# AMYLOIDOSIS: lecture materials for students

# Definitions (according to different sources)

- Amyloidosis is a systemic disease, characterized by deposition of specific fibrillar protein, localizing perireticularly or around the collagen fibers, that leads to affected organs impairment.
- Amyloidosis is a disorder of protein metabolism, which may be either acquired or hereditary, characterized by extracellular deposition of abnormal protein fibrils.
- **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 (2006).**

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

# What are β-pleated sheets

- Every fibril consist of stacks of anti-parallel β-pleated sheets
- Sheets are arranged with the long axes perpendicular to the long axis of the fibril
- This structure resembles the structure of silk (which is also proteinase resistant)
- Amyloid structure is visible by x-ray diffraction)

# 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

# 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

# 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: 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"

# 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<br>-Oedematic-proteinuric<br>-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

# *parenchymal nodular*

- associated with focal clonal immunocyte 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

# 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, sometimes called Familial Renal)

- **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)

# Inflammatory amyloidosis

- Amyloid A (AA) amyloidosis is the most common form of systemic amyloidosis worldwide. It is characterized by extracellular tissue deposition of fibrils that are composed of fragments of serum amyloid A (SAA) protein, a major acute-phase reactant protein, produced predominantly by hepatocytes

- AA protein is a single non-glycosylated polypeptide of mass 8000 Da containing 76 residues corresponding to the N-terminal portion of the 104 residue serum amyloid A protein (SAA)
- **SAA is an apolipoprotein of high density lipoprotein particles** and is the polymorphic product of a set of genes located on the **short arm of chromosome 11. SAA is a major acute phase protein**
- **Produced**: mostly - by **hepatocytes**; transcriptional regulation by cytokines, especially **interleukin 1(IL-1), interleukin 6 (IL-6), and TNF**, acting via nuclear factor χB-like and possibly other transcription factors.

- **The circulating concentration** can rise from normal levels (3mg/l) to over 1000mg/;l within 24 to 48h of an acute stimulus; **in presence of chronic inflammation the level may remain very high**.
- SAA as an exquisitely sensitive acute phase protein (more sensitive than CRP)
- 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:**

- Increased hepatic transcription of the messenger ribonucleic acid (mRNA) for SAA (up to1000-times)
- High SAA level in serum
- macrophages: SAA proteolytic cleavage

- **AA-peptide in blood**
- **In presence of amyloid synthesis accelerating factor:**

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

- **Amyloid synthesis**

# Causes

- chronic inflammatory disorders
- chronic inflammation due to bacterial infections
- malignant neoplasms; proliferative diseases of blood system
- 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
- **More rare:**
- - systemic lupus erythematosus
- - ulcerative colitis

# Chronic bacterial infections

- tuberculosis; leprosy
- chronic osteomyelitis
- bronchiectasis
- chronic abscess (different localizations)
- chronically infected burns
- chronic ulcers of lower extremities
- other chronic bacterial infections

# Malignant neoplasms; proliferative diseases of blood system

- **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

- Development of AA amyloidosis was reported in subcutaneous drug abusers in some cities in the United States.
- Pathogenesis of this is not clear (drug related or chronic inflammation due to some contaminating substance)

# 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, which is not related with any infection
- **Liver and spleen affection** (hepatosplenomegalia)
- **Thyroid enlargement**
- **Involvement of the 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 outcome is 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 may develop as a complication, which makes prognosis more unfavorable
- Fibrinous-purulent peritonitis, accompanying by pain and ascitis, is a rare complication.

# 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; hyperaemia and cellular infiltrate (neutrophils, lymphocytes, monocytes, sometimes, plasma cells and eosinophils)
- synovia: pannus formation and extensive intra-articular damage is possible
- vascular changes - thickening of the basement membrane; reduplication of the basement membrane is possibly due to 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 decade 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.
- course: accompanied by fever with high peaks lasting 1-3 days; in 5% acute attack fails to resolve and the symptoms may persist several weeks or even months before they abate with no residual damage.

# *A. most common:*

- **asymmetric, non-destructive mono- or oligoarthritis affecting the large joints**; knees and ankles (3 times more often than hips, shoulders, feet, wrists; involvement of small joints is very rare).
- **joints are painful and swollen** without marked local redness and heat.
- **1-2 large joints** are affected at a time;
- in frequent attacks involvement of one joint may start before previous joint improves, so impression of migratory arthritis is present

# *B. Rarely (2%):*

- chronic destructive mono- or oligoarthritis affecting most frequently the hip or the knee.
- permanent organic damage results from one protracted attack or from repeated short attacks.
- **C. Also had been described:**
- - sacroiliitis, frequently asymptomatic

# Investigations

- **Synovial fluid**

- usually turbid; forms a good mucin clot
- white count ranges between 15000 and 30000 polymorphonuclears per mm3
- is always sterile.

