Diagnosis of Amyloidosis

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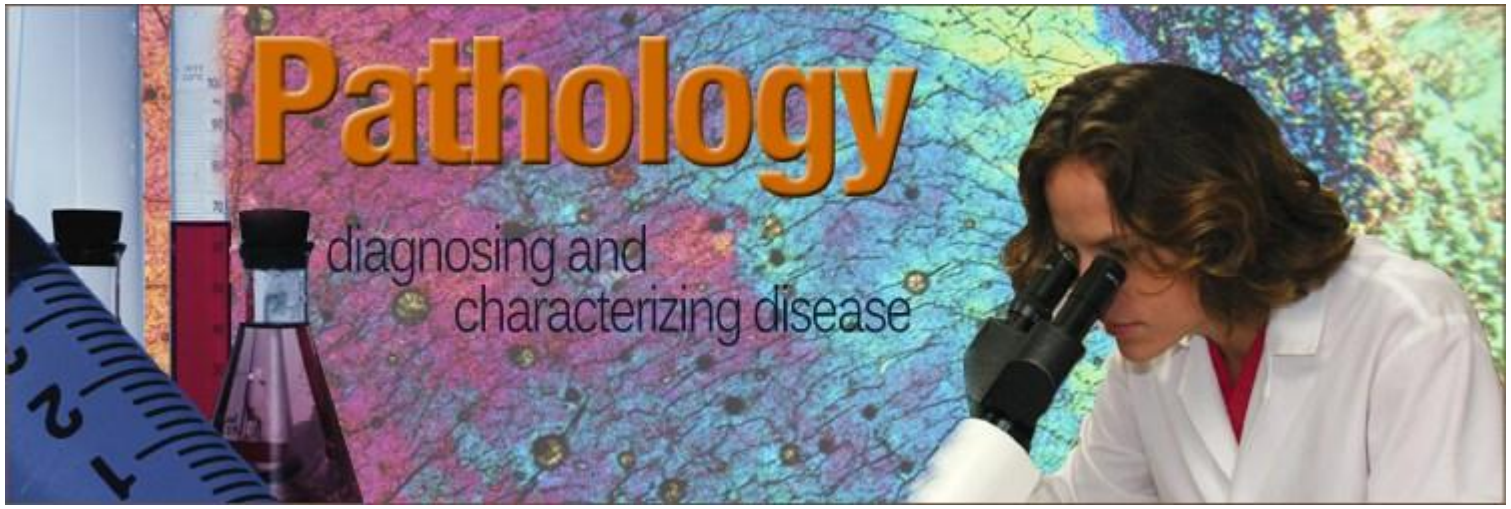
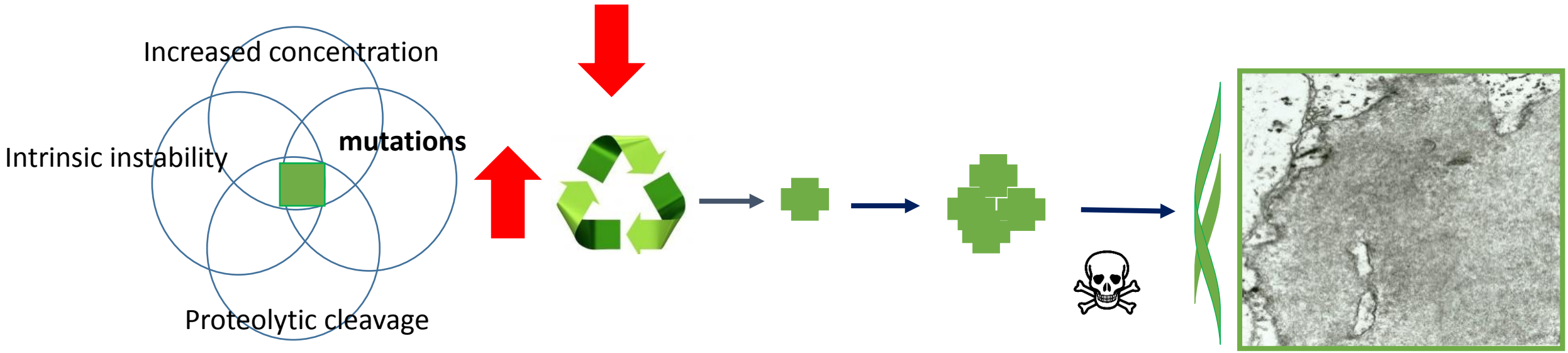


