AMYLOIDOSIS

Definitions

- **Amyloidosis is a clinical disorder caused by extracellular deposition of insoluble abnormal fibrils that injure tissue. The fibrils are formed by the aggregation of misfolded, normally soluble proteins**

Common features of all definitions

- presence of systemic protein metabolism disorder (acquired or hereditary)
- extracellular deposition of abnormal protein fibrils
- impairment of affected organs due to amyloid deposition

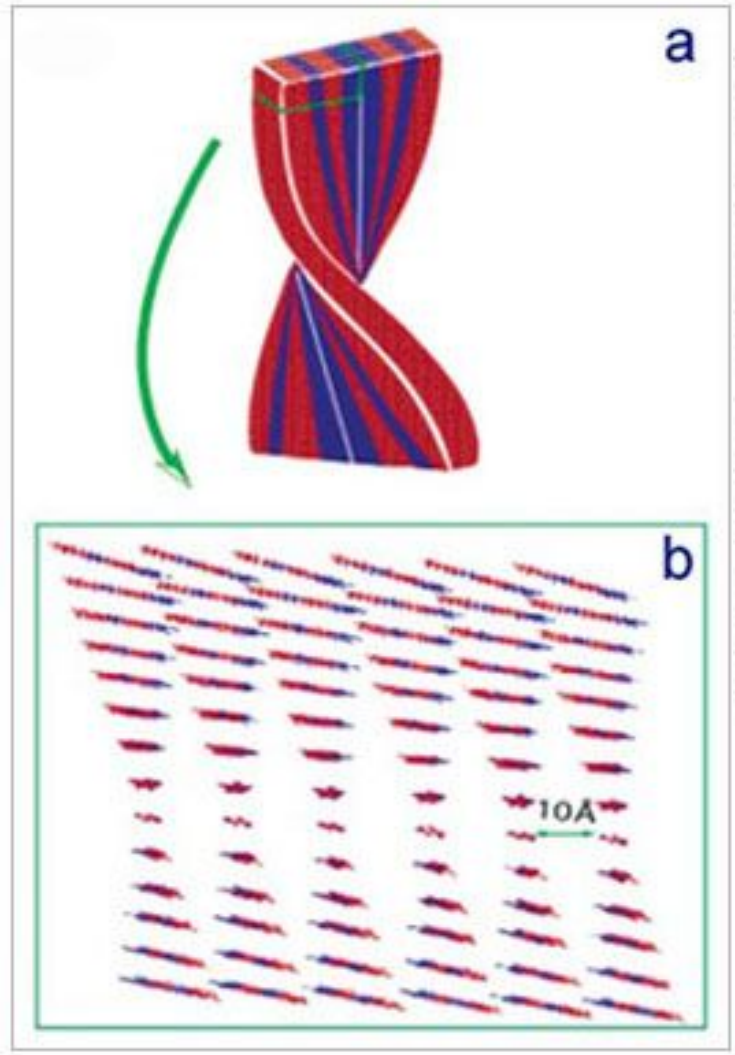
What is amyloid? (physical properties)

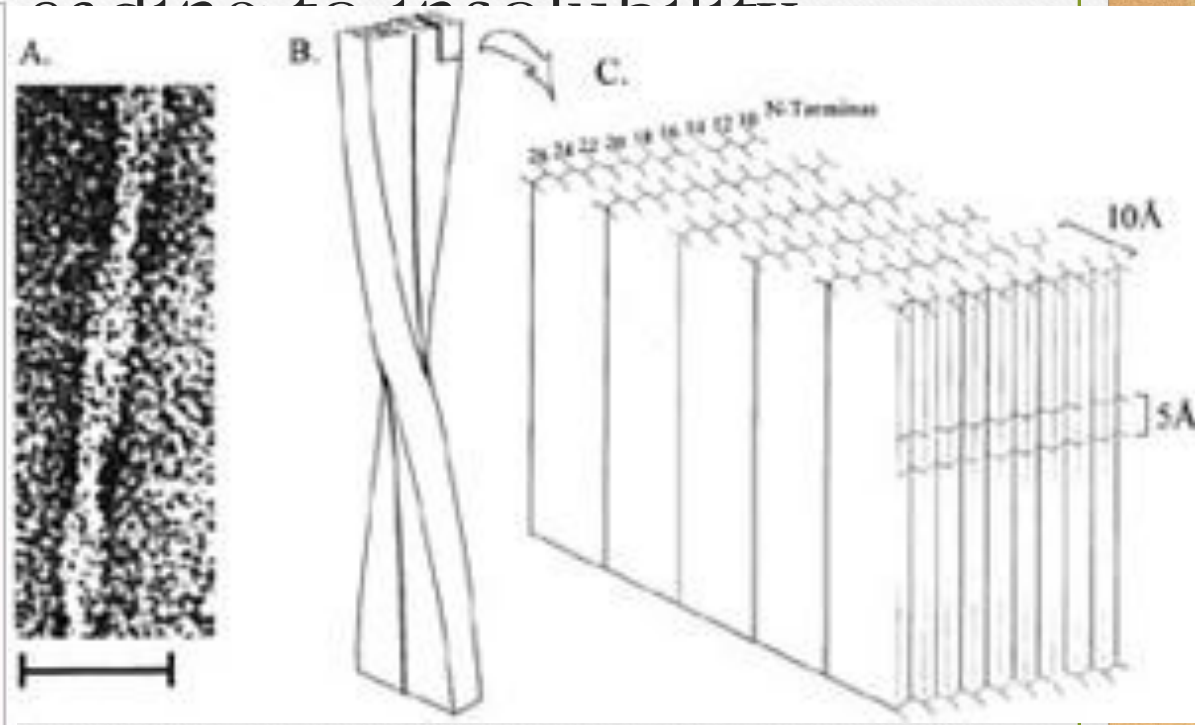
- straight, rigid, non-branching, of indeterminate length and 10 to 15nm in diameter; regular fibrillar structure
- consisting of β-pleated sheets
- aggregates are insoluble in physiological solutions
- relatively resistant to proteolysis

What are β-pleated sheets

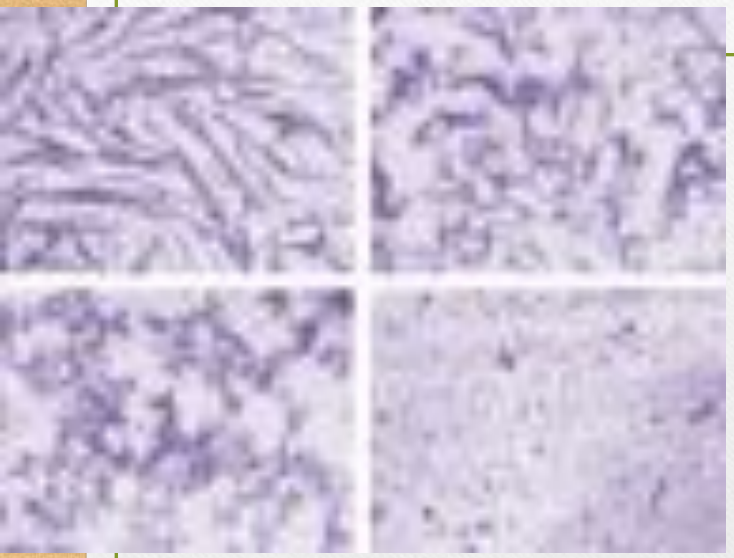
- single amyloid fibril consists
- of stacks of anti-parallel β-pleated sheets
- arranged with their long axes perpendicular to the long axis of the fibril,
- resembling structure of silk, which, like amyloid, is proteinase resistant (can be revealed by x-ray diffraction)

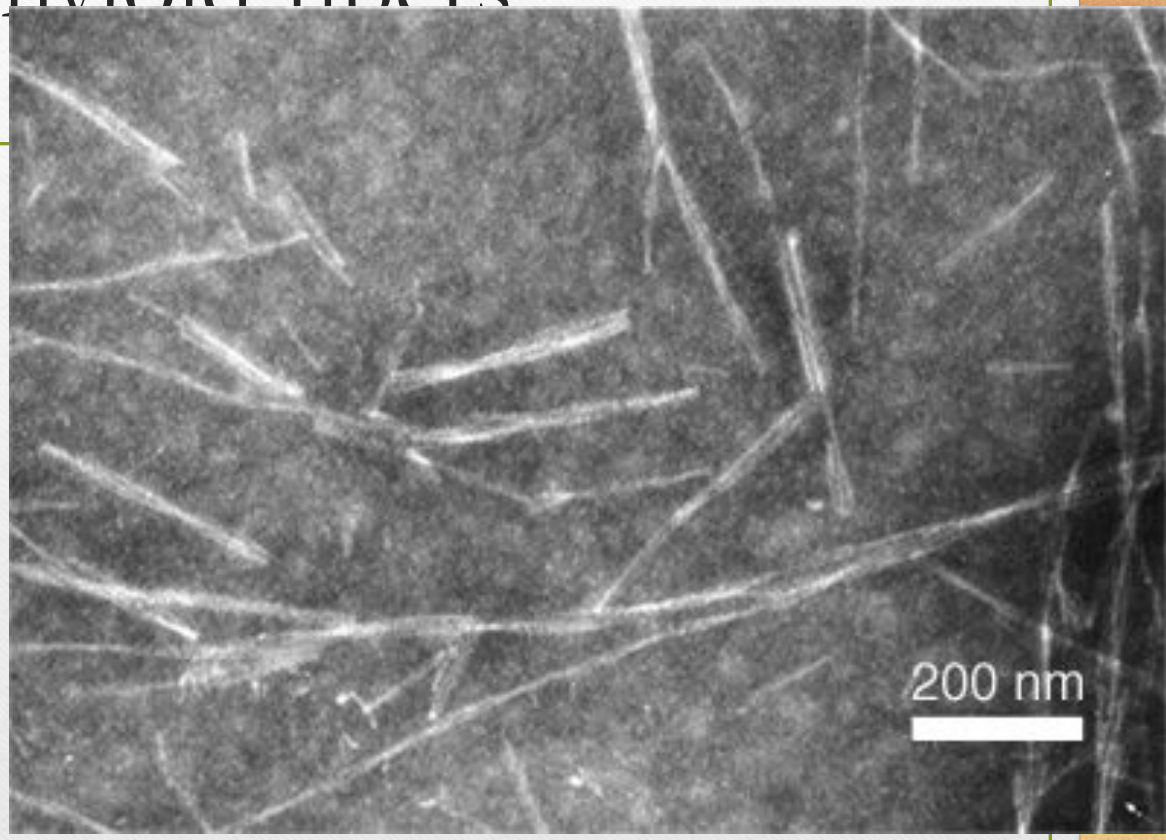
Tertiary structure of amyloid



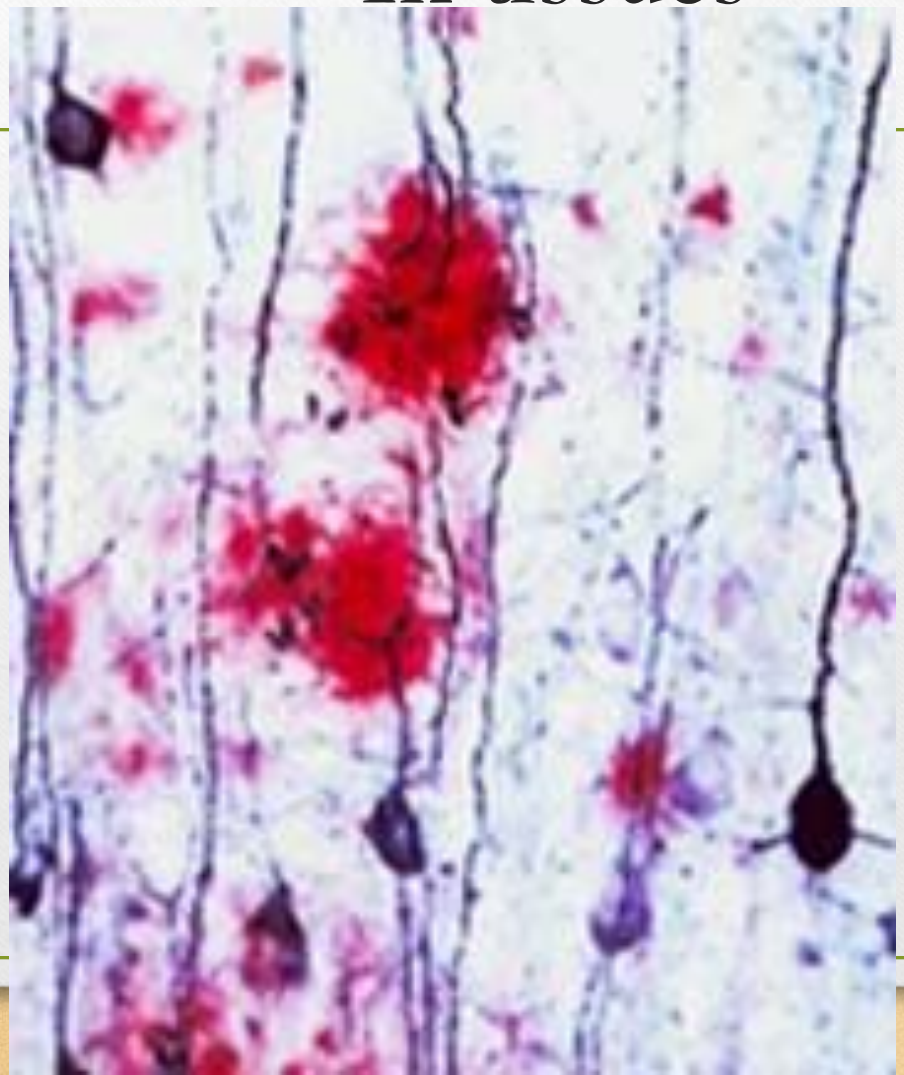


Amyloid fibers





In tissues



Chemical properties (main components)

- **Proteins and their derivates**
- **Glucosaminoglycans**
- **amyloid P component**
- Other proteins in amyloid deposits: α1-antichymotrypsin, some complement components, apolipoprotein E, various extracellular matrix or basement membrane proteins. Significance of these findings is unclear

Main protein precursors (total 22)

- serum amyloid A protein (SAA)
- AL proteins (monoclonal light and heavy chains Ig - whole or part of the variable (VL, VH) domains)
- Transthyretin (TTR) with normal aminoacids sequence or genetically abnormal TTR
- β2-microglobulin
- β-amyloid protein precursor; abnormal atrial natriuretic factor; IAPP insular amyloid polypeptide (amylin)
- Cystatin C; Gelsolin; Lysozyme; Apolipoproteins AI and AII; Prion protein; ADan and ABri precursor proteins; Lactoferrin; Keratoepithelin; Calcitonin; Prolactin; Keratin; Medin etc

Glycosaminoglycans

- significance in amyloid is unclear
- participate in organization of some normal structural proteins into fibrils; may have fibrillogenic effects on certain amyloid fibril precursor proteins.
- may be ligands to which serum amyloid P component binds.

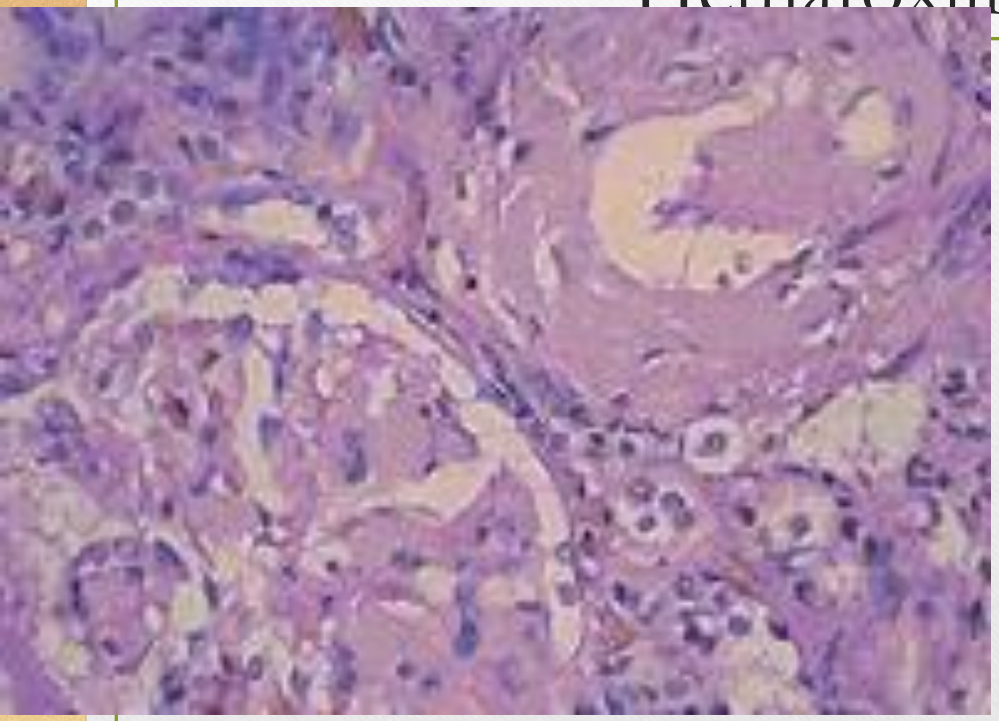
amyloid P component and serum amyloid P component

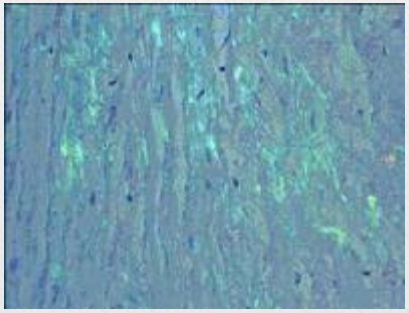
- amyloid deposits in all different forms of the disease contain the non-fibrillar glycoprotein amyloid P component (AP)
- ins role remains unclear

Morphology and staining: common features for all types

- Amorphous eosinophilic appearance on light microscopy after hematoxylin and eosin staining
- Bright green fluorescence observed under polarized light after Congo red staining

Typical staining for amyloid (right – heart Congo red, left – kidney Hematoxilin/eosin)





Classifications: before 1993

- AA (inflammatory)
- AL (light chains related)
- AF (familial)
- AS (senile)
- AD (dermal)
- AH (haemodyalysis-related)

WHO (1993): biochemical structure-based classification. Systemic variants

- **AA (ApoSAA):** chronic inflammatory diseases; periodical fever; Muckle-Wales
- **AL (Systemically produced monoclonal light chains Ig:** Aλ(λVI); Aχ(χIII): primary (idiopathic) or associated with gammapathies
- **ATTR**

- **normal TTR:** senile systemic amyloidosis with gradual heart involvement
- **Met30:** Family amyloid polyneuropathy
- **Met111:** Family amyloid cardiopathy

- **Aβ2M (β2-microglobulin):** haemodialysis-associated systemic amyloidosis

WHO (1993): local variants

- **AL (Locally produced monoclonal Ig):** local urogenital; skin, eyes, respiratory
- **Aβ (β-amyloid protein precursor):** cerebral; cerebrovascular; Alzgeimer-associated
- **AANF (abnormal atrial natriuretic factor):** local atrial
- **AIAPP (IAPP insular amyloid polypeptide):** Langerhans insuli amyloidosis in II type of diabetes mellitus

- From 1993 to nowadays new precursors and new variants were found (2006 – 22 precursors).
- So, new approaches to biochemistry-based classification became necessary.

