ "invasion" of muscle fibers by presence of endomysial cytotoxic CD8+ T-cells
- ✓ widespread upregulation of MHC class I

Table 2
Proposed diagnostic criteria following ENMC Workshop 2011 [50]

Clinical & laboratory features	Classification	Pathological features
Duration >12 months Age at onset > 45 yrs Quads weakness ≥ hip flex and/or FF weakness > should abd sCK no greater than 15xULN	**Clinicopathologically defined IBM**	Endomysial inflammation & Rimmed vacuoles & Protein accumulation (amyloid or other proteins)* or 15-18nm filaments
Duration >12 months Age at onset > 45 yrs Quads weakness ≥ hip flex and FF weakness > should abd sCK no greater than 15xULN	**Clinically defined IBM**	*One or more of:* Endomysial inflammation or ↑MHC1 or Rimmed vacuoles or Protein accumulation (amyloid or other proteins)* or 15-18nm filaments
Duration >12 months Age at onset > 45 yrs Quads weakness ≥ hip flex or FF weakness > should abd sCK no greater than 15xULN	**Probable IBM**	*One or more of:* Endomysial exudate or ↑MHC1 or Rimmed vacuoles or Protein accumulation (amyloid or other proteins)* or 15-18nm filaments

* Amyloid or other protein accumulation by established methods: for amyloid Congo red, crystal violet, thioflavine T/S, and for other proteins p62, SMI-31, TDP-43.

Adapted from Machado P, Brady S, Hanna MG. Update in inclusion body myositis. Curr Opin Rheumatol. 2013 Nov;25(6):763-71.

Sporadic inclusion-body myositis (s-IBM)

The most common progressive muscle disease of older persons than 50 years

It leads to severe disability, and there is no enduring treatment currently available.

TDP-43 and p62

the most sensitive markers, accumulating in

all definite IBM

MUSCLE BIOPSY

- Endomysial lymphocytic infiltration (mainly CD8þ T cells) with or without nonnecrotic muscle fiber invasion
- rimmed vacuoles
- intracellular protein aggregates, and tubulofilaments
- MHC class I immunopositivity as a marker for inflammation facilitate early-stage disease detection in clinically defined-IBM and probable IBM
- mitochondrial abnormality (cytochrome c oxidase-negative fibers or ragged red fibers
- subsarcolemmal or perivacuolar positivity immunohistochemistry for p62 or TDP-43),

•The relevance of proteins that accumulate and aggregate in the muscle fibers of patients sIBM is unknown.

• Many of these proteins also aggregate in other disorders, including Alzheimer's disease, leading to speculation that sIBM pathogenesis has similarities to neurodegenerative disorders.

Amyloid deposits immunoreact against:

• amyloid precursor protein,

• amyloid-β42,

•apolipoprotein E,

•α-synuclein,

•presenilin,

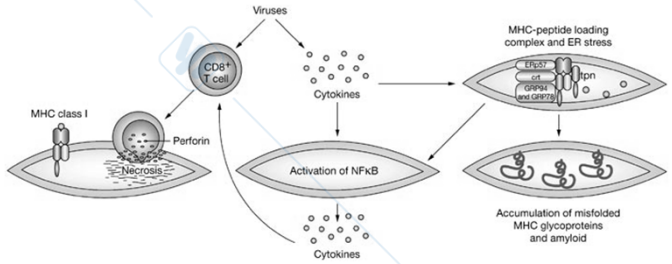
•ubiquitin,

•phosphorylated tau,

In vitro evidence suggests that amyloid-β42 and its oligomers are involved in the pathway of intracellular toxicity, but it remains unclear how these proteinaceous aggregates, which are also seen in other vacuolar myopathies, induce an inflammatory and degenerative myopathy and what triggers disease, inflammation, or protein aggregation.

Compelling evidence suggests that aging, abnormal proteostasis, impaired autophagy, cell stress induced by MHC class I or nitric oxide, long-standing inflammation, and proinflammatory cytokines such as interferon-γ and interleukin-1β may cumulatively trigger or enhance degeneration, leading to further accumulation of stressor molecules and misfolded proteins

Proposed mechanism for the pathogenesis of inclusion body myositis



Dalakas MC (2006) Sporadic inclusion body myositis—diagnosis, pathogenesis and therapeutic strategies *Nat Clin Pract Neurol* **2:** 437–447 10.1038/ncpneuro0261

nature CLINICAL PRACTICE NEUROLOGY

Pathogenetic mechanism

Autoimmune

- cytotoxic T cells
- IBM autoantibody

Degenerative

- lack of IBM response to immunomodulatory therapies
- protein aggregates often associated with other neurodegenerative diseases
- impairment of autophagy

next generation sequencing

Non-coding RNAs regulating the transcription, translation and turnover of mRNA

- house-keeping (transfer RNAs and ribosomal RNAs)
- short (<200 nucleotides) (RNAsmall interfering RNAs and microRNAs)
- long non-coding RNA (actions upon mRNA expression through regulating protein-protein and protein-DNA interactions)

miRs

(Controlli vs Miositi)

- miR-126 ↓
- miR-146 a/b ↓
- miR-21 ↓
- miR-206 ↑
- miR-223 ↓
- miR-7-5p ↓

(Inoue et al, 2013; Okada et al, 2014; Oshikawa et al, 2013; Shimada et al, 2013; Tang et al, 2014)

La *card A* presenta sonde specifiche che riconoscono miRs comuni, ben caratterizzati, descritti nei recenti studi.

La *card B* è composta da sonde che riconoscono miRs recentemente scoperti e meno caratterizzati.

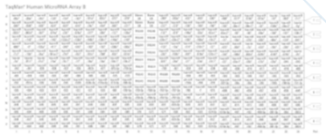


Tabella 2

miRs	fold-change
miR-23a	-2.1
miR-942	-2.3
miR-93-3p	-2.6
miR-18b	-2.7
miR-411-5p	-4.3
miR-125a-5p	-4.5
miR-200c	-4.8
miR-425-3p	-4.8
miR-30e-3p	-6.2
miR-409-3p	-20.1

Muscle dystrophies

Genetic disorders, characerized by primary and progressive involvement of skeletal muscles

- Molecular genetics
- Specific protein loss

Classification based on clinical/genetic/molecular criteria

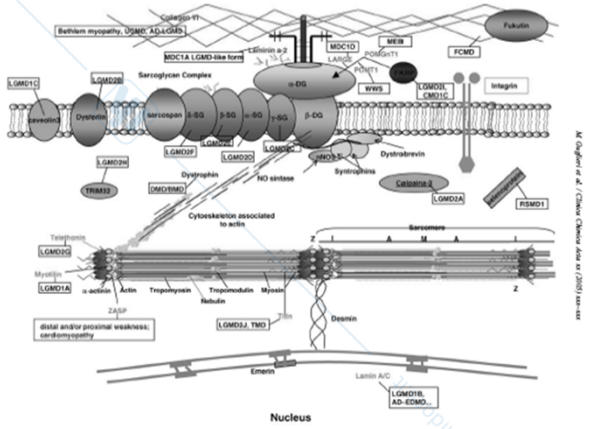
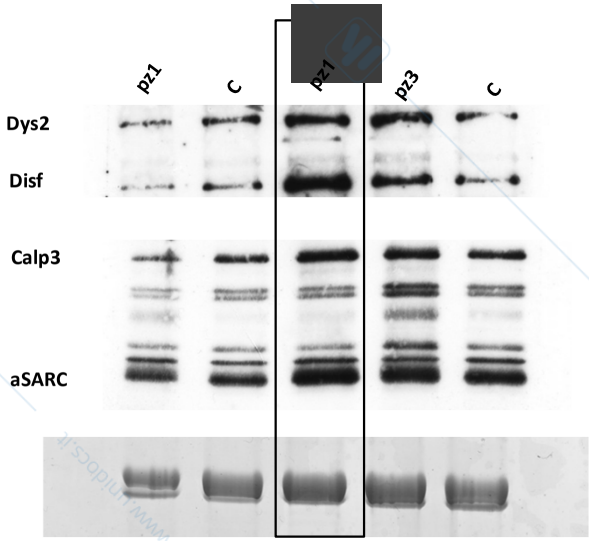


Fig. 1. Schematic diagram illustrating the location of proteins from the extracellular matrix, the sarcolemma, the sarcomere, the cytosol and the nucleus, involved in muscle limb girdle muscular (proteins in orange color) and congenital muscular dystrophies (proteins in green color). Proteins in red color are related to both disorders.

M. Guglieri et al. / Clinica Chimica Acta xx (2005) xxx–xxx



ANALISI PROTEICA – Western Blotting

Classification

X-related muscle dystrophies

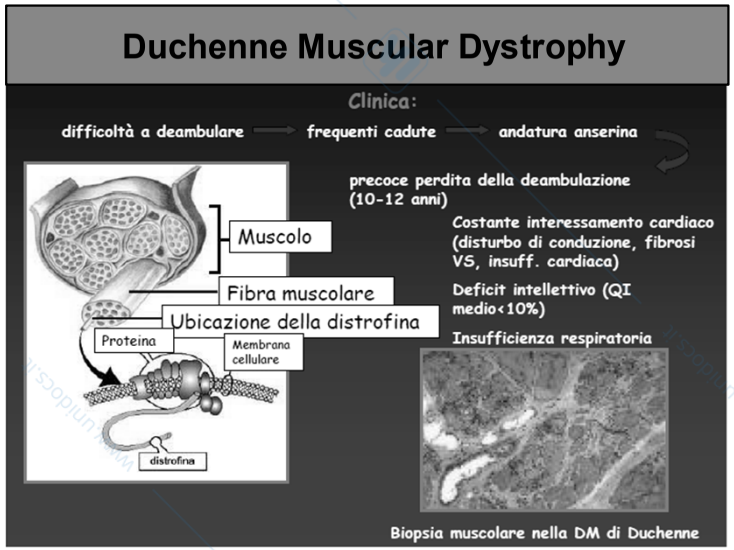
1. Duchenne Dystrophy
2. Becker Dystrophy
3. Benign with contractures (Emery-Dreyfuss)

Recessive autosomal Dystrophies

1. Congenital muscle dystrophies
2. Limb Girdle Muscle Distriphies, LGMD, 2A-2L

Dominant autosomal dystrophies

1. Limb Girdle Muscle Distrophies, LGMD, 1A-1F
2. Facio-Scapulo-Humeral Dystrophy, FSH
3. Emery-Dreyfuss syndrome
4. Distal Muscular Dystrophy(Welander)
5. Oculo-pharyngeal Dystrophy



Duchenne Muscular Dystrophy

•Clinical features

X-linked, recessive, male

Chromosome xp21 - dystrophin

•Early onset <4yrs

•cognitive impairment

•Limb girdle weakness

•Anserine walk, lordotic posture

•ECG altered

•Pseudohypertrophic muscle

•75% death before 20yrs

Duchenne Muscular Dystrophy

Pathology

• Hyalin fibres

• necrosis and phagocytosis

•small and basophilic Fibres, regenerating

•Hypertrophic Fibres, splitting

•Inflammatory infiltrate, fibroadipose substitution

•NADH normal (hyaline overstained)

•ATP-ase not differentiating

•EM: not diagnostic

•ICC: dystrophin negative

Becker Muscular Dystrophy

CLINICAL FEATURES

- **X-linked, recessive, male**
- **Chromosome Xp21 – dystrophin, altererd molecular structure and/ot lower molecular weight**
- **Late onset , progressive**
- **Survival >40yrs**

Table 1
Comparison of the previous LGMD nomenclature to the proposed classification system after the definition has been applied to the current list of LGMD. Conditions which are no longer considered LGMDs are highlighted in grey with a reason for their exclusion given.