Outline
Diagnosis of amyloidosis
Fat pad
Other

Amyloidoses – protein folding disorders

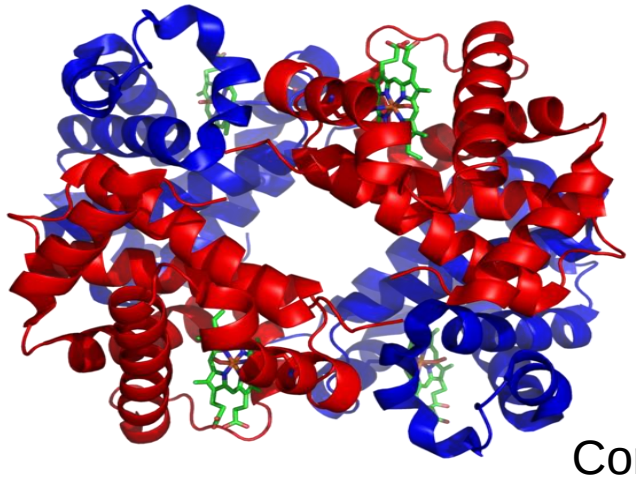
protein quality control systems:
intracellular (proteasomes)
extracellular (macrophages)



precursor protein
misfolded protein
protofibril
mature fibrils

Amyloidoses

α helix



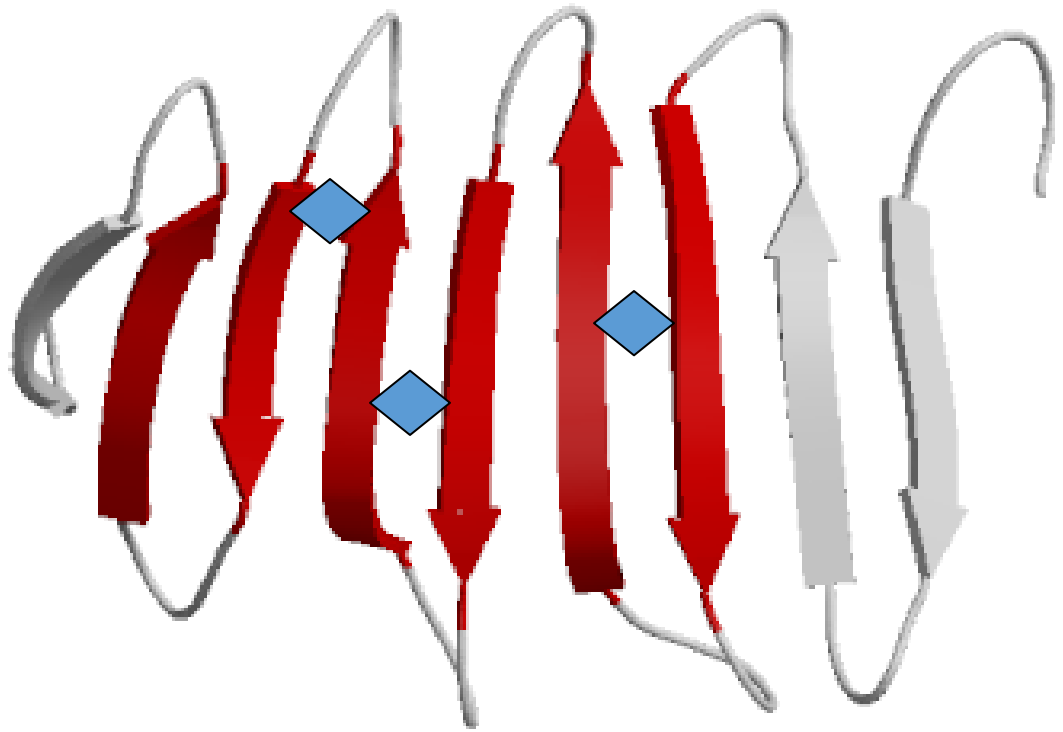
β pleated sheet

Amyloid formation

Conformational shift to
β-pleated sheet 2^0 structure