Systemic

- **Ig light chains** (plasma cell disorders)
- **Transthyretin** (Familial amyloidosis, senile cardiac amyloidosis)
- **A amyloidosis** (Inflammation, Mediterranean fever
- **Beta2 –microglobulin** (Dialysis-associated)
- **Ig heavy chains**(Systemic amyloidosis)

Hereditary
(Familial systemic amyloidosis)

- **Fibrinogen alpha chain**
- **Apolipoprotein AI**
- **Apolipoprotein AII**
- **Lysozyme**

CNS amyloidosis

- **Beta protein precursor** (Alzheimer syndrome, Down syndrome, hereditary cerebral hemorrhage with amyloidosis - Dutch type)
- **Prion protein** (Creutzfeldt-Jakob disease, Gerstmann-Strussler-Scheinker disease, fatal familial insomnia)
- **Cystatin C** (hereditary cerebral hemorrhage with amyloidosis - Icelandic type)
- **ABri precursor protein** (Familial dementia British type)
- **ADan precursor protein** (Familial dementia Danish type)

Ocular

- **Gelsolin** (Familial amyloidosis; Finnish type)
- **Lactoferrin** (Familial corneal amyloidosis)
- **Keratoepithelin** (Familial corneal dystrophies)

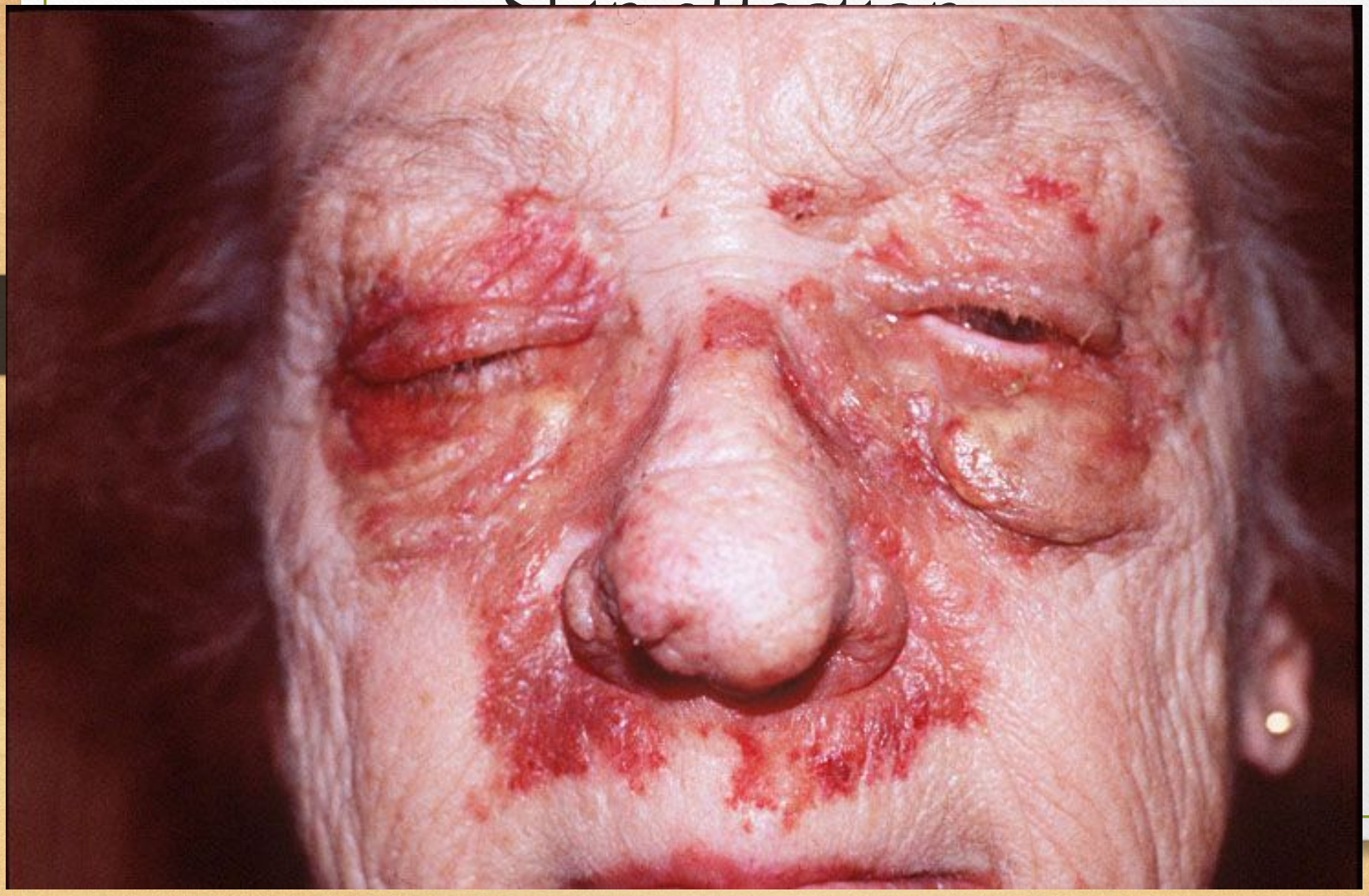
Localized

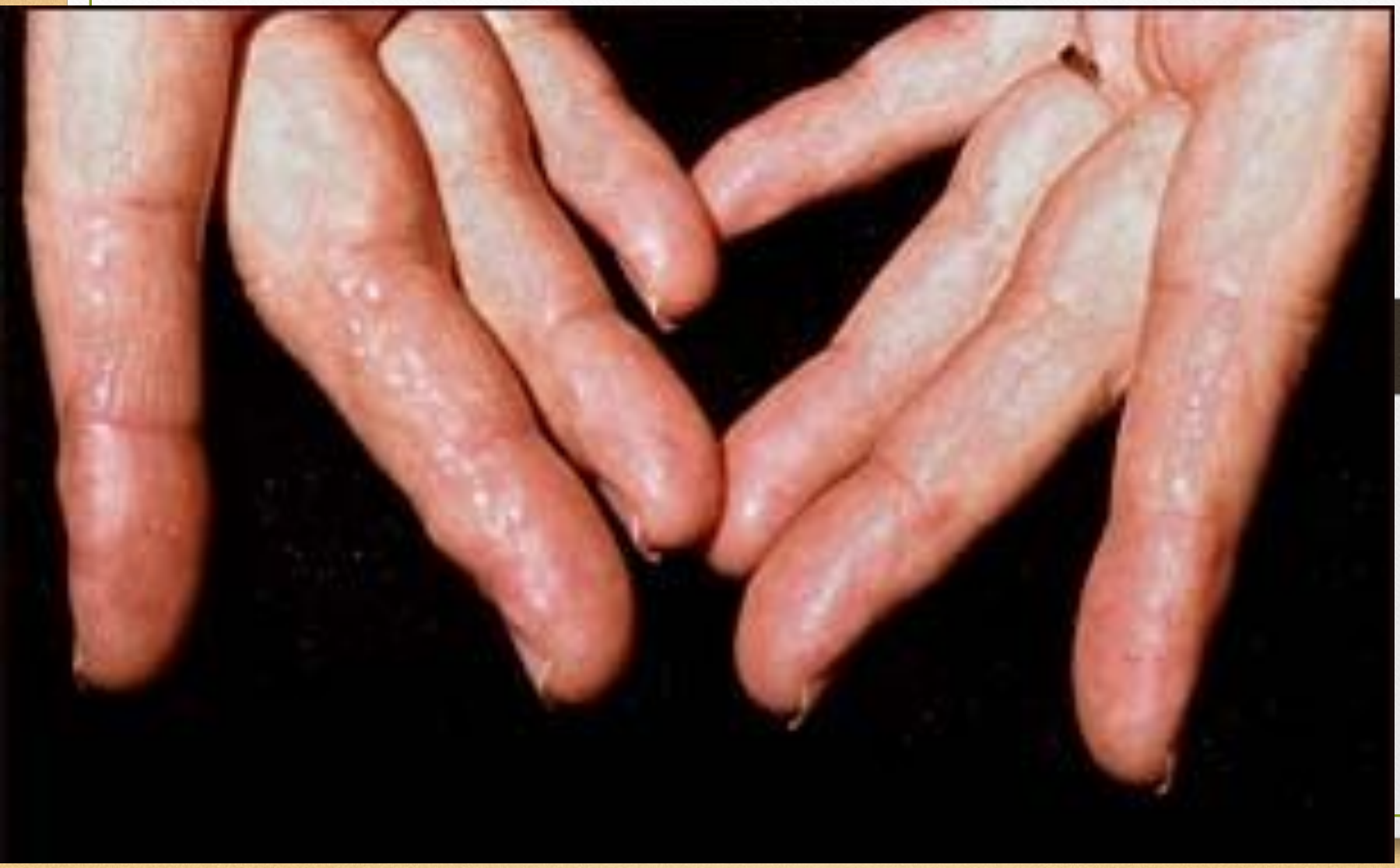
- **Calcitonin** (Medullary thyroid carcinoma)
- **IAAP= Amylin** (Insulinoma, type 2 diabetes)
- **Atrial natriuretic factor** (Isolated atrial amyloidosis)
- **Prolactin** (Pituitary amyloid)
- **Keratin** (Cutaneous amyloidosis)
- **Medin** (Aortic amyloidosis in elderly)

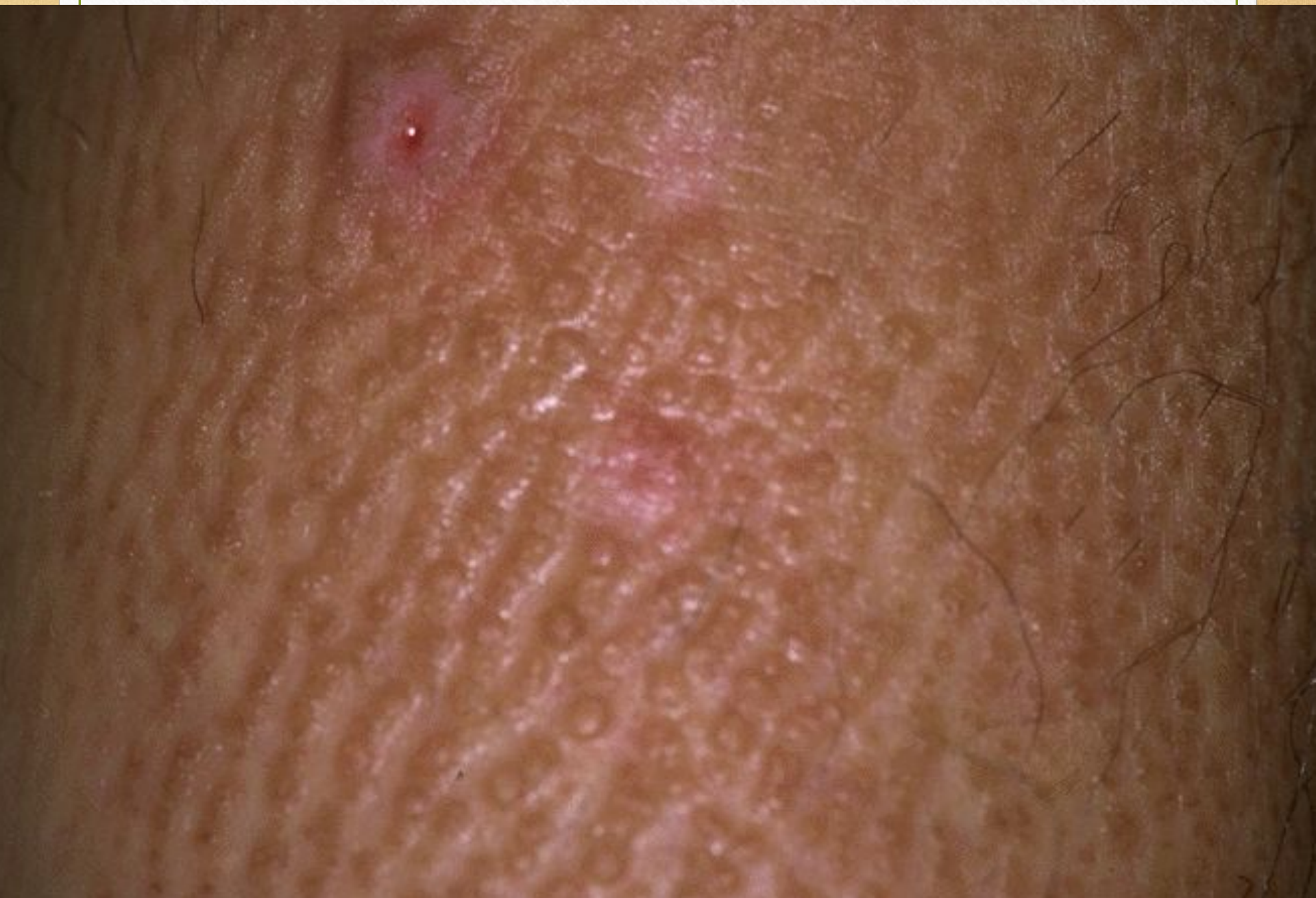
Clinical syndromes related to amyloidosis

- **General symptoms and intoxication**: weakness, fatigue, sometimes fever and weight loss (not common)
- **Skin**: itching; urticar rash, papules, nodules, and plaques usually on the face and upper trunk; involvement of dermal blood vessels results in purpura occurring either spontaneously or after minimal trauma

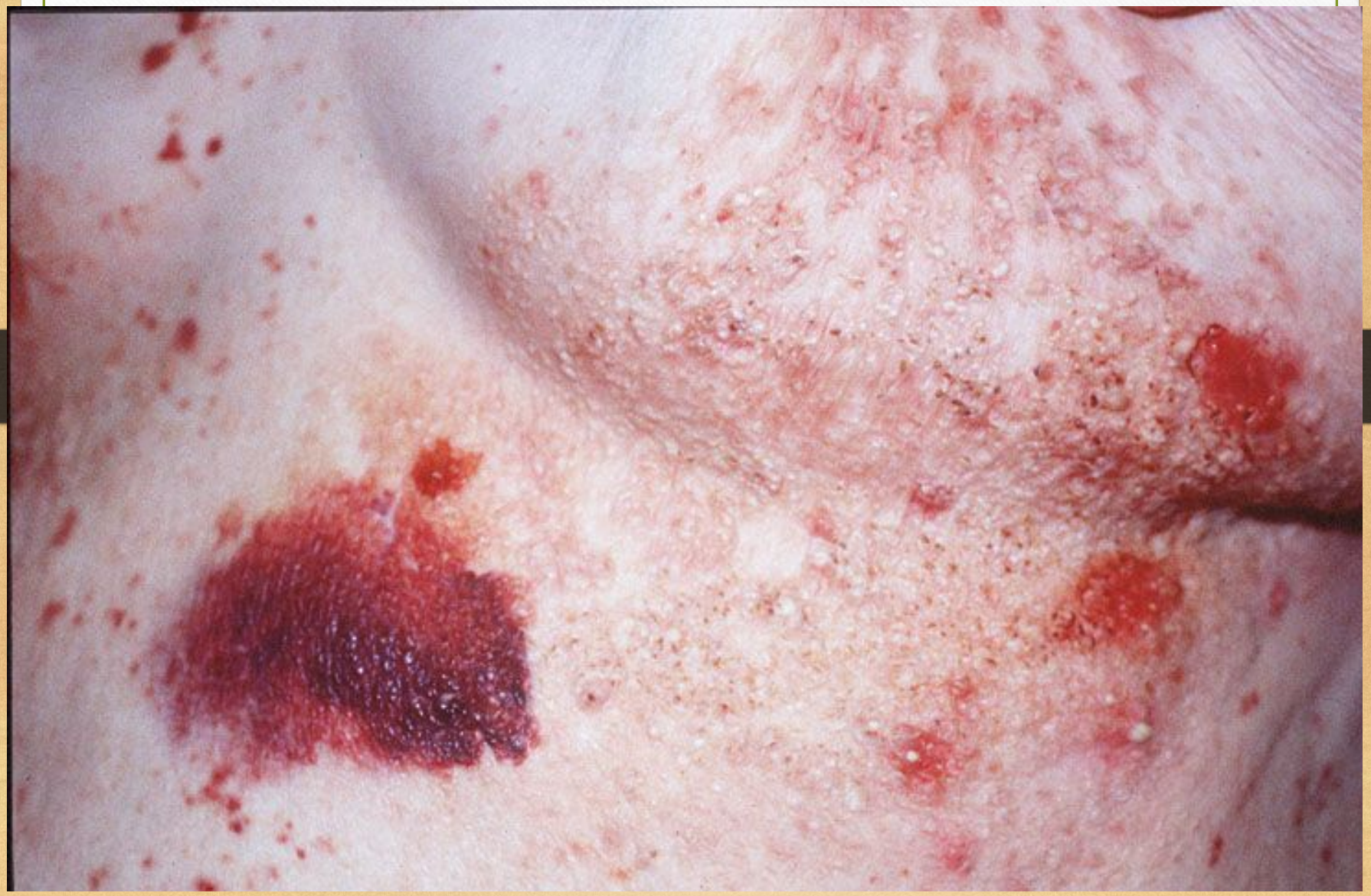
Skin affection



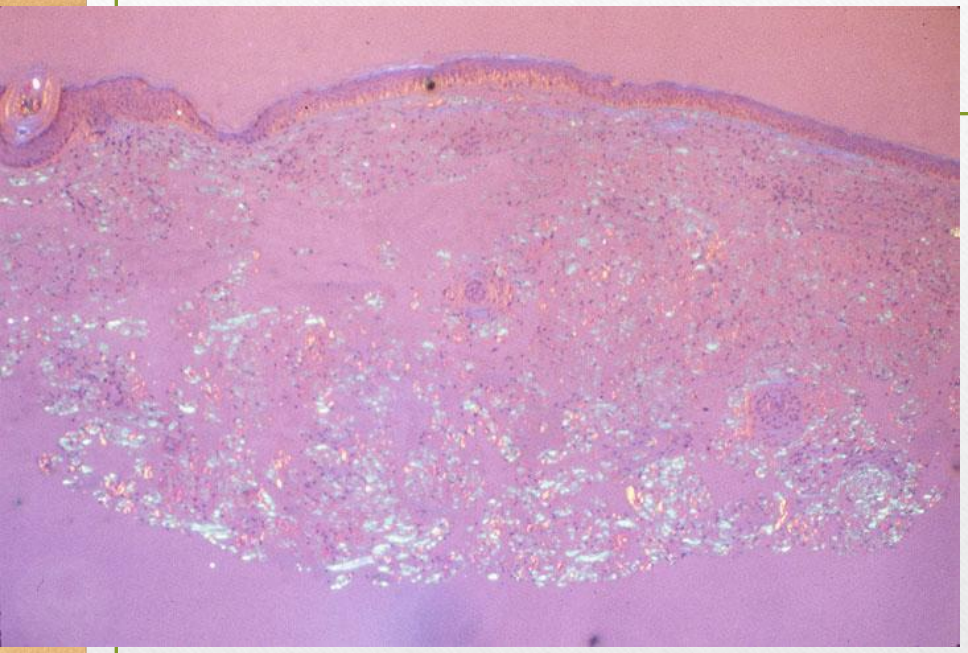


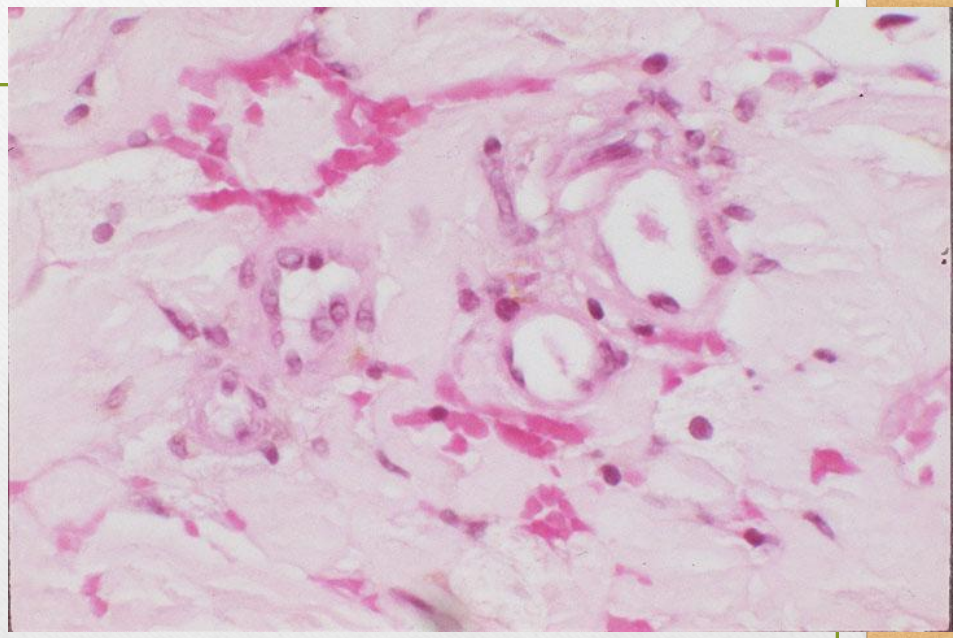


Skin: hemorrhages and papules



Skin microscopy





Periphreral nervous system:

