AMYLOIDOSIS

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LESSON PLAN

- HISTORY
- INTRODUCTION
- PHYSICAL & CHEMICAL NATURE OF AMYLOID
- CLASSIFICATION
- PATHOGENESIS
- CLINICAL FEATURES
- STAINING CHARACTERISTICS
- MORPHOLOGICAL FEATURES
- DIAGNOSIS
- PROGNOSIS

History

- First described by Rokitansky in 1842.
- **Term** first used by **Rudolf Virchow** in 1854 based on the color after staining it with crude iodine-staining techniques.
- Later recognized as **Protein** by ***Friedreich and Kekule*** 5 years later.

INTRODUCTION

- It is derived from the word-**amylum** in Latin, **amylon in Greek;** means cellulose or starch like.

- **Definition :** Amyloid refers to an abnormal deposit **of insoluble polymeric protein fibrils in** tissues and organs.This condition of deposition of amyloid in tissues is known as Amyloidosis.

- The fibrils are formed by the aggregation of misfolded, normally soluble proteins .

- Deposition of amyloid fibrils is usualy extracellular.

- Amyloidosis or “protein aggregation diseases” should not be considered as single disease entity its rather a group of inherited & inflammatory disorders which are resposible for tissue damage and functional compromise.

PHYSICAL NATURE OF AMYLOID

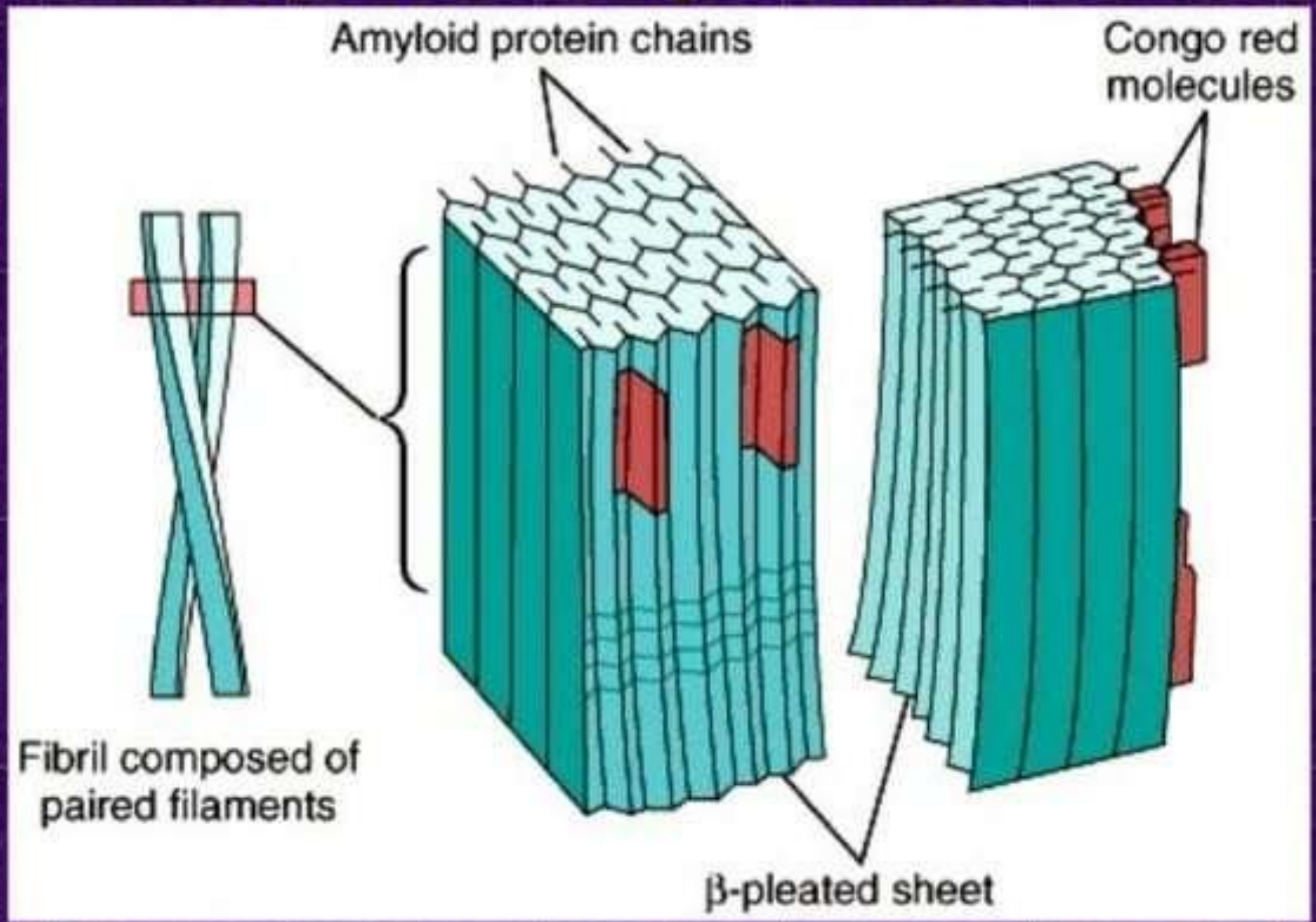
On Electron Microscopy

- These fibrils are continous, non-branching,insoluble , linear, rigid and measures 7.5 - 10 mm in diameter .

On X-Ray Crystallography & Infrared spectroscopy

- Characteristic **Beta pleated** sheet confirmation.

Amyloid – Physical nature



Chemical nature of amyloid

The main components of amyloid are:

- **A fibrillary protein** (95%) which is characteristic for each different type of disease.
- **Amyloid P component** (5%) consists of stacks of doughnut-shaped proteins. All different types of amyloid possess this protein.
- **A glycosoamynoglycan**. This is the part of the molecule responsible for the positive reaction with iodine.

The most common forms of amyloid **fibril** proteins are:

1. **Amyloid light chain(AL)-**made up of complete immunoglobulin light chain, derived from the *lambda light chain.*
2. **Amyloid associated(AA)-**derived from a unique non-Ig protein made by the liver, derived from larger precursor protein *SAA(serum amyloid associated protein)*

others

3. **Aβ2 Microglobulin(AβM)-**seen in patients on long term hemodialysis.

4. **Transthyretin(TTR)** - serum protein synthesized in liver & transports thyroxine and retinol.

5. **Amyloid β-peptide(Aβ)-** seen in Alzheimers disease.

6. **Prion proteins(APrP)**

7. **Precursor of AA=SAA(Serum Amyloid Associated Protein)**

NON FIBRILLAR AMYLOID PROTEINS ARE

- 1.Amyloid P(AP) component-found in all forms of amyloid.
- 2.Apolipoprotein-E (apoE)
- 3.Sulfated glycosaminoglycans(GAGs)

TABLE 4.10: Classification of Amyloidosis.

Category	Associated Disease	Biochemical Type	Organs Commonly Involved
A. SYSTEMIC (GENERALISED) AMYLOIDOSIS			
1. Primary	Plasma cell dyscrasias	AL type	Heart, bowel, skin, nerves, kidney
2. Secondary (Reactive)	Chronic inflammation, cancers	AA type	Liver, spleen, kidneys, adrenals
3. Haemodialysis-associated	Chronic renal failure	$A\beta_2M$	Synovium, joints, tendon sheaths
4. Heredofamilial			
i. Hereditary polyneuropathies	—	ATTR	Peripheral and autonomic nerves, heart
ii. Familial Mediterranean fever	—	AA type	Liver, spleen, kidneys, adrenals
iii. Rare hereditary forms	—	AApoAI, AGel ALys, AFib, ACys	Systemic amyloidosis
B. LOCALISED AMYLOIDOSIS			
1. Senile cardiac	Senility	ATTR	Heart
2. Senile cerebral	Alzheimer's, transmissible encephalopathy	Aβ, APrP	Cerebral vessels, plaques, neurofibrillary tangles
3. Endocrine	Medullary carcinoma type 2 diabetes mellitus	Procalcitonin Proinsulin	Thyroid Islets of Langerhans
4. Tumour-forming	Lungs, larynx, skin, urinary bladder, tongue, eye	AL	Respective anatomic location

(AL= Amyloid light chain; AA= Amyloid-associated protein; $A\beta_2M$= Amyloid β_2-microglobulin; ATTR= Amyloid transthyretin; APrP=Amyloid of prion proteins, Aβ= β-amyloid protein).

Biochemical Structure-based classification

SYSTEMIC VARIANTS

AA (SAA): chronic inflammatory diseases; periodical fever; Mediterranean fever

AL (Systemic monoclonal light chains Ig): multiple myeloma, Waldenstroms macroglobulinemia, B cell lymphoma

Transthyretrin

Normal TTR: senile systemic amyloidosis with gradual heart involvement

Met30: Familial amyloid polyneuropathy

Met111: Familial amyloid cardiopathy

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

Local Variants

AL (Locally produced monoclonal Ig): urogenital; skin, eyes, respiratory

AANF (abnormal atrial natriuretic factor): atria

Medin :Aortic amyloidosis in elderly

Insular amyloid polypeptide/ Amylin Insulinoma, type 2 diabetes

Calcitonin :Medullary thyroid carcinoma

Prolactin : Pituitary amyloid

Keratin : Cutaneous amyloidosis

Ocular

Gelsolin :Familial amyloidosis; Finnish type

Lactoferrin :Familial corneal amyloidosis

Keratoepithelin: Familial corneal dystrophies

Other variants

Hereditary (Familial systemic amyloidosis/ Familial Renal)

Fibrinogen alpha chain

Apolipoprotein AI

Apolipoprotein AII

Lysozyme

CNS amyloidosis

Beta protein precursor :Alzheimer's disease,

Down syndrome

Prion protein : Creutzfeldt-Jakob 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

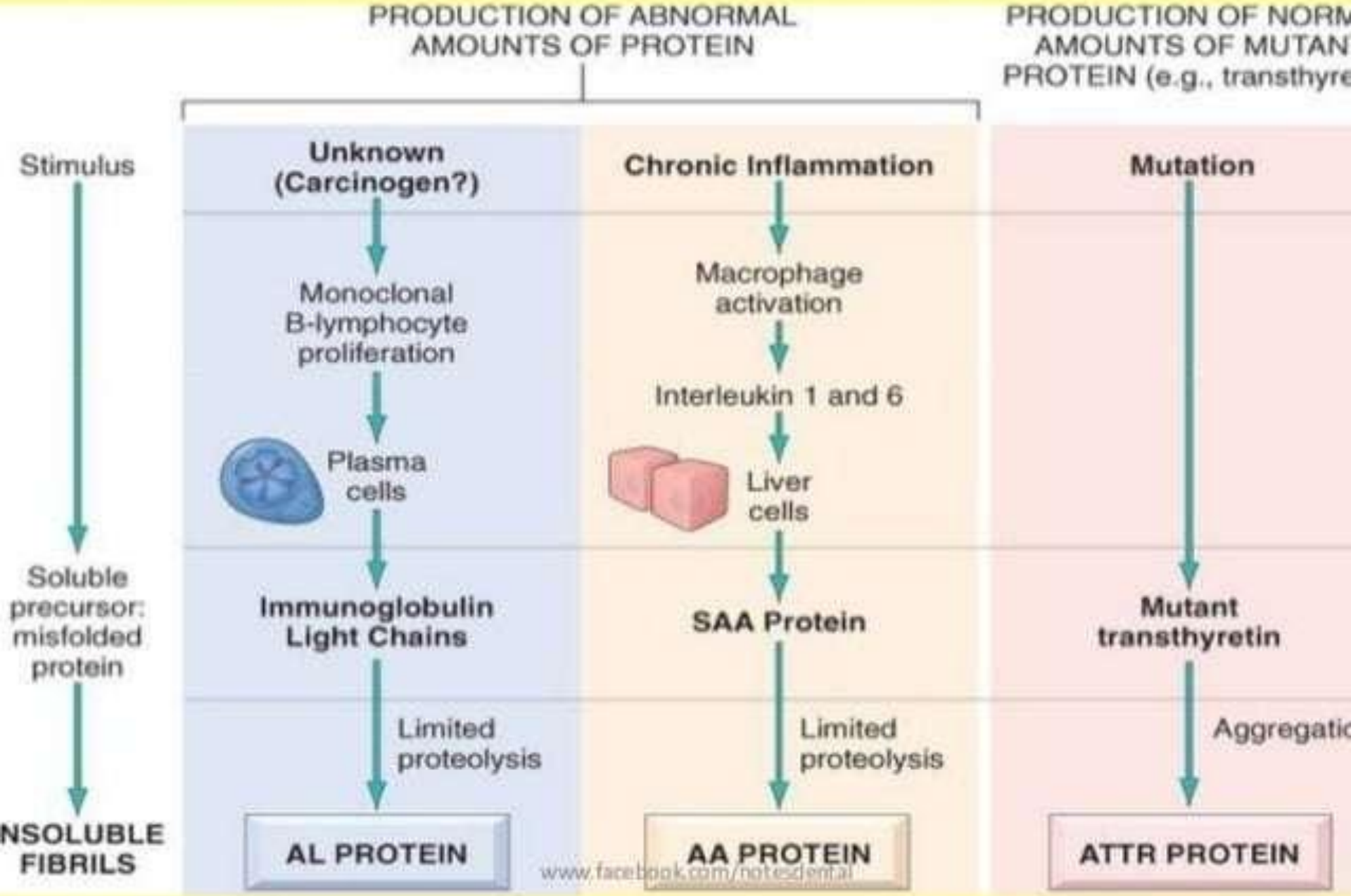
PATHOGENESIS

- Amyloidosis results from abnormal folding of proteins, which become insoluble, aggregate, and deposit as fibrils in extracellular tissue.
- Normally, misfolded proteins are degraded intracellularly by proteasomes or extracellularly by macrophages.
- In amyloidosis the quality control mechanism fail so,

there is rise in level of precursor of fibrillary protein(AL in primary and SAA in secondary form) followed by partial degradation by reticuloendothelial cells.