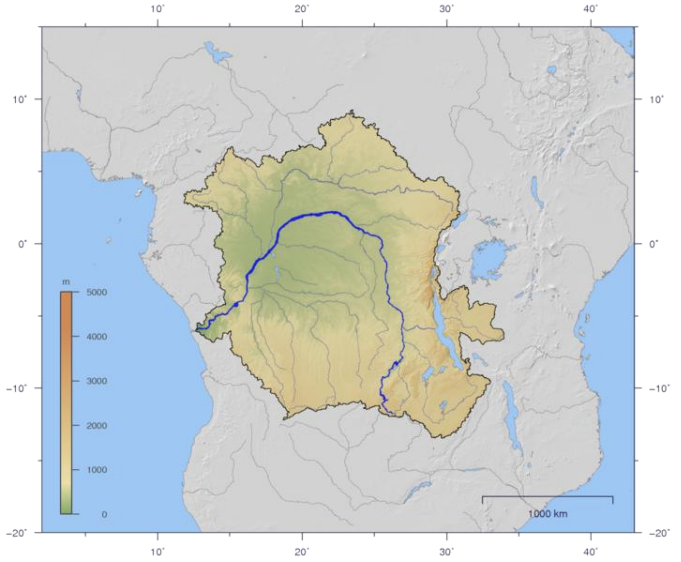
β-pleated sheet conformation confers affinity to Congo red
<u>common to ALL types of amyloid</u>

<u>Diagnosis of amyloid requires biopsy</u>



Congo red binding sites

Congo red = bright red color:



- synthesized in 1883 by Paul Bottiger, Friedrich Bayer Company, Germany

- textile dye

- patent sold to the AGFA company of Berlin

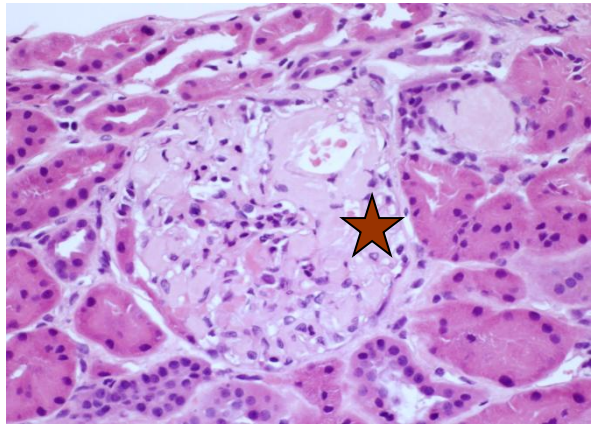
- AGFA marketed the dye under the name "Congo red"