- **axonal peripheral neuropathy with subsequent demyelination:**

- paresthesiae, numbness, muscular weakness; begin from lower extremities and ascending over time
- feeling constraint in the whole body
- painful sensory polyneuropathy (usually symmetrical, usually affecting lower extremities) with early loss of pain and temperature sensation followed later by motor deficits

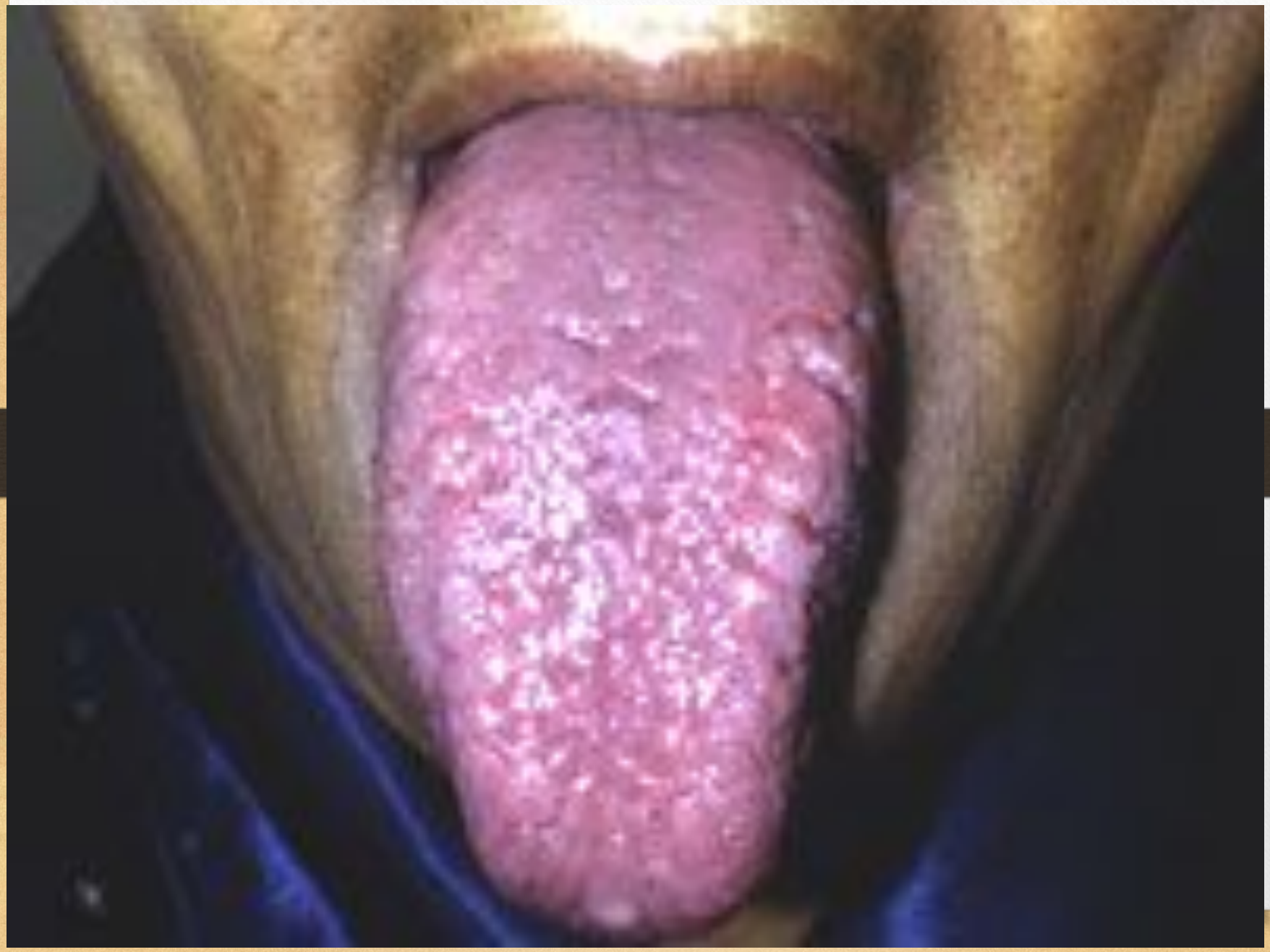
- **carpal tunnel syndrome**
- **autonomic neuropathy:** orthostatic hypotension, impotence, poor bladder emptying and gastrointestinal disturbances may occur alone or together with the peripheral neuropathy

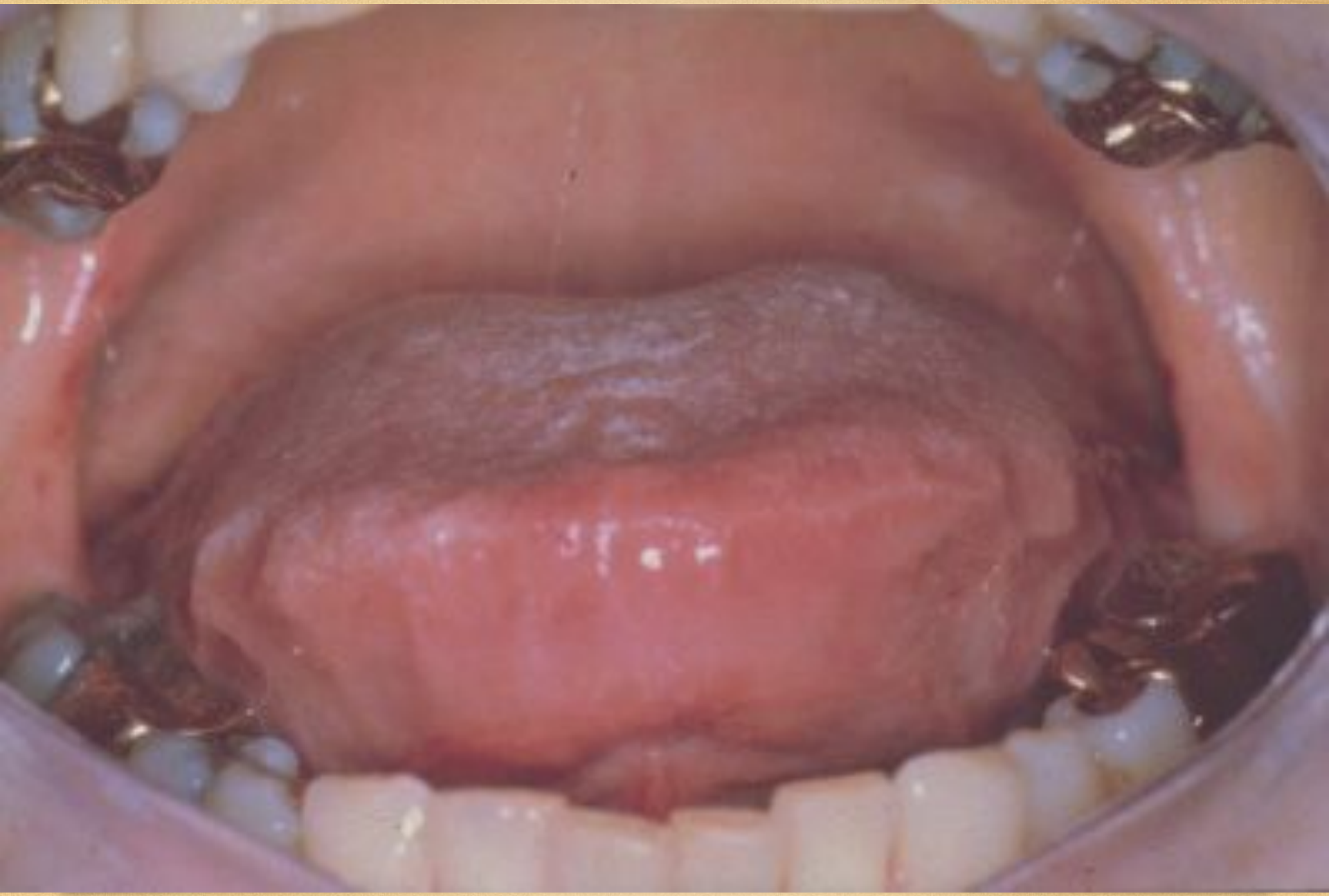
Central nervous system

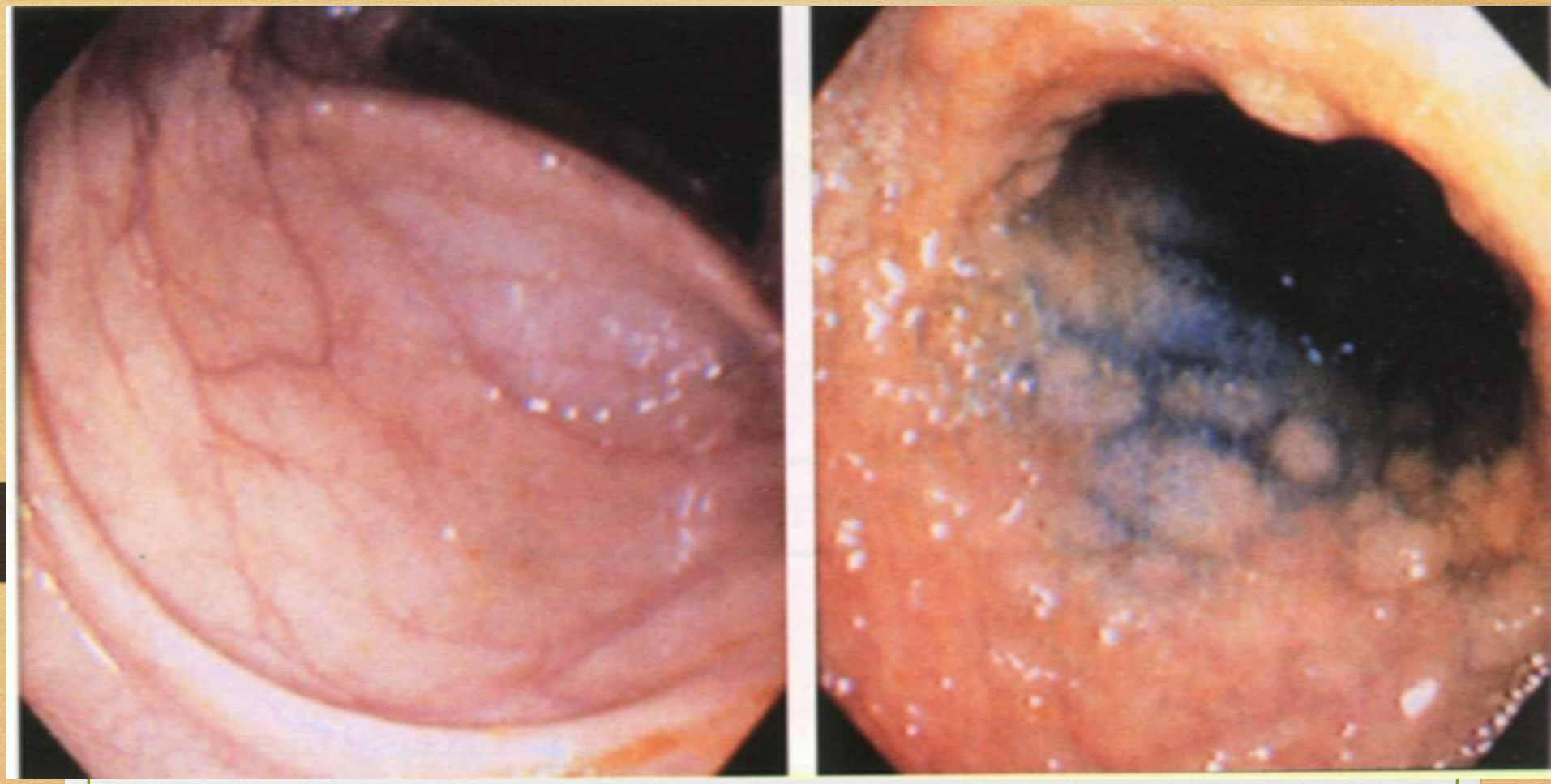
- cerebral blood vessels affection
- recurrent cerebral hemorrhages
- intracerebral plaques
- progressive dementia

Gastrointestinal disorders:

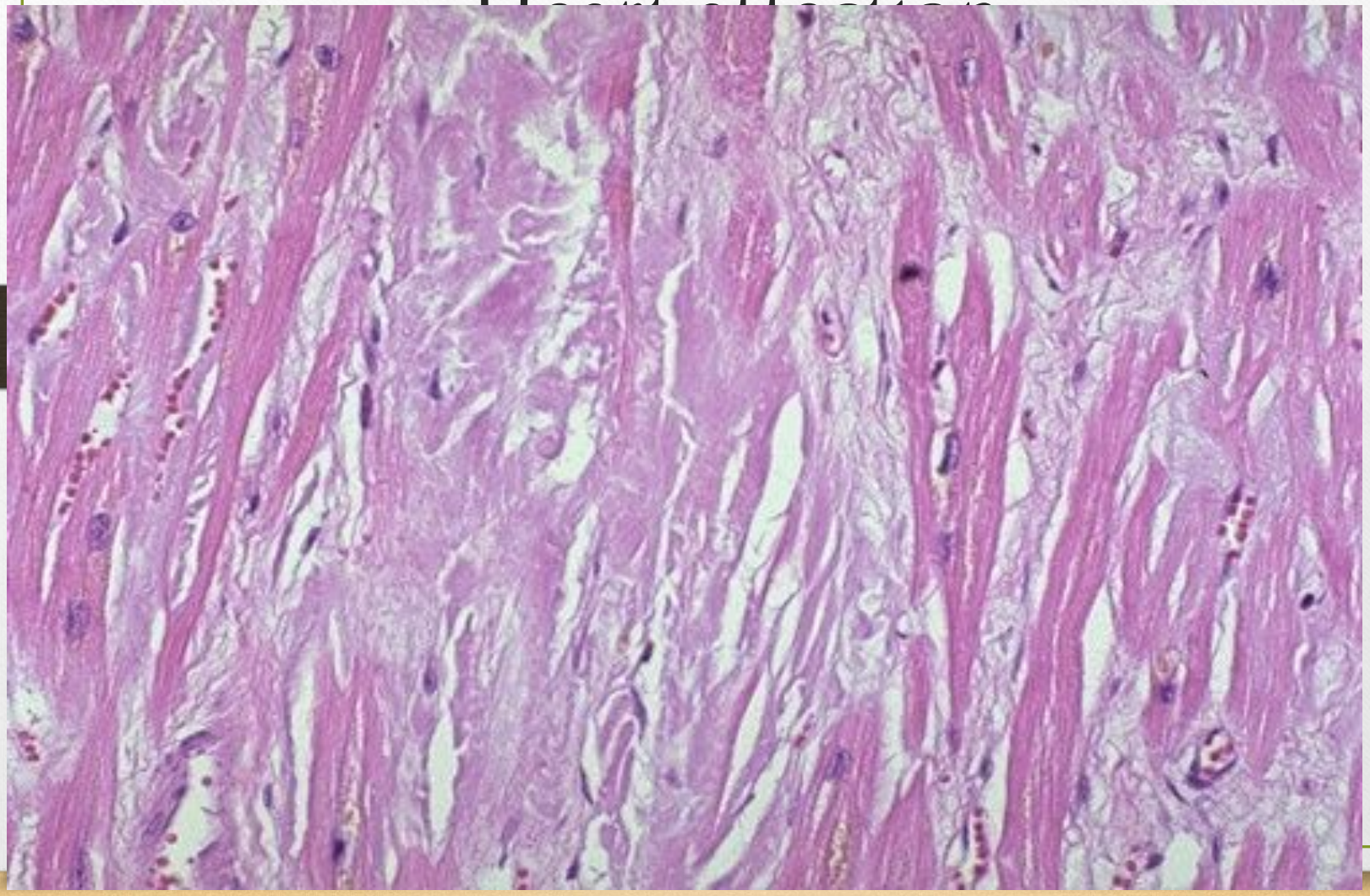
- **Tongue:** increased, dense, red or purple; so that it can't go in mouth; tooth imprints, ulcers and fissures; speech is difficult – disarthria; difficulties in swallowing (dysphagia); excessive salivation
- **Stomach**: early satiety, chronic nausea, vomiting
- **Gut:** diarrhea and/or constipation; malabsorption, *obstruction* or pseudo-obstruction (both due to mucosal deposition); *perforation; haemorrhage, infarction* (the last one is due to vascular deposits and is mostly localized in descending and sigmoid colon)
- **Motility disturbances** (often secondary to autonomic neuropathy) may affect stomach and gut

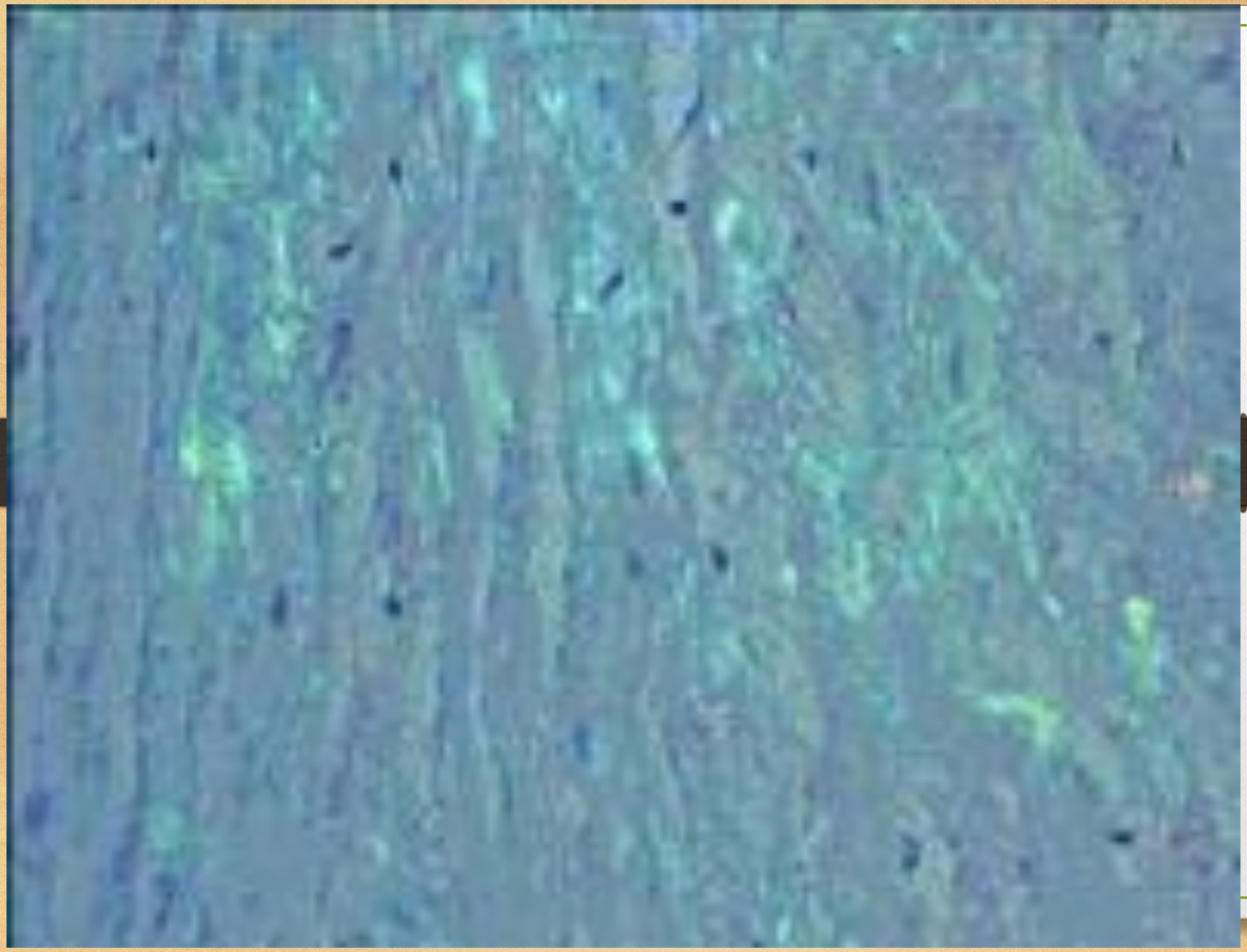






Heart affection





Heart: myocardium

- increase of relative cardiac dullness, soft heart sounds, systolic murmur at the apex and diastolic at aorta (relative valves insufficiency in dilated heart);
- congestive heart failure (with up to 50% of fatal cases); hypotonia
- restrictive cardiomyopathy with signs and symptoms of right ventricular failure
- cardialgias

ECG: heart muscle affection

- ECG – decrease of voltage, plain or inverted T, scars, pseudoinfarction QS complexes in precordial leads.