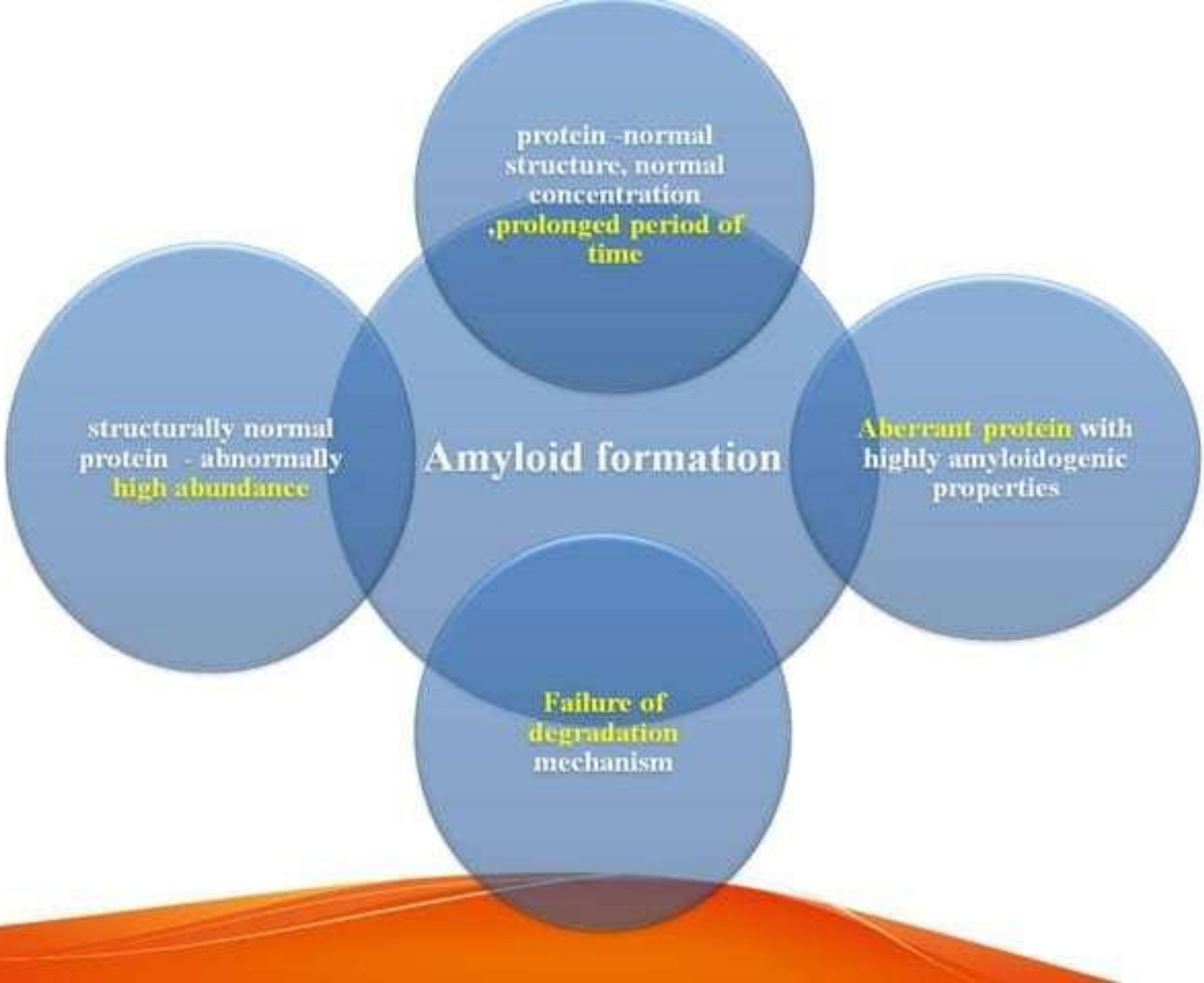
- Non-fibrillary proteins facilitate aggregation and protection against solubilisation.
- So all these factors result in deposition of misfolded protein outside the cells.

PATHOGENESIS OF AMYLOIDOSIS



- The proteins that form amyloid falls in 2 categories:

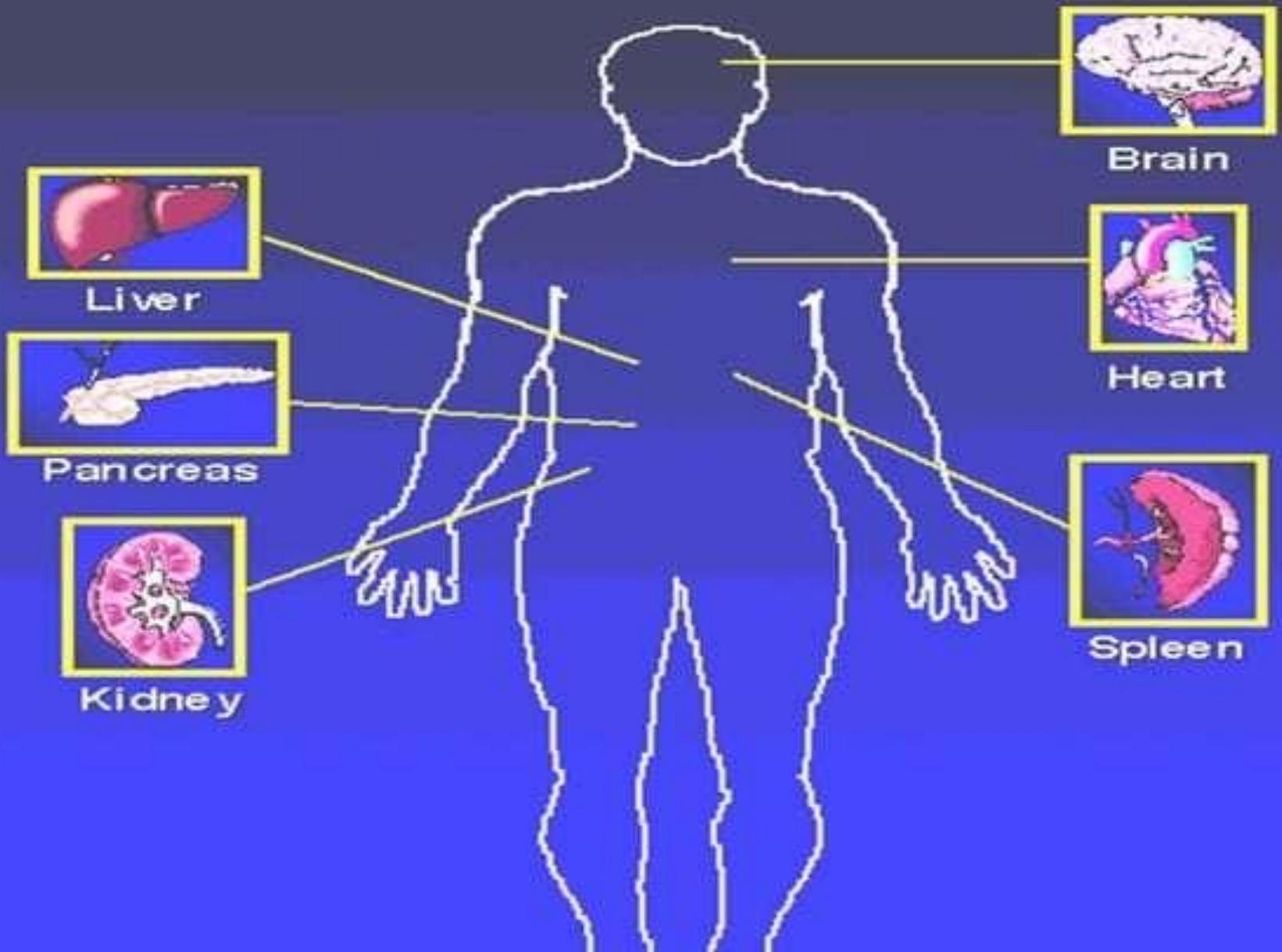
1. **Normal proteins**- that have inherent tendency to fold improperly and form fibrils when increased in number.
2. **Mutant proteins**- that are prone to misfolding and aggregation.


protein -normal structure, normal concentration ,prolonged period of time
structurally normal protein - abnormally high abundance
Amyloid formation
Aberrant protein with highly amyloidogenic properties
Failure of degradation mechanism

Clinical features

- May produce no clinical manifestations or may cause serious problems & even death.
- At first features are non specific like weakness, weight loss, light headedness or syncope.

Organs Affected by Amyloid



- Liver-hepatomegaly, ↑ alkaline phosphatase.
- Spleen-splenomegaly & splenic dysfunction.
- Heart-congestive heart failure, restrictive cardiomyopathy, constrictive pericarditis & amyloid deposits in valves.

KIDNEYS

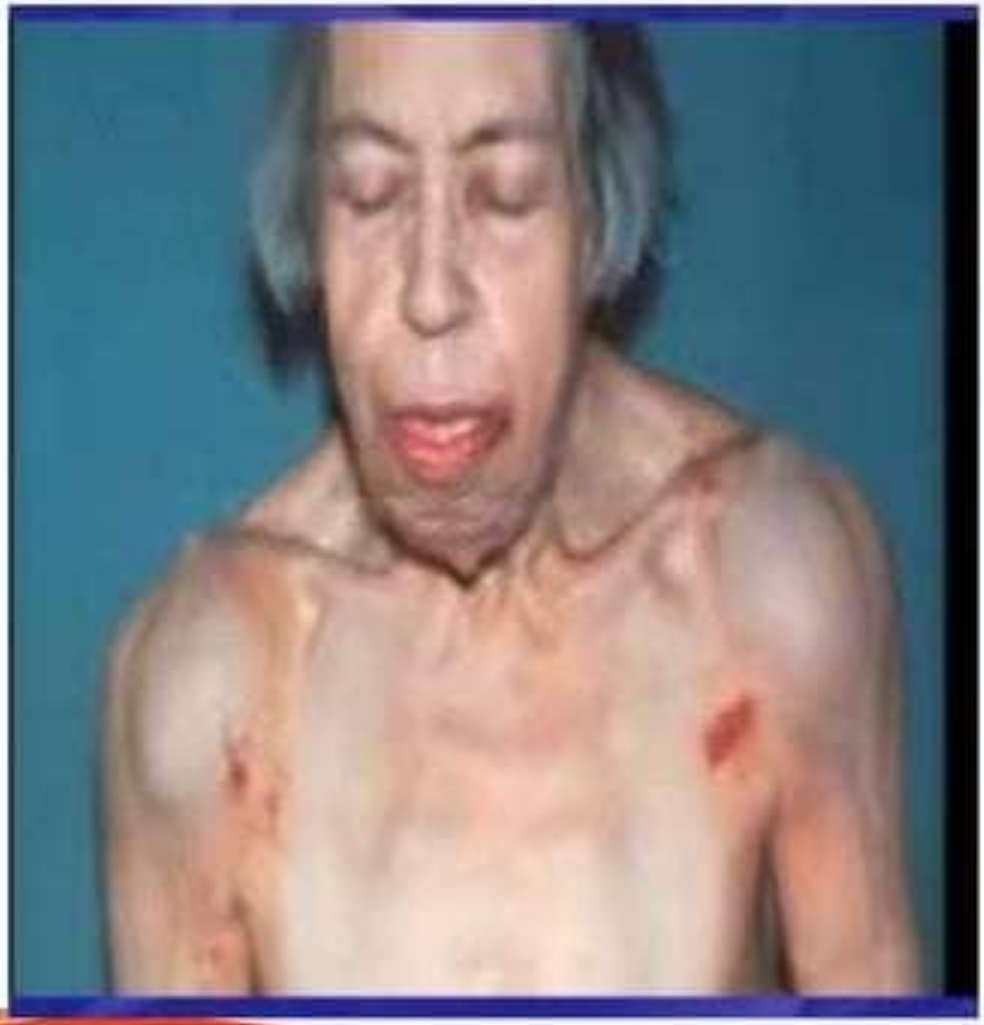
stage	phase	course
initial	Proteinuria	Slowly progressing
Clinical manifestations	Nephrotic syndrome Oedema,proteinuria Hypertensive (rare)	Rapidly progressing
terminal course	Chronic renal failure	Relapsing

- **Central Nervous System**
  Dementia(Alzheimers disease)
  Hemorrhagic strokes
- **Peripheral nervous system**
  Peripheral neuropathy
- **Endocrine organs**
  hypothyroidism due to infiltration.