1884 Berlin West Africa Conference

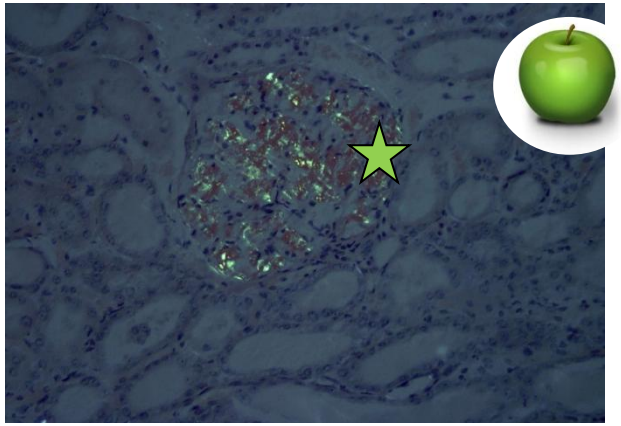
Diagnosis of amyloidosis

tissue diagnosis:
- biopsy of an affected organ
- “surrogate” site

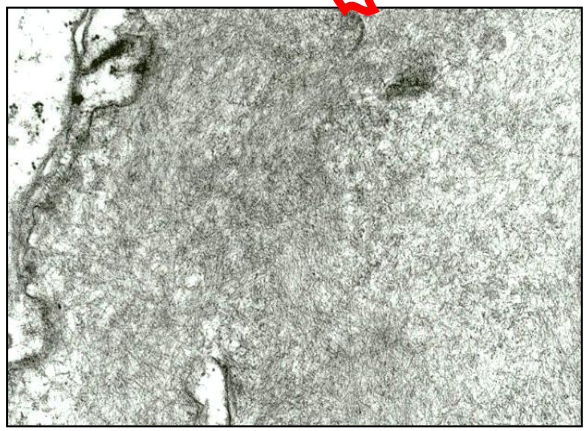
think – amyloid
order Congo red stain



Routine stain: extracellular “amorphous” deposits, not-specific for amyloid

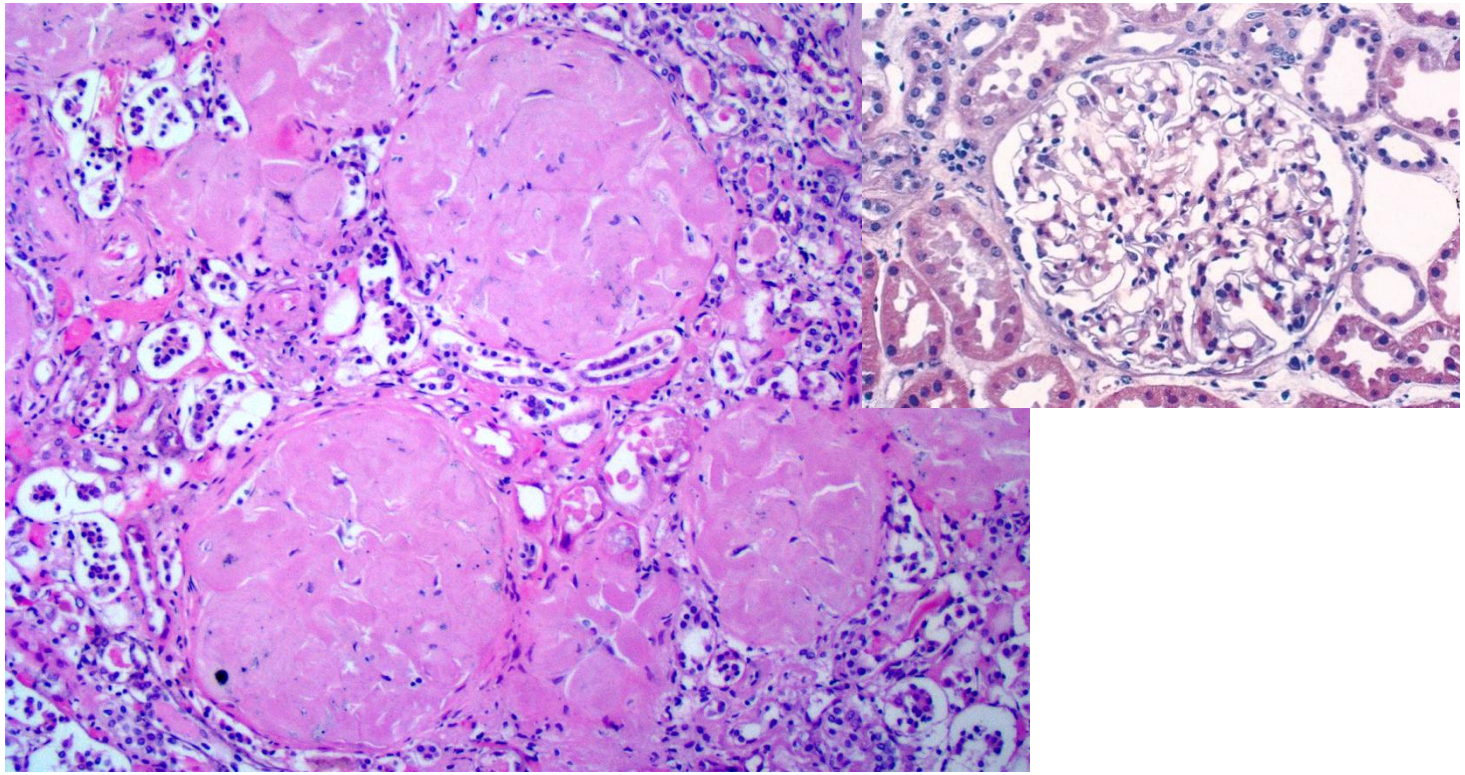


Need Congo red stain with green birefringence under polarized light [“apple green” birefringence] = diagnostic



Amyloid is fibrillary only by electron microscopy

Detection:



Late diagnosis (left); normal glomerulus on right