- **Radiographic changes:**

- loss of cortical definition
- sclerosis with or without bone erosion
- fusion of the joints.

# 4. **Chest (pleurisy) pain – more than 50%**

- sharp and stabbing, localized in the lower part, mostly on the right side; radiating to abdomen and shoulders; patients splint their respiratory excursions
- suppression of breath sounds over the affected side is usual but pleural friction is exceptional
- small effusion may be in the 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 of pain vary from mild discomfort to that in severe generalized peritonitis
- constipation and vomiting are frequent
- symptoms of acute peritonitis - exquisite abdominal tenderness, involuntary rigidity, rebound tenderness, and diminished peristalsis.
- attack usually reaches its peak in 12h; resolves spontaneously and is usually over within 24 to 48h; then pain subsides gradually.

# 6. Skin rash – 10-20%

- typical is erysipelas; skin becomes bright-red, hot, swollen, and painful; rash is usually unilateral and its border may or may not be sharply defined
- localization - over extensor surfaces of the legs below he knees, over the ankle joints, or the dorsum of the foot.
- symptoms intensify rapidly and then disappear within 2-3 days without any therapy.
- on biopsy:  mild acanthosis and hyperkeratosis, dermis contains an inflammatory exudate consisting of polymorpho-nuclear cells, lymphocytes, and some histiocytes concentrated mainly around the blood vessels.
- nodular rashes, Schonlein-Henoch purpura, and urticaria are also reported.

# 7. Other organs affection

- attacks of pericarditis (occasionally)
- severe headache may occur during attacks
- transient ECG signs of myopericarditis and non-specific EEG abnormalities during paroxysms.
- severe myalgia; muscle atrophy at affected joints
- numerous attacks in children may lead to growth retardation.
- colloid bodies are often found in the eye grounds
- spleen is palpable in more than 33% of patients.

# 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

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

# Immunoglobulin-related amyloidosis (AL)

- Immunoglobulin-related amyloidosis is a monoclonal plasma cell disorder in which the secreted monoclonal immunoglobulin protein forms insoluble fibrillar deposits in 1 or more organs
- Mostly related to light chains (AL-amyloidosis)
- In few reported patients amyloid deposits contained immunoglobulin heavy (H) chains amyloid H-chain type (AH).
- Light chains consist of the whole or part of the variable (VL) domain, more commonly derived from λ chains than from χ chains
- associated with gammapathies

# 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.
- It circulates as a tetramer of 4 identical subunits of 127 amino acids each.
- TTR formerly was called prealbumin because it migrates anodally to albumin on serum protein electrophoresis, but this name was misleading because TTR is not a precursor of albumin.
- 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. Its gene is located on chromosome 18 and contains 4 exons.

# 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

# Clinical manifestations

- **1. Neurological 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 Systemic (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%)

# 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

# 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 absence of increased number of plasma cells

# Biochemistry

- concentration of normal Ig is often decreased
- amyloid A type is mostly 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 P component scanning
- Bone imaging: plasma cell infiltration
- Chest radiography: 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
- Radiolabeled P component scanning
- 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 erosions**
- **may be used 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) may be present
- retention of synovial fluid may be present

# Scintigraphy

- radiolabeled P-component scans

# 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

# Treatment: AA

- primary inflammatory disease treatment
- tumor necrosis factor-a inhibitors and interleukin-1 inhibitors (arthritis, FMF)
- Colchicine – FMF

New approaches

- Anti–IL-6R therapy (clinical studies)
- anionic sulphonates (clinical studies)
- NC-503: interfers fibril formation and deposition of amyloid by inhibiting interaction of SAA with glycosaminoglycans (at study)
- palindromic compound (CPHPC) triggers: dimerization of human SAP molecules (vivo pre-clinical studies)

AL:

- melphalan plus prednisone
- melphalan, prednisone, and colchicine
- Other chemotherapeutic regimens used for multiple myeloma (vincristine, carmustine, melphalan, cyclophosphamide, prednisone scheme etc)

# Pharmacologic therapy to solubilize amyloid fibrils

**anthracycline analogue of doxorubicin, 4-iododoxorubicin (Idox)**: in vivo and clinical studies (solubilize amyloid L-chain type deposits) 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 reported 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
-

# Familial systemic (familial renal)

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