Journey into the world of pathology

Back to important events: historical landmarks in pathology

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Prerequisite knowledge

• In order to understand the topic of this lecture, you should:
  – know the basics of histology and histophysiology
  – know the basics of immunology
Learning objectives

• After watching this lecture, you should be able to:
  – describe briefly the pathologist’s job
  – define the basis of disease nomenclature
  – define tissue homeostasis
  – give examples of loss of tissue homeostasis
  – describe the pre-analytical procedure for a microscopic evaluation of a tissue
  – describe the Koch’s postulates
  – give a simple account of immunofluorescence methods
  – define the « clusters of differentiation »
  – decribe a method to detect gene amplification at the tissue level
Pathological Anatomy in 2014

• The Pathologist’s job:
  – diagnosis of diseases based on the morphological interpretation of lesions (gross examination, microscopy, immunohistochemistry, molecular biology…)
  – determination of prognostic clues as well as eligibility (or non eligibility) of patients for targeted therapies (neoplastic diseases)

• How to become a Pathologist (in France)?
  – get a M.D. degree
  – perform successfully a 5-Y training (internship) in pathology departments
The basis of « general pathology »

- General pathology is concerned with the loss of tissue homeostasis in relation with stimuli or stresses, that underlie all diseases.
- Tissue homeostasis is defined as the maintenance of structural and functional tissue integrity.
Chapter 1: Diagrammatic representation of general pathology

- *Space-time diagram* of an individual’s life
- *Developmental pathology*
- physiological tissue alterations: *aging*
- tissue homeostasis
- loss of tissue homeostasis
- *Illustrations of loss of tissue homeostasis*
Space-time representation of an individual’s life: the pathologist’s point of view

Widening of the cone: tendency toward deviation from standard pattern (young adult)

Development: Tissue and organ morphogenesis: narrowing of the cone

Time (years)

Reproductive age

Limit of compatibility With life

Toward tissue alterations => « level of issue disorganization »

birth

elderly

Limit of compatibility With life
Space-time representation of an individual’s life: 
the pathologist point of view

Widening of the cone: tendency toward deviation from standard pattern (young adult)

Development: Tissue and organ morphogenesis

Time (years)

A biopsy is a slice at a time point recounting the present and often the past and future.

Radar chart of Tissue organization/disorganization

- Reproductive system
- Circulatory system
- Respiratory system
- Digestive system

Limit of compatibility
With life
Space-time representation of an individual’s life: the pathologist’s point of view

Tendency to desorganization

Developmental pathology: Example: miscarriage due chromosomic aberration or viral infection leading to developmental defect

Toward organization / Development: Tissue and organ morphogenesis

Out of limits

Limit of compatibility With life
Deviation from «normal» over time is a physiological process: aging

Tendency to disorganization

Time (years)

elderly

Reproductive age

birth

Tissue (des)-organization
Tissue homeostasis: maintenance of (or return to) the structural and functional integrity of tissues facing a stress (bacterial, viral, carcinogenic...)

1 - Integrative systems maintain tissue homeostasis:
   - Nervous
   - Hormonal (endocrine)
   - Immune (innate and adaptive immunity)

2 - The immune system tends to restore tissue homeostasis through an inflammatory reaction in face of infection

Biopsy: a slice in time

Time (years)

birth

Tissue (des)-organization

repair
Aging (senescence) affects the integrative systems and contributes to the loss of homeostasis

- Senescence of the immune system (immunosenescence) weakens the immunosurveillance of tumors. Explains the increased frequencies of tumors over time.
- Hormonal senescence (menopause)

**Definition:** Immunosurveillance of tumors is the ability of the immune system to detect and destroy nascent tumors.
loss of tissue homeostasis may be due to inappropriate proliferation

1- loss of tissue homeostasis may result in uncontrolled cell growth and eventually death

2 – gastric carcinoma

Biopsy: a slice in time
Loss of tissue homeostasis can result from altered immune control: autoimmune thyroiditis

- **Autoimmune thyroiditis**, is a disease in which the body interprets the thyroid glands and its hormone products T3, T4 and TSH as threats, therefore producing special antibodies that target the thyroid’s cells, thereby destroying it.
Loss of tissue homeostasis due to poor oxygenation results in cell necrosis

1- loss of tissue homeostasis through ischemia (improper tissue oxygenation) or anoxia leads to tissue necrosis (infarct)

Consequence: Sudden death
Scar leading to heart failure
Chapter 2: the pioneers...