- **Musculoskeletal**

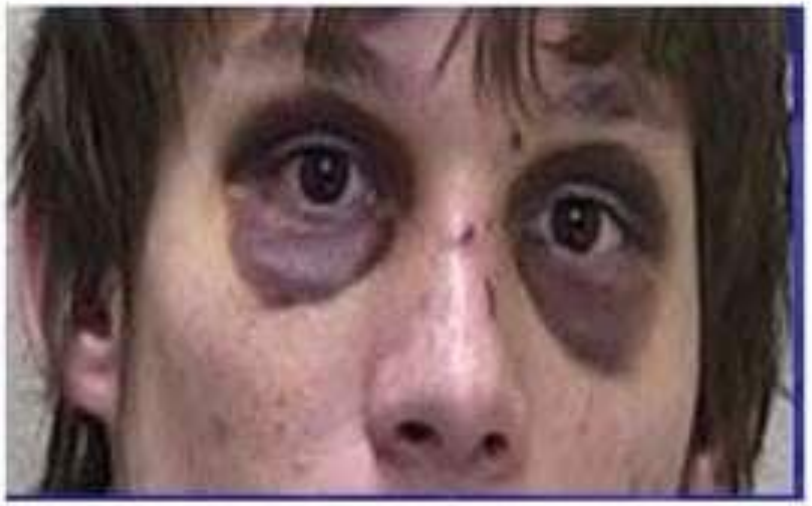
"Shoulder pad sign"

- enlargement of the anterior shoulder due to amyloid deposition in periarticular soft tissue.
- Carpal tunnel syndrome.

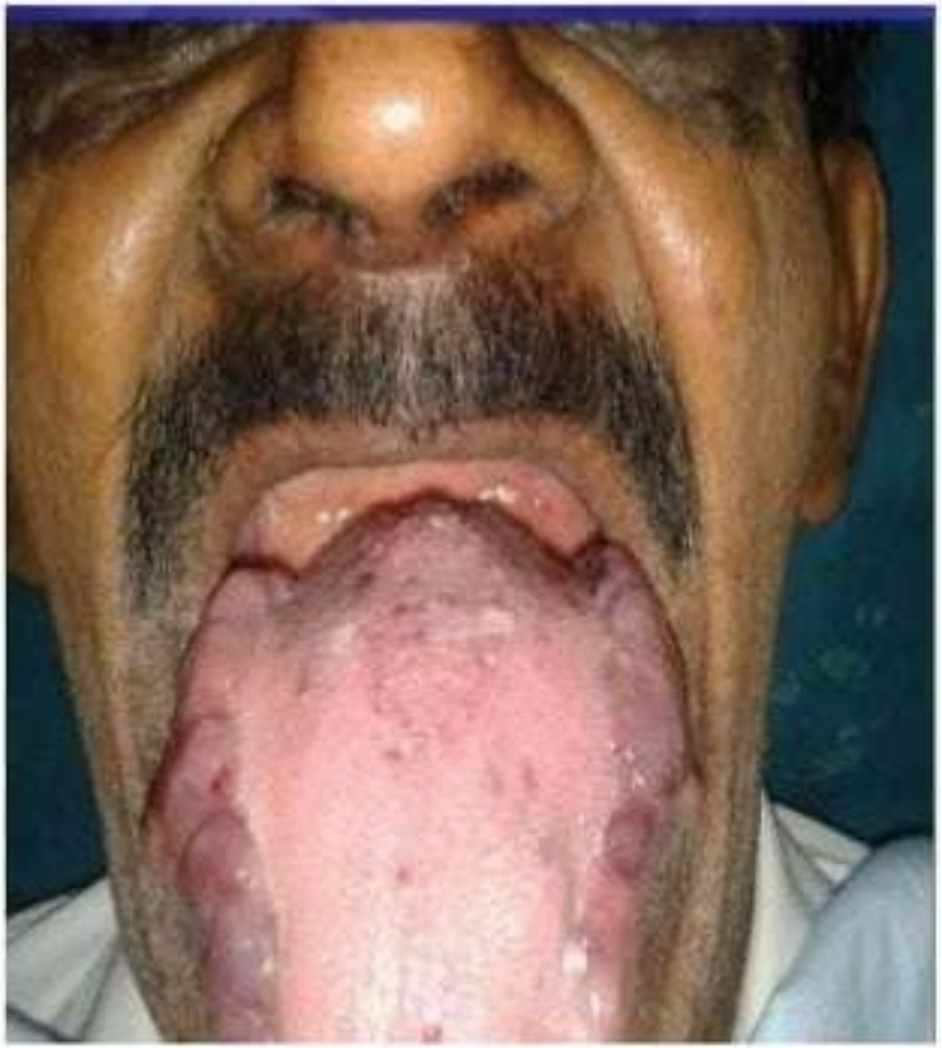


Blood vessels

- Increase susceptibility to bruising-typical **Raccoon eyes**



- **Gastrointestinal amyloidosis-** macrglossia which may hamper speech.
- In stomach and intestine may lead to malabsorption.

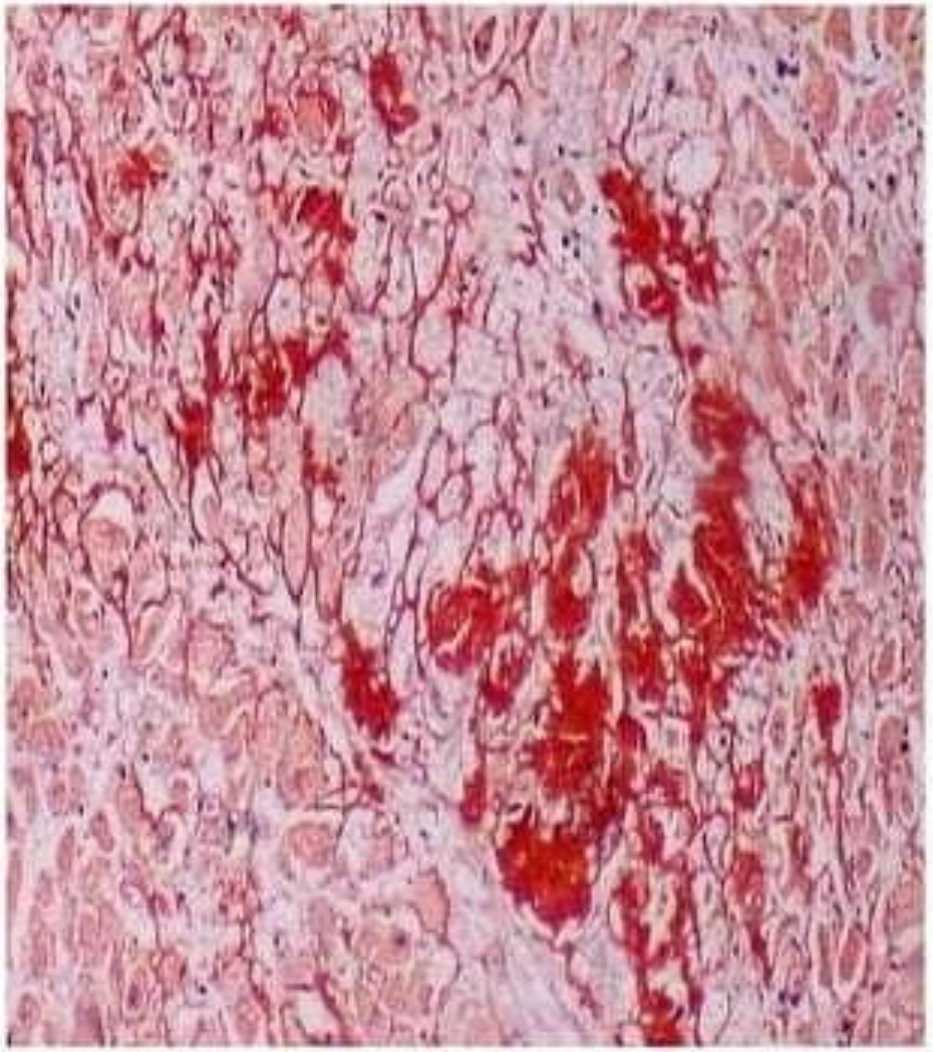


Staining chararcteristics of Amyloid

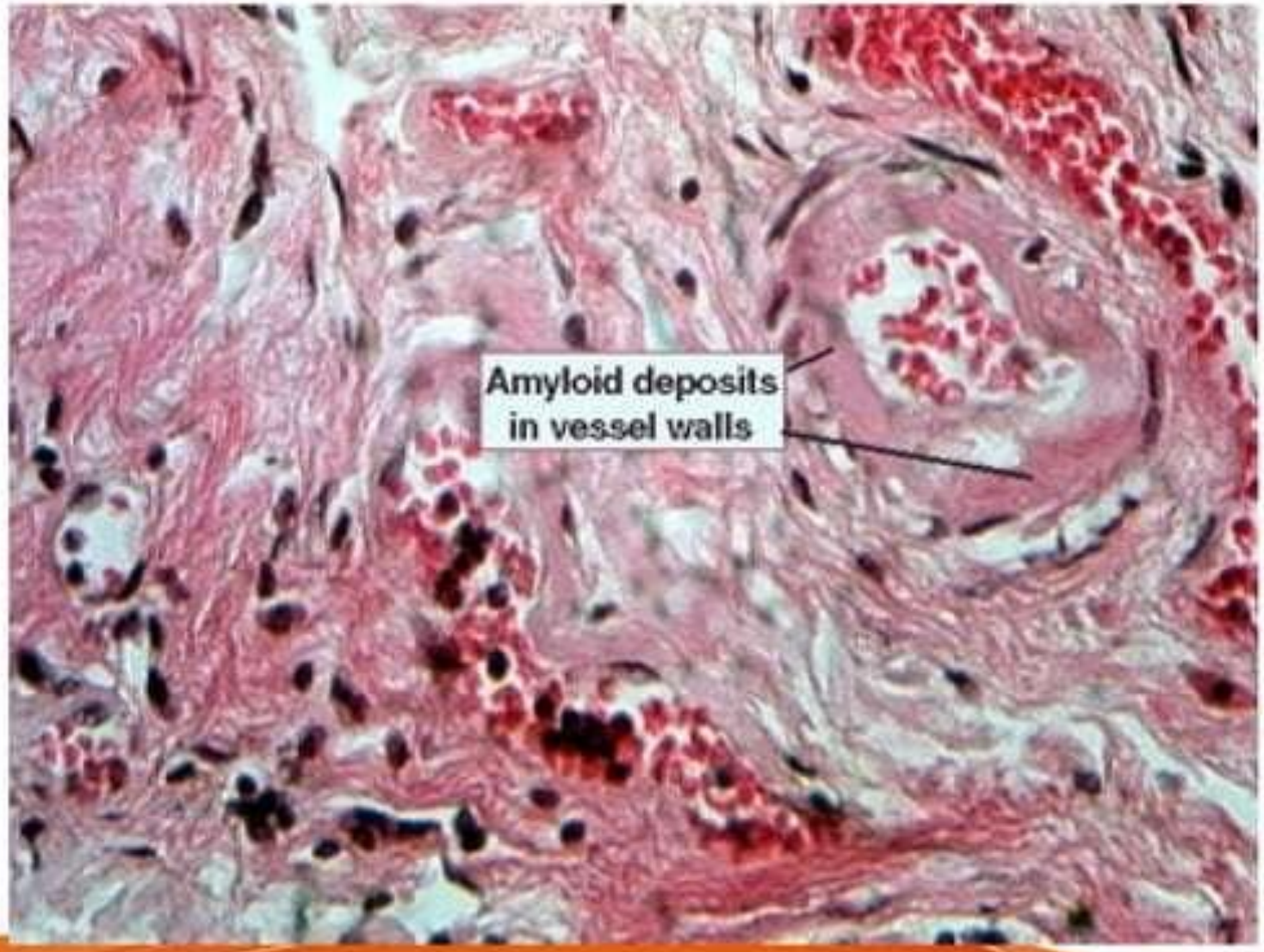
1. **Stain on Gross-** oldest method used by Virchow on cut section of gross specimen is **Lugols Iodine** which imparts **mahogany brown** colour to the amyloid deposit which on addition of sulfuric acid turns **blue.**

2. In routine histological sections **(hematoxylin and eosin stains)** amyloid appears amorphous, eosinophilic, hyaline, extracellular substance.