Old name	Gene	Proposed new nomenclature	Reason for exclusion
LGMD 1A	*Myot*	Myofibrillar myopathy	Distal weakness
LGMD 1B	*LMNA*	Emery–Dreifuss muscular dystrophy (EDMD)	High risk of cardiac arrhythmias; EDMD phenotype
LGMD 1C	*CAV3*	Rippling muscle disease	Main clinical features rippling muscle disease and myalgia
LGMD 1D	*DNAJB6*	LGMD D1 DNAJB6-related	
LGMD 1E	*DES*	Myofibrillar myopathy	Primarily false linkage; distal weakness and cardiomyopathy
LGMD 1F	*TNP03*	LGMD D2 TNP03-related	
LGMD 1G	*HNRNPDL*	LGMD D3 HNRNPDL-related	
LGMD 1H	?	Not confirmed	False linkage
LGMD 1I	*CAPN*	LGMD D4 calpain3-related	
LGMD 2A	*CAPN*	LGMD R1 calpain3-related	
LGMD 2B	*DYSF*	LGMD R2 dysferlin-related	
LGMD 2C	*SGCG*	LGMD R5 γ-sarcoglycan-related[a]	
LGMD 2D	*SGCA*	LGMD R3 α-sarcoglycan-related	
LGMD 2E	*SGCB*	LGMD R4 β-sarcoglycan-related	
LGMD 2F	*SGCD*	LGMD R6 δ-sarcoglycan-related	
LGMD 2G	*TCAP*	LGMD R7 telethonin-related	
LGMD 2H	*TRIM32*	LGMD R8 TRIM 32-related	
LGMD 2I	*FKRP*	LGMD R9 FKRP-related	
LGMD 2J	*TTN*	LGMD R10 titin-related	
LGMD 2K	*POMT1*	LGMD R11 POMT1-related	
LGMD 2L	*ANO5*	LGMD R12 anoctamin5-related	
LGMD 2M	*FKTN*	LGMD R13 Fukutin-related	
LGMD 2N	*POMT2*	LGMD R14 POMT2-related	
LGMD 2O	*POMGnT1*	LGMD R15 POMGnT1-related	
LGMD 2P	*DAG1*	LGMD R16 α-dystroglycan-related	
LGMD 2Q	*PLEC*	LGMD R17 plectin-related	
LGMD 2R	*DES*	myofibrillar myopathy	Distal weakness
LGMD 2S	*TRAPPC11*	LGMD R18 TRAPPC11-related	
LGMD 2T	*GMPPB*	LGMD R19 GMPPB-related	
LGMD 2U	*ISPD*	LGMD R20 ISPD-related	
LGMD 2V	*GAA*	Pompe disease	Known disease entity, histological changes
LGMD 2W	*PINCH2*	PINCH-2 related myopathy	Reported in one family
LGMD 2X	*BVES*	BVES related myopathy	Reported in one family
LGMD 2Y	*TOR1AIP1*	TOR1AIP1 related myopathy	Reported in one family
LGMD 2Z	*POGLUT1*	LGMD R21 POGLUT1-related	
Bethlem myopathy recessive	*COL6A1, COL6A2, COL6A3*	LGMD R22 collagen 6-related	
Bethlem myopathy dominant	*COL6A1, COL6A2, COL6A3*	LGMD D5 collagen 6-related	
Laminin α2-related muscular dystrophy	*LAMA2*	LGMD R23 laminin α2-related	
POMGNT2-related muscular dystrophy	*POMGNT2*	LGMD R24 POMGNT2-related	

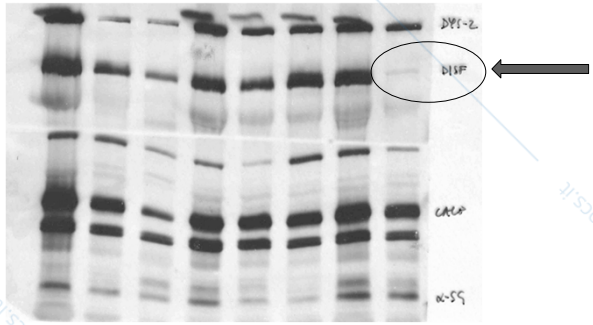
[a] Sarcoglycan-related LGMDs rationalised based on order of gene discovery.

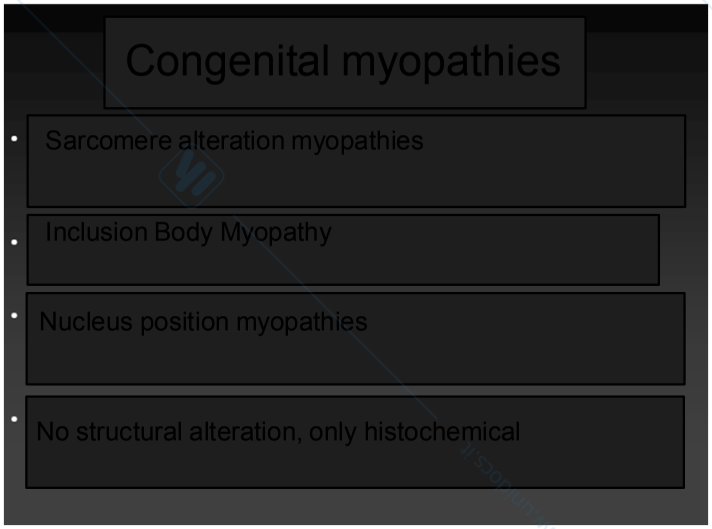
Becker Muscular Dystrophy

Pathology

- Few hyalin fibres
- **necrosis and phagocytosis**
- **small and basophilic Fibres, regenerating**
- **Hypertrophic Fibres, splitting**
- Infiltrato inf., sostituzione fibro-adiposa
- Angulated fibres, NADH-overstained, in cluster
- ATP-asi weakly differentiating
- EM: **not diagnostic**
- ICC: irregular positivity of dystrophin

Dysferlin Deficiency (LGMD 2B)





CONGENITAL MYOPATHIES

•Nemaline, Rods

Alpha-tropomyosin, nebulin, sarcomeric actin, beta-tropomyosin

Ovoids or rods (fibres type 1 and 2)

•Centronuclear o myotubular

Myotubularin

No myofubrils around the nucleus, central nuclei (ATPase -, NADH-)

•Congenital fibre-type disproportion

Tipe 1 predominant, small or normal

Tipe 2 hypertrophic

CONGENITAL MYOPATHIES

•Central core e multicore

Rhyanodin receptor

Central area NADH – e PAS – (mainly in type 1 fibres)

•Structured Cores, normal ATPase, hypercontraction, z-band smeared

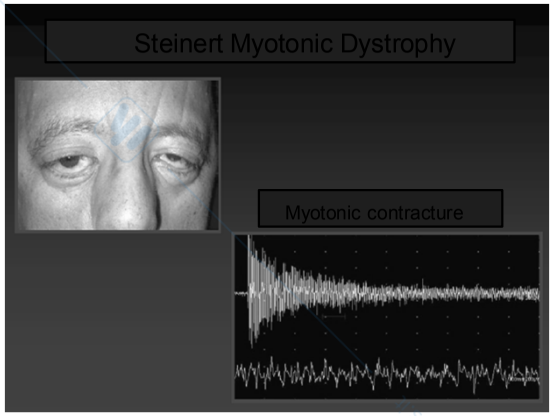
•Unstructured Cores ATPasi –

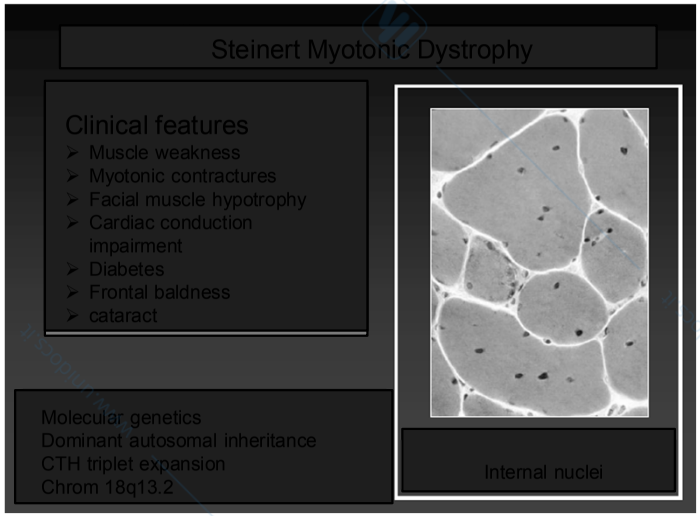
•Vacuolar myopathies

- **desminopathy**
- **Desmin-related (spheroid bodies, cytoplasmic bodies)**
- **Actinopathies**

Channelopathies and Myotonies

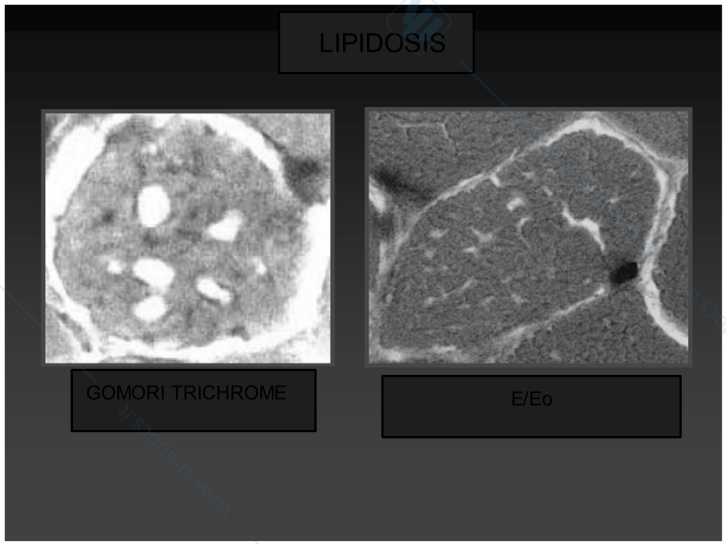
- **Myotonic dystrophy**
- Sodium channelopathy
- Calcium channelopathy
- Potassium channelopathy
- Chloride channelopathy

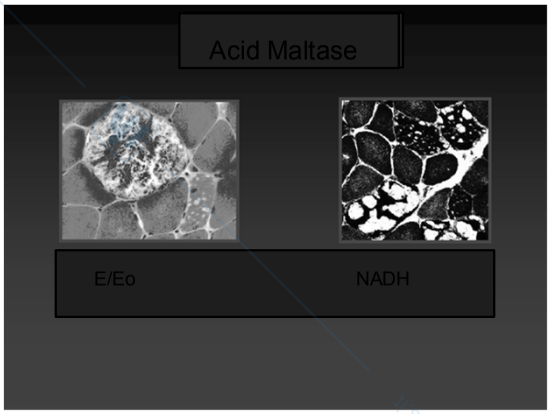




Metabolic myopathies

- Lipid metabolism
- Charbohydrate metabolism
- Purine metabolism defect





Mitochondrial myopathies

- CPEO
- Sindrome di Kearns-Sayre
- MELAS
- MERRF
- NARP
- Neuropatia ottica ereditaria di Leber
- Sindrome da deplezione del DNA mitocondriale
- Sindrome di Leigh

Transmission Electron Microscopy

Nacked eyes: two different points can be seen as distinct points at a distance of 0.1-0.4mm; if the distance is less than 0,1 mm the two points are not more distinguishable but they appear as confused in only one point.

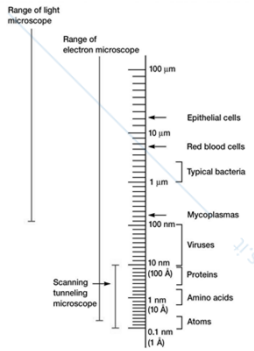
What is Electron Microscopy?

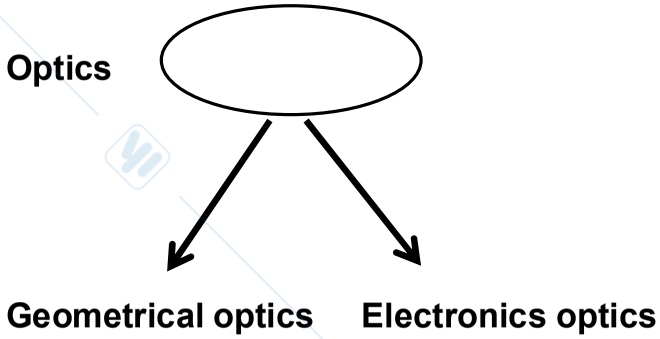
- A beam of electrons is used to create an image of a sample
- Can view samples at higher magnifications than light microscopes and gives better resolution
- Smaller objects can be seen in finer detail

Why electrons instead of light?

Light microscope
- up to x1000 mag
- resolution of 200 nm

Electron microscope
- up to x450,000 mag
- resolution of 0.5 – 4 nm





Geometrical optics

•**Geometrical optics**, or **ray optics**, is a model of optics that describes light propagation in terms of rays. The simplifying assumptions of geometrical optics include that light rays:

•propagate in straight-line paths as they travel in a homogeneous medium

•bend, and in particular circumstances may split in two, at the interface between two dissimilar media

•follow curved paths in a medium in which the refractive index changes

•may be absorbed or reflected

- Refrection
- Reflaction
- Absorbtion

Electron Optics

❖ It studies the electronic emission such as the production of free electrons in the vacuum

❖ **Thermionic emission** is the liberation of electrons from an electrode by virtue of its temperature (releasing of energy supplied by heat). This occurs because the thermal energy given to the carrier overcomes the work function of the material. The charge carriers can be electrons or ions, and in older literature are sometimes referred to as thermions.

❖ **A field emission gun** is a type of electron gun in which a sharply pointed emitter is held at several kilovolts negative potential relative to a nearby electrode, so that there is sufficient potential gradient at the emitter surface to cause field electron emission. Emitters are either of cold-cathode type, usually made of single crystal tungsten sharpened to a tip radius of about 100 nm

❖ **Secondary emission**, ejection of **electrons** from a solid that is bombarded by a beam of charged particles. Some **electrons** within the surface of a material are given enough energy to break free from the attractive force holding them to the surface by a transfer of kinetic energy from the bombarding particles.