Let’s go back to 1802...
Pinel’s classification of diseases is in some way the « gold standard »: based on « clinical scenes »

Classification (rank hierarchy) according to Linné:
Classes => Orders => genera => species (simple or complicated)
The limitations of existing classifications of diseases (1)

- Very complicated classifications (see Pinel)
- Diseases often named from the symptoms (a narrative by the patient) : how to detect the liars? (many people want to escape their military duties…) (Corvisart)
The limitations of the existing classifications of diseases (2)

• terms that are used to name diseases are too vague

• case in point: « phtisis »: literally « consumption » has several meanings:
  – is the last phase in the course of progressive diseases (e.g. cancer) associated with dramatic weight loss, anorexia, dramatic weakness: phtisis is then the evolutive convergence of several diseases
  – for others, should be restricted to pulmonary diseases causing weight loss, fever, hemoptysis…
  – finally there are young patients dying of phtisis without internal lesions (called phtisis nervosa) (now referred to as anorexia nervosa)
And then came Laennec: founder of pathological anatomy…

- His rationale:
- The systematic autopsies of patients who died in the hospital show a limited variety of lesions
- there is a close correlation between the morphology (texture) of the lesions, their evolution, and the outcome of the patients.
Seminal lecture (december 27th 1804) published in the « journal de médecine, chirurgie, pharmacie, 1805 »

360  ANATOMIE

NOTE

SUR L'ANATOMIE PATHOLOGIQUE (1);


A l'aspect des altérations nombreuses dont l'anatomie nous révèle l'existence, et dans lesquelles elle nous fait voir la source, ou les effets, des maux qui affligent l'homme physique, on serait tenté de croire que l' nature, si constante dans la marche qu'elle suit pour la production des êtres organisés, devient sujette, lorsqu'elle en opère la destruction, à
Laennec: the metaphor of the acorn and oak

• Apparently unrelated lesions are merely different stages of a unique disease: in other terms, a lesion encapsulates the disease process.

• The pathologist’s job is to regroup lesions that are evolutionary stages of a unique disease process.
Laennec introduces a morpho-chronological classification of lesions

• classification based on the assumption that the « texture » of a lesion recapitulates the disease process

• Introducing a «morpho-chronological » denomination of lesions ex. « the tubercle » encompasses several lesional patterns.

Pulmonary Tubercle = yellowish non translucid substance (crude state) becoming soft and friable on softening. May lead to a cavity upon evacuation or to infiltrates.

=> Phtisis becomes « tuberculosis »

*Important notice: this nomenclature omits any causal element because too speculative
Tubercle: unites lesions with different morphologic and topographic presentations (nodules, infiltrates, caverns...)
Definition of pathological anatomy by Laennec

« Science whose aim is to gain the knowledge of the visible alterations that the disease state produces in the human body. The opening of corpses is the way to get this knowledge. However in clinical practice, the pathological anatomy should be connected with the observation of symptoms or the alterations of functions associated with alteration of organs » (In Panckoucke 1812)
Laennec’s legacy

• The morphology of a given lesion is predictive of its evolution and of the clinical outcome.

• The disease name should be based only on the representative anatomo-pathological lesions

• example: the term « phtisis » becomes only anatomical and will be replaced by « tuberculosis »
Main opponent to Laennec: Broussais (examen des doctrines médicales et des systèmes de nosologie)

- A nomenclature of diseases based on pathological anatomy is irrelevant to the medicine as the name of the disease can be obtained only by the autopsy…
- No therapeutic relevance of this nomenclature
- No causal relationship
Answer 1: translating an anatomo-pathological classification into medical diagnosis: anatomoclinical correlations

• Laennec: Stethoscopic semiology* defines « pectoriloquy » that indicates a cavity in the lung

* Semiology: the science which deals with signs
The renowned French school of medicine, led mainly by anatomists, refuses the microscope.....