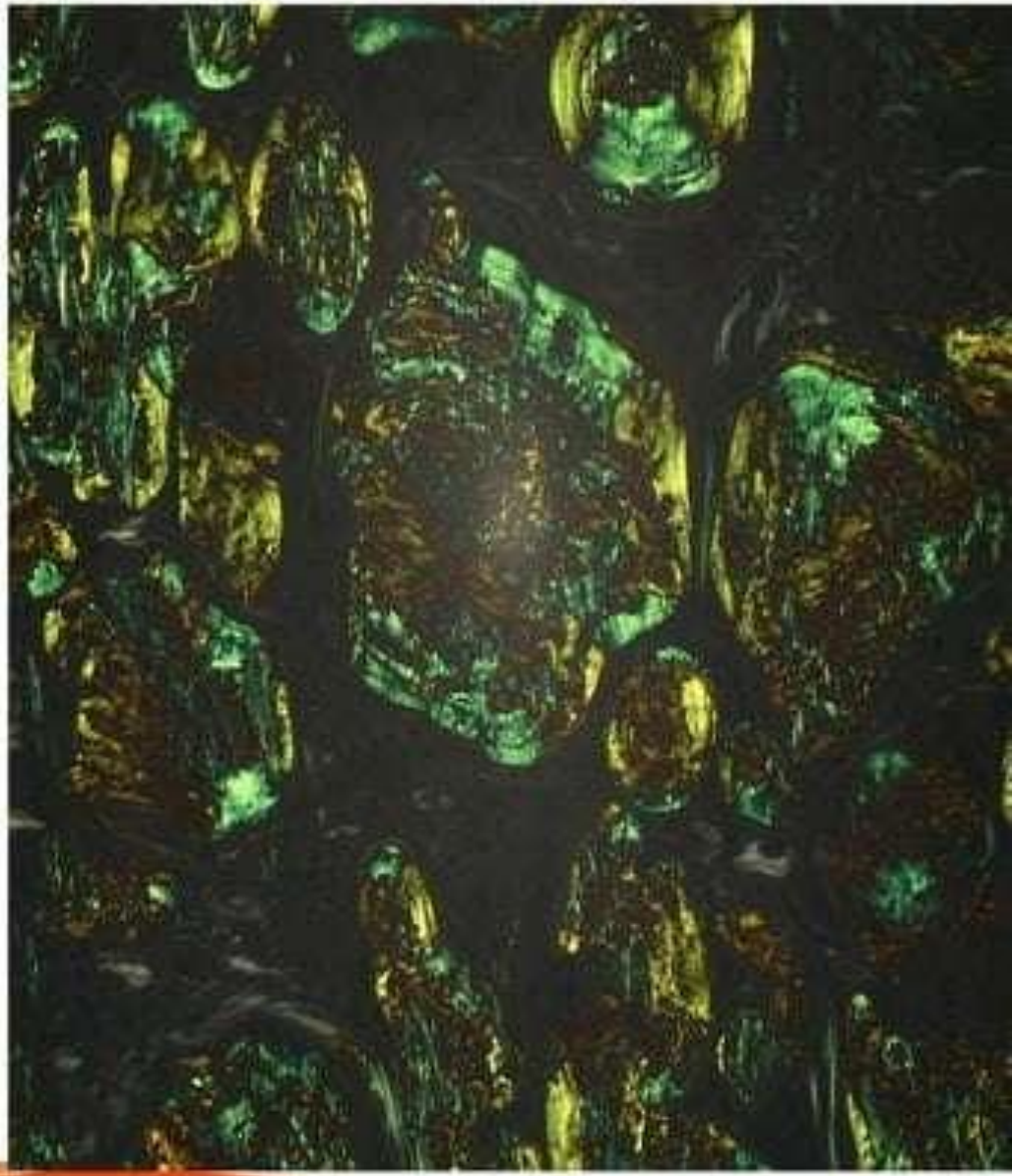
- However all proteins are stained pink by eosin and thus this stain is not specific.



- 3.All amyloids stain pink-red with the **Congo Red** stain.

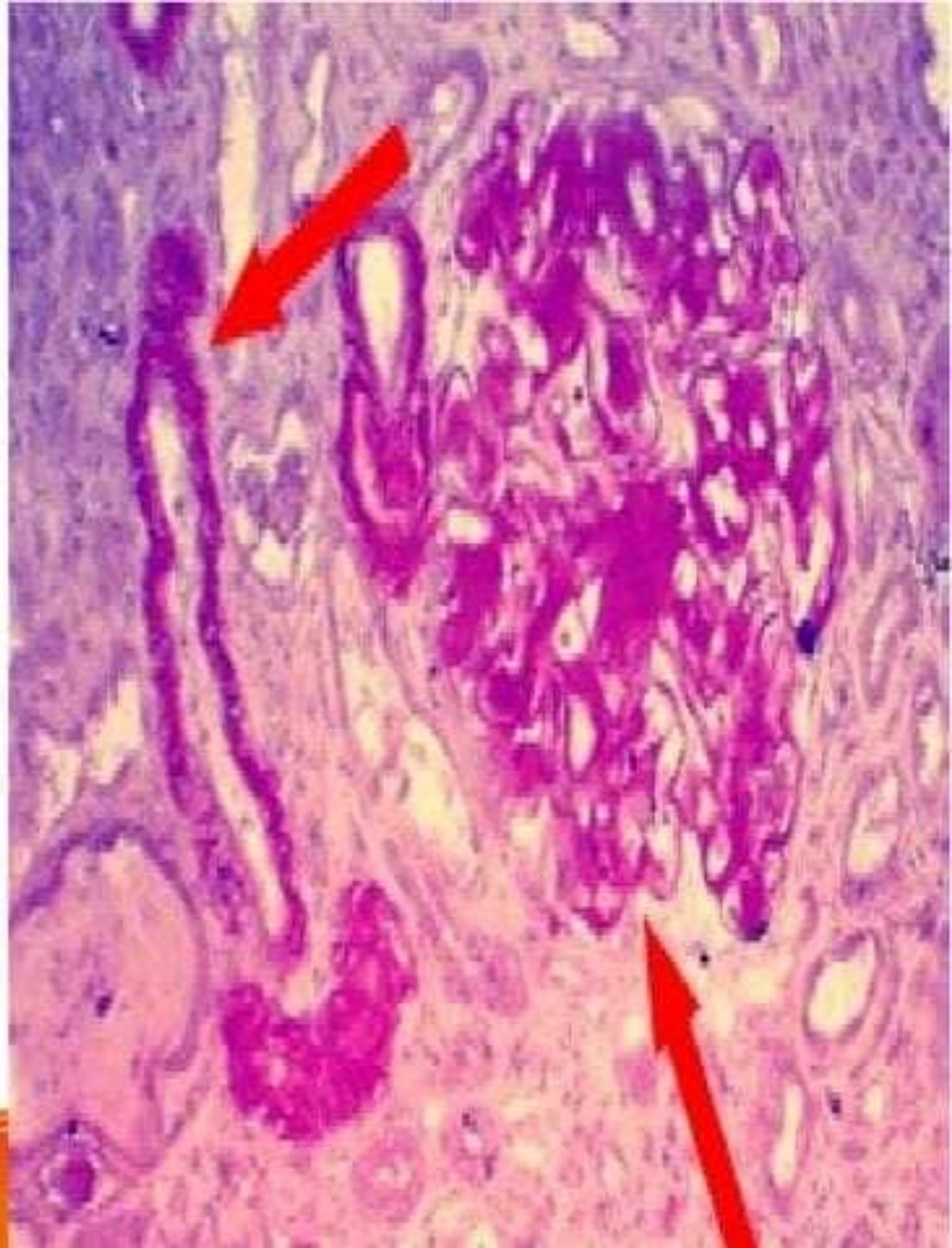


But when these sections are viewed with *polarized light* they exhibit a *apple green birefrigence*. This feature of amyloid can be used to identify it in tissue sections.



- **Loss of Congo Red staining** caused by **pretreatment** of the tissue **with potassium permanganate** is a useful tool for the diagnosis of amyloidosis AA.

4. **Metachromatic stains(**rosaniline dyes) i.e dye reacts with amyloid and undergoes color change-**methyl voilet/crystal voilet** imparts rose pink color.



5.Flourescent stains-

Thioflavin-T/Thioflavin S- on ultravoilet light imparts **yellow flourescence.**

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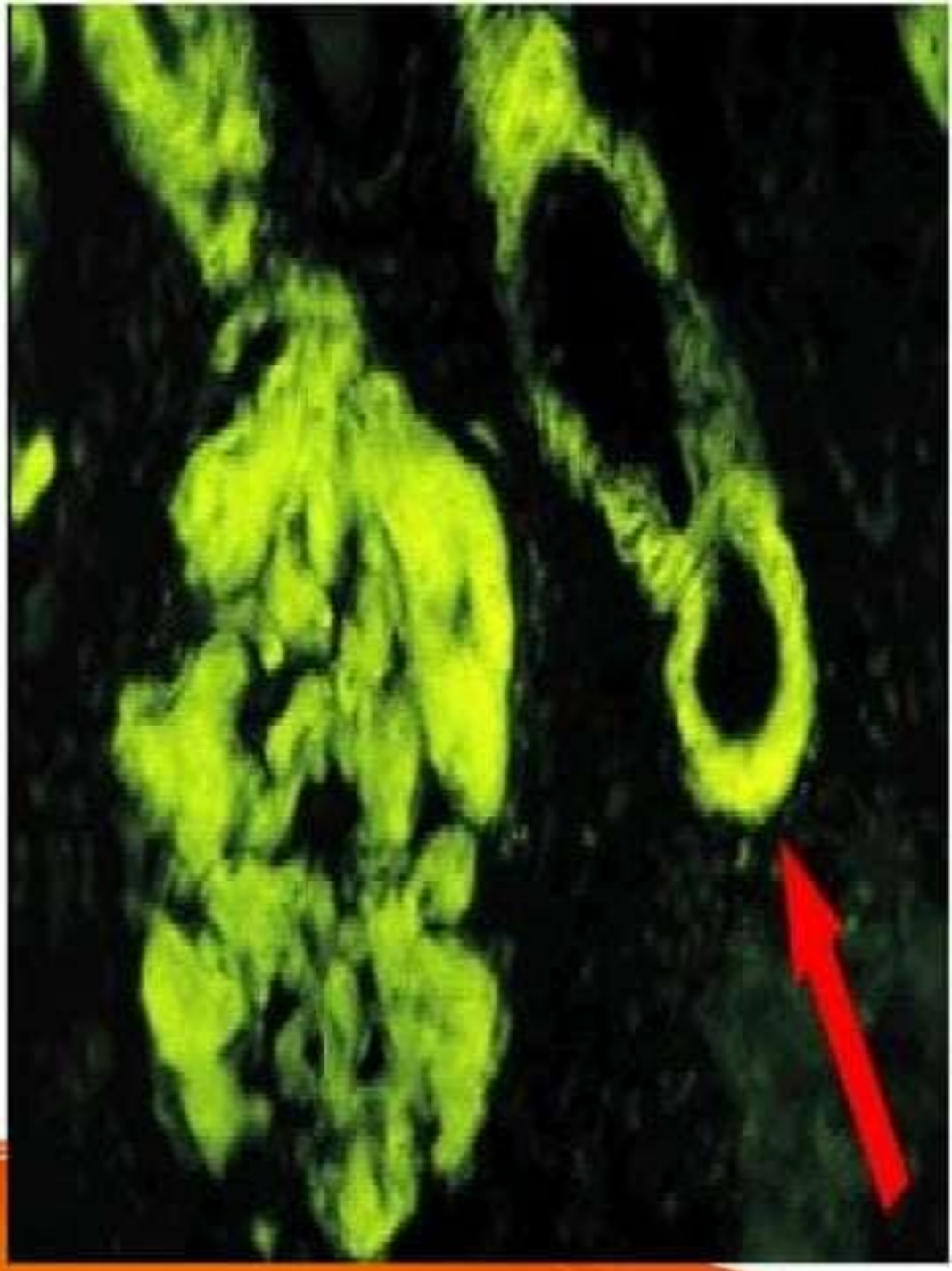
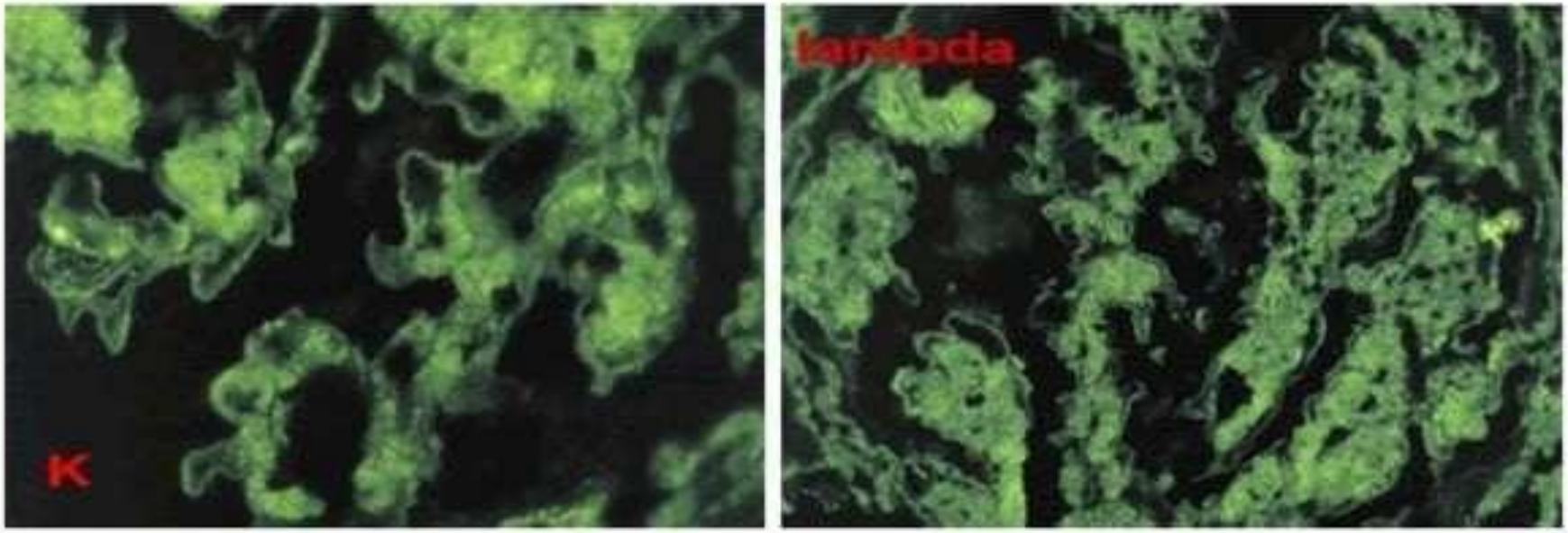


- **6.Immunohistochemistry** stains-positive with anti-AA stain



DIAGNOSIS

- **Presence of amyloid:**

- Evaluation of organ involvement (In-vivo tests & Imaging)
- Tissue Biopsy and its histology
- Congo red staining

- **Type of amyloid:** Immunohistochemistry.
- **Mutation type:** amino acid sequence analysis.