Heart: coronary arteries

- secondary coronary syndrome and myocardial infarction.
- more marked affection of intramyocardial arteries; angiographic changes may not be revealed

Rhythm and conductivity disorders

- conductivity disorders in sinus node, AV node and left bundle branch with dizziness, syncopes, bradycardia, SA block and lower automaticity centers activation
- predisposition to cardiac arrest (especially ATTR)
- sensitivity to digoxin also may cause fatal arrhythmias

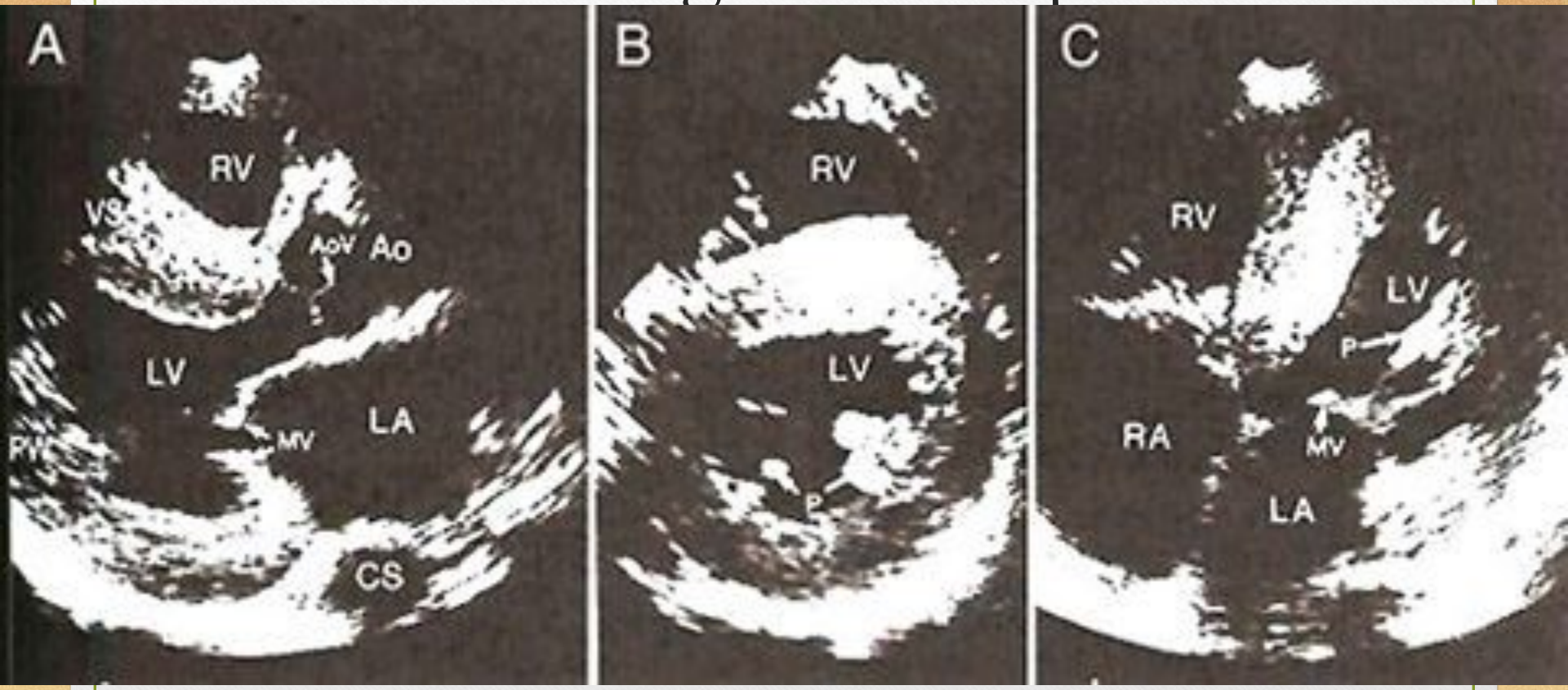
Pericardium and endocardium affection

- Pericardium deposits – constrictive pericarditis
- Valves affection (amyloid deposits in valves): mild stenosis due to valve rings infiltration
- Endomyocardial thrombi with embolisms

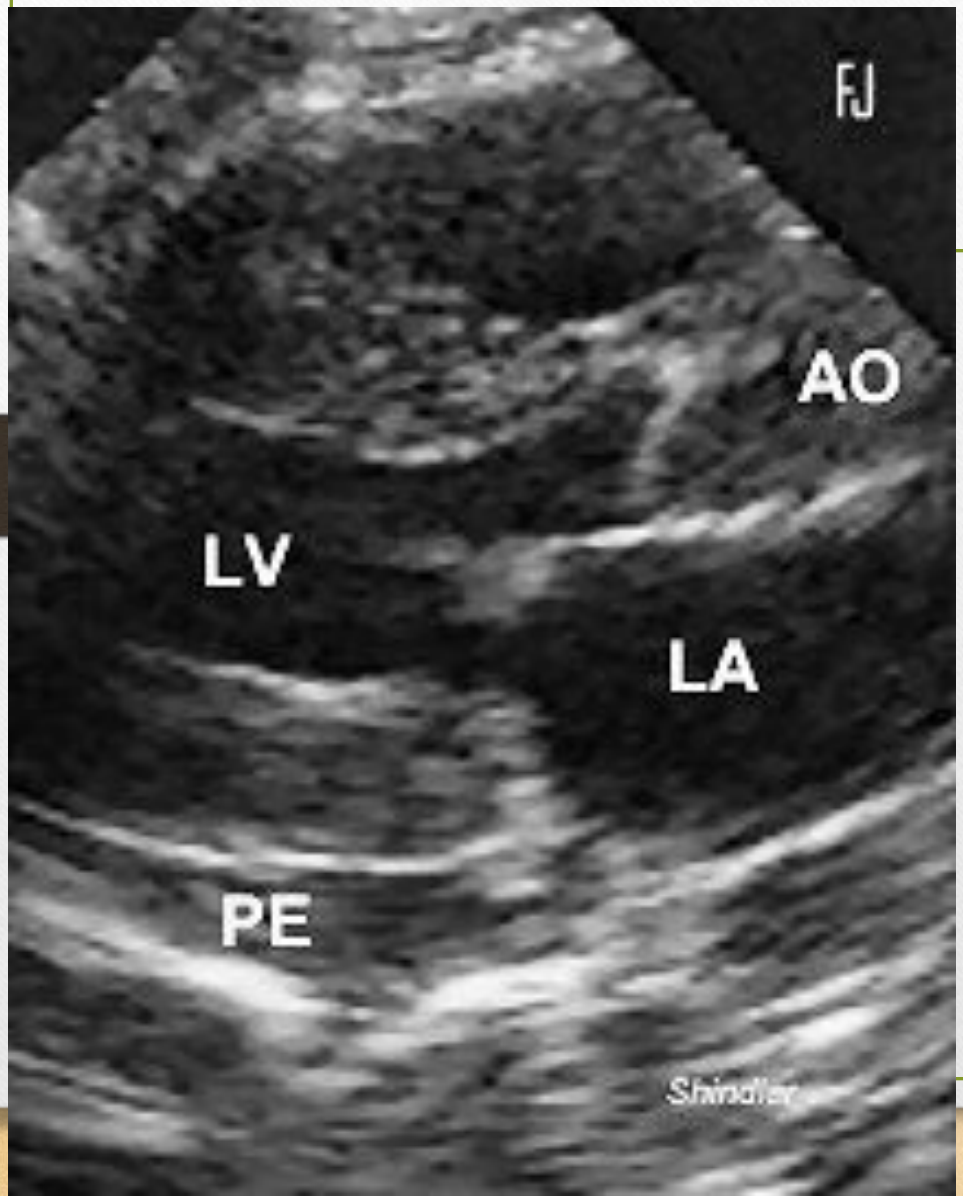
Echo

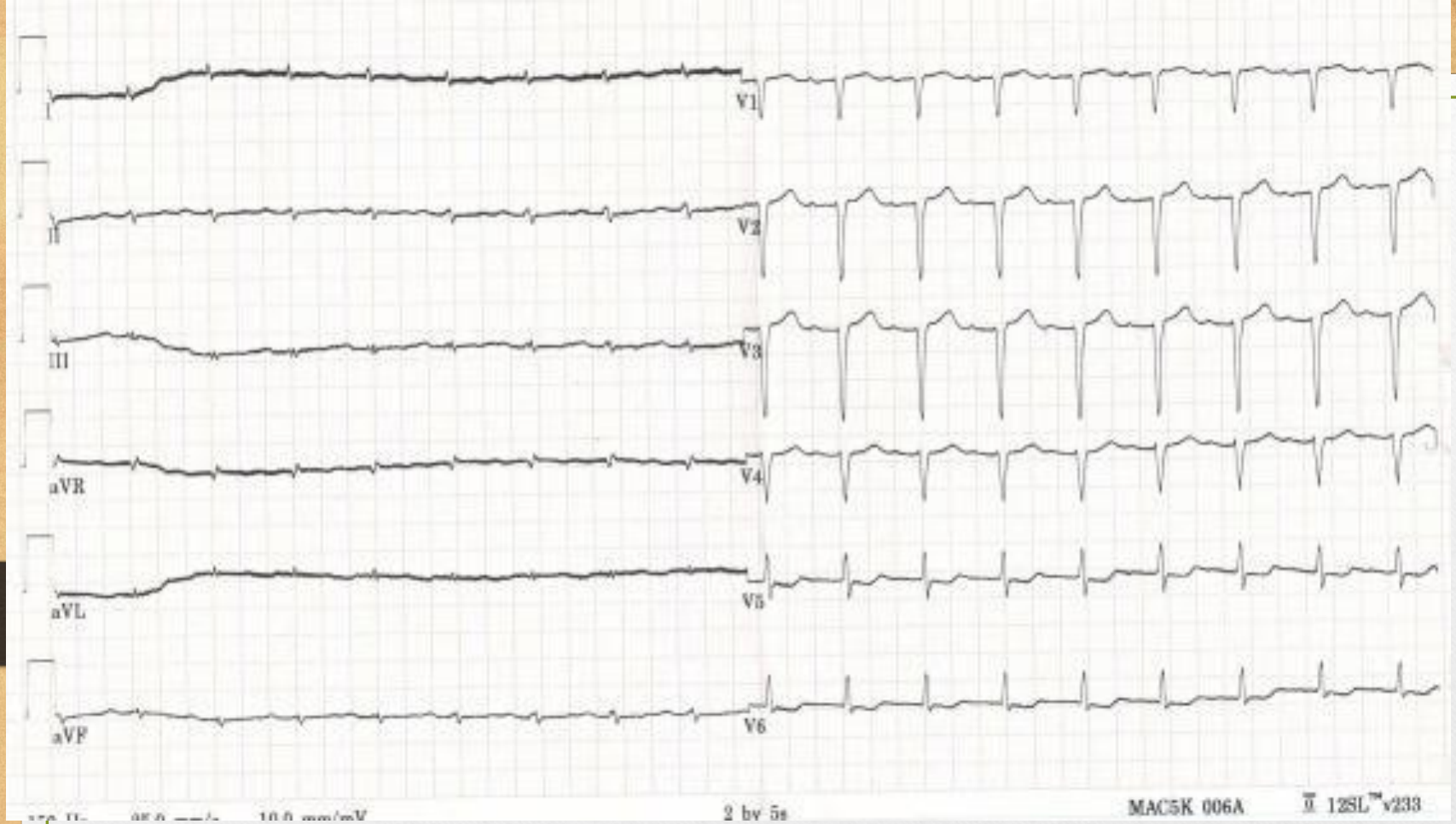
- The most common: thickening of the intraventricular septum (usually 15 mm and more; normal values being <12 mm)
- granular "sparkling"