- Do I need a microscope to tell the difference between an apple and a pear?..... ?
Chapter 3: early times of microscopic studies
Chapter 3: Virchow’s lectures on cellular pathology Feb-April 1858 Berlin

- Attempts to give an interpretation of lesions based on their microscopic examination

- «Omnis cellula e cellula»: every tissue is made of cells (Schwann); all cells derive from parent cells. All lesions comprised of cells (including purulent inflammation, tuberculosis, and tumors) are only deviations of cell multiplication/differentiation… there is no alien tissue

- However, Virchow proposes that inflammatory (pus) and cancer cells evolve from the same local cells

Virchow
Does not pay attention to the preparation of tissues
Tissue processing is a prerequisite for Microscopic examination of tissues

• Pathological diagnosis based on gross examination does not need a specific preparation/processing of tissues

• Microscopic examination of tissues requires a preparation/processing i.e. 1) tissue fixation (formalin), 2) tissue hardening (paraffin), 3) thin sectioning, 4) staining of tissue sections

• This pre-analytical phase is a prerequisite for the elaboration of a « microscopic semiology » needed for a microscopic diagnosis.
Tissue processing: paraffin embedding
sectioning
Staining: « Bloodwood » *Haematoxyllum campechianum*

*haima* being for blood and *xulon* for wood: use for nuclear staining
Chapter 4: delineating the pathological characteristics of inflammatory and neoplastic diseases
Villemin: « studies on tuberculosis » 1868

- Tuberculosis is contagious, and can be transmitted by inoculation to specific animals (rabbit, guinea pigs) of tuberculous lesions, or blood or saliva.
- Tuberculosis is due to a transmissible « agent », external to the patient, that induces a response by the organism.
- He confirms that the variety of lesions termed « tuberculosis » have a unique cause.
Koch’s conference: March 22, 1882, Berlin

- Gives evidence (proof) that tuberculosis is due to a living microorganism according to the so-called Henle-Koch postulates:

- A disease can be assigned to a specific agent if:
  - the pathogenic agent is always present in the lesion (and never in the healthy subject)
  - the pathogenic agent is purified by culture
  - the cultured agent reproduces the disease in an experimental animal
  - the cultured agent can be recovered from the diseased animal
Koch’s postulates

- establish the principle of subordination of a lesion to a « specific » agent
- question the validity of a classification of diseases based on the morphology of the lesions…
- However, an organism can harbor a pathogenic agent without being ill (healthy carrier)
Cohnheim, a gifted pathologist: a pathologist must be also be an experimental pathologist/ the crucial experiment

- method: frog’s tongue or mesentery (transparent tissues): irritation causes the following sequence:
  - dilation of arterioles
  - increased blood flow
  - plasma leakage
  - leukocyte margination
  - leukocyte diapedesis
  -=> microscopic findings that correlate with « acute inflammation »:
  - Vasodilation=rubor (redness)
  - Increased flow= calor (heat)
  - fluid accum= tumor (swelling)
Metchnikoff: « leçons sur la pathologie comparée de l’inflammation » Pasteur Institute 1891

- The inflammation is primarily a salutary reaction
- Two types of leukocytes, mononucleated cells and neutrophils, have phagoytic properties
• **Acute Inflammation**: «*In an acute inflammation, there is a vascular dilatation, an activation of vascular endothelium and a exsudate with diapedesis, i.e. three steps that result in the accumulation of phagocytes in the injured area*»

• **Chronic Inflammation**: *ex. tuberculosis*: «*the tubercle is only made of phagocytic cells (derived from mononuclear cells)*». It does not result from the multiplication of cells, but only from the recruitment of cells….. The so-called epithelioid cells (comprised of mononucleated cells) fuse in order to form giant cells…..
Chapter 4 : conclusion (1)

- New concepts drawn from observation and experimentation:
  - **Inflammatory lesions**: lesions associated with the local recruitment of blood-derived cells - causative agents are external, transported by the blood: it is the dissemination of the agent that causes the extension of the disease
Chapter 4 : conclusion 2: nomenclature of inflammation

• By the end of the 19th century the microscopic tools allow to tell the difference between inflammation and neoplasia. Inflammation has its own nomenclature.

**Acute inflammation**
(vasodilatation /congestion/, oedema, Polynuclear leukocytes)

**Binominal classification**
Acute Gastritis
Acute Appendicitis

**Chronic Inflammation**
(monorucleated phagocytes)

**Chronic Gastritis**

**Morpho-chronological nomenclature**

**Spécificity:** pathogenic agent that can be revealed in situ by specific stains
Chapter 4 : conclusion (3)

• New concepts drawn from observation and experimentation:

• **neoplastic lesions**: lesions associated with the local multiplication of resident cells - causative agents are (probably) internal to the cells. The metastasis of a malignant neoplasm is due to the dissemination of malignant cells.
Chapter 4 : conclusion 4: building a microscopic nomenclature of neoplasia

- **Law of homology**: the homology between a neoplastic tissue and healthy tissue, as assessed by microscopy, establishes the tumor genealogy and its nomenclature.