INDICATIONS FOR TESTS

- Nephrotic range proteinuria with or without renal insufficiency
- Unexplained kidney failure
- Non-dilated cardiomyopathy
- Peripheral or autonomic neuropathy
- Hepatomegaly or splenomegaly
- Malabsorption

Particular vigilance should be maintained in patients with multiple myeloma.

IN VIVO CONGO RED TEST

Intravenous injection of Congo red dye of a known quantity

↓

Dye gets bound to the amyloid deposits

↓

Serum levels of the dye are decreased.

Disadvantage : risk of anaphylaxis

Imaging techniques

- **Technetium scan**: Tc 99m pyrophosphate binds avidly to many types of amyloid.

-strongly positive in pt's with severe disease.

- **Technetium labeled aprotinin** more sensitive.
- **Quantitative scintigraphy**

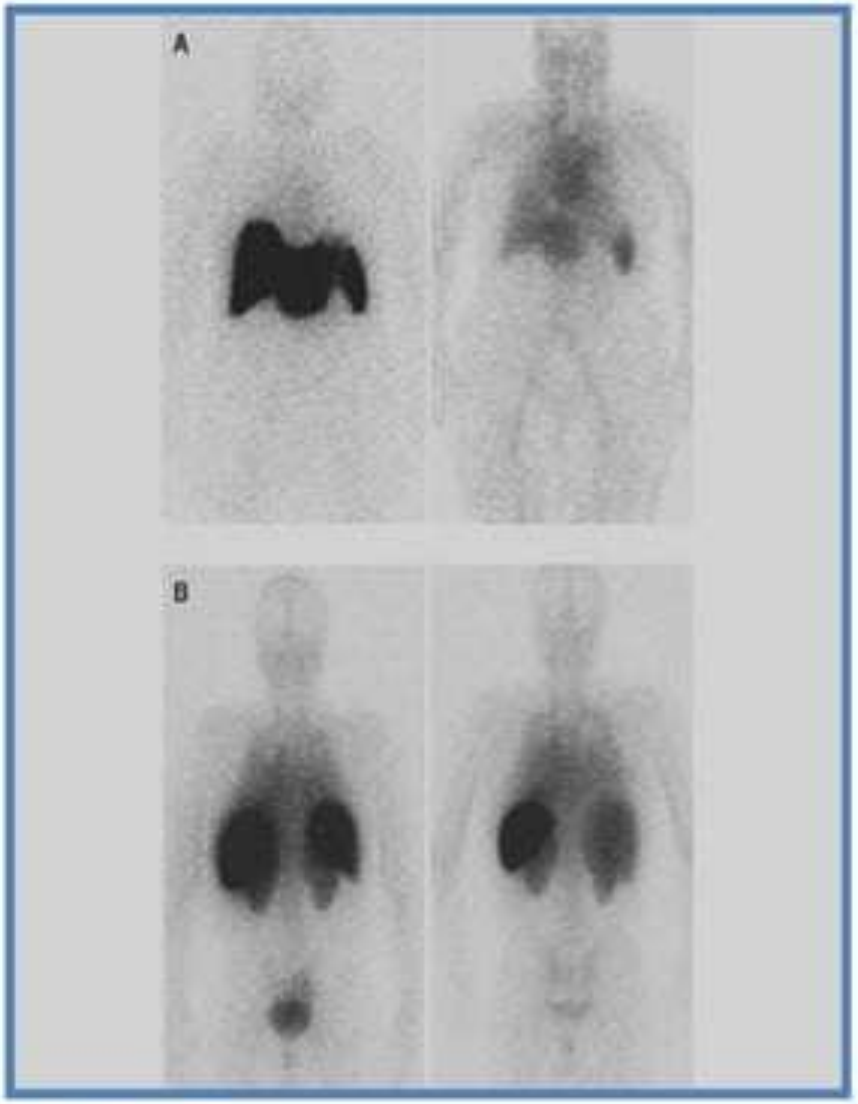
-Done with iodine-123- labeled serum amyloid P component (sensitive for AL, ATTR and AA)

- Cardiovascular magnetic resonance imaging (CMR)
- **Research:**
  - Magnetic Resonance Microimaging
  - Near infrared imaging using an oxazine-derivative probe

- **Scintigraphy with radiolabeled serum amyloid(SAP-SERUM AMYLOID P component) -**component is rapid & specific test since SAP binds to amyloid, it gives measure of extent of amyloidosis.

SAP SCANNING

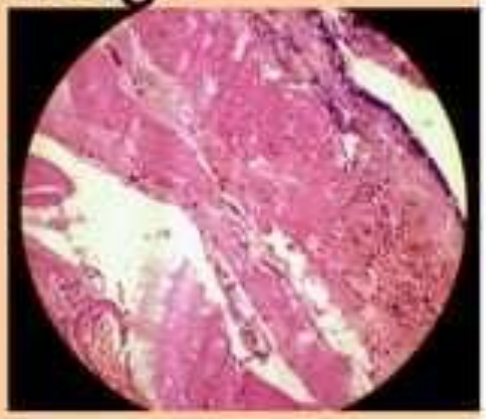
- Iodine-123-labelled SAP injected.
- Visualize the scintigraphy
- 24hr whole-body retention of I-123 is visualised.
- SAP is higher in pts with Amyloidosis
- Distribution of organ involvement is seen.



TISSUE DIAGNOSIS

Tissues for biopsy

Subcutaneous fat aspiration (provides enough material for all investigations) – 60%



Rectal biopsy

Gums

Bone marrow

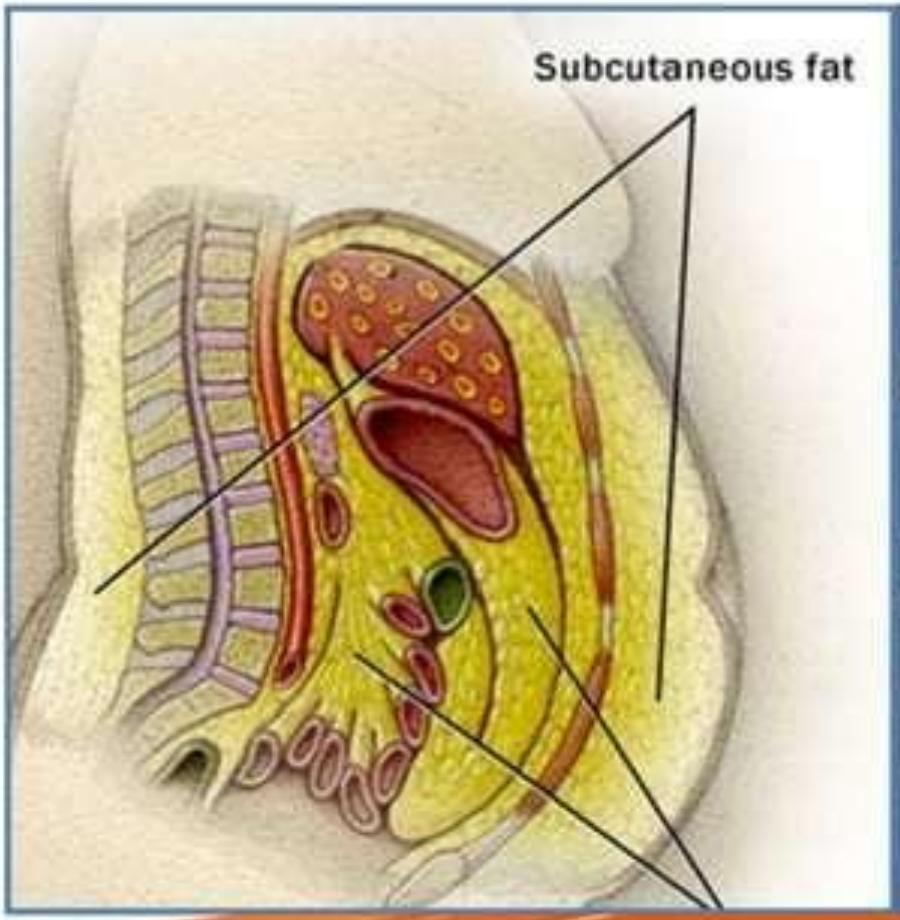
Others: kidneys, nerves, heart, liver

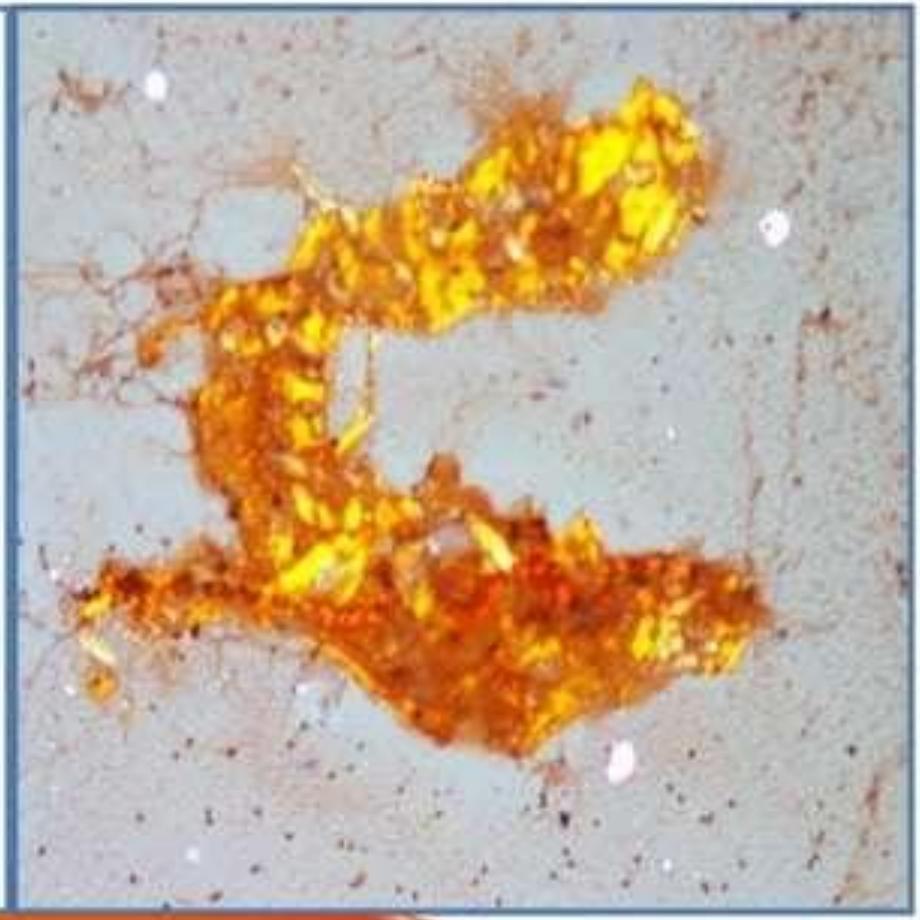
Organ biopsy: if subcutaneous fat investigation did not provide diagnosis

Kidney biopsy to determine the cause of nephrotic syndrome (informativity is 100%)

ABDOMINAL FAT ASPIRATION

- Technique used : FNAC
- (fine needle aspiration cytology)





Morphological features of amyloidosis of organs

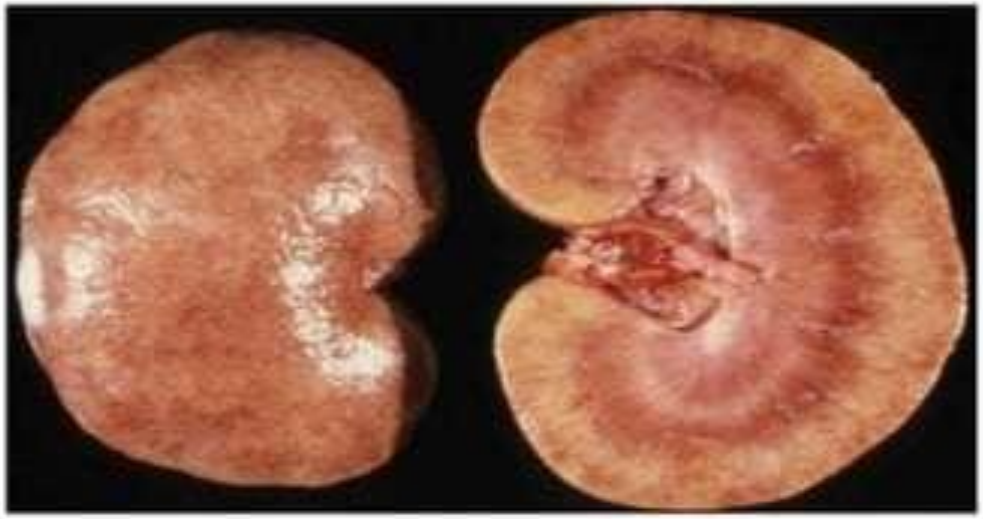
Amyloidosis of different organs show variation in morphologic patterns, ***general*** features are:

Grossly-affected organ is large, grey, waxy and rubbery(firm consistency).
Microscopically,deposits are always extracellular,begins between the cells close to the basement membrane and are amorphous,eosinophilic.