Thickening of the septum



Pericardial changes

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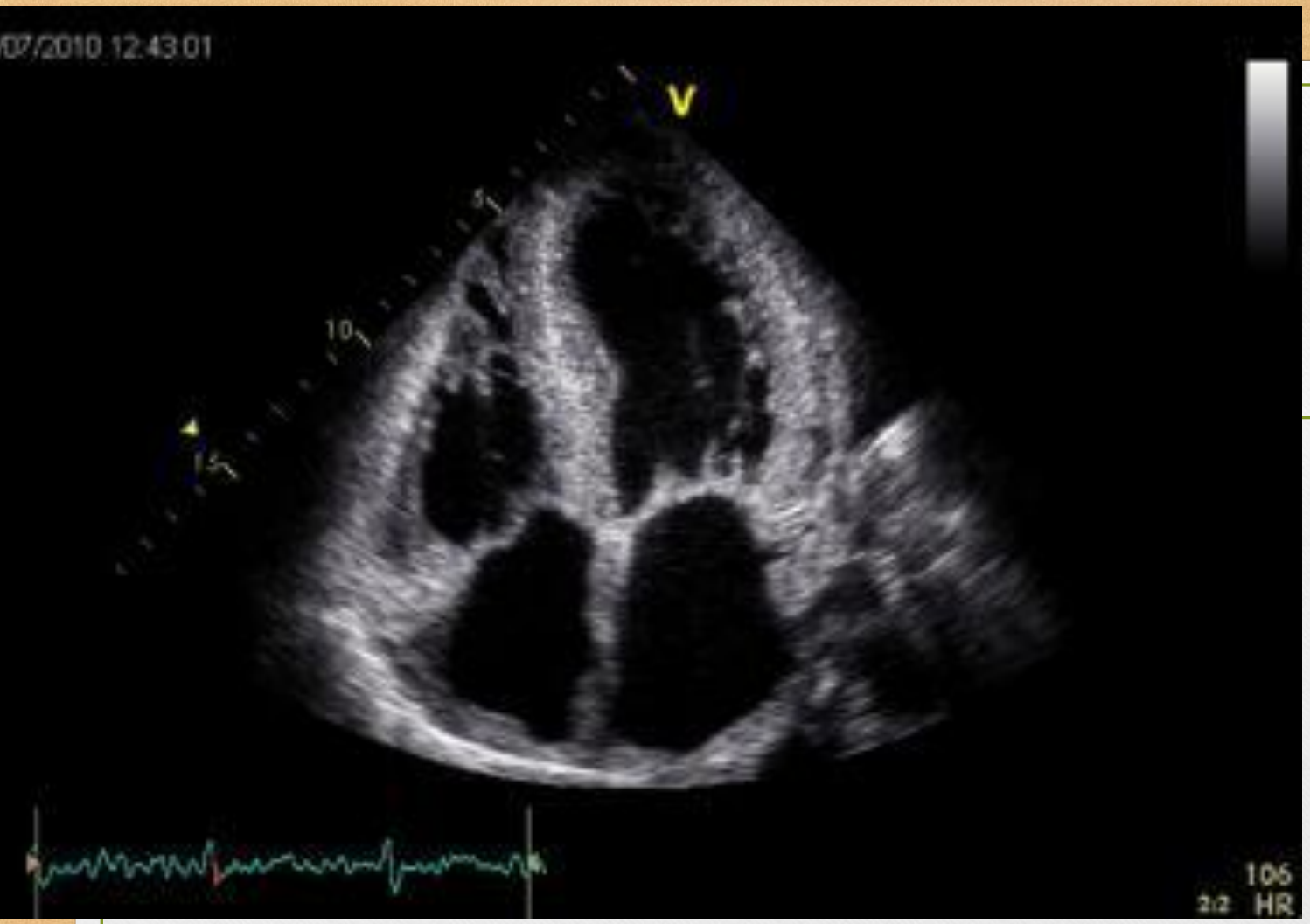
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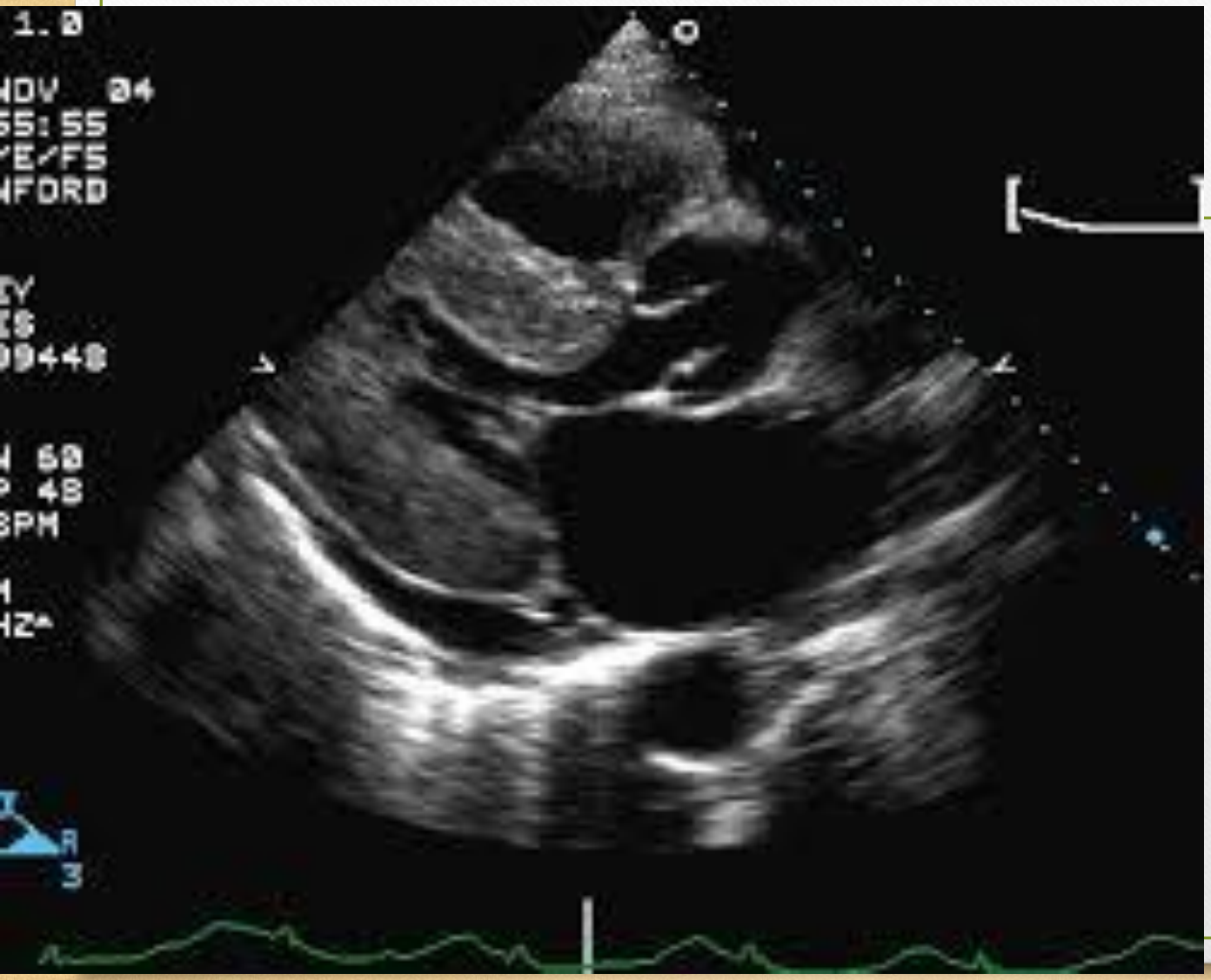
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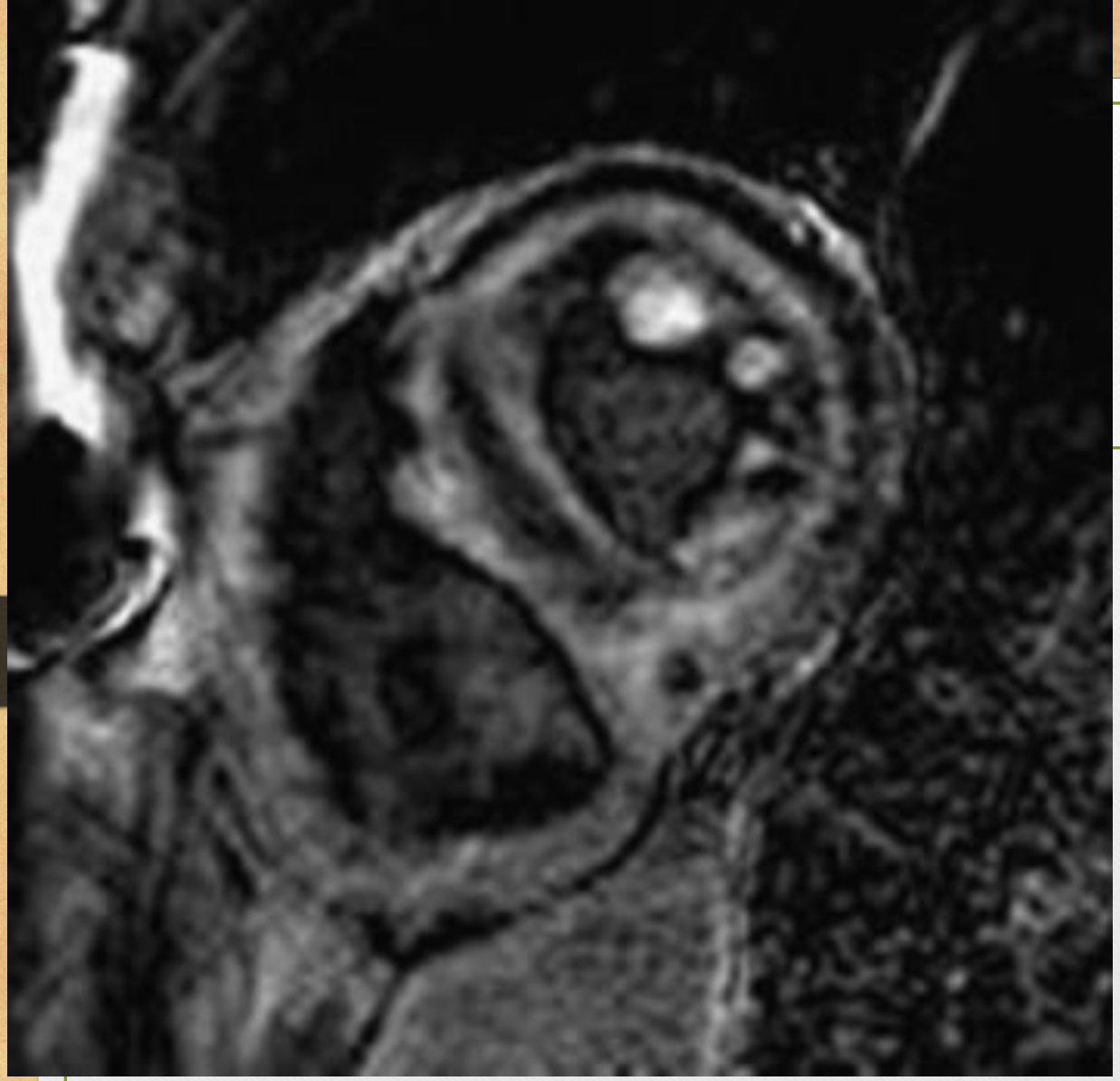
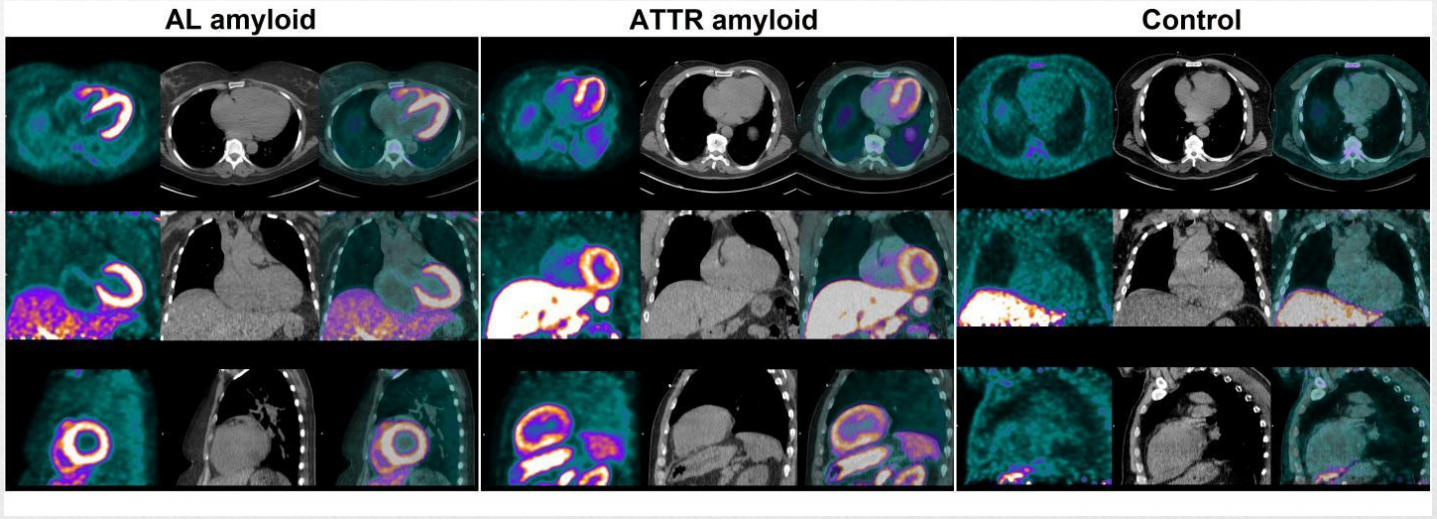
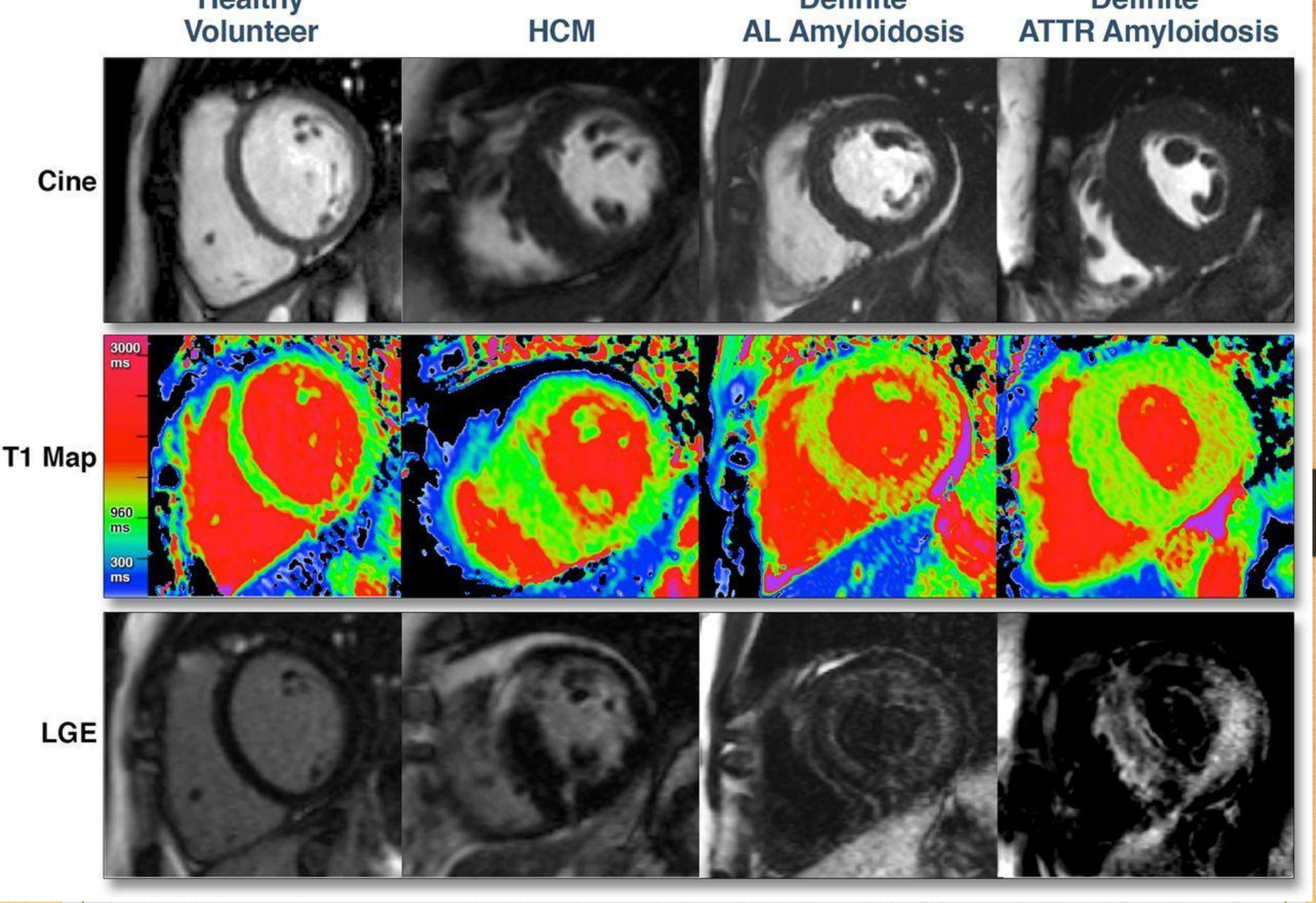



AL amyloid
ATTR amyloid
Control


Healthy Volunteer
HCM
Definite AL Amyloidosis
Definite ATTR Amyloidosis
Cine
T1 Map
3000 ms
960 ms
300 ms
LGE

WHO staging system for cardiac amyloid

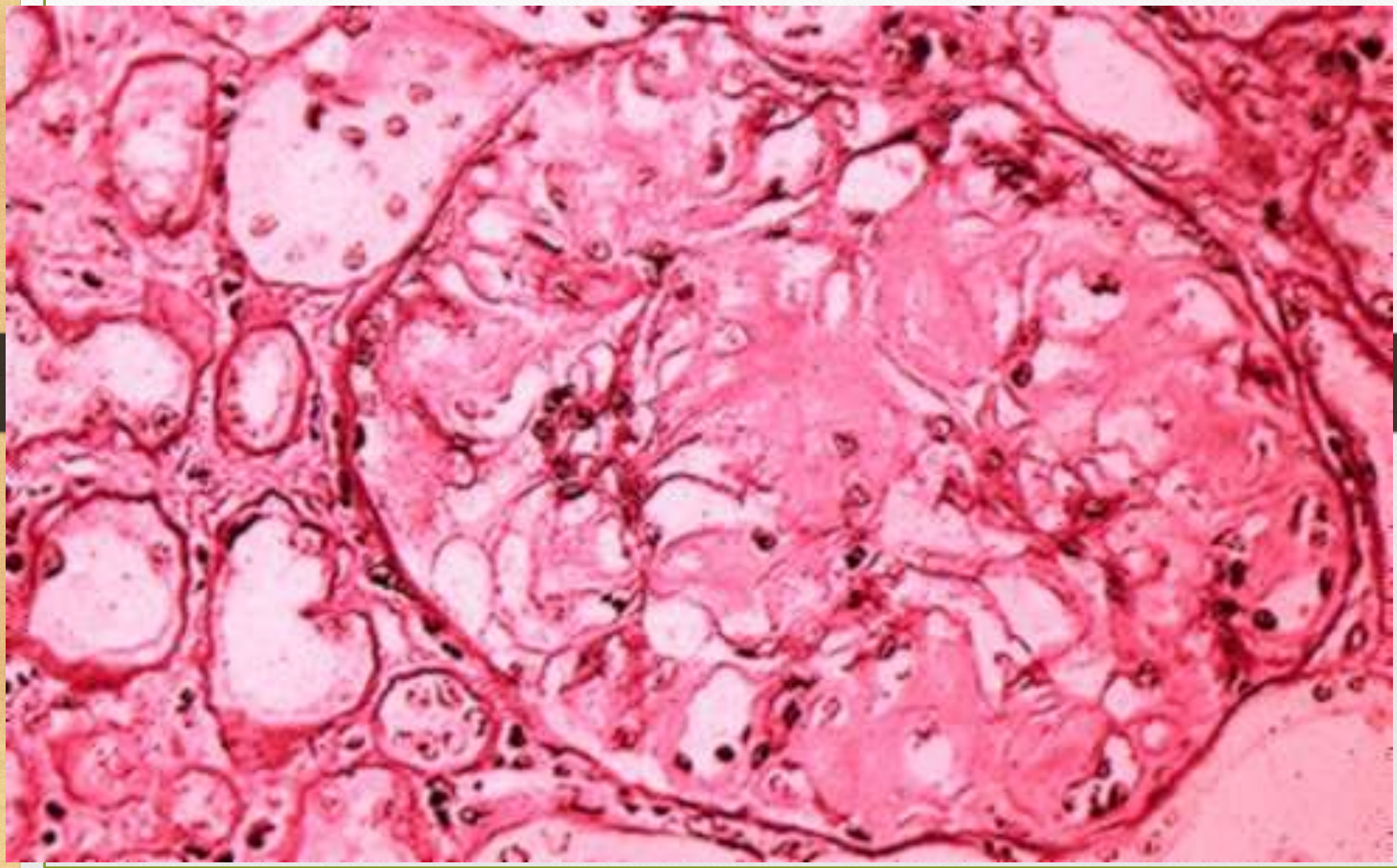
- 1 – no symptomatic or occult cardiac amyloid by biopsy or non-invasive testing
- 2 – asymptomatic cardiac involvement by biopsy or non-invasive testing eg wall thickness > 1.1 cm in the absence of prior hypertension or valvular disease, unexplained low voltage of ECG
- 3 – compensated symptomatic cardiac involvement
- 4 – uncompensated cardiomyopathy

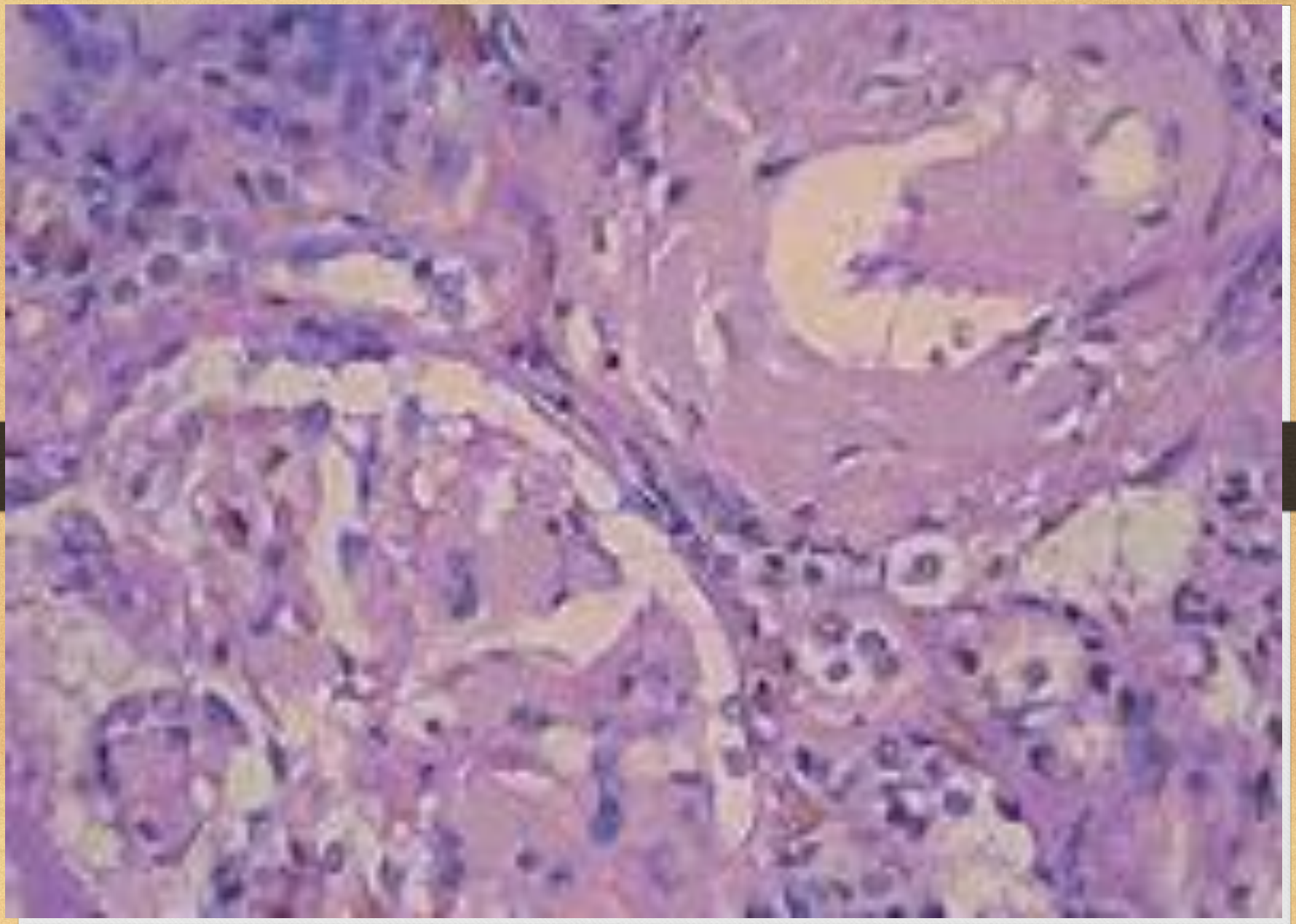
Vessels

- **capillaries in the subcutaneous fat**
- **dermal capillars**
- **coronary and brain arteries (coronary syndrome, recurrent strokes)**
- **aorta**
- **rare – pulmonary artery**

Liver and spleen

- Hepatomegaly, usually elevation of alkaline phosphatase is revealed with near normal levels of transaminases and bilirubin
- Jaundice due to cholestasis
- Splenomegaly
- Rarely - portal hypertension; liver failure





Kidneys: symptoms

- Proteinuria (usually – with nephrotic syndrome)
- Chronic renal failure
- Acute renal failure due to tubules affection

Kidneys: staging system

Stage	**Phase**	**Course**
Initial	Proteinuric	Slowly progressing
Clinical manifestations	-Nephrotic -Oedematic-proteinuric -Hypertensive (rare)	Rapidly progressing
Terminal	Chronic renal failure	Relapsing

Joints affection

- usually occurs in association with myeloma
- mimic acute polyarticular rheumatoid arthritis affecting large joints
- asymmetrical arthritis affecting the hip or shoulder.
- infiltration of the glenohumeral articulation occasionally with characteristic shoulder pad sign.

Blood

- **Acquired bleeding diathesis:**

- deficiency of factor X and sometimes factor IX, or increased fibrinolysis: (AL)

- in all variants may be serious bleeding in the absence of any identifiable factor deficiency.