Transmission Electron Microscopy (TEM)

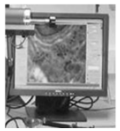
Used to view the internal ultrastructure of cells and tissues

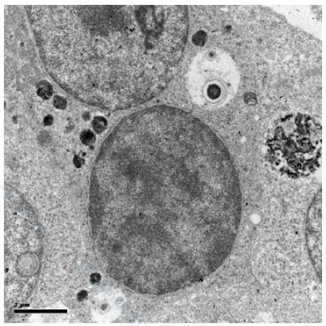
TEM

•A beam of electrons passes through an ultrathin sample (less than 100nm) and interact with it

•An image is formed from the interaction of the electrons with the sample

•Image can be viewed on the fluorescent screen of the TEM or on a computer screen

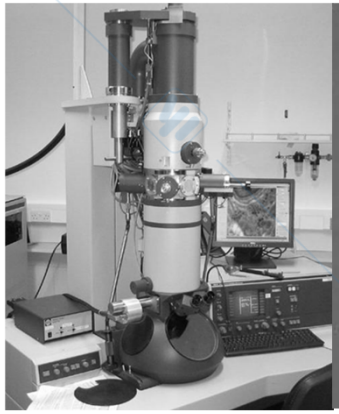




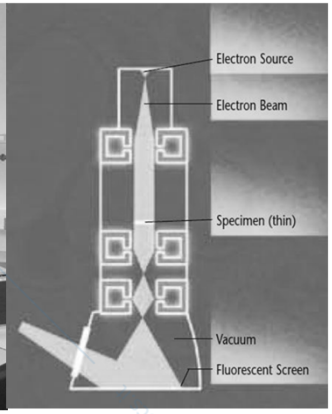
TEM

•Under high vacuum
•Sample has to be completely dry
•Magnification range x18 – x450,000
•High resolution ~ 0.5nm in biological samples - 400 times improvement compared to LM resolution of 0.2 microns (200nm)
•Cannot view living specimens

How does the TEM work?



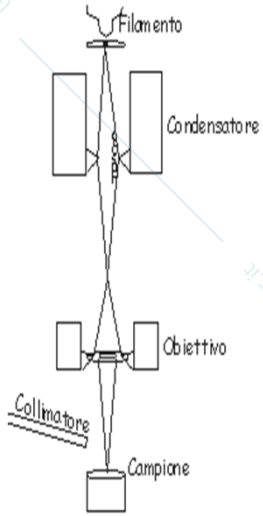
Philips CM10 transmission electron microscope



Path of electrons in a TEM

TEM is composed by an electron gun (filament) and by a cathod giving strenght acceleration to the electrons produced by the gun and attracted by an anode giving a high tension (20 to 100.000 **Volt.**

The electron guns crosses the condenser and meet the sample : after it has been collected by an objective lens (electromagnetic lens) and inetracts with a fluorescent screen thus forming the final image on the eye



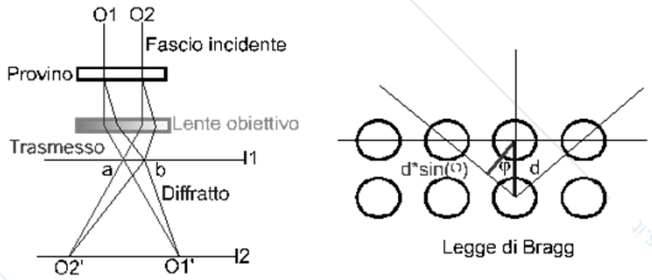
The electron microscope is quite silimial to a light microsoe using light with a very low wavelenght The norml light microscopes are not able to modify the electron pathway in the electron microscope there aren't glass lenses , but only electromagnetic lenses which are able to modify the electric charge of electrons changing their path.

The described mechanism is possible only in the ultra high vacuum.

	LM	TEM	SEM
	Surfaces and section	Thin sections	Surface structures Bulky specimen
Resolution (nm)	200-500	**0,2-1**	3-6
Max Magnification	10-1500	**200-500000**	20-100000
Gun	light	**electrons**	**electrons**
Propagation medium	aria	High vacuum	High vacuum
lens	Glass	Electromagnetic	Electromagnetic
Contrast	Light absorbance	Electron diffusion	Electron diffusion
Sample procedure	Easy	**Difficult**	Easy

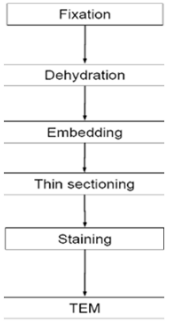
Vacuum system

- Rotative pump (**10-1-10-2Pa)**
- Diffusion pumps (**10-7-10-8Pa)** and turbomolecular pumps **(10-1–10-8Pa)**



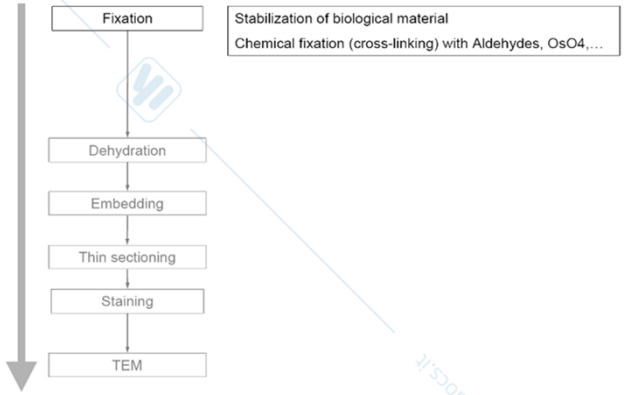
TEM functioning and geometric incidence of electrons on atom surfaces

Sample Preparation for TEM



The specimen must be crossed by electrons showing a very short wavelenght. The thickness of the section to be crossed must be very thin (70nm/700A)

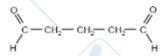
The most difficult step in specimen procedure is represented by the cutting step



Fixation

•Glutaraldehyde – fixes proteins and stabilises structures. Depth of penetration 2mm/hour
•Paraformaldehyde –depth of penetration 10mm/hour, but takes much longer to stabilise the tissue
•Osmium Tetroxide – stabilises lipids and acts as a stain. Depth of penetration 1mm/hour. Extended times will cause extraction of proteins

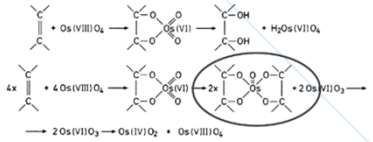
Glutaraldehyde versus Formaldehyde



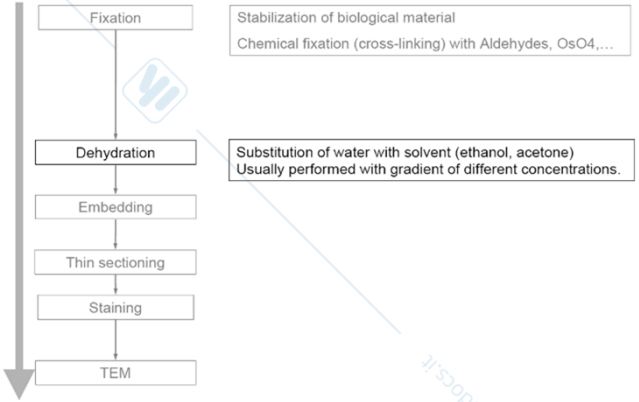
- Mainly used for electron microscopy
- Irreversible fixation and polymerisation
- Destroys antigens considerably: Not suitable for subsequent immunolabelling
- Needs to be prepared freshly (prevent polymerisation prior to fixation)
- Needs to be buffered (reaction causes drop of pH)

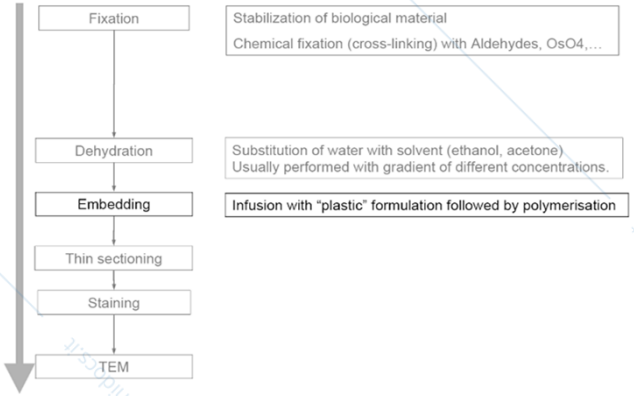
- Mainly used for LM
- Reversible fixation and polymerisation
- Destroys much less antigens than Glutaraldehyde
- Used for EM only in combination with Glutaraldehyde
- Needs to be prepared freshly (prevent polymerisation prior to fixation)
- Needs to be buffered (reaction causes drop of pH)

Osmiumtetroxide



- Cross linker mainly of unsaturated lipids, some proteins & phenolic compounds
- Main used as secondary fixative
- Causes elastic electron scattering
- Can solubilise some proteins





Plastic formulations consist of monomers, hardener, accelerator

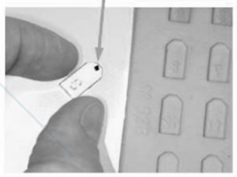
Polymerization by heat or UV light

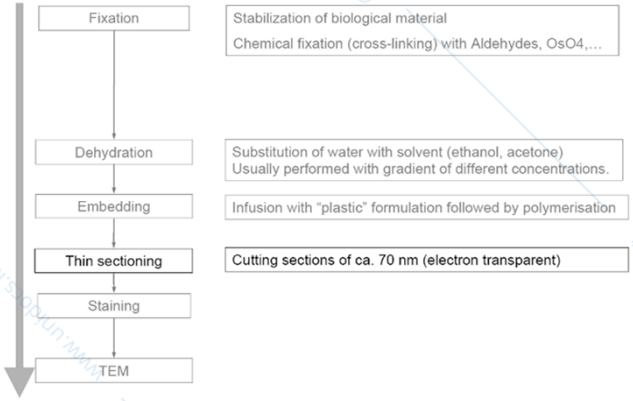
Epoxy resins, acrylic resins

Note: Resins are toxic and allergenic

Embedding molds

Specimen embedded in plastic (Epon)





Typical protocol

•2.5% Glutaraldehyde in 0.1M phosphate buffer pH 7.4

•1% Osmium Tetroxide in distilled water

•Dehydrate in ethanol series (70%, 80%, 90%, 100%)

•100% ethanol:resin (1:1)

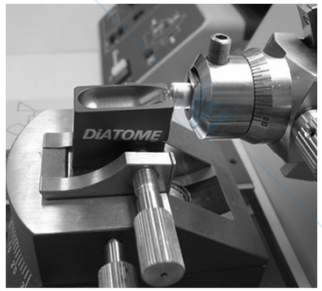
•Fresh Resin

•Embed and polymerise at 60**°C for 24 - 48 hours**

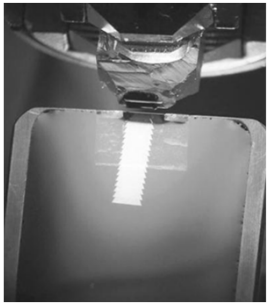
Thin sectioning

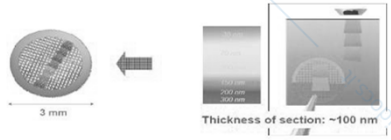
•A microtome is a sectioning device for cutting extremely thin slices of material (sections)

Diamond knife cutting ultra thin sections



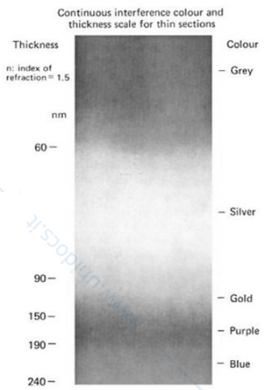
Sections floating out onto water



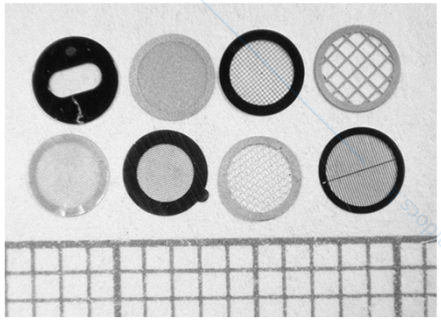


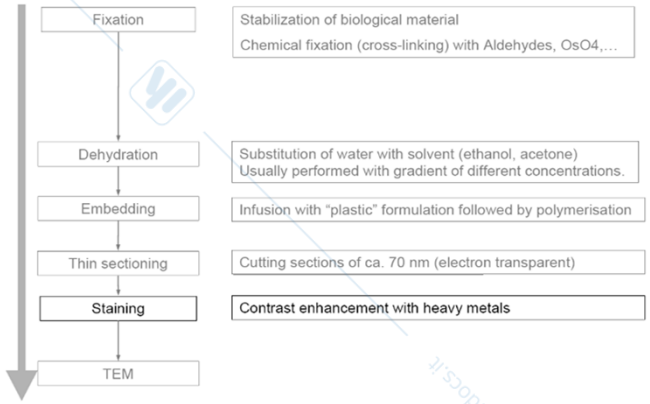
Pick up of thin sections onto grids

Section Thickness



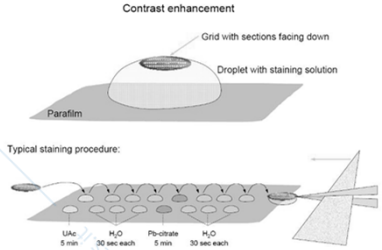
Grids used to pick up sections for viewing in TEM

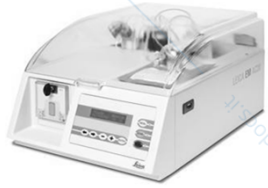




Heavy metal staining

- Uranyl Acetate – stains nucleic acids and proteins.
- Lead Citrate – gives high contrast staining to cellular and tissue components.