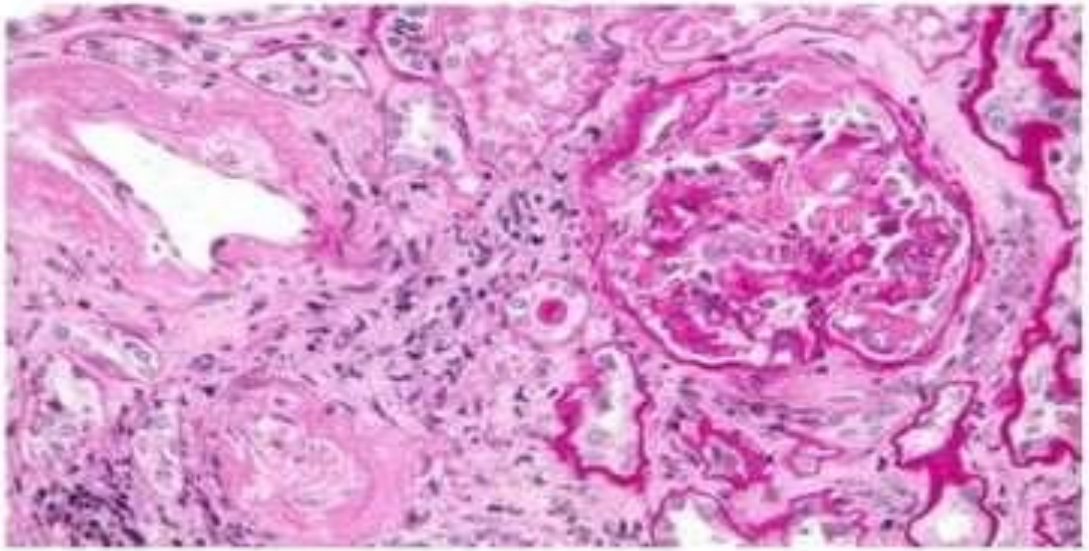
Amyloidosis of KIDNEY

- Most common and serious form.
- ***Grossly***,kidneys may be normal-sized, enlarged or shrunken in advance cases because of ischemia.
- **C/S** is pale, waxy,translucent.
- ***Microscopically,*** amyloid deposit primarily in glomeruli, but arteries, arterioles and peritubular tissues are also affected.

Macroscopic



Microscopic



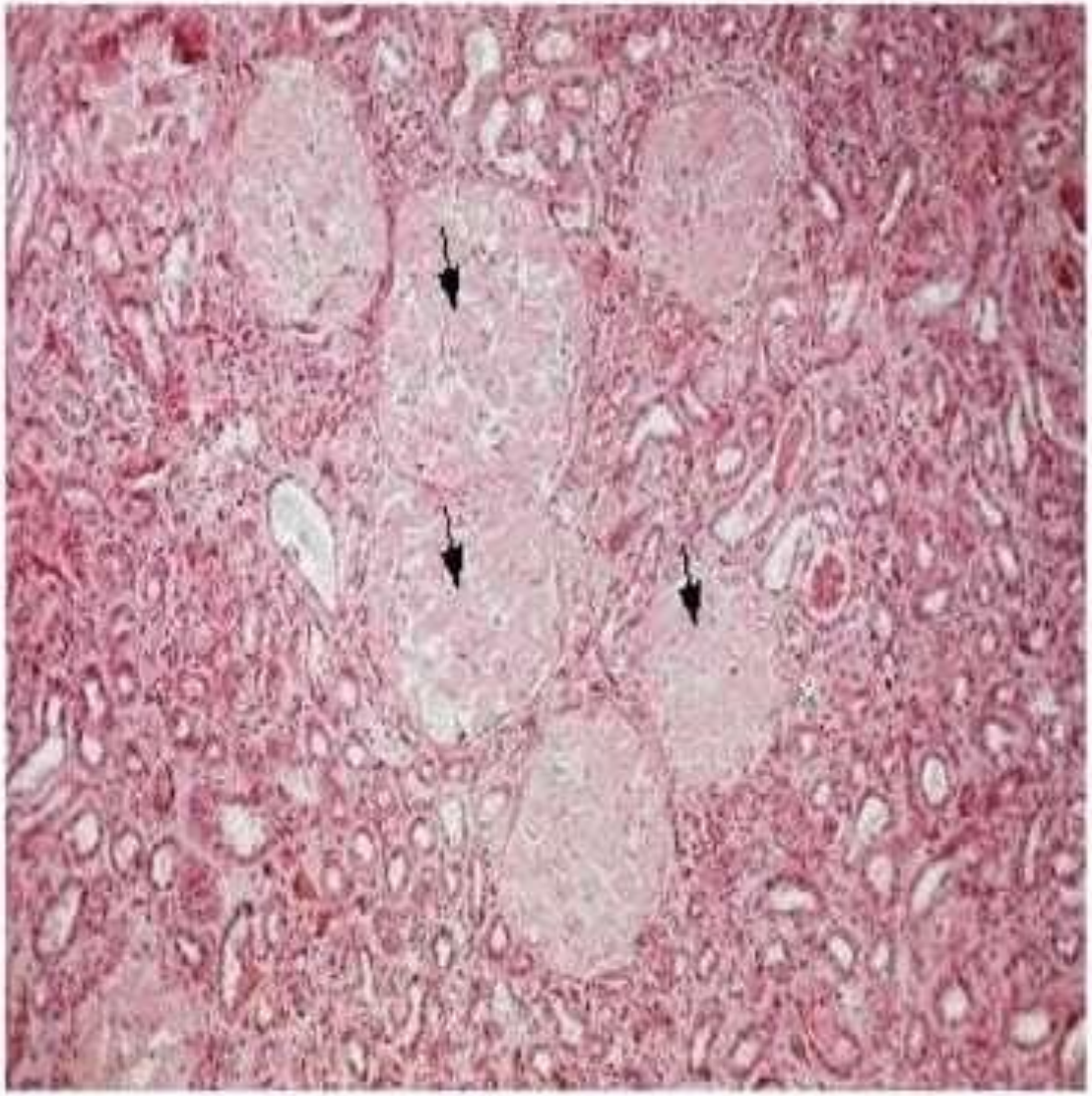
Glomeruli:

-Begins in mesangium extending into capillary walls

glomerulus is flooded by confluent masses or

interlacing ribbons of amyloid.

- Homogenous amorphous eosinophilic deposits: H&E

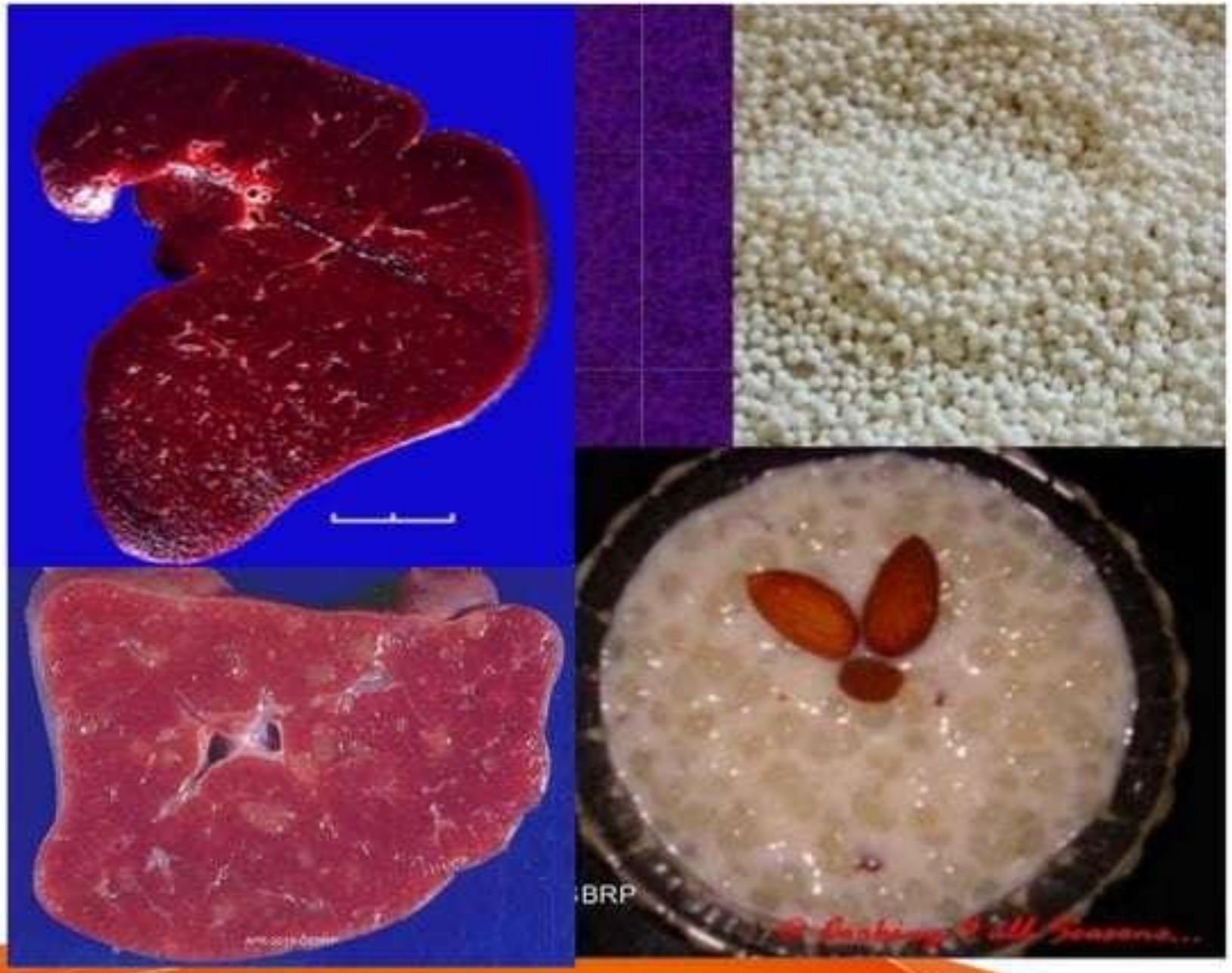


Histology of the kidney in amyloidosis. Deposits are present in the vessels of the renal glomeruli.

Amyloidosis of spleen

- Amyloidosis of the spleen has **two** different anatomical patterns.

- Most commonly, the amyloid deposition is limited to the splenic follicles, resulting in the gross appearance of a moderately enlarged spleen dotted with gray nodules (so called "**sago" spleen).** .


BRP

Alternatively, the amyloid deposits may spare the follicles and mainly infiltrate the **red pulp sinuses**, producing a large, firm spleen mottled with waxy discolorations showing map like areas ("lardaceous" spleen)

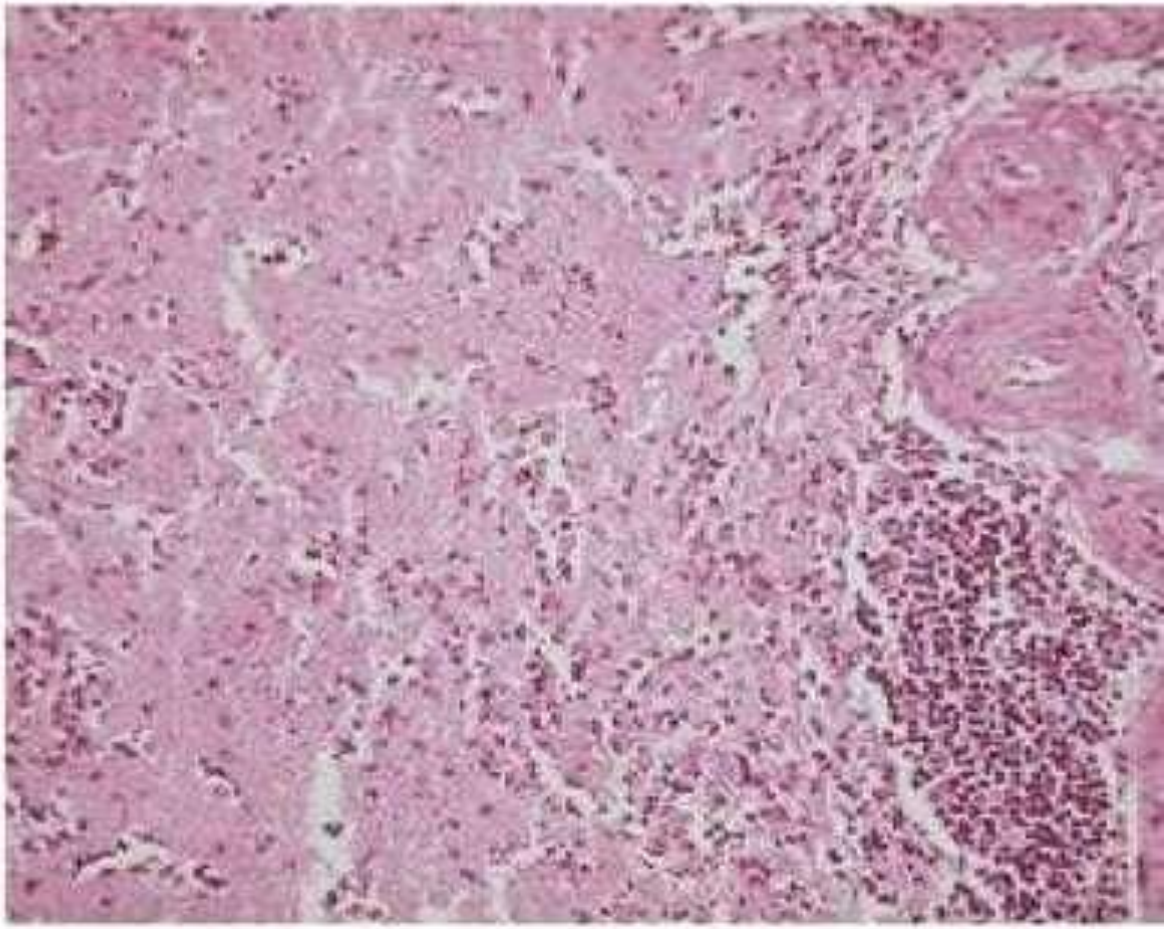
Lardaceous spleen

- fat from the abdomen of a pig, especially as prepared for use in cooking

Microscopicaly

-deposits involve the red pulp in the wall of splenic sinuses,

small arteries and connective tissue.