- lymphadenopathy
- bone marrow affection
- splenomegaly

Respiratory system

vocal cord infiltration

- associated with focal clonal immunocyte dyscrasia
- nodular or diffuse infiltrative form
- manifested by a hoarse voice

tracheobronchial

- associated with focal clonal immunocyte dyscrasia
- nodular or diffuse infiltrative
- manifested by dyspnea, cough
- Occasionally - haemopthysis; distal athelectasis with recurrent pneumonias

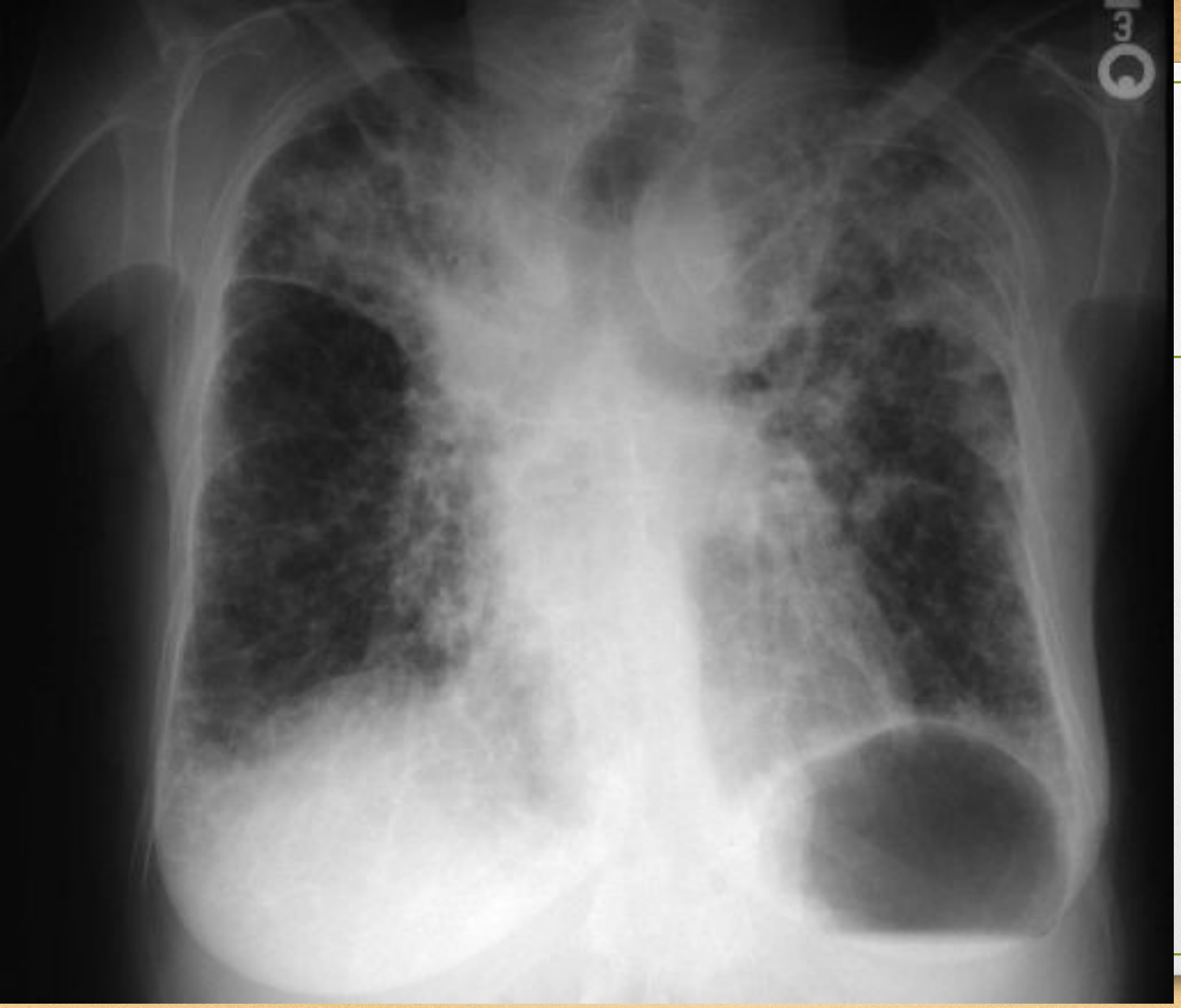
- associated with *parenchymal* monoclonal dyscrasia
- solitary (amyloidoma) or multiple nodules in lung parenchyma; usually peripheral or subpleural, more frequently in lower lobes; may be bilateral; diameter ranges from 0.4 to 15sm;
- grow slowly
- frequently cavitate or calcify
- larger nodules can occasionally produce space occupying effects

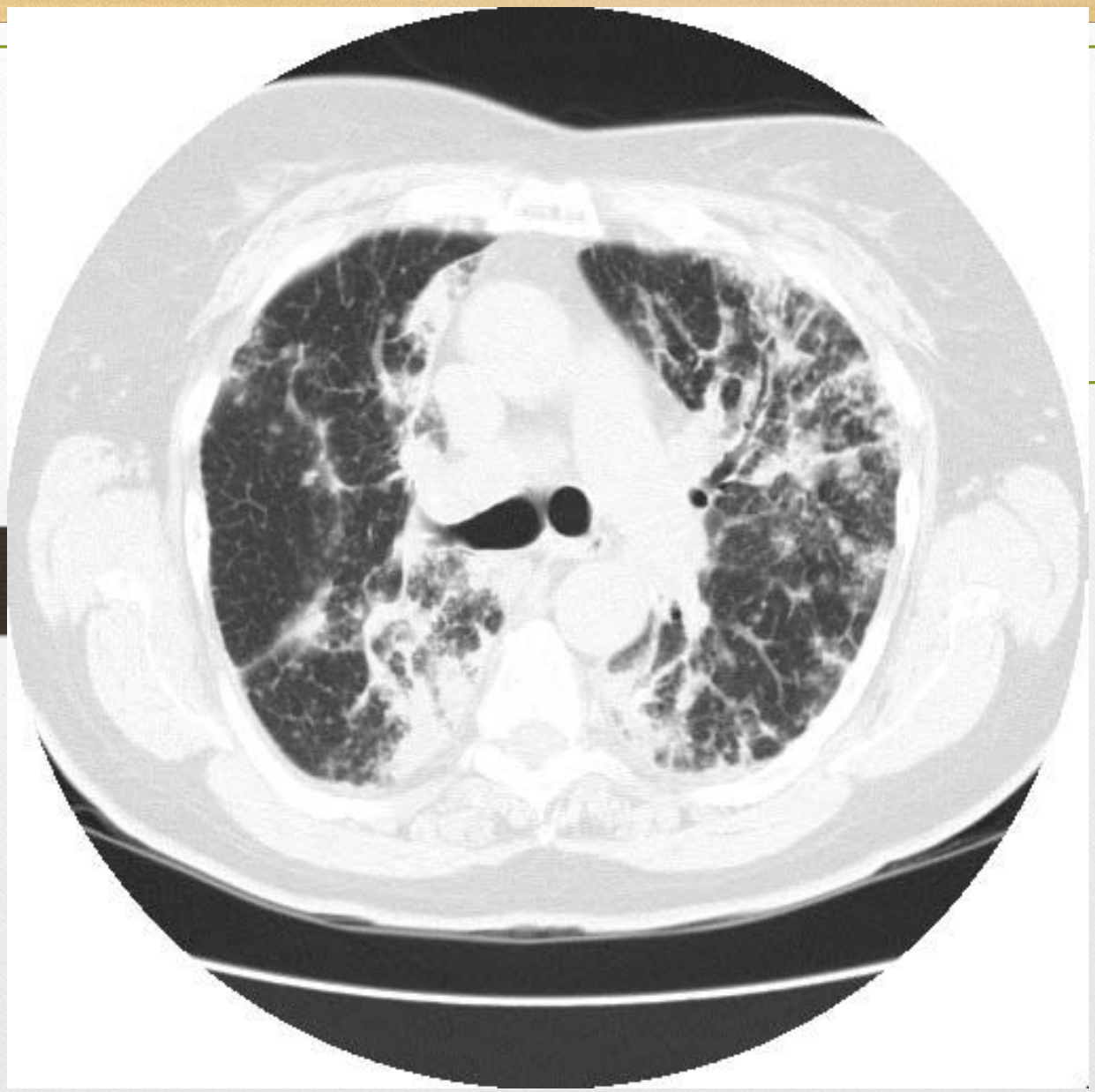
diffuse alveolar septal

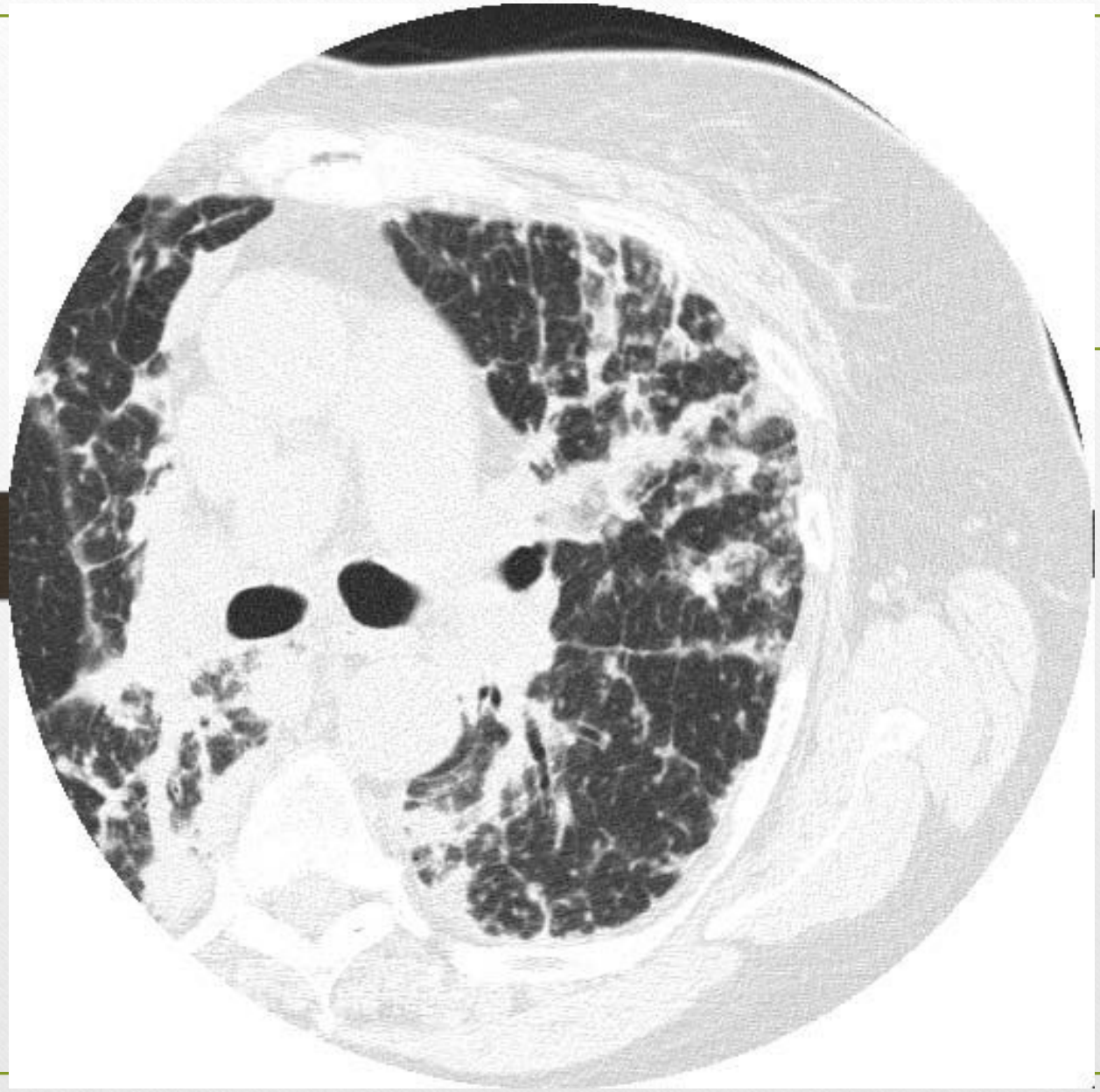
- usually is a manifestation of systemic AL amyloidosis associated with low grade monoclonal gammopathy, myeloma; ATTR, AA-variants etc
- restrictive respiratory symptoms
- restrictive functional tests changes and impared gas exchange
- radiological changes may be absent

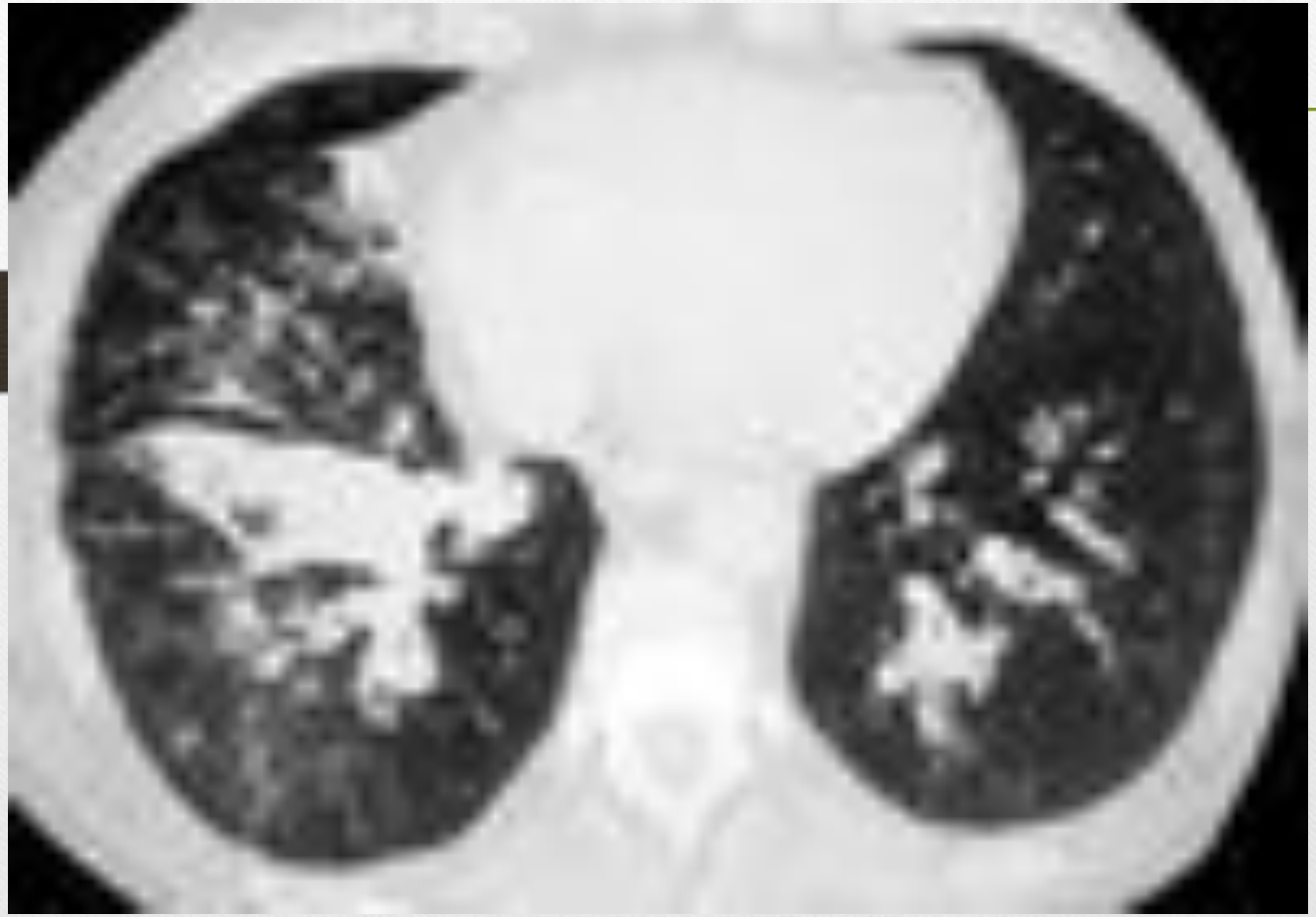
intrathoracic lymphadenopathy

- usually manifestation of systemic AL amyloidosis (hilar or meduastinal amyloidosis)
- is uni- or bilateral
- may be asymptomatic
- may calcify
- may cause tracheal compression or vena caval obstruction.









Eye

- visible or palpable periocular mass or tissue infiltration
- ptosis
- periocular discomfort or pain
- proptosis or globe displacement
- limitations in ocular motility
- recurrent periocular subcutaneous hemorrhages
- diplopia

Endocrine and exocrine glands

- adrenal gland infiltration (hypoadrenalism)
- thyroid infiltration (hypothyroidism)
- IAAP – progressive loss of insular production
- corpora amylacea of the prostate (β2-microglobulin)
- seminal vesicles
- salivary glands

Inflammatory amyloidosis

- Amyloid A (AA)
- most common form of systemic amyloidosis worldwide.
- characterized by extracellular tissue deposition of fibrils that are composed of fragments of serum amyloid A (SAA) protein

- **SAA is an apolipoprotein of high density lipoprotein particles**
- **SAA is a major** exquisitely sensitive **acute phase protein,** more sensitive than CRP

- **Produced**: mostly - by **hepatocytes**
- transcriptional regulation by cytokines, especially **interleukin 1(IL-1), interleukin 6 (IL-6), and TNF**
- regulators act via nuclear factor χB-like and possibly other transcription factors.

- **The circulating concentration:**

- Normal - 3mg/l

- Rise to over 1000mg/l within 24 to 48h

- **in ongoing chronic inflammation remains persistently high**

- AA protein is derived from circulating SAA by proteolytic cleavage by macrophages and by a variety of proteinases

Pathogenesis

- **Inflammation**
- **Macrophages activation:** IL-1, 6
- **IL-1,6:**

- hepatic transcription of the messenger RNA for SAA
- High SAA level in serum
- macrophages: SAA proteolytic cleavage

- **AA-peptide in blood**
- **amyloid synthesis accelerating factor:**

- macrophages' surface: amyloid fibrils synthesis (membrane-binding enzymes)

- **Amyloid synthesis**

Causes

- chronic inflammatory disorders
- chronic local or systemic microbial infections
- malignant neoplasms and blood system diseases
- subcutaneous drug abuse

Chronic inflammatory disorders

- **Very often:**
- rheumatoid arthritis and juvenile rheumatoid arthritis – in 10% of arthrites cases
- Becchet disease
- ankylosing spondylitis
- Psoriatic arthritis
- Crohn's disease
- **Exceptionally rare:**
- - systemic lupus erythematosus
- - ulcerative colitis

Chronic local or systemic microbial infections

- tuberculosis and leprosy
- chronic osteomyelitis
- bronchiectasis
- chronic abscesses
- chronically infected burns
- decubitus ulcers as well
- other chronic microbial infections
- chronic pyelonephritis of paraplegic patients

malignant neoplasms and blood system diseases

- **The most frequent:**
- diseases, causing fever, other systemic symptoms, and a major acute phase response (SAA protein) or increased IL-6 production
- - Hodgkin's disease
- - renal carcinoma
- Occasionally: atrial myxomas, renal cell carcinomas, Hodgkin disease, hairy cells leukemia, carcinomas of the lung and stomach

Subcutaneous drug abuse

- AA amyloidosis was frequently observed among subcutaneous drug abusers in some cities in the United States.
- Was this related to drug or to some contaminating substance causing chronic inflammation is not clear.

Clinical symptoms

- **Relating to the main disease**
- **General:** weakness, weight loss
- **Kidneys affection** (up to renal failure)
- **GI symptoms:** dyspepsia (nausea, episodes of vomiting, loss of appetite); diarrhea
- **Liver and spleen affection** (hepatosplenomegalia)
- **Thyroid enlargement**
- **Heart:** Echo-signs in 10% ; doesn't cause severe impairment.

Course:

- Initially, disease is manifesting only by transient proteinuria, increasing in cases of main disease exacerbations.
- Course is progressive and is terminated by chronic renal failure development
- Course is determined by the efficacy of the main disease treatment

Outcomes and complications:

- Main - chronic renal failure (end-stage - 5-10 years from 1st symptoms); the first proteinuric period is the longest – 2-4 years; marked clinical manifestations period lasts about 1 year, then chronic renal failure develops).
- Renal vessels thrombosis: makes prognosis more unfavorable
- Fibrinous-purulent peritonitis, accompanying by pain and ascitis - rare

Prognosis

- Depends on the course of the main disease
- Survival: 50% of patients die within 5 years of the amyloid being diagnosed.
- Availability of chronic hemodialysis and transplantation prevents early death from uraemia
- Renal vessels thrombosis makes prognosis more unfavorable

Familiar Mediterranean fever (recurrent polyserositis)
and AA-amyloidosis

- **Epidemiology:**
- **Incidence:** in families with healthy parents: 18%; with one affected parent – 36%
- **Nationality:** most often in non-Ashkenazi Jews, Armenians, Anatolian Turks, and Levantine Arabs; prevalence doesn't depend on place of settlement of these nationalities representatives.
- **Inheritance:** autosomal recessive
- **Sex:** M:F 1.7:1

Morphology

- serosa: non-specific inflammation with hyperaemia and a cellular infiltrate
- synovia: pannus formation
- vascular changes - thickening of the basement membrane; its reduplication (repeated episodes of cell death and regeneration).