- Maintenance of a binominal morpho-chronological nomenclature of neoplasia, based on the genealogy of the neoplastic tissue:

  - **Colonic adeno-ma**: Colonic tumor developed from the colonic glandular Epithelium, benign
  
  - **Colonic adenoc-arcinoma**: Colonic tumor developed from the colonic glandular Epithelium, unstable, that metastasizes
building a microscopic nomenclature of neoplasia: eponymous diseases

- The status of some lesions remained undetermined for a long time: case in point Hodgkin’s disease: What is the cell of origin? is it inflammatory or neoplastic?....
Chapter 5: the morphological nomenclature of diseases becomes therapeutically relevant

Halsted
Implementing safe invasive procedures thanks to the work of the big three…

- Pasteur
- Lister
- Semmelweiss
Invasive procedures validate the morphological nomenclature of diseases / surgery becomes safe.

- Biopsy and excisional biopsy: removal of a tissue fragment (or the whole lesion: excisional biopsy) at a given site to perform a morphological (microscopic) diagnosis.
- Practically all organs and lesions are amenable to biopsy: the criticism to a pathological classification of diseases becomes outdated.
Pathological diagnosis dictates the therapeutic strategy in oncology

- Most **malignant epithelial tumors** are primarily treated by **oncologic surgery**
- **Lymphomas** (malignant tumors of the lymphoid tissue) are sensitive to chemotherapy and are not eligible for surgery
- **choriocarcinomas** (highly malignant tumors of the placenta) are sensitive to **chemotherapy**
- **seminoma** (malignant tumor of the testis or ovary) is highly radiosensitive
Chapter 6: complementary techniques: protein and DNA imaging
Seeing the invisible... Introducing imaging at the molecular level: proteins

- Coon’s story (1940-50): antibodies with a fluorescent tag allow to see at the molecular level (protein, glycoprotein) in a tissue.
- Antibodies are polyclonal (produced by injecting an animal, rabbit, goat, with a purified antigen)
Seeing the invisible… Introducing the molecular imaging of proteins

- Monoclonal antibodies: Köhler and Milstein
  - Production of unlimited amounts of antibodies specific for a predetermined antigen
  - Allows cell immunophenotyping. Heterogeneity of the nomenclature of antibodies and redundancy of specificity have led to a denomination of antigens based on a standard classification: Clusters of Differentiation (CDs)

Example CALLA antigen (Common Acute Lymphoblastic Leukemia Antigen) recognized by the J5 monoclonal was designated CD10. Now known as a type II transmembrane protein found on pre-B cells, germinal-center B cells, some neutrophils, kidney cells, T-cell precursors, and epithelial cells that acts as a zinc metalloprotease cleaving peptide bonds on the amino side of hydrophobic amino acids; expressed in acute lymphocytic leukemia and follicular-center-cell lymphomas.
Seeing the invisible… introducing molecular pathology via in situ hybridization: reading the DNA code

Oncogenesis (cancerogenesis) proceeds partly via an accumulation of genomic errors or repeats (amplification) of some normal genes (called proto-oncogenes) that become oncogenic (called oncogenes)…. 
The approach used by the pathologist to « see » genomic alterations: metaphor of the Braille method for blind people

Letters are replaced by raised dots
A fluorescent paper tag with tiny holes that fit a sequence of raised dots can be used to detect a specific word or its repetition in a text.
Now replace the paper tag by a fluorescent sequence complementary to a gene sequence.... And you can observe gene amplifications.
The role of the pathologist: top-down tissue analysis:

• Depending on the tissue available for examination, the pathologist uses 3 levels of analysis (semiology):
  – **gross examination**: form, texture, weigh of sample (tumor, organ, biopsy etc…) dimensions
  – **Microscopic examination**: tissue architecture, type of cells (inflammatory cells, tumor cells…)
  – **Complementary methods**:
    • Immunohistochemistry: tumor immunophenotype
    • In situ hybridization: deciphering the abnormalities of the DNA code (gene amplification, translocations…)
Chapter 6 conclusion: The role of the pathologist

• Laennec’s legacy:
  – the diseases get their names from the morphology of the underlying lesions
  – the morphological diagnosis dictates the therapeutic strategy
  – Anatomic pathology is the gold standard for the diagnosis and treatment of neoplastic diseases
Pathological anatomy: the diagnostic « gold standard »
That's all Folks!