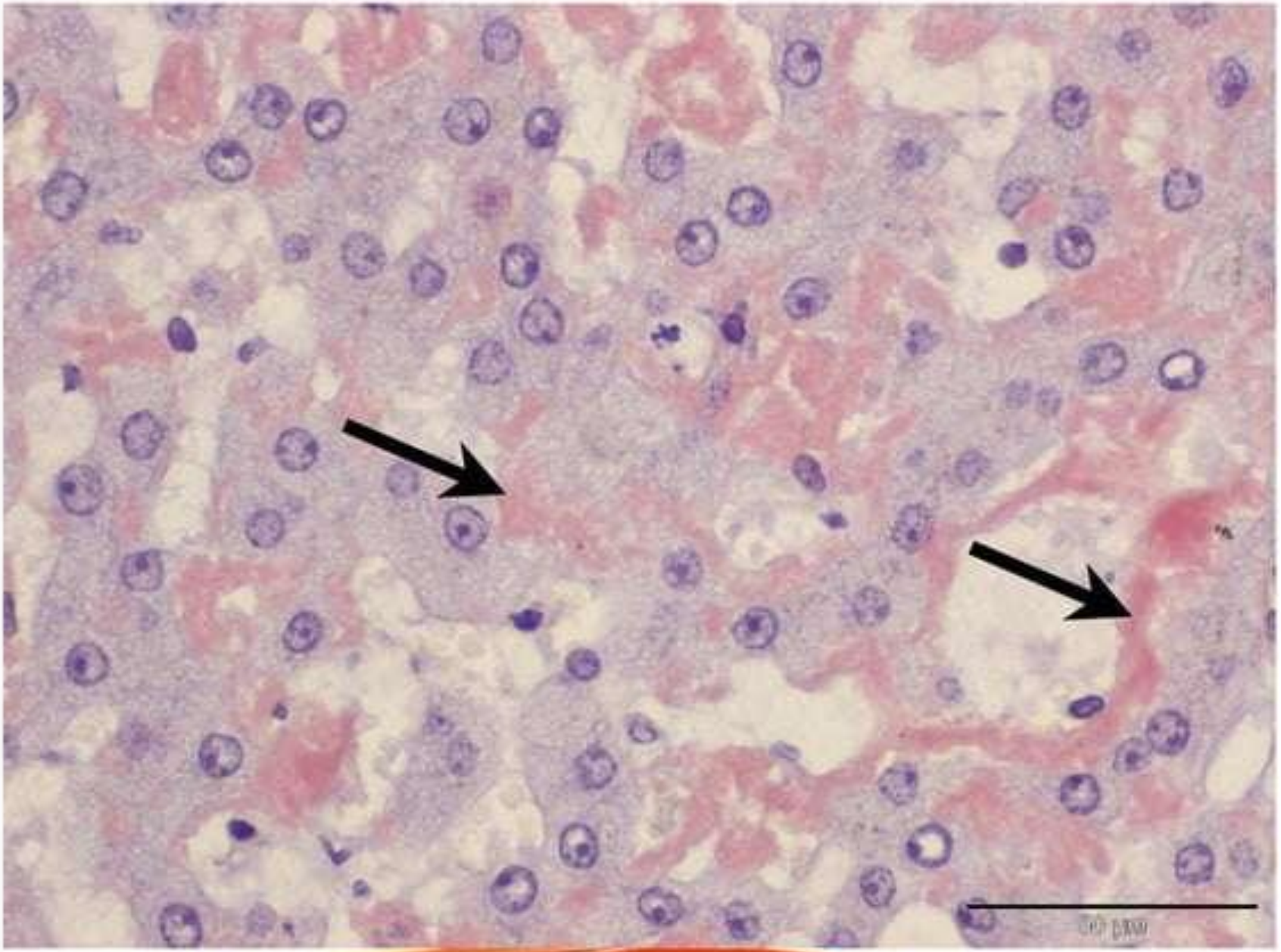
Deposition of amyloid in the white pulp of the spleen. The large pink area on the left side of the image consists of amyloid.

Amyloidosis of liver

Grossly, liver is enlarged, pale, waxy & firm.

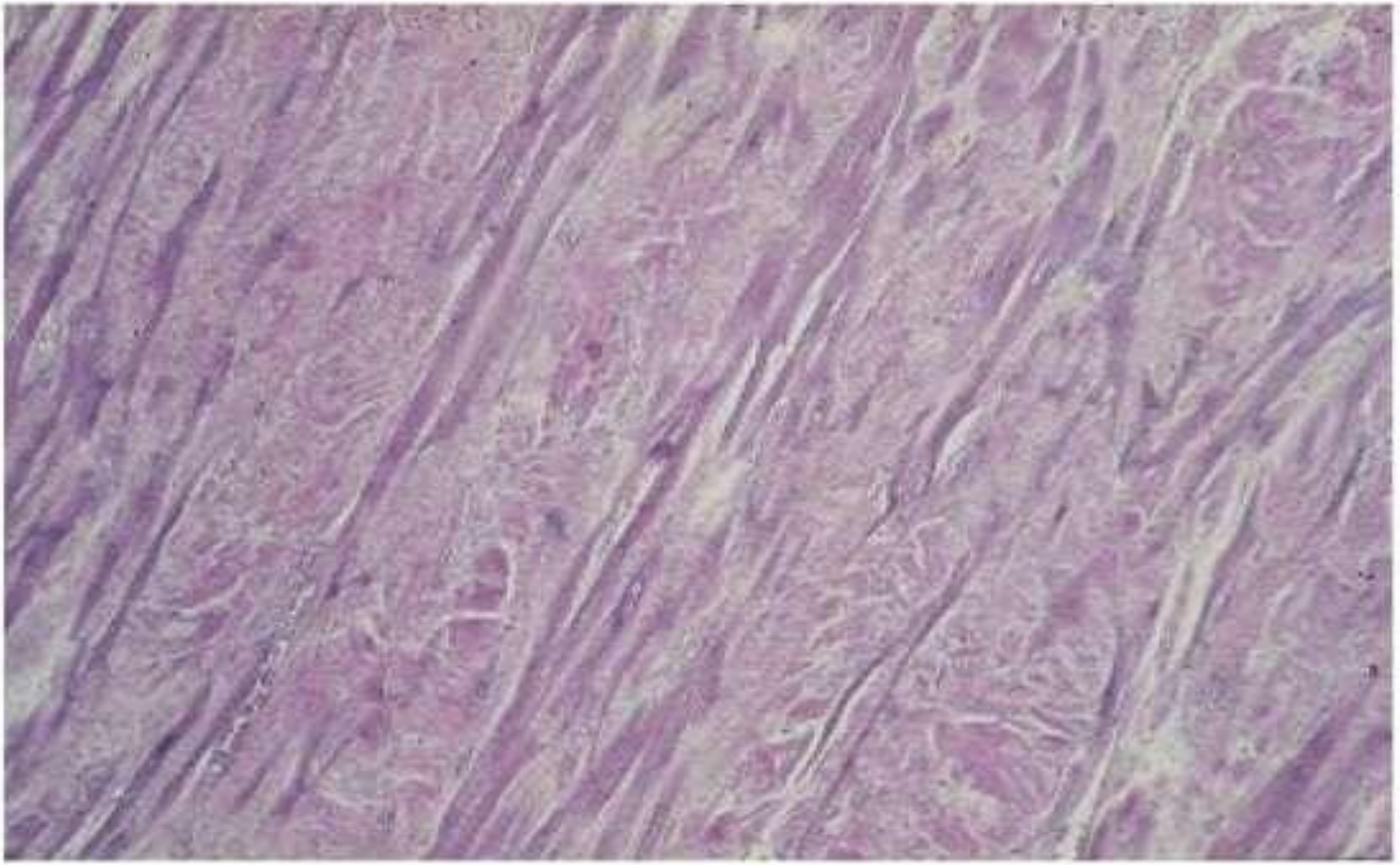
Microscopically-

- Amyloid initially appears in space of disse(space b/w the hepatocytes & sinusoidal endothelial cells).
- Later,disappearence of hepatocytes occur due to pressure atrophy.
- Vascular involvement & deposits in kupffer cells are frequent.



Amyloidosis of heart

- It may occur in any form of systemic amyloidosis.
- ***Grossly***,heart is enlarged and firm.
- Epi/endocardium and valves show tiny nodular deposits.
- ***Microscopically-****focal* subendocardial accumulations,in primary form,deposits are seen around myocardial fibres in ring forms also known as **ring fibres**,
- In localized,deposits seen in left atrium.



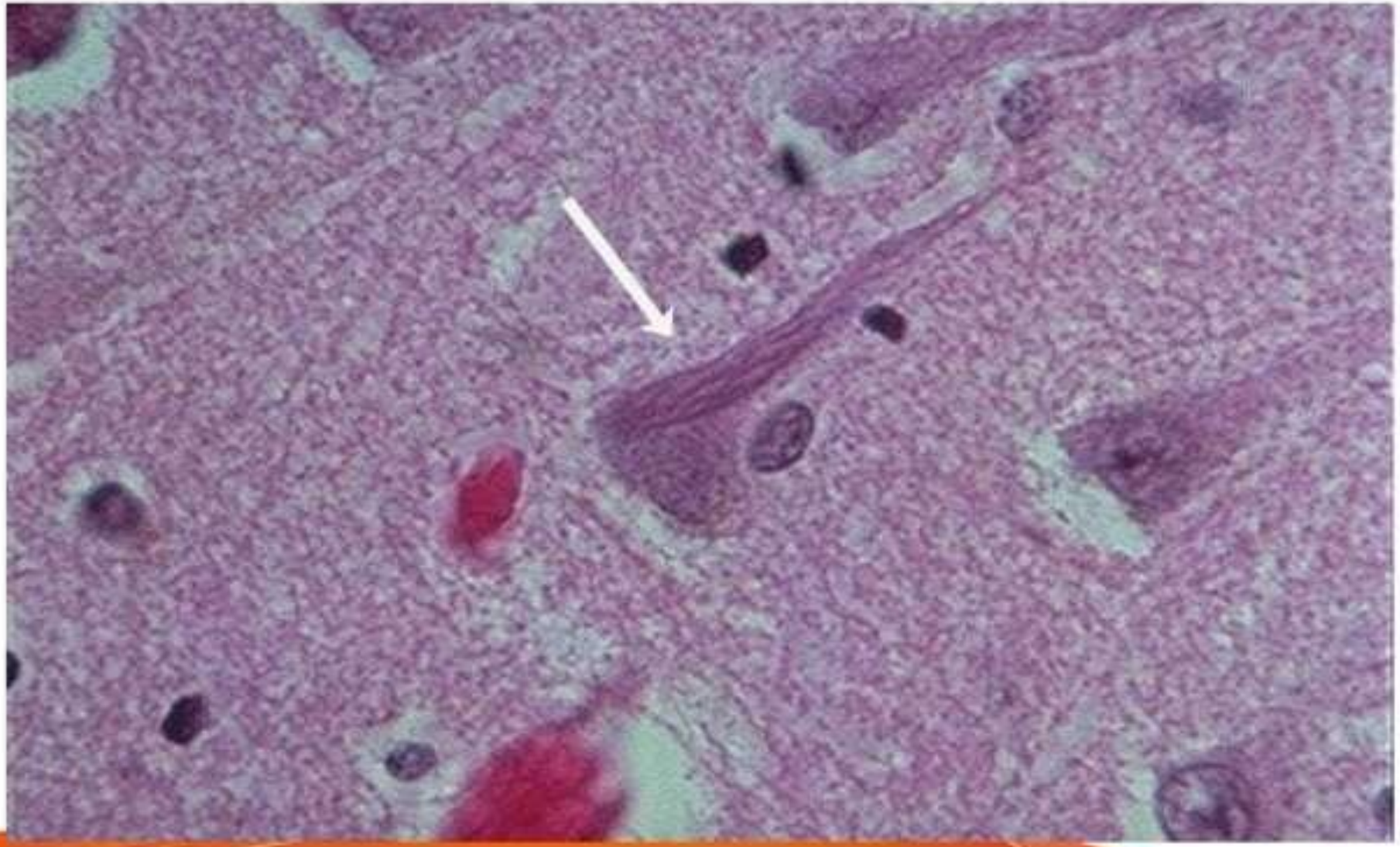
Heart histology (hematoxylin and eosin stain). The homogeneous pink material deposited between the atrophic cardiac muscle fibers is amyloid.