Pathogenesis

- **genetic nature**
- **immunological disturbances** (higher incidence of autoimmune diseases and allergy in patients with Mediterrhanian fever; high serum Ig and circulating immune complexes levels)
- **involvement of vascular system**
- **C5a-inhibitor** deficiency in joint and peritoneal fluids may have a role in the pathogenesis of the attacks (result in severe inflammatory attacks following the accidental release of C5a).

Clinical manifestations and syndromes

- **1. Onset:** in childhood (1st year of life – 50%; before 20 -80%; over 40 – 1% only)

2. **Fever:**

- may be even asymptomatic (afebrile mild attacks)
- abdominal pain attacks with fever up to 38-40C with tachycardia, and (in 25%) – chills; temperature returns to normal after 12hours - 3days.
- in arthritis high fever peak lasts for 1-3 days

3. **Joints affection:**

- **from arthralgia to arthritis (24-84%, mean 55%)**
- symptoms increase during the first 24-48h; may last about a week.
- accompanied by fever with high peaks lasting 1-3 days
- in 5% symptoms persist several weeks or even months
- usually no residual damage

- **asymmetric, non-destructive mono- or oligoarthritis affecting the large joints**; knees and ankles (small – rare)
- **1-2 large joints** affected at a time;
- in frequent attacks - impression of migratory arthritis is present
- chronic destructive mono- or oligoarthritis: mostly hip or knee – 2%
- sacroiliitis, mosttly asymptomatic - rare

4. **Chest (pleurisy) pain**

- **more than 50%**
- pleural friction rub - rare
- small effusion in costophrenic angle.

5. Abdominal pain - almost in all patients

- attacks originate in one area, spread over whole abdomen within few hours; patients flex their thighs and lie motionless to relieve the pain;
- Intensity: from mild discomfort to that in severe peritonitis
- Peritoneal symptoms – rare
- constipation and vomiting - frequent
- attack reaches peak in 12h; acute pain resolves spontaneously in 24 to 48h; then subsides gradually.

6. Skin rash – 10-20%

- localization - extensor surfaces of legs; below knees, over ankle joints or dorsum of foot.
- typical: bright-red, hot, swollen, painful
- usually unilateral
- border may or may not be sharply defined
- symptoms intensify rapidly and disappear in 2-3 days without therapy.
- other rashes – urticaria, purpura etc also possible

7. Other organs affection

- attacks of pericarditis (occasionally)
- severe headache during attacks
- transient ECG changes (myo-, pericarditis like)
- severe myalgia; muscle atrophy at affected joints
- numerous attacks in children: growth retardation.
- colloid bodies in eye grounds
- palpable spleen - more than 33%

8. **Kidneys: AA-amyloidosis**

- at the late stages
- the first sign is massive albuminuria;
- within several years - nephrotic syndrome
- progresses to chronic renal failure

Amyloid deposits in other organs

- intestine
- adrenals
- heart
- ovaries
- pancreas
- muscles
- deposits are mostly perivascular.

Clinical variants

- with abdominal; thoracic, joint and fever syndromes dominating
- may vary in different life periods of the individual

Course

- first symptoms: sudden onset of asymptomatic fever, arthralgia, chest and abdominal pain.
- last for days or weeks and relieve by themselves with no objective symptoms revealed.
- attacks recur at irregular periods of several days to several months; spontaneous remissions may last years.
- further progression: recurrent episodes with increasing frequency; shortening of asymptomatic periods.

Factors influencing exacerbations

- physical exertion
- stress
- walking and standing
- pregnancy.

Outcomes

- end-stage chronic renal failure and death.
- adequate treatment can delay (but not stop) the disease development
- rapid progression is observed after the first signs of asotemia appearance

Immunoglobulin-related amyloidosis (AL)

- monoclonal plasma cell disorder associated with gammapathies
- mostly related to light chains (AL-amyloidosis)
- In few reported patients - heavy (H) chains amyloid H-chain type (AH).
- Light chains consist of whole or part of the variable (VL) domain, more commonly derived from λ chains than from χ chains

Conditions causing AL-amyloidosis

- Multiple myeloma
- Waldenstrom disease
- Monoclonal gammapathy of undetermined significance (MGUS)

Pathogenesis

- In L chains certain amino acid and glycosylation characteristics predispose to amyloid formation (why - remains unknown).
- probably these changes promote aggregation and insolubilization
- amyloidogenicity of particular monoclonal light chains was confirmed in an in vivo model (injection of isolated Bence Jones proteins into mice, who developed typical amyloid deposits)
- *In some patients with monoclonal gammapathy monoclonal proteins accumulate in various organs, but the deposits do not form fibrils. Patients with this form are described as having* ***nonamyloid monoclonal immunoglobulin deposition disease (MIDD).***

Epidemiology

- **Incidence:** annually, 1-5 cases per 100 000 people occur (may be higher basing on myeloma incidence – underdiagnosis?)
- **Race:** probably not related (no comparative investigations)
- **Sex:** M:F 2:1
- **Age:** It is revealed usually in aged (in UK – 66% were between 50 and 70 years old at diagnosis; 4% - less than 40 years. Median age – **64** years old (Mayo clinic)

Symptoms

- Major systemic amyloidosis with affection of most organs described (except CNS)
- **Most common initial symptoms**: peripheral edema, hepatomegaly, purpura, orthostatic hypotension, peripheral neuropathy (10-20%), carpal tunnel syndrome (20%), and macroglossia (10%)
- Hepatosplenomegaly is revealed in 25%
- Heart is affected in about 90%
- Kidneys in 33-40%

Localized amyloid L-chain type

- most commonly in respiratory tract
- often remains localized
- may involve ureter or urinary bladder (hematuria)
- Amyloidomas may be also in soft tissues, including the mediastinum and the retroperitoneum
- Skin involvement can manifest as plaques and nodules
- Isolated heart affection (not common in AL)

Complications

- **congestive heart failure, arrhythmias, or both (cause of death more than 50%)**
- **renal failure**
- **bleedings**

Course and prognosis

- In the absence of chemotherapy always progressive course
- Rapid development of heart or renal failure
- Treatment of heart and renal failure is usually ineffective.
- Survival: 18 months-10 years; mean – 18-20 months; 1-year survival rate is 51%, 5 – 16%; 10 – 4.7%
- Heart affection is the most unfavorable sign (mean survival after symptoms appearance – 6 months).

ATTR –amyloidosis

- TTR is a serum protein that transports thyroxine and retinol-binding protein.
- TTR monomer contains 8 antiparallel beta pleated sheet domains.
- TTR is synthesized primarily in the liver, as well as in the choroid plexus and retina.

Normal-sequence TTR

- senile cardiac amyloidosis (SCA).
- microscopic deposits are also found in many other organs - senile systemic amyloidosis (SSA)

Clinical manifestations; SSA

- **in 25% of old patients clinically silent** microscopic, systemic deposits of transthyretin (TTR) amyloid involving the heart and blood vessel walls, smooth and striated muscle, fat tissue, renal papillae, and alveolar walls are revealed.
- spleen and renal glomeruli are rarely affected
- brain is not involved.
- occasionally more extensive deposits in the heart, affecting ventricles and atria and situated in the interstitium and vessel walls, cause significant impairment of cardiac function and may be fatal.

Clinical manifestations; SCA

- may be silent or accompanied by significant impairment of cardiac function

TTR mutations

- accelerate the process of TTR amyloid formation
- mutations destabilize TTR monomers or tetramers and allow molecule to more easily attain amyloidogenic intermediate conformation
- more than 85 amyloidogenic *TTR* variants cause **systemic familial amyloidosis.**
- **Mostly autosomal dominant inheritance**

Variants of TTR systemic familial amyloidosis

- **FAP (family amyloid polyneuropathy) –Val30Met (Valin to Metionin in 30 position)**
- **Cardiac amyloidosis (Leu111Met, Dutch)**
- **Cardiac amyloidosis V122I** (late-onset (after age 60) cardiac amyloidosis, most common)
- **late-onset systemic amyloidosis T60A** with cardiac, and sometimes neuropathic, involvement (northwest Ireland)
- **amyloidosis of carpal ligament and nerves of the upper extremities L58H** (Germany, MidAtlantic region)
- In total, 100 variants of TTR, about 98 are amyloidogenic

Epidemiology

- **Incidence:**

- cardiac ATTR with normal sequence – 15% of all the autopsies after 80 years old

- for mutant TTR - depends on the type (V122I in USA - 2%-3.9%)

- **Race and region**: types of mutations are region-related
- **Sex:** all *TTR* variants encoded on chromosome 18, so M=F; for unknown reasons, penetrance is more and age of onset earlier in males.
- **Age**: depending on the mutation and region (age of onset in V30M in Portugal, Brazil, and Japan is 32, in Sweden – 56); *normal TTR – after 60; rapid increase after 80.*

Clinical manifestations

- **General** - cachexia
- **Skin:** purpura (vascular fragility due to subendothelial deposits)
- **Heart:** heart failure, arrhythmias (blocks, PVC, VT, postural hypotension (subendothelial deposits in peripheral vessels)
- **GI** – gastric symptoms, diarrhea and/or constipation
- **Liver:** hepatomegaly

Neuropathy: axonal degeneration of small nerve fibers due to deposits

- sensorimotor impairment (V30M - lower limb neuropathy; I84S, L58H - primarily upper limb neuropathy).
- hyperalgesia; altered temperature sensation
- carpal tunnel syndrome – most typical for L58H, may be in normal TTR
- autonomic dysfunction (sexual or urinary – common for V30M)
- cranial neuropathy
- eye: deposits in corpus vitreum

FAP (family amyloid polyneuropathy) V30M

- major foci - Portugal, Japan, Sweden; age 20-70
- **Clinical manifestations include**:
- **progressive peripheral and autonomic neuropathy;** vitreous and cornea of the eye affection;
- **Varying degrees of visceral involvement**: kidneys, thyroid, adrenals
- **General symptoms:** weight loss etc
- **Heart** affection is not typical, but predisposition to sudden heart stoppage exists
- **Course and prognosis:** progression; disorder is fatal. Death results from the effects and complications of peripheral and/or autonomic neuropathy, or from cardiac or renal failure.

Beta2 –microglobulin
(Dialysis-associated)

- Beta-2-microglobulin amyloidosis is a condition affecting patients on long-term hemodialysis or continuous ambulatory peritoneal dialysis (CAPD). Patients with normal or mildly reduced renal function or those with functioning renal transplant are not affected.

Pathogenesis

- Beta-2-microglobulin is a component of beta chain of HLA class I molecule and is present on the surface of most of the cells
- In normally functioning kidney, beta-2-microglobulin is filtrated by glomerulus
- In renal failure , impaired renal catabolism causes an increase in beta-2-microglobulin synthesis leads to 10- to 60-times increase of its level
- Role of IL-6 stimulation by dialysis is discussed

Epidemiology

- 1st symptoms – 4-8 years after haemodialysis onset (in 20%)
- 10 years after – in 70% of cases
- 15 years after – in 95% of cases
- 20 years after – in 100% of cases
- Race, age and sex: no differences

- **1. Neurological manifestations:** Clinical syndromes:
- **carpal tunnel syndrome – most common** (deposits in hands ligaments compress the nerves)
  - bilateral and progressive
  - numbness, paresthesias, pain, swelling in the region of the distal median nerve
  - worse during dialysis and at night
  - progresses to contraction of the hand and atrophy of the muscles

Joints and bones affection

- Flexor tenosynovitis
- Scapulohumeral arthropathy - shoulder pain worse in supine position
- Spondyloarthropathy (more – cervical)
- Bone cysts (thin-walled; in carpal bone, femoral heads, humerus, acetabulum, spine), cause stiffness and/or pain.
- Pathological fractures (femoral neck mostly common)

Systemic manifestations

- **after 10-15 years, usually asymptomatic**
- **GI:** macroglossia, dysphagia, small bowel ischemia, malabsorption, and pseudoobstruction
- **Cardiovascular:** Myocardium, pericardium, valves; small pulmonary arteries and veins
- **Kidneys:** renal and bladder calculi containing beta-2-microglobulin deposits
- **Reproductive:** prostate and the female reproductive tract
- **Spleen** deposits

Familial Renal (FRA)

- Syndrome of familial systemic amyloidosis with predominant nephropathy
- First described in 1932 by Ostertag, former name - Non-neuropathic systemic amyloidosis, Ostertag type
- Autosomal dominant
- Age – from first decade to old age but most typically in mid adult life

Amyloid precursors

- *Lysozyme*
- Apolypoprotein I
- *Apolipoprotein AII*
- *Fibrinogen A alpha-chain*

Lysozyme Ile56Thr, Asp67His, Try64Arg

- *Renal: Proteinuria and renal failure*

- *GI tract - Bleeding and perforation*
- *Liver and spleen - Organomegaly and hepatic hemorrhage*
- *Salivary glands – Sicca syndrome*
- Petechial rashes may occur

Apolypoprotein I

- **Proteinuria and renal failure – almost in all**
- **Peptic ulcers** (*Gly26Arg*)
- **Progressive neuropathy** (*Gly26Arg*)
- **Liver and spleen** – varying from organomegaly to liver failure (*Trp50Arg* ; deletions *60-71*)
- **Heart failure** (*Leu90Pro; Arg173Pro* etc); aggressive early IHD (*deletion Lys107)*
- **Retina** - Central scotoma (*deletion 70-72*)
- **Skin:** Infiltrated yellowish plaques (*Leu90Pro);* acanthosis nigricans-like plaques (*Arg173Pro)*
- **Larynx** – dysphonia (*Arg173Pro*)
- **Males reproductive:** infertility (*Ala175Pro*)

Apolipoprotein AI with normal sequence

- Causes amyloid deposits in human aortic atherosclerotic plaques
- Found in 20-30% of elderly individuals at autopsy

Apolipoprotein AII

- Proteinuria and renal failure

Fibrinogen A alpha-chain

- Proteinuria and renal failure
- In Glu526Val variant hepatosplenomegaly and liver failure may occur (late sign)

CNS amyloidosis

- **Beta protein precursor** (Alzheimer syndrome, Down syndrome, hereditary cerebral hemorrhage with amyloidosis - Dutch type)
- **Prion protein** (Creutzfeldt-Jakob disease, Gerstmann-Strussler-Scheinker disease, fatal familial insomnia)
- **Cystatin C** (hereditary cerebral hemorrhage with amyloidosis - Icelandic type)
- **ABri precursor protein** (Familial dementia British type)
- **ADan precursor protein** (Familial dementia Danish type)

Hereditary cerebral haemorrhage with amyloidosis; hereditary cerebral amyloid angiopathy

- **Icelandic type**
- autosomal dominant; symptoms early adult life.
- cerebrovascular deposits (cystatin C)
- recurrent major cerebral haemorrhages
- appreciable but clinically silent amyloid deposits are present in the spleen, lymph nodes, and skin.
- no extravascular amyloid in the brain.
- multi-infarct dementia is common

Dutch type

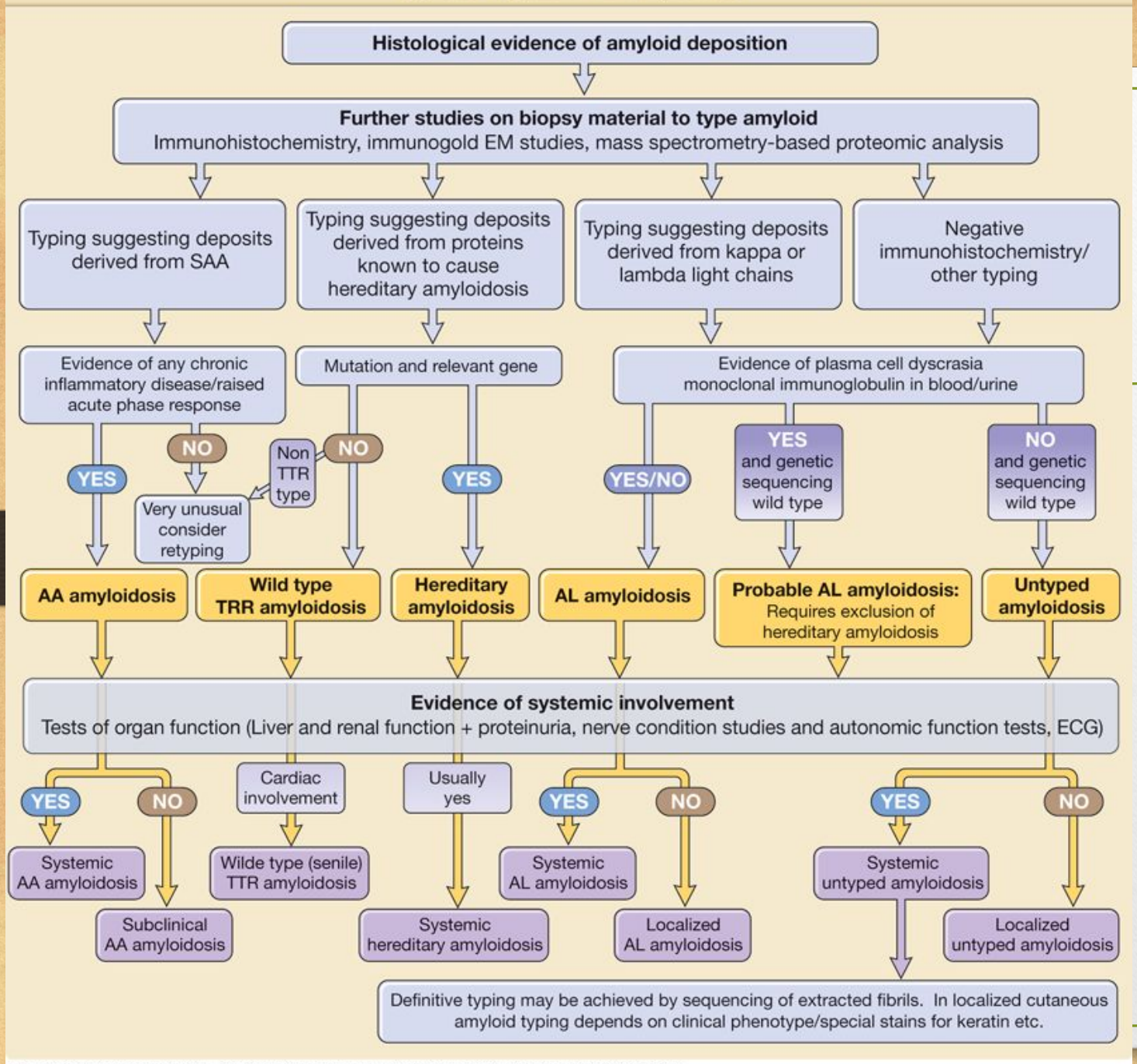
- autosomal dominant; starts at middle age
- β-protein deposits
- recurrent normotensive cerebral hemorrhages
- Multi-infarct dementia; some patients become demented in the absence of stroke.
- Amyloid outside the brain has not been reported

Diagnosis of amyloidosis

- **1. Presence of amyloid: congo red staining**
- **2. Type of amyloid: immunohistochemistry**
- **3. Mutation type: amino acid sequence analysis**

Tissues for biopsy

- **subcutaneous fat aspiration (provides enough material for all investigations) – 60%**
- rectal biopsy 80-85%
- cheek biopsy 60%
- organ biopsy: if subcutaneous fat investigation didn't not provide enough information for diagnosis
- Anyway, kidney biopsy is usually performed to determine the cause of nephrotic syndrome (informativity is 100%)