Scanning electron microscopy

Scanning Electron Microscopy (SEM)

A microscopy technique used to view the surface features of a sample

Surface microvilli

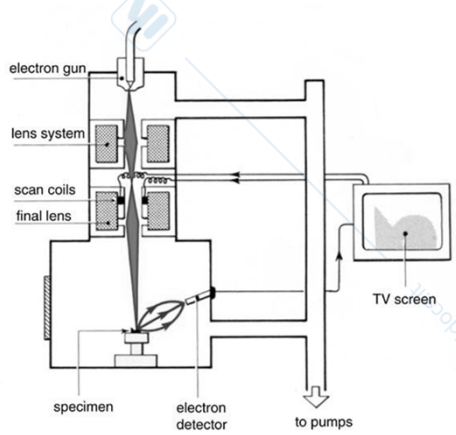
What is SEM?

- The sample is scanned with a beam of electrons
- The electrons interact with the sample and are picked up by detectors
- Image can be viewed on the computer screen

SEM

- Microscope under high vacuum
- Sample has to be dry
- Able to see surface detail
- Magnification range x20 – x50,000
- Resolution ~ 5nm. 40 times improvement compared to LM resolution of 0.2 microns (200nm)
- Cannot view living specimens

• An electron gun emits a beam of electrons onto the sample surface
• The beam is then scanned across the sample
• As the electrons hit the sample, secondary electrons are emitted and picked up by a secondary electron detector
• Backscattered electrons are reflected from the sample and can be picked up by a backscatter detector



Typical Sample Preparation for SEM

•Fix in 2.5% glutaraldehyde in 0.1M phosphate buffer

•1% Osmium Tetroxide in distilled water

•Wash in distilled water

•Dehydrate in ethanol series (70% - 100%)

•Critical Point Dry, HMDS (Hexamethyldisilizane) or air dry

•Attach to aluminium specimen stub and sputter coat with gold.

Gold Sputtering

Samples need to be coated in a thin layer of gold to make them conducting

Emitech K550 gold sputter coater

Cilia

Immuocytochemistry

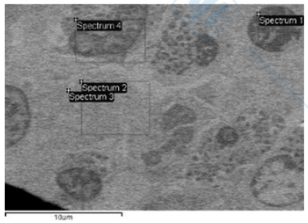
Cytochemistry

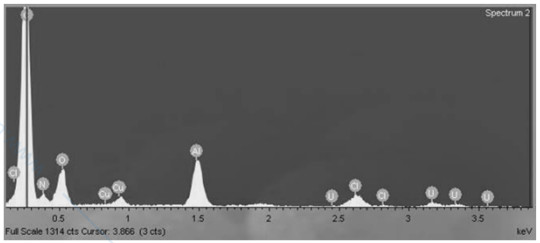
Ultrastructural in situ hybridization

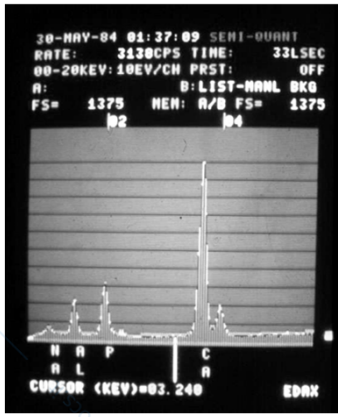
•CMV

X ray microanalysis

- Hydroxyapatite crystals



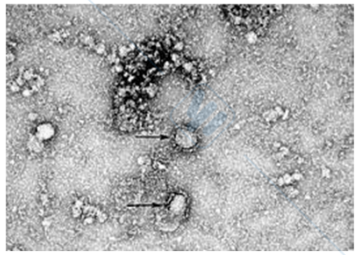


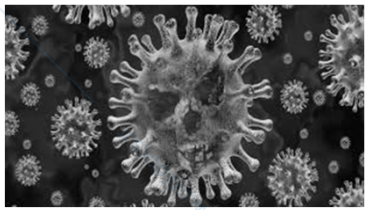


Neagtive staining

- Collagen fibrils
- viruses

2019-nCoV





Osmic maceration technique

•Nuclear membrane

•Hepatocyte cytoskeleton

•Skeletal muscle cell cytoskeleton

Osmic maceration technique

SEM

•Mitochondria

•Enzymatic protein (high resolution SEM)

Ultrastructural Cytology

Ultrastructural markers
Neoplastic and non-neoplastic

- Intercellular relationship
- Basal lamina
- Intercellular Junctions
- Cytoplasmic granules
- Cytoplasmic filaments
- Vacuoles and vesicles
- Organelle pattern
- Nucleus and nucleolus
- Intercellular stroma

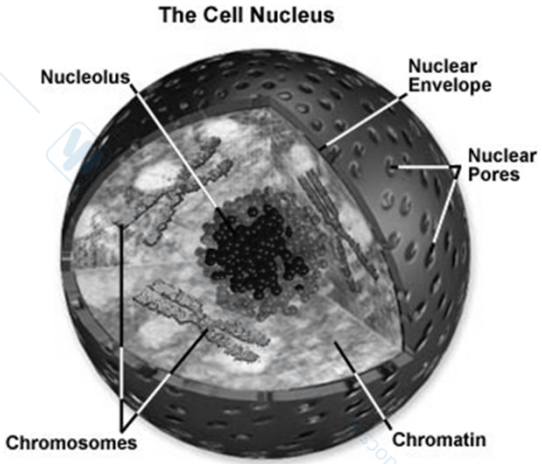
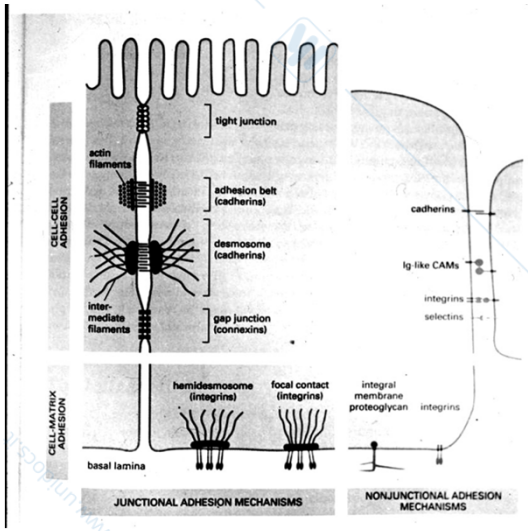
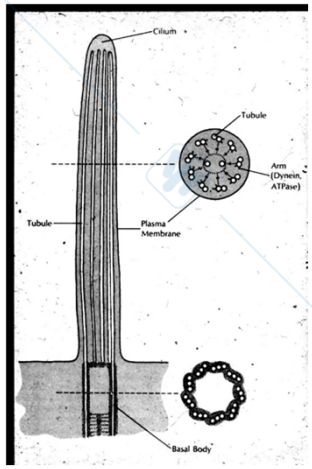
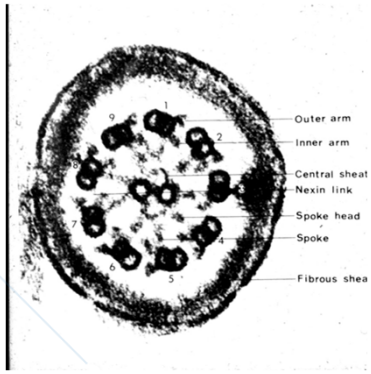
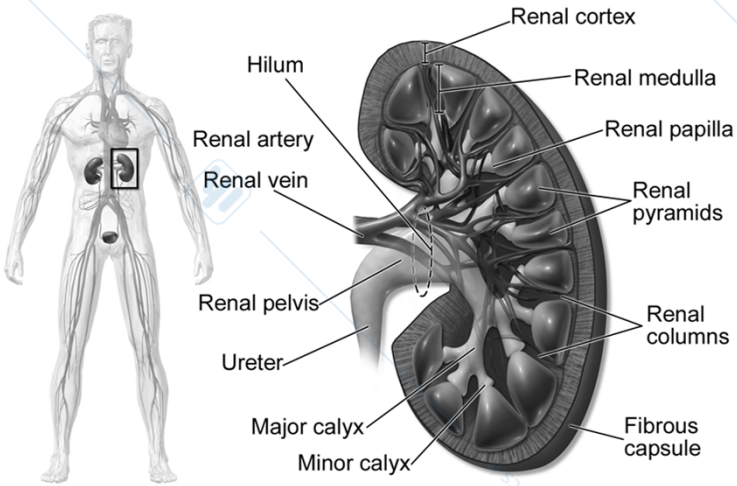


Figure 1

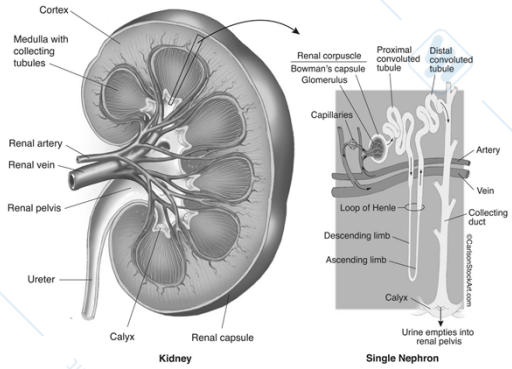








Kidney Anatomy



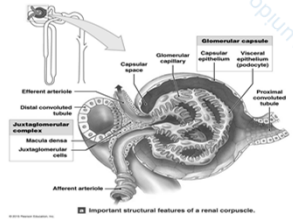
Nephron

Vascular component
- glomerulus

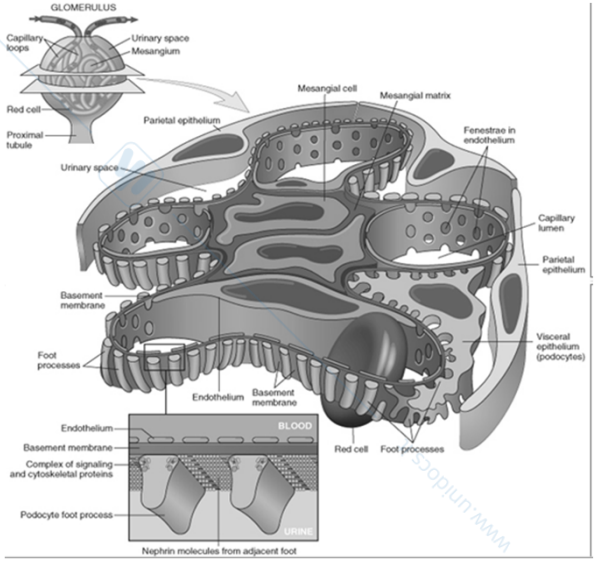
Epithelial component
- Bowman's capsule

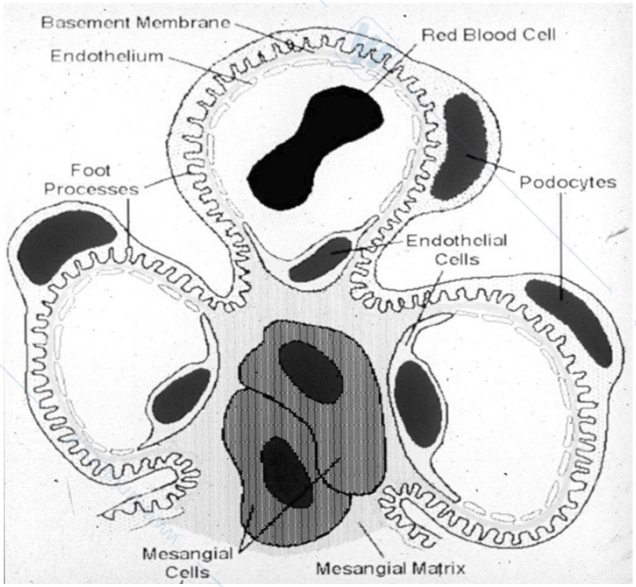
Tubular component
- convoluted proximal tubule
- loop of Henle
- convoluted distal tubule
- collecting duct

1. Blood filtration in glomeruli
2. Selective reabsorbption in renal tubules
3. Urine formation



Important structural features of a renal corpuscle.