Brain

Alzheimers disease.

- AD and many other neurodegenerative disorders belong to the family of protein misfolding diseases, characterized by protein self-aggregation and deposition.
- In vivo detection of amyloid plaques & neurofibrilary tangles in the brain enables early identification of AD.
- **Molecular PET imaging** using beta-sheet binding agents has the potential to be extended to these wide spectrums of protein misfolding diseases.

Neurofibrilary Tangles-Alzheimers disease



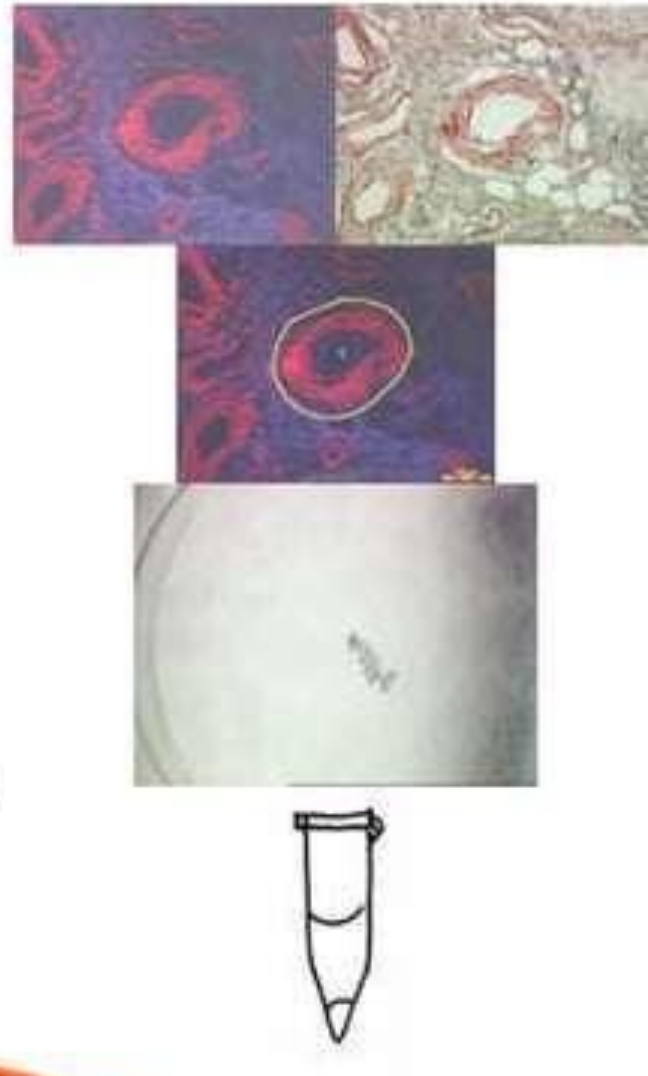
Other organs

- Alimentary tract-may occur at any level from oral cavity to anus, deposits initially in vessel wall and then adjacent layers of bowel wall.In tongue can cause macroglossia.
- Respiratory tract- involved focally or diffuse from larynx to bronchioles.

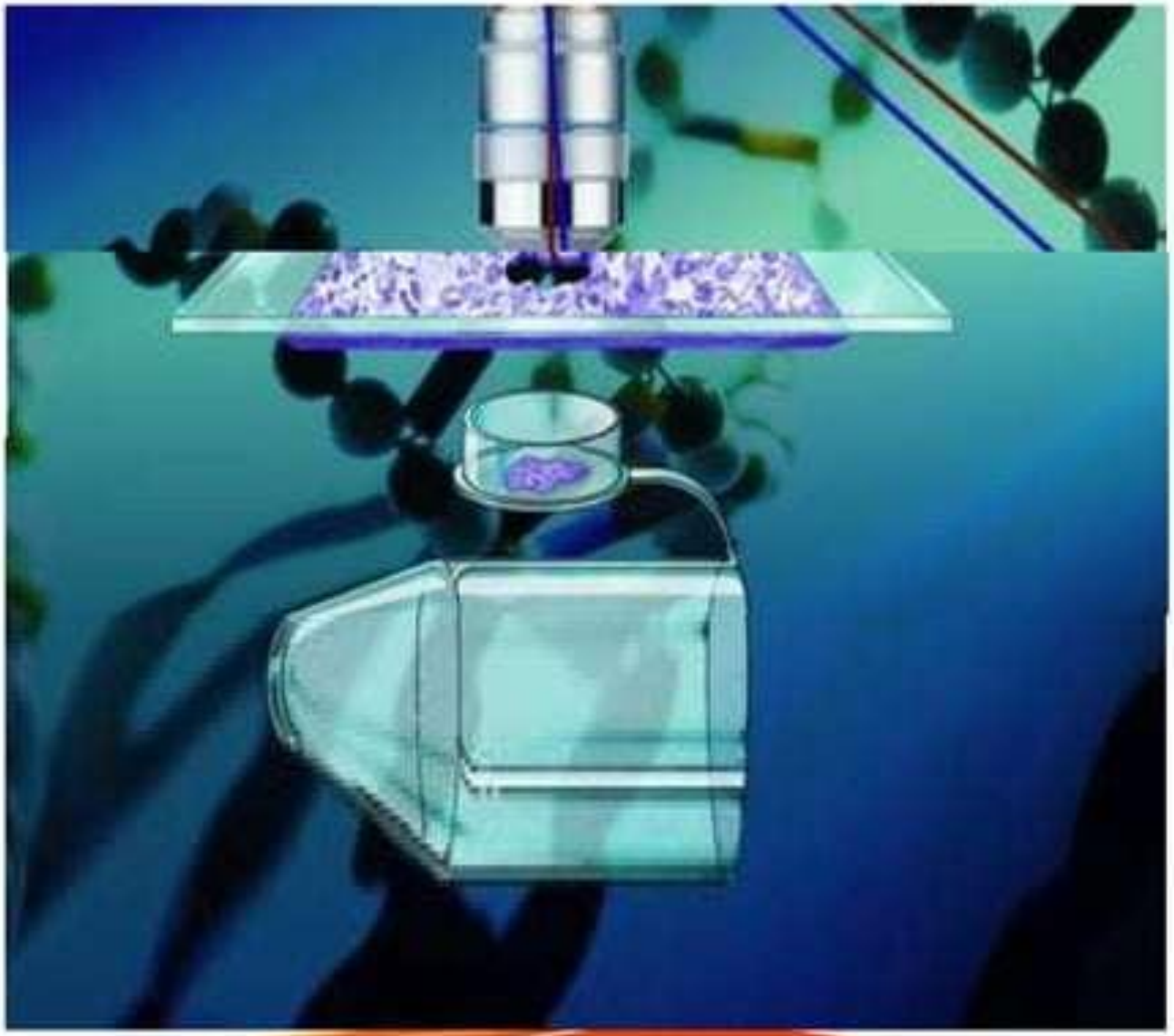
LASER Micro-dissection system

Histological examination of tissue section identifying amyloid deposits

- Identify amyloid deposit
- Laser cut around amyloid deposit
- Tissue drops into microfuge cap
- Tissue is fragmented into peptide strands and electrophoresed; nature of protein studied.



Laser Micro-dissection equipment



Instrumental methods

- **Ultrasonography**: kidney's 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

√ Most useful in AA amyloidosis because the major sites of deposition are accessible to the imaging agent

Beta-2-microglobulin

- **Carpal tunnel syndrome** – most common (deposits in hands ligaments compress the nerves).
- **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.

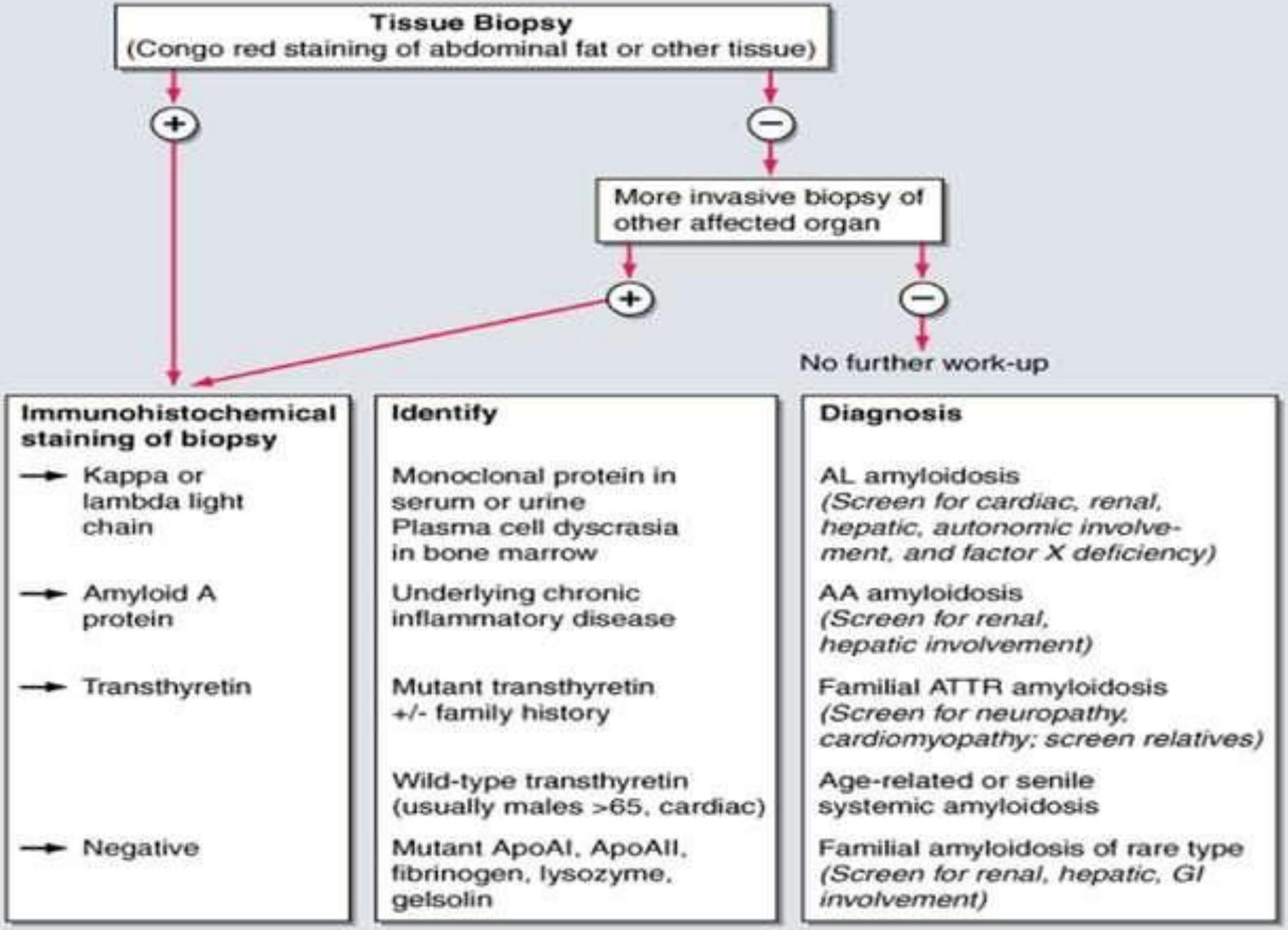
OTHER TESTS

- **AL-Diagnosis**-protein electrophoresis immunoelectrophoresis of serum and urine.
- bone marrow aspiration.
- Serum immunoglobulins (to exclude AL)
- **In AA amyloidosis** : polyclonal hypergammaglobulinemia
- IL-1,6 levels
- **SAA** as an exquisitely sensitive acute phase protein (more sensitive than CRP)
- **Immunohistochemistry**- to know the type of amyloid.

  Example-anti-AA stain.

APPROACH TO AMYLOIDOSIS

Clinical Suspicion of Amyloidosis



PROGNOSIS

- Prognosis with generalized amyloidosis is poor.
- those with AL amyloidosis have a median survival rate of 2 years after diagnosis.

KEY CONCEPTS

- Amyloidosis is a disorder characterized by extrecellular deposits of misfolded proteins that aggregates to form insoluble proteins.

- 3 factors causing misfolding-

a)normal proteins-excessive production

b)mutant proteins

c)defective proteolytic degradation

- **Characteristical features**

√Fibrillar appearance(Electron microscopy)

√β pleated sheet structure(X-ray diffraction)

√Amorphous eosinophilic appearance(H & E)

√Apple green bifringence(Congo red staining)

- Amyloidosis may be systemic or localized.

  AL is the most common type in western countries and AA is the most common worldwide.

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thank you!