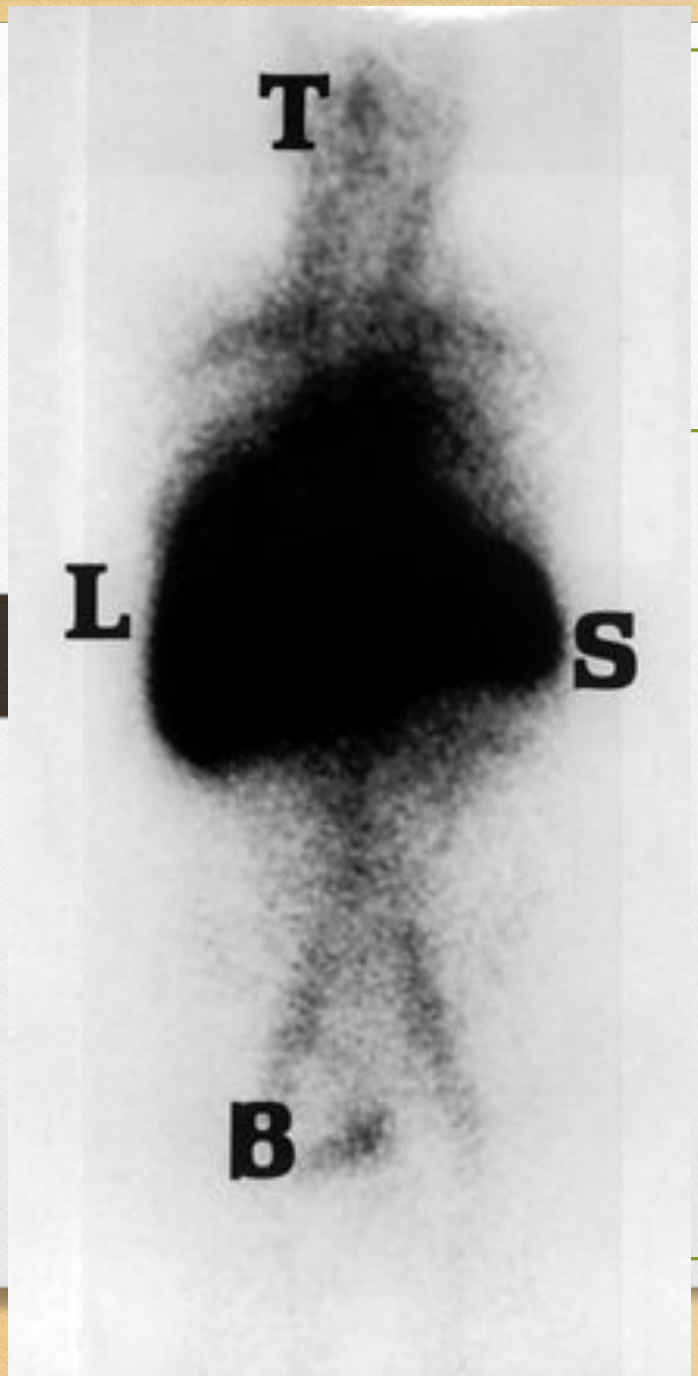
Source: Goldsmith LA, Katz SI, Gilchrest BA, Paller AS, Leffell DJ, Wolff K: *Fitzpatrick's Dermatology in General Medicine, 8th Edition*: www.accessmedicine.com

AA

- SAA precursor level in blood
- Serum immunoglobulins (to exclude AL;in AA amyloidosis usually polyclonal hypergammaglobulinemia is presentdue to underlying inflammation)
- Kidney function (urine analysis, daily proteinuria, GFR)

Instrumental methods

- **Avoid IV pyelography** if amyloidosis is suspected (more frequent renal failure)
- **Ultrasonography:** kidneys' size (non-specific)
- **CT scanning**: with technetium which binds to soft-tissue amyloid deposits (to monitor progression)
- **Radiolabeled P-component gamma scanning:** total body burden of amyloid and its disappearance after successful treatment of the primary disease. most useful in AA amyloidosis because the major sites of deposition are accessible to the imaging agent


T
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B

AL

- Monoclonal immunoglobulin L chain - in the serum or the urine of 80-90%

- immunoglobulin free light chain (FLC); kappa and lambda chains
- bone marrow: in 40% of patients more than 10% plasma cells
- L-chain immunophenotyping of the marrow, even in the absence of increased numbers of plasma cells

Biochemistry

- concentration of albumin is often decreased
- hypogammaglobulinemia + proteinuria suggests a diagnosis of amyloid L-chain type or MIDD.
- In contrast: amyloid A type is associated with hypergammaglobulinemia due to persistent inflammation and interleukin 6 production.

Functional systems tests

- clotting system abnormalities
- kidney function tests
- liver function tests
-

Instrumental

- Echocardiography
- Radiolabeled pentagonal (P) component scanning: total body burden of amyloid
- Bone imaging: to reveal plasma cell infiltration of the bones
- Chest radiography: to reveal pulmonary deposits

ATTR

- subcutaneous fat aspiration
- sural nerve biopsy
- rectum, stomach, myocardium biopsy
- Congo red; antiserum against TTR

Instrumental

- Echocardiography
- Nerve conduction studies to monitor course of disease and assess response to treatment
- Genetic studies (*TTR* variant)

Familial systemic (renal)

- Biopsy: amyloid confirmation
- Affection of organs
- SAP component scintigraphy; iodine I123 –labeled SAP
- DNA analysis obligatory in all patients with systemic amyloidosis who cannot be confirmed absolutely to have the AA or AL type.

beta-2-microglobulin

- reference range of serum beta-2-microglobulin concentration of is 1.5-3 mg/L; can be elevated to values of 50-100 mg/L.
- Beta-2-microglobulin levels correlate with elevated serum creatinine levels and are inversely related to the glomerular filtration rate

Radiologic:

- joint erosions (usually large joints)
- lytic and cystic bone lesions (typically juxta-articular)
- pathological fractures
- spondyloarthropathies
- vertebral compression fractures
- May precede the pain appearance

CT

- amyloid deposits: intermediate attenuation.
- identification pseudotumors and pseudocystic areas in the juxta-articular bone.
- best method for detecting small areas of osteolysis in cortical bone or osseous erosion
- may be helpful in the assessment of the distribution and extent of destructive changes.

MRI

- differentiating destructive spondyloarthropathies from inflammatory processes and infections.

Ultrasound

- tendon thickness.
- rotator cuff thickness greater than 8 mm, thickening of joint capsules (especially of the hip and knee), and retention of synovial fluid may be observed

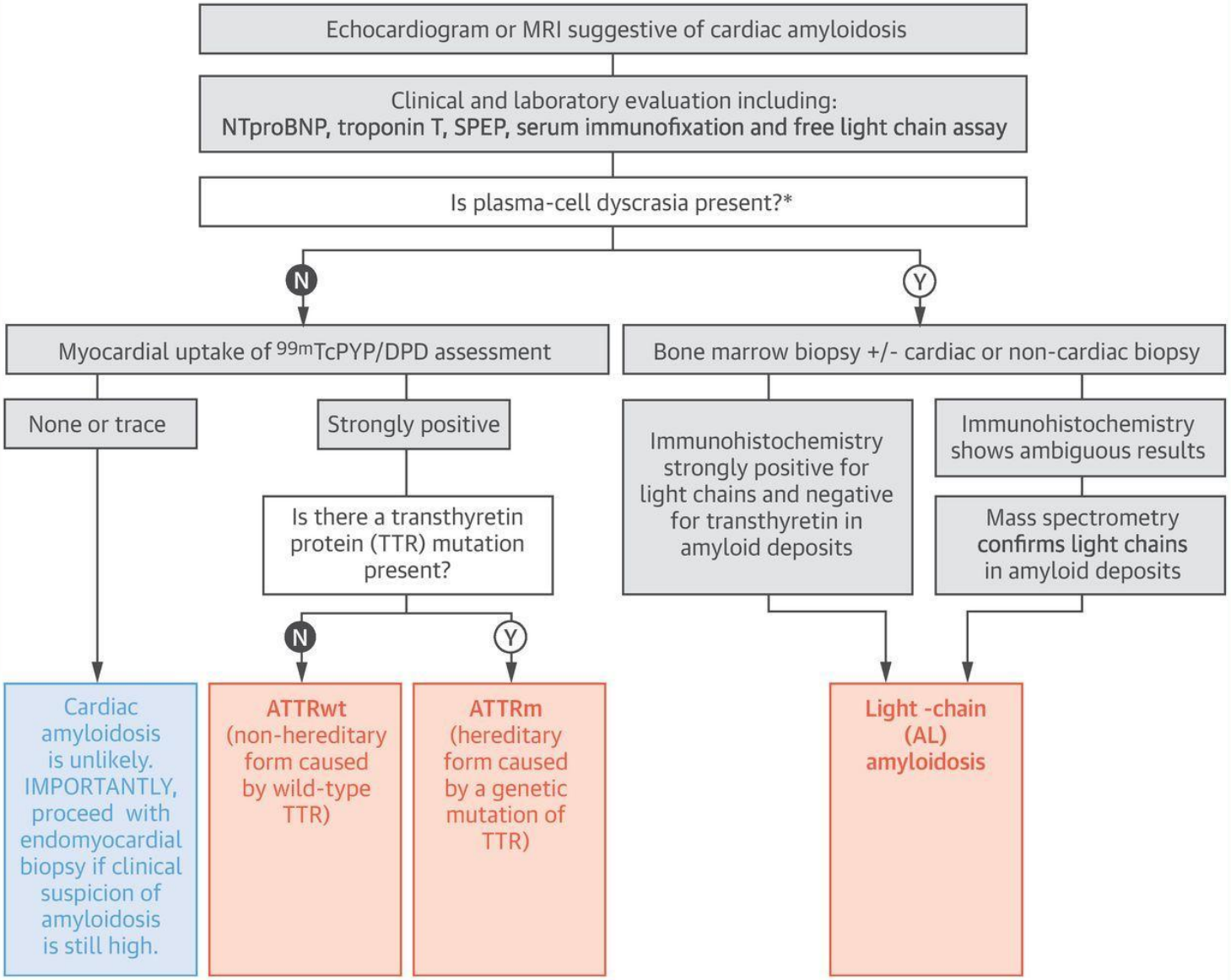
Scintigraphy

- radiolabeled P-component scans:
- iodine I 123 serum amyloid P
- iodohippurate sodium I 131 beta-2-microglobulin
- I 111 beta-2-microglobulin

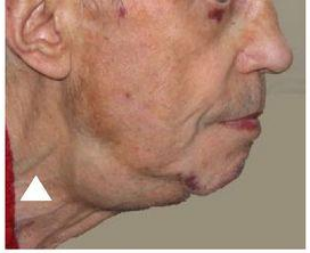
Biopsy with Congo red staining and with immunostaining

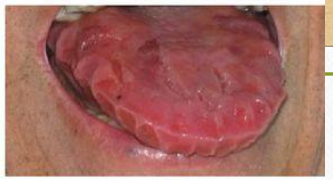
- centrifuged synovial fluid sediments
- cystic bone lesions biopsy
- synovia biopsy
- most common site for biopsies: sternoclavicular joint.
- rectal biopsy and subcutaneous fat aspiration are of little value.
- antisera to beta-2-microglobulin

CENTRAL ILLUSTRATION: Diagnosing and Typing Cardiac Amyloidosis in a Patient With Unexplained Heart Failure



Falk, R.H. et al. J Am Coll Cardiol. 2016;68(12):1323–41.





Signs and Symptoms of AL

Lethargy, fatigue
Weight loss
Peripheral edema
Heart failure
Diarrhea/constipation
Peripheral &/or autonomic neuropathy
Postural hypotension
Purpura

Patient with suspected systemic AL amyloidosis

Red Flags for AL
(in MGUS/MM)

Unexplained high NT-proBNP
Albuminuria

Confirm diagnosis by tissue biopsy (Congo red)

Amyloid typing (IHC or LC-MS)

Detect PC clone

Serum and urine IF, FLC, bone marrow biopsy, iFISH, imaging for bone lesions

Assess extent of organ involvement and stage disease

ECG, Echocardiogram, NT-proBNP, Troponin-T

eGFR, 24 hour proteinuria, Alkaline phosphatase, clotting

Excellent PS,
Limited organ involvement,
Good renal function,
Troponin-T <0.06 ng/ml and
NTproBNP <5000 ng/L

Reasonable PS,
NT-proBNP <8500 ng/L

NT-proBNP >8500 ng/L

"Low Risk"

Consider ASCT with HDM (Mel 200 mg/m^2) or

Dose attenuated HDM-ASCT with bortezomib consolidation

"Intermediate Risk"

Combination chemotherapy MDex

or CTD

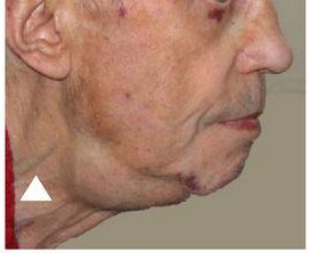
(CyBorD or BMDex)

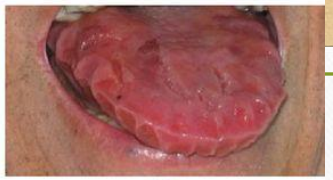
"High Risk"

Cautious chemotherapy with dose attenuated regimens and close monitoring

Treatment: AA

- primary inflammatory disease treatment
- tumor necrosis factor-a inhibitors and interleukin-1 inhibitors (arthritis, FMF)
- colchicine (0.6 mg tid) – FMF
- low–molecular-weight sulfonated molecule interfering with fibril formation and deposition of amyloid by inhibiting interaction of SAA with glycosaminoglycans (NC-503): the amount of amyloid deposits.
- dimerization of human SAP molecules in vivo with a palindromic compound (CPHPC) triggers
- Anti–IL-6R therapy appears promising
- anionic sulphonates (clinical studies)





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Assess extent of organ involvement and stage disease

ECG, Echocardiogram, NT-proBNP, Troponin-T

eGFR, 24 hour proteinuria, Alkaline phosphatase, clotting

Excellent PS,
Limited organ involvement,
Good renal function,
Troponin-T <0.06 ng/ml and
NTproBNP <5000 ng/L

Reasonable PS,
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NT-proBNP >8500 ng/L

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Dose attenuated HDM-ASCT with bortezomib consolidation

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Combination chemotherapy MDex

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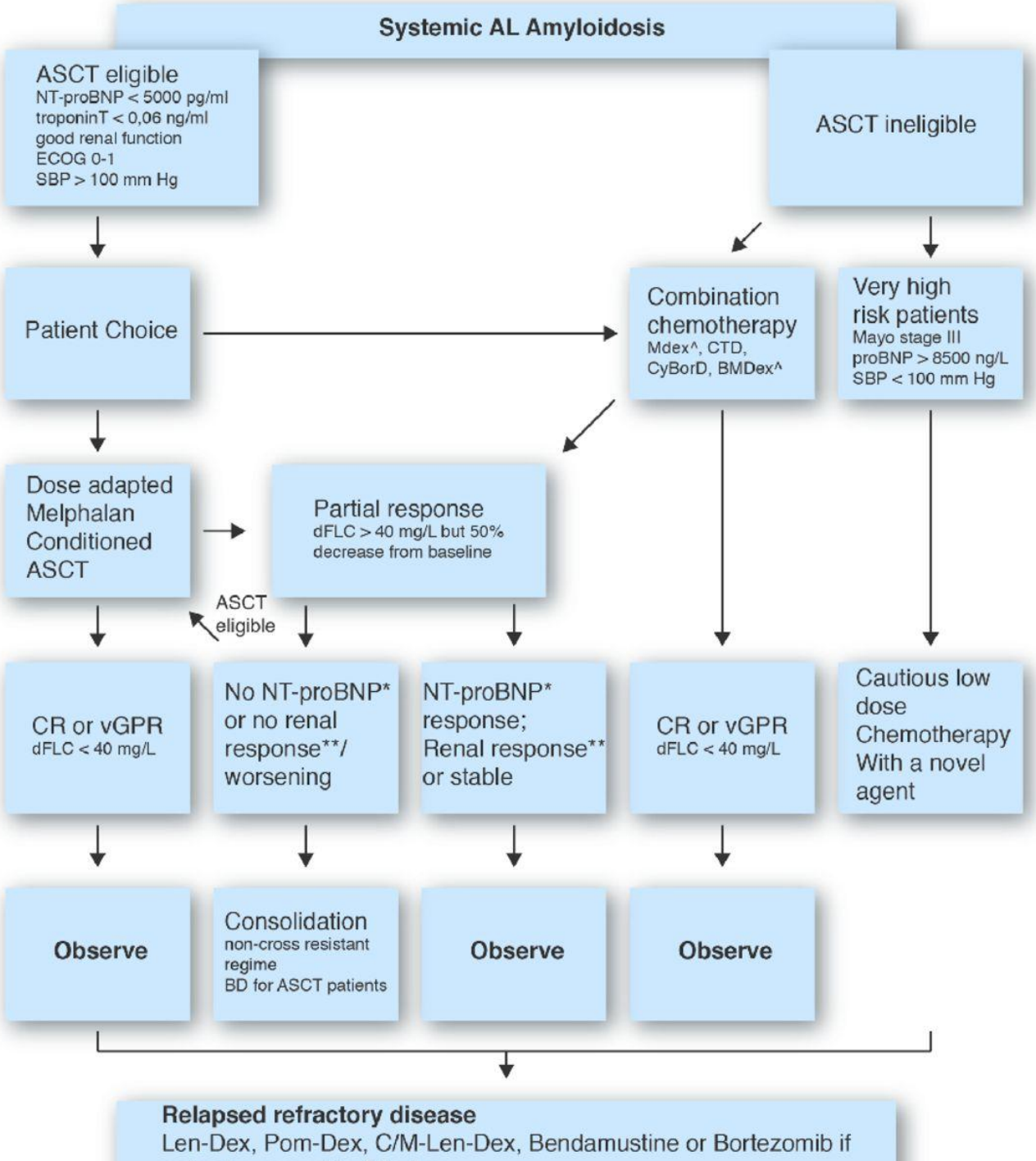
Systemic AL Amyloidosis
ASCT eligible
NT-proBNP < 5000 pg/ml
troponinT < 0,06 ng/ml
good renal function
ECOG 0-1
SBP > 100 mm Hg
ASCT ineligible
Patient Choice
Combination chemotherapy
Mdex^, CTD, CyBorD, BMDex^
Very high risk patients
Mayo stage III
proBNP > 8500 ng/L
SBP < 100 mm Hg
Dose adapted Melphalan Conditioned ASCT
Partial response
dFLC > 40 mg/L but 50% decrease from baseline
ASCT eligible
CR or vGPR
dFLC < 40 mg/L
No NT-proBNP* or no renal response**/ worsening
NT-proBNP* response; Renal response** or stable
CR or vGPR
dFLC < 40 mg/L
Cautious low dose Chemotherapy With a novel agent
Observe
Consolidation
non-cross resistant regime
BD for ASCT patients
Observe
Observe
Relapsed refractory disease
Len-Dex, Pom-Dex, C/M-Len-Dex, Bendamustine or Bortezomib if

- melphalan plus prednisone
- melphalan, prednisone, and colchicine
- Other chemotherapeutic regimens used for multiple myeloma are also expected to benefit
- 5-drug myeloma regimen (vincristine, carmustine, melphalan, cyclophosphamide, prednisone
- chemotherapy is usually continued for 1-2 years
- Pharmacologic therapy to solubilize amyloid fibrils
- anthracycline analogue of doxorubicin, 4-iododoxorubicin (Idox), is the first small molecule found with in vivo activity to solubilize amyloid L-chain type deposits.
- The ideal use of small molecule amyloid inhibitors, such as Idox, likely lies in combination with cytotoxic chemotherapy

Treatment of localized amyloid L-chain type

- has not been studied systematically
- chemotherapy is not indicated
- Localized radiation therapy aimed at destroying the local collection of plasma cells producing the amyloid L-chain type can be administered when a plasma cell collection can be identified
- **Local collections of amyloid L-chain type in the genitourinary tract, even in the absence of an identified clonal plasma cell collection, can cause hematuria. In these patients, surgical resection of amyloidomas may be required to control the bleeding.**

TTR

- Digoxin and calcium channel blockers are contraindicated
- Liver transplantation
- patients with cardiac, leptomeningeal, gastrointestinal, or ocular involvement often progress despite transplantation
- **Combined heart and liver or liver and kidney transplantation has been performed in a very few patients, with variable success**
- no pharmacologic therapy is available for ATTR. A number of small molecules that may have the potential to inhibit or reverse TTR amyloid formation are under preclinical study

beta-2-microglobulin

- no adequate treatment (symptomatic)
- Improvement of dialysis membranes
- Online hemodiafiltration
- Direct hemoperfusion-type adsorption column (Lixelle):

Familial renal

- Transplantation: liver (in case of liver failure), kidney, heart