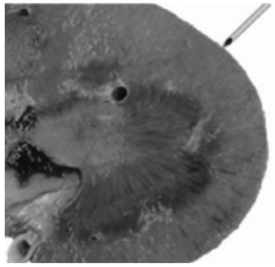




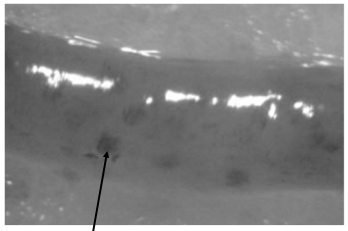
RENAL BIOPSY SUGGESTS ESSENTIAL INFORMATIONS ABOUT:

- **TYPE**
- **NATURE**
- **LOCATION**
- **EXTENSION**
- **EVOLUTION**

OF KIDNEY DISEASE

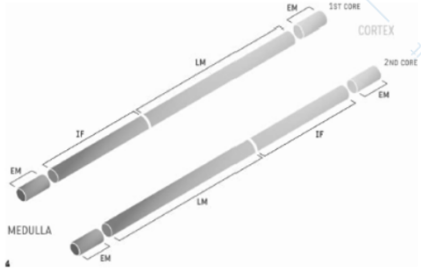


- **PREDICTION OF PROGNOSIS**
  - **grade**
  - **severity**
- **GUIDE FOR TREATMENT**
  - **progression/regression of disease**
  - **drug toxicity**



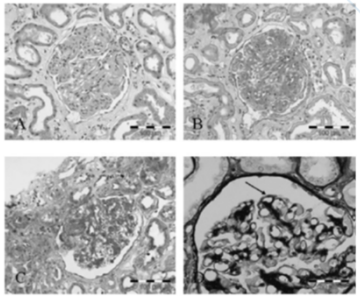
The kidney biopsy

Renal glomerulus

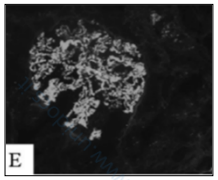


LIGHT MICROSCOPY

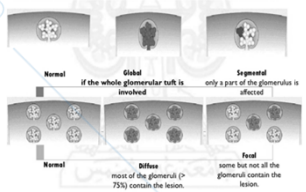
- Haematoxylin-eosin (A)
- Periodic Acid Schiff's (B)
- Trichrome (C)
- Silver staining (D)



IMMUNOFLUORESCENCE



DEFINITIONS



- **DIFFUSE engaging the majority of glomeruli (≥ 50%)**
- **FOCAL minority of glomeruli (< 50%)**

- **SEGMENTAL part of glomerulus involved**
- **GLOBAL entire glomerulus involved**

- **HYALINOSIS acellular matrix PAS+ PASM-**
- **SCLEROSIS increase of matrix or other extracellular components PAS+ PASM+**

- **CRESCENT extracapillary proliferation of > 2 cell layers occupying 25% or more of glomerular capsular circumference with epithelial cells, mixture of cellular and fibrous component, interstitial cells or macrophages**
- **SYNECHIA localized narrow bridges of connective tissue between glomerular tuft and Bowman capsule with or without increased glomerular extracellular matrix**

PRIMARY IDIOPATHIC GLOMERULAR DISEASES

MINIMAL CHANGE DISEASE o LIPOID NEPHROSIS

- Nephrotic syndrome
- Age > 2 years
- Selective proteinuria (albuminuria)
- M>F

PATHOGENESIS

Mediator produced by T-cell – abnormality of selective permeability of circulating molecules transport - proteinuria

MINIMAL CHANGE DISEASE o LIPOID NEPHROSIS

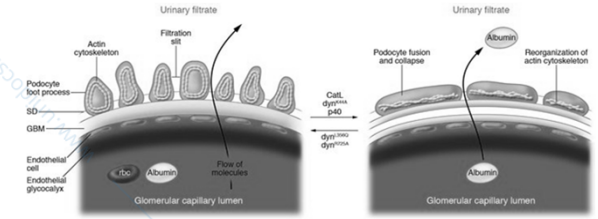
- cytoplasmic vacuolization
- No mesangial hypercellularity or matrix proliferation

PROGNOSIS – complete remission after steroid therapy

MINIMAL CHANGE DISEASE o LIPOID NEPHROSIS

IF – no immunoreactivity– IgM e C3 may be scantly present

EM – extensive effacement of foot processes resulting in a continuos epithelial layer, smooth and without bans, podocyte cell bodies frequently display microvillous transformation and focal lipid droplets (vacuoles) in the urinary space.



Minimal Change Disease– Proliferative Mesangial Nephropathy – Focal Segmental Glomerulosclerosis

are a continuum

PROLIFERATIVE MESANGIAL NEPHROPATHY

Clinically indistinguishable from MCD except for the constant steroid therapy they must receive to keep urine free from proteins

LM –mesangial hypercellularity may occur; normal mesangial matrix;

IF – IgM and sometimes C3 deposits; impairment of all glomeruli

EM – effacement of foot processes with irregular and not spread pattern; intraepithelial vacuoles and increase of cytoplasmic organelles; mesangial electron dense deposits.

PROLIFERATIVE MESANGIAL NEPHROPATHY

PROGNOSIS

Complete remission of 30-50% cases under steroid therapy

FOCAL SEGMENTAL GLOMERULOSCLEROSIS

•Sclerosis involves some glomeruli (focal) and only a portion of glomerular tuft (segmental)

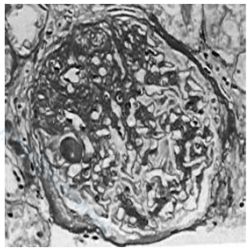
•It is the most common cause of idiopathic nephrotic syndrome in adults

•Reactivity to steroid therapy (lipoid nephrosis complication)

•Some patients are therapy resistant and risk progression of renal disease:: high risk of recurrence in transplants

•Sclerosis after post-infective GN as "scar" = benign sclerotic lesion

•Secondary to HIV infection or heroin nephropathy

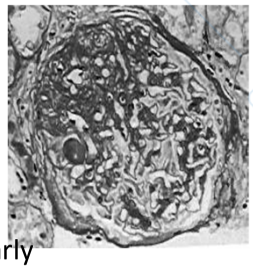


FOCAL SEGMENTAL GLOMERULOSCLEROSIS

ETIOPATHOGENESIS

- pathogenesis is not well known yet
- a reduced number of anionic charges at the lamina rara externa of the glomerular basement membrane leads to a defective permeability for proteins from blood to pre-urine which is responsible of the increased proteinuria
- it is demonstrated the presence of a blood circulating factor in individuals with kidney transplant which had a recurrence of the previous focal segmental glomerulosclerosis
- this factor can be similiar to a lymphokine: with a m.w. between 30 and 50 kD and it can be responsible for the reduction of the anionic charges as, if it is injected in a rat it induces elevated proteinuria within 6-24 hours.

FOCAL SEGMENTAL GLOMERULOSCLEROSIS



MORPHOLOGY:

- juxtamedullary glomeruly affected early
- hyalinosis and sclerosis at the periphery of the glomerulus
- tuft-capsule adhesions
- vacuolar degeneration of podocytes
- arteriolar hyalinosis=negative prognosis
- IgM, IgG and C3 deposits only in the sclerohyalinosis areas

FOCAL SEGMENTAL GLOMERULOSCLEROSIS

FACTORS THAT FAVOR FSGS

Protein-based diet, hypertension, growth factors, arachidonic acid metabolism

alterations of the basal membrane components

Non selective proteinuria

FOCAL SEGMENTAL GLOMERULOSCLEROSIS

LM – many normal glomeruli, slight increase in mesangial cellularity and matrix

Sclerosis associated with loss of capillary loops

PAS+ Hyaline deposits

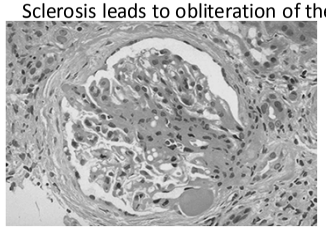
Foam cells and lipid vacuoles

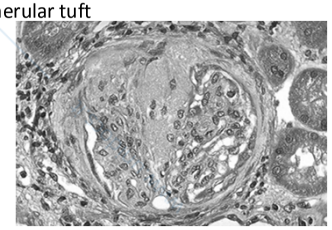
random synechia

multilayered Bowman capsule in correspondence of synechia

Segmental lesion

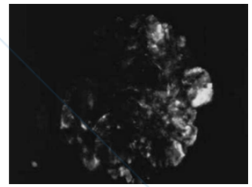
Sclerosis leads to obliteration of the glomerular tuft





FOCAL SEGMENTAL GLOMERULOSCLEROSIS

IF – IgM + C3 in segmental scars



EM –

Non sclerotic areas- effacement and expansion of foot processes; cytoplasmic vacuoles, pseudocystis, increase of cytoplasmic organelles

Sclerotic areas- increase of the matrix which encompasses the basal lamina; matrix forms bridges between tuft and Bowman capsule; shrunken and conctracted GBM; sclerotic mesangial areas are hypocellular with cellular residues; hypertrophic endothelial cells

PROGNOSIS – favorable without nephrotic syndrome; progression with no response to steroid

MEMBRANOUS GLOMERULONEPHRITIS

Continuos deposits of immunocomplexes on the epithelial side of glomerular basement membrane

Pattern shared with LES, hepatitis B and C virus infection, poisoning by heavy metals (mercury, gold), drugs

PATHOGENESIS – autoantibodies against podocyte antigens with complement activaction; activation may be induced by antigens from the circulation or derived from local biosynthesis

CLINICAL ISSUE –

- Nephrotic syndrome
- > 30 years

MEMBRANOUS GLOMERULONEPHRITIS

STAGE I

LM – normal glomerular basement membrane; granular aspect with PAS because of negativity of deposits

IF – presence of deposits

EM – subepithelial deposits; normal glomerular basement membranes; short spikes.

STAGE II

LM – thickening of the glomerular basement membrane, spikes

IF – granular IgG pattern, rare IgA e IgM, normal mesangium

EM – subepithelial deposits with evident spikes, increased podocyte cytoplasm with numerous organelles

MEMBRANOUS GLOMERULONEPHRITIS

STAGE III

LM – spread thickening of capillary loops with irregular aspects due to evident spikes and focally fused

IF – deposits progressively embedded within basal lamina; persistent in obsolescent glomeruli;

EM – lamina densa distinctly thickened with irregular outline; intramembranous and subepithelial electrondense deposits;

STADIO IV

LM – obsolescent and sclerotic glomeruli; thickened basal membranes; focal hyalinosis, sclerosis and synechia

EM – lamina densa extensively and strongly thickened, less evident deposits and replaced by electronlucent areas (membrane with bugs)

MEMBRANOUS GLOMERULONEPHRITIS

Associated with LES, RA, infectious or parasitic diseases, drugs, renal vein thrombosis

PROGNOSIS

- **complete and spontaneous remission**
- **50% progression with renal insufficiency within 2-3 years**
- **favorable prognostic factors: age, female, absence of nephrotic syndrome, normal renal function, normotension**

IgA GLOMERULONEPHRITIS

(BERGER DISEASE)

- **diffuse IgA mesangial deposits**
- **focal and segmental glomerular lesions**
- **(secondary deposition: Henoch-Schonlein purpura, LES, cirrhosis)**
- **Clinical issues: young-adults; M>F; macroscopic hematuria; favourable prognosis in children**
- **Pathogenesis: strong genetic factors; often exacerbated by upper respiratory infections: unknown nature of the circulating immunocomplexes antigen; recurrence after transplant**

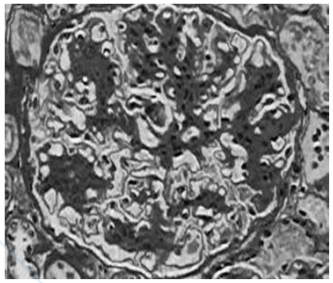
IgA GLOMERULONEPHRITIS

(BERGER DISEASE)

LM focal and segmental mesangial proliferation; slight increase of mesangial matrix; rare sclerosis and cellular proliferation, inflammatory infiltrate, localized crescents

Lately fibrotic crescents

5 classes, depending on the lesions



MEMBRANOPROLIFERATIVE GLOMERULONEPHRITIS

- **Endocapillary hypercellularity with thickened capillary walls**
- **Idiopathic or secondary to systemic or infectious (hepatitis C virus) disease**
- **Clinical issues children and teenagers; nephrotic syndrome with haematuria; rare nephritic syndrome and gross haematuria**

Pathogenesis and classification

Membranoproliferative GN I, II, III

Unknown pathogenesis: hypotetic role of hepatitis C virus;

Deposit's nature is still unknown

MEMBRANOPROLIFERATIVE GN

TYPE I

- **LM** enlarged glomerulus with hypercellularity with thickened capillary walls and increased mesangial substance; irregularly thickened lamina densa with"tram tracks," "double contours," or GBM duplication secondary to GBM synthesis (macrophagic cells with interdigitations between two levels of basal lamina);
- **IF C3, IgG, IgM**
- **ME** mesangial and subendothelial deposits; "doubled" lamina densa: between two glomerular basement membranes there are cytoplasmic debries and mesangial matrix
- **Type III + subepithelial deposits with spikes**

MEMBRANOPROLIFERATIVE GN.

II type (Dense Deposit Disease, DDD)

•LM enlarged glomerulus, diffuse and regular thickening of the glomerular basement membrane due to immunocomplexes (PAS +, PASM+), mesangial sclerosis;

•IF C3 (linear deposits)

•EM intramembranous hyperdense deposits with alteration of the architecture of the capillary loop; peritubular and basement membrane of the Bowman capsule, deposits; increase of the mesangial matrix; foot effacement and microvilli

MEMBRANOPROLIFERATIVE GN.

•PROGNOSIS

Type I slowly progressive

Type II 50-60% with renal insufficience after 10 years

The evidence of progression is the extraglomerular proliferative GN (crescent)

Type II can recur in transplants

RAPIDLY PROGRESSIVE GN.

Crescentic glomerulonephritis

Extracapillary proliferative glomerulonephritis

Characterized by crescents (pariethal epithelial cells + macrophages) in numerous glomeruli and by a rapid progression into renal insufficiency (days or weeks)

PATHOGENESIS

Some types can be associated with:

- antibodies against glomerular basement membrane (20%)
- Glomerular immunocomplexes (40%)
- No immune (no immunocomplexes) with autoantibodies anti-neutrophils (ANCA)o ANCA neg (40%)

RAPIDLY PROGRESSIVE GN.

- CLINICAL ISSUE

1. adults (patients with HLA DR2 phenotype, most frequent)
2. M>F
3. oliguric o anuric renal insufficiency;
4. Possible previous flu episode

- HISTOLOGY

1. Filling of the Bowman's space
2. Crescents characterized by large epithelial cells often similar to macrophages; fibroblasts invasion in the capsular basal lamina; fibrosis; glomeruli rapidly obsolescent

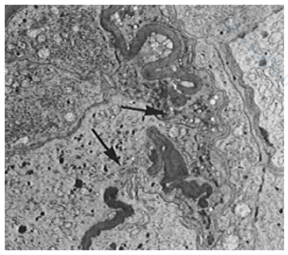
RAPIDLY PROGRESSIVE GN.

- IF

1. Type I autoantibodies anti-basal membrane: linear IgG along basal membrane
2. Type II caused by immunocomplexes: mesangial, subendothelial and subepithelial deposits (IgG, IgA, IgM), fibrin e fibrinogen
3. Type III non immune: fibrin and fibrinogen

RAPIDLY PROGRESSIVE GN.

- TRANSMISSION ELECTRON MICROSCOPY
- Glomerular capillary loops collapse
- Cellular proliferation
- lamina rara interna widening and endothelial swelling
- Fibrin
- BL fragmentation
- Inflammatory cells and fibroblasts in capsule
- Matrix like BL or collagen containing



RAPIDLY PROGRESSIVE GN.

- PROGNOSIS
- DEPENDS ON IRREVERSIBLE GLOMERULAR LESION GRADE
- WORST PROGNOSIS IF THERE ARE CRESCENT
- STEROID THERAPY OR BY IMMUNOSOPPRESSORS

INHERITED PRIMITIVE GLOMERULOPHATIES

- CONGENITAL NEPHROTIC SYNDROME
- FAMILIAR BENIGN HAEMATURIA
- ALPORT'S SYNDROME

GENETIC

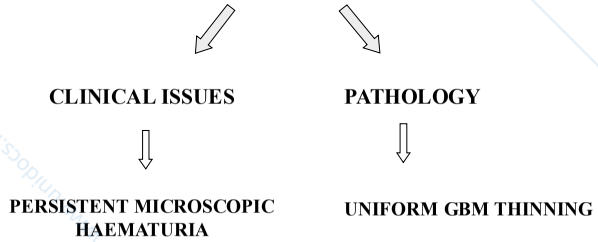
NMBS and ALPORT are genetic diseases caused by mutations in genes encoding the α3/α4/α5 chains of the collagen IV

collagen IV is synthesized by EC and podocytes

6 chains of collagen IV are genetically different encoded by 6 genes in 3 different chromosomes

THIN BASEMENT MEMBRANE DISEASE

HEREDITARY NEPHROPATHY



FAMILIAR BENIGN HAEMATURIA
or thin basement membrane disease

- Persistent microscopic or gross haematuria
- Favorable clinical course. Associated with IgA nephropathy, mesangial proliferative, GSFS
- Membrane width: 191 nm (361 nm, normal values)
- Genetic alteration of the type IV collagen synthesis

FAMILIAR BENIGN HEMATURIA
or this basement membrane disease

LM Normal

IF C3 deposition sometimes

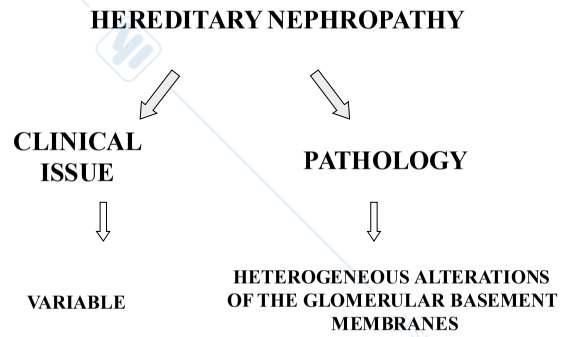
EM

- Segmental thinning of the glomerular basement membranes
- Thickening of the mesangial matrix, deposits

PROGNOSIS

Excellent prognosis in patients without other associated diseases

ALPORT SYNDROME



ALPORT SYNDROME

- 85-90% X-linked
- 10-15% Autosomal dominant or recessive inheritance
- Genetic defect in association with the 5-alpha chain of the collagen IV

CLINICAL ISSUES

- Microscopic haematuria
- Progressive sensorineural deafness
- Eye abnormalities

ALPORT SYNDROME

LM
Mild mesangial hypercellularity
Progressive fibrous interstitium with foam cells

IF
Negative
Anti-collagen antibodies (alpha-3,-4,-5 chains of the collagen IV)

EM
At the beginning minimal alterations visible by EM;
Focal and widespread splitting of the basal lamina; all BL are thinner than normal; electronlucent spaces are filled by granular material (cellular fragments)

ALPORT SYNDROME

PROGNOSIS

- symptoms onset < 10 years
- progression toward renal insufficiency
- some families show a late onset (after 30 years)

GLOMERULAR DISEASE WITH KNOWN ETIOLOGY

- Bacterial infection
- Viral infection
- Parasite
- Drug nephropathy

POSTSTREPTOCOCCAL GLOMERUNEPHRITIS

- Diffuse, proliferative glomerulopathy
- After 8-14 days *Streptococcus pyogenes* infection of respiratory tract or skin; nephritogenic *Streptococcus* strains exist
- PATHOGENESIS

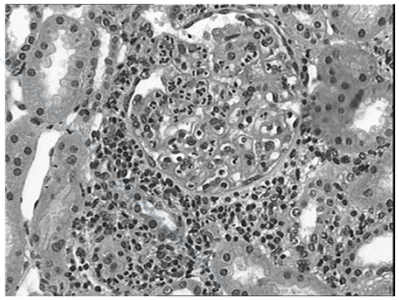
  Deposition of antibody-antigen complexes in glomerulus; antibiotics can reduce the onset of this nephritis which is highly persistent in developing country
- CLINICAL ISSUE

  Haematuria, edema, hypertension, azotemia

POSTSTREPTOCOCCAL GLOMERULONEPHRITIS

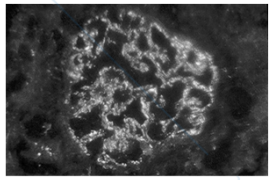
LIGHT MICROSCOPY

- Enlarged glomerulus, diffuse increase of cellularity, neutrophils infiltrate
- Mesangial and endothelial cells involved in proliferation
- Occlusion of the capillary loops in the early stages
- Rare splitting of the GBM
- Subepithelial "Humps" deposits



POSTSTREPTOCOCCAL GLOMERUNEPHRITIS

- IMMUNOFLUORESCENCE
- Small granular subepithelial IgG deposits in correspondence of "humps"



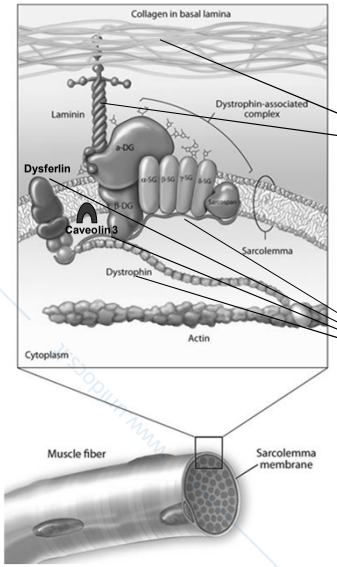
TRANSMISSION ELECTRON MICROSCOPY

- Endothelial proliferation
- Hypertrophic endothelial cells with numerous organelles
- Mesangial hypercellularity
- Subepithelial deposits separated from lamina densa by electronlucent halo; possible
- mesangial deposits
- Hypertrophic podocytes: foot process effacement, microvilli

POSTSTREPTOCOCCAL GLOMERUNEPHRITIS

- **PROGNOSIS**
- **Usually excellent; exudative response is short-lived; only in some cases may progress towards renal failure**

Multiplex Western Blotting: a system for the analysis of muscular dystophy proteins



Extracellular matrix proteins:

Collagen VI,

α2-laminin (or merosin)

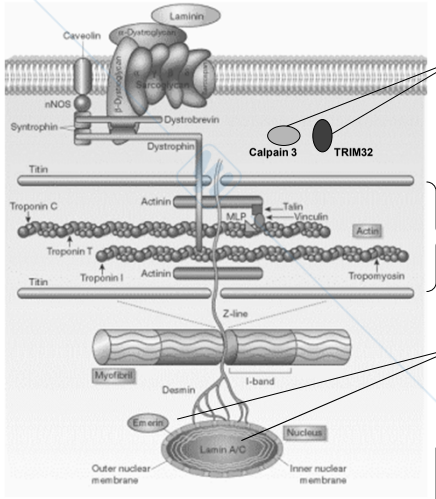
Sarcolemmal proteins:

Dystrophin,

Dysferlin,

Sarcoglycans,

Caveolin-3



Sarcoplasmic proteins:

Calpain 3, TRIM32

Sarcomeric proteins:

Telethonin, myotilin, titin, nebulin

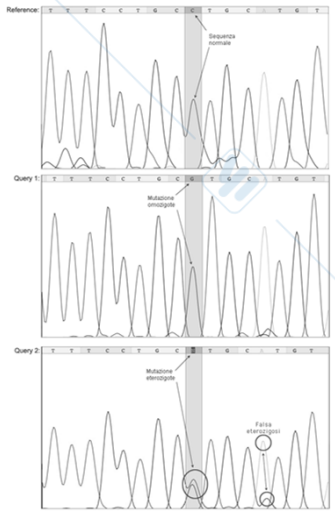
Nuclear proteins:

Emerin, Lamin A/C

Proteins linked to glycosylation process:

fukutin, fukutin-related protein

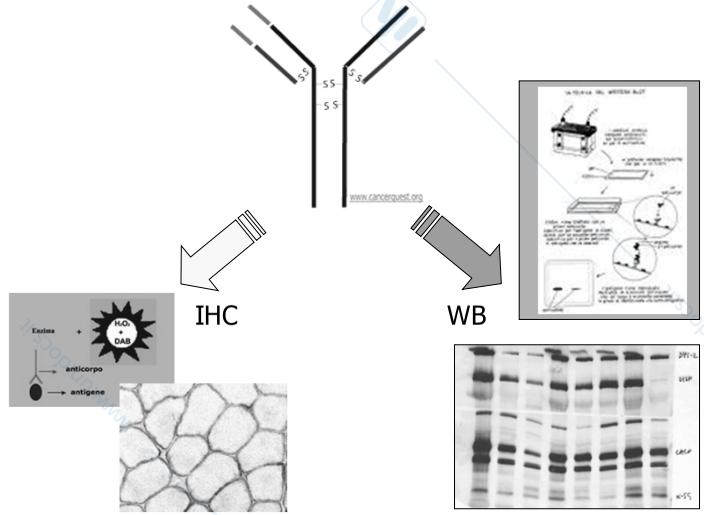
Proteins	Dystrophy	Hereditary	Gene
Extracellular matrix			
Alpha2 laminin (merosin)	alpha2 laminin/merosin deficient CMD	AR	6q2
Collagen VI	Ullrich CMD/Bethlem myopathy	AR	21q22
Sarcolemmal			
Dystrophin	DMD/BMD	XL	Xp21
alpha-sarcoglycan	LGMD2D	AR	17q12-q21.33
beta-sarcoglycan	LGMD2E	AR	4q12
Gamma-sarcoglycan	LGMD2C	AR	13q12
Delta-sarcoglycan	LGMD2F	AR	5q33-34
Dyspherlin	LGMD2B/Myoshi myopathy	AR	2p13
Caveolin 3	LGMD1C	AD	3p25
Sarcoplasm			
Calpain 3	LGMD2A	AR	15q15.1
TRIM-32	LGMD2H	AR	9q31-34
Sarcomeric			
Titin	LGMD2J	AR	2q31
Myotilin	LGMD1A	AD	5q31
Telethonin	LGMD2G	AR	17q11-q12
Nuclear			
Lamin A/C	LGMD1B	AD	1q11-q21
Emerin	Emery-Dreifuss MD	XL	Xq28
Involved in Glycosylation			
Fukutin	LGMD2M/CMD Fukuyama	AR	9q31
FKRP	LGMD2I/CMD 1C	AR	19q1



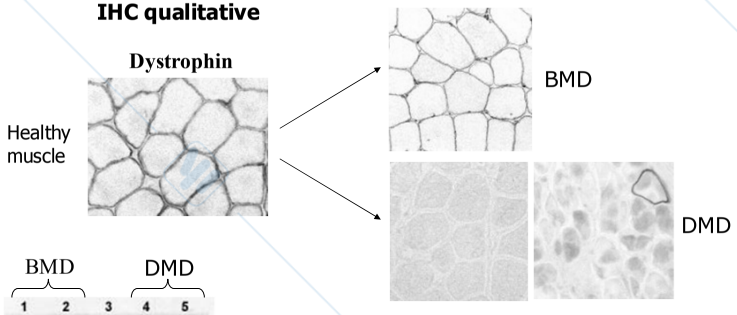
Sequence analysis gives the final diagnosis, but:

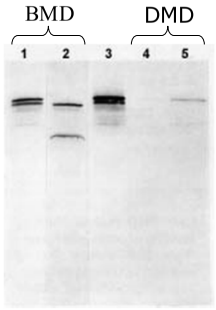
- Expensive
- Many genes involved in the pathogenesis of muscle dystrophies
- Frequently more mutations in one gene
- Identifications of genetic variants that could be not associated to a muscle dystrophy: difficult data interpretation

Techniques for protein study



IHC qualitative





WB qualitative and semi-quantitative

1. Is there the predicted protein?
2. What size is it?
3. How much protein is there?

Western blot of dystrophin from dystrophinopathies.
Lane 1: Becker dystrophy; Dystrophin has reduced abundance but normal size.
Lane 2: Becker dystrophy; Dystrophin has reduced size and abundance.
Lane 3: Normal; Dystrophin has normal size and amount.
Lane 4: Duchenne dystrophy; Almost no protein is present.
Lane 5: Duchenne outlier; Dystrophin has severely reduced abundance.

Western Blot

WB separates proteins on the base of their size (kDa); this method uses a biphasic polyacrylamide gel to separate proteins and then they are blotted on a nitrocellulose membrane; membranes are immunoblotted using specific antibodies.

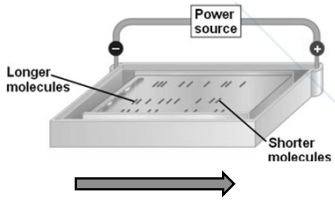
Western Blot

WB steps:

1. Sample preparation: protein extraction from cells or tissues
2. Protein amount quantification
3. Polyacrylamide gel electrophoresis
4. Transfer from gel to membrane (*blotting*)
5. Protein immuno-detection

Gel electrophoresis

Electrophoresis is a separation method based on the different migration speed of molecules of different size and charge.



Molecules are separated by applying an electric field which permits to the negatively charged molecules to move through the matrix of polyacrylamide.
Shorter molecules move faster and migrate farther than longer ones.

It' s a good separation method for macromolecules and in particular for proteins and DNA fragments.

Sample preparation

WARNING

1. During cell/tissue lysis and protein extraction it is necessary to avoid protein denaturation in our samples (high temperatures, cleanser, extreme pH could easly cause protein denaturation).

2. Protein extraction should be performed as quickly as possible in order to not trigger degradation processes.

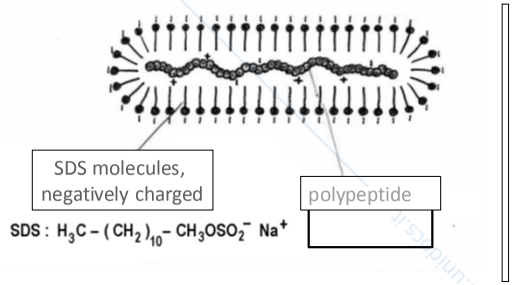
Polyacrylamide gel electrophoresis for proteins in SDS (SDS-PAGE)

Gels are prepared by polymerization of monomers of acrylamide plus small amounts of bis-acrylamide.
Polymerization starts adding ammonium persolfate (APS) e N,N,N',N'-tetramethylethylenediamine (TEMED).

The size of pores in gels depends on the amount of acrylamide/bis-acrylamide (3-15%) used in creating a gel.

SDS-PAGE

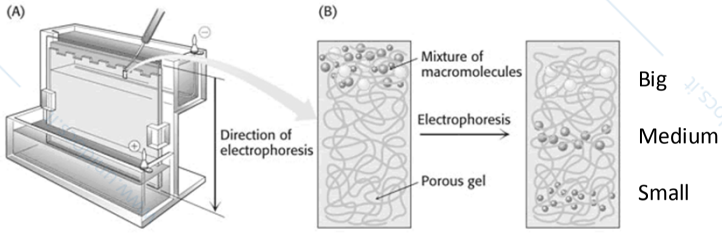
SDS (sodium dodecyl sulfate) is a strong reducing agent able to denature proteins removing secondary and tertiary structures; so proteins remain as linear polypeptides negatively charged.



SDS-PAGE

Proteins are separated according to their size.

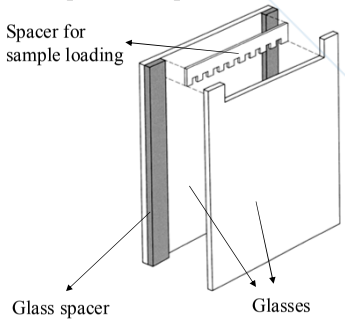
Big proteins move slowly, small proteins migrate faster.



Polyacrylamide gel preparation

Laemmli method (two different gels)

1- Between the two vertical spaced glasses used to polymerize the gels, we pour the first gel solution: the running gel which will separate the proteins.



2- On the running gel we pour the second gel solution: the stacking gel which will concentrate the samples so they will start the migration from the same starting point.

3- Before the polymerization of the stacking gel, we add the specific spacer that create the wells for sample loading.

Polyacrylamide gel preparation

WARNING!

1. Take care when creating this type of gel: acrylamide is a neurotoxin!!!

2. Sometimes gels do not polymerize correctly.

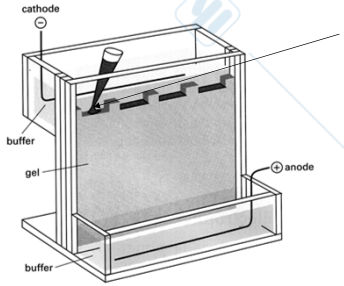
⬇

Now: precast polyacrylamide gels designed to give optimal separation of a wide range of proteins under denaturing conditions.

Preparazione gel di poliacrilammide

Laemmli method (two different gels)

4- Once the gels polymerize, they are inserted in the electrophoretic chamber that will be filled with running buffer.



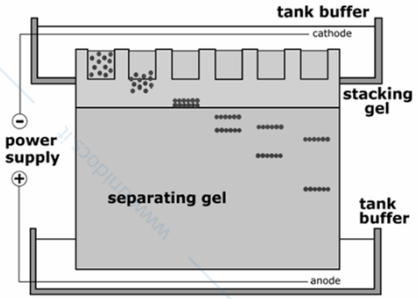
5- Samples are resuspended in loading buffer containing SDS, glycerol and bromophenol blue and loaded in the wells

6- One lane is reserved for a marker, a mixture of proteins of known molecular weights

Running buffer : 0,1% SDS, Tris 25mM, Glycin 0,192M pH 8,3

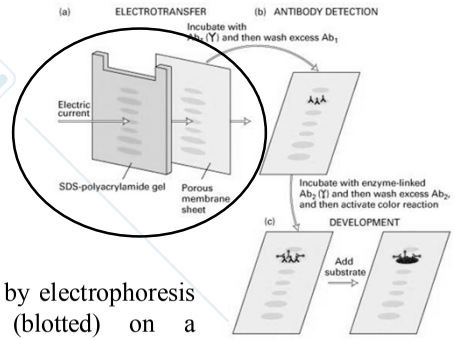
SDS-PAGE

7- When voltage is applied, proteins migrate through it at different speeds dependent on their size.



8- The different rates of advancement separate into bands in each lane. Protein bands can then be compared to the marker bands and protein's molecular weight can be estimated.

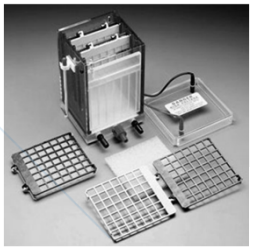
Transfer



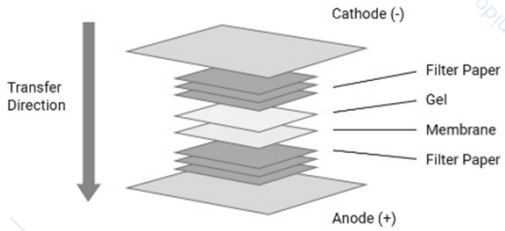
Protein separated by electrophoresis are transferred (blotted) on a membrane (nitrocellulose, PDVF or nylon) applying an electric current.

Transfer

To transfer the protein into the membrane we have to create a sandwich made with:

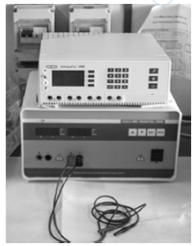


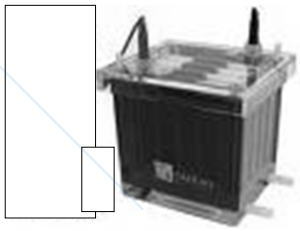
- sponge
- filter paper
- gel
- membrane
- filter paper
- sponge



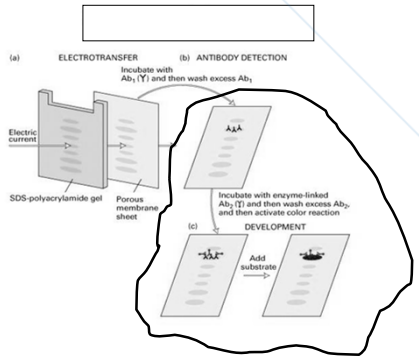
Transfer

Protein blotting is realized applying an electric current to pull the negatively charged proteins from the gel towards the positively charged anode and into the membrane.





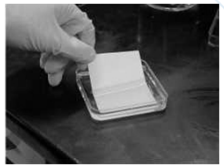
Detection

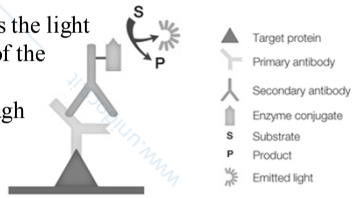


The proteins blotted on the membrane are immunolabelled using specific antibodies.

Detection

1- Wash in TBS-Tween
2- blocking of non-specific binding with TBS-T + milk + BSA
3- Overnight incubation with primary antibody
4- wash with TBS-T
5- incubation with secondary antibody linked to a reporter enzyme (such as HRP)
6- wash with TBS-T
7- adding of chemiluminscent substrate that is cleaved by HRP, resulting in the production of luminescence
8- CCD camera/photographic film captures the light from the reaction and creates an image of the antibodies bound to the blot.
9- OD of each band can be measured through image analysis softwares (ImageJ)



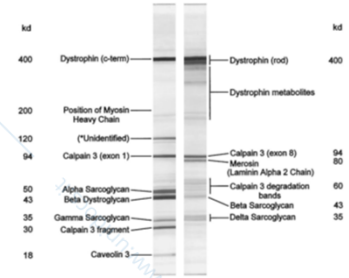


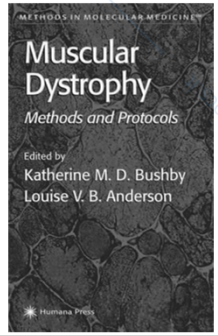
American Journal of Pathology, Vol. 154, No. 4, April 1999

Technical Advance

Louise V. B. Anderson and Keith Davison
From the Neurobiology Department, University Medical School, Framlington Place, Newcastle upon Tyne, United Kingdom

Multiplex Western Blotting System for the Analysis of Muscular Dystrophy Proteins

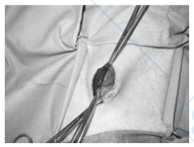




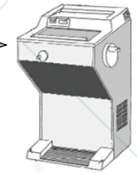
Muscle sample preparation

(Padua protocol)

Muscle biopsy



Snap frozen muscle sample



~ 30-40 slices 10 µm

COLD !!!

100µl of Loading buffer (E. Hoffman): 50mM dithiothreitol, 0.1M EDTA, 0.125 TRIS, 4% SDS, 10% glycerol, few mg of bromophenol blue. pH=8.0

Muscle sample preparation

(Padua protocol)

Vortex

Heat 99°C 5 min

12000 rpm 10 min

~ 1 [redacted] of proteins

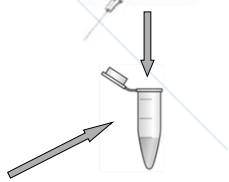
Muscle sample preparation

(Newcastle protocol)

Muscle sample weighed before freezing (Ex. 20mg) + 19 volumes of loading buffer (Ex. 380 µl)

Homogenizer

2 x 15 sec

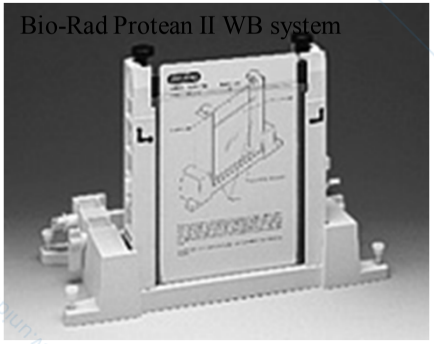


Loading buffer (L. Anderson): 10% beta-mercaptoethanol, 4M urea, 0,125M TRIS pH=6.8, 4% SDS, 10% glycerol, 0,001% bromophenol blue. pH=8.0

~ 7 [redacted] of proteins

Running gel prepration

Gel with polyacrylamide gradient **3.5% [redacted] 10%**



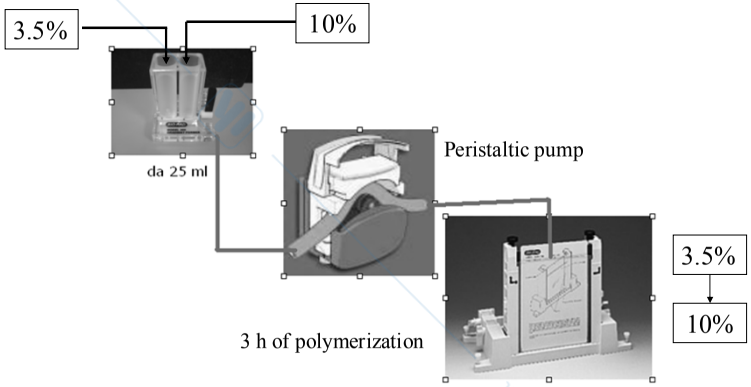
3.5%

10%

16cm x 14cm x 1,5mm

Running gel preparation

Gel with polyacrylamide gradient **3.5%** ➡ **10%**

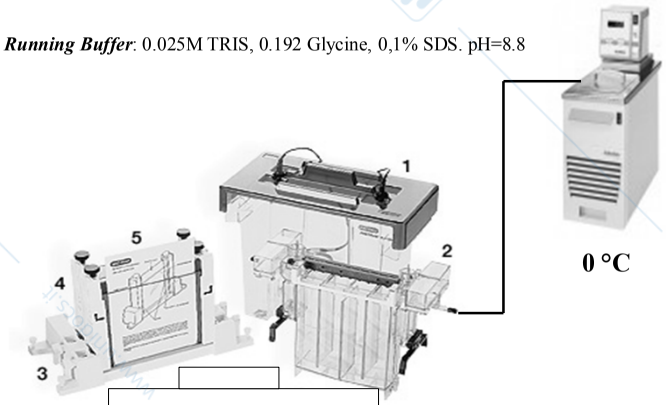


Stacking gel preparation

Gel with 1,5% polyacrylamide

Electrophoresis

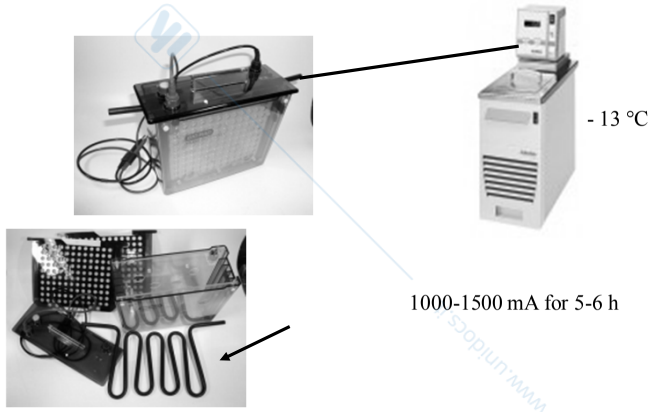
Running Buffer: 0.025M TRIS, 0.192 Glycine, 0,1% SDS. pH=8.8



110V for 16-18 h (or overnight)

Blotting on nitrocellulose membrane

Transfer Buffer: 0.025M TRIS, 0.192 Glycine. pH=8.3 + MetOH 20%



1000-1500 mA for 5-6 h

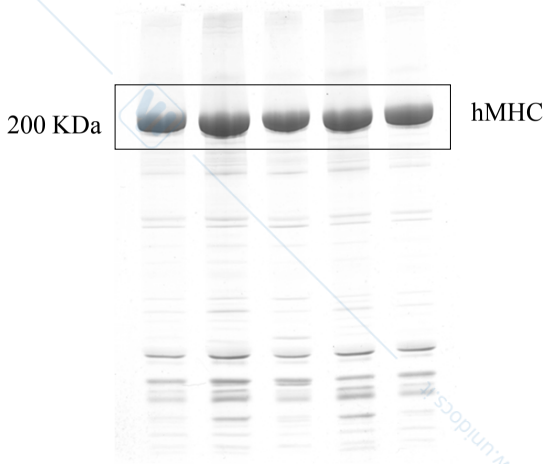
Detection

Immuno labelling of membrane using different sets of antibodies

MEMBRANE 1	MEMBRANE 2
1. DYS C-ter	1. DYS Rod
2. DYSFERLIN	2. CALP 3 (EX 8)
3. CALP 3 (EX 1)	3. LAMININ α2
4. α SARC	4. β DYSTRO
5. CAV3	

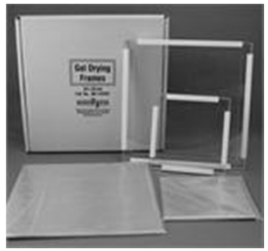
Gel staining

Coomassie Blue stains all proteins in the gel

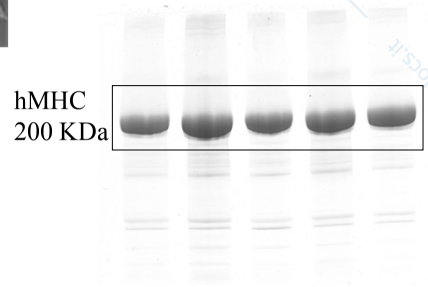


Gel drying

Gel Drying Frames are used to keep the gels.



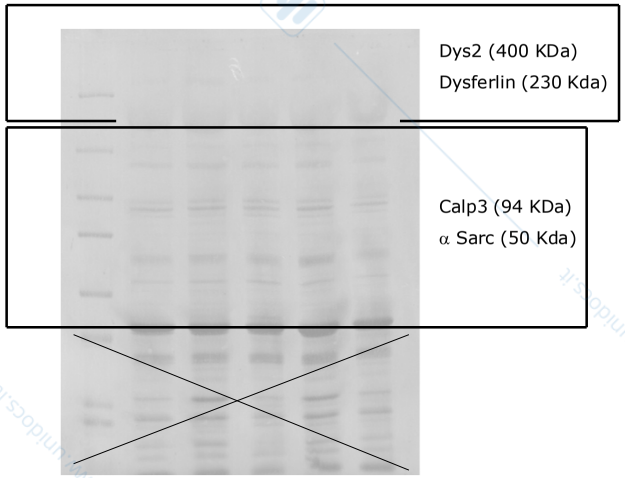
Once gels are completely dry, the myosin (hMHC) band will be used as normalizer for calculating the amount of the other muscle proteins detected on the membranes.



Membrane staining

Ponceau Red stains all proteins in the membrane

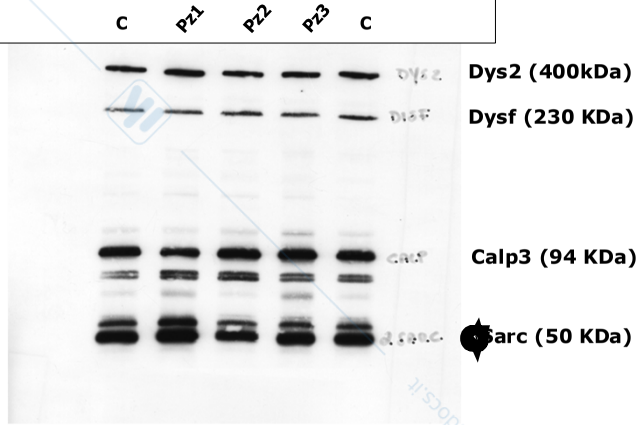
Detection



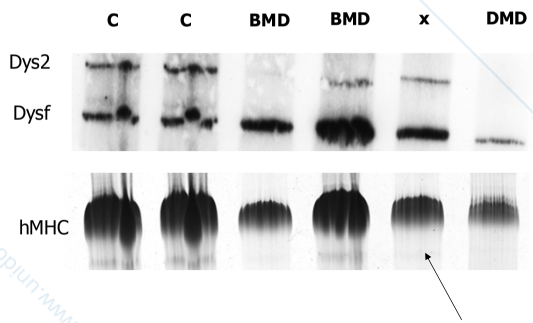
Different combinations of antibodies are used at the same time for immunoblotting

Photographic films

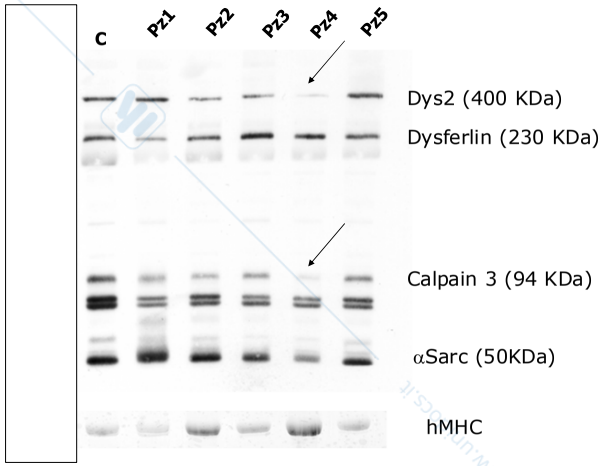
Films capture the light from chemiluminescent reaction



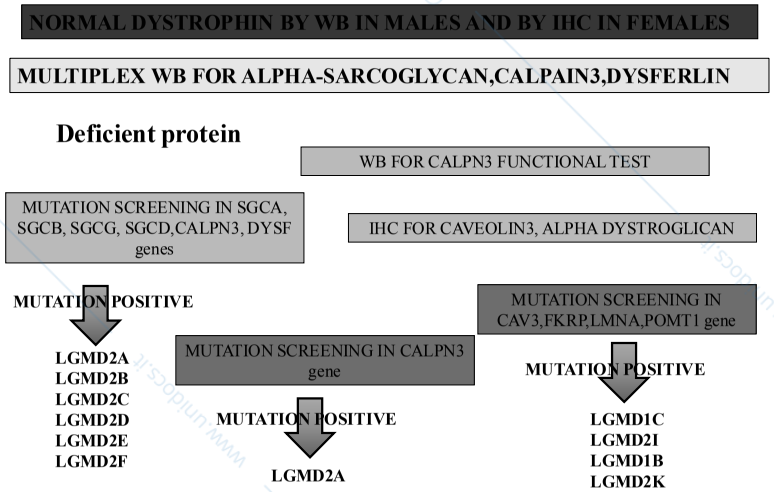
Densitometric analysis



C: control (healthy muscle)



DIAGNOSTIC ALGORITHM OF LGMD



Multiplex Western Blotting System

Analysis on protein expression is useful for:

- evaluating the effect of newly identified mutations.

- dystrophynopaties: phenotype is more linked to the protein expression than the genotype.

-Analysis of proteins is essential to evaluate the effect of researches or clinical trials, such as gene therapy, whose aim is restoring the missing/mutated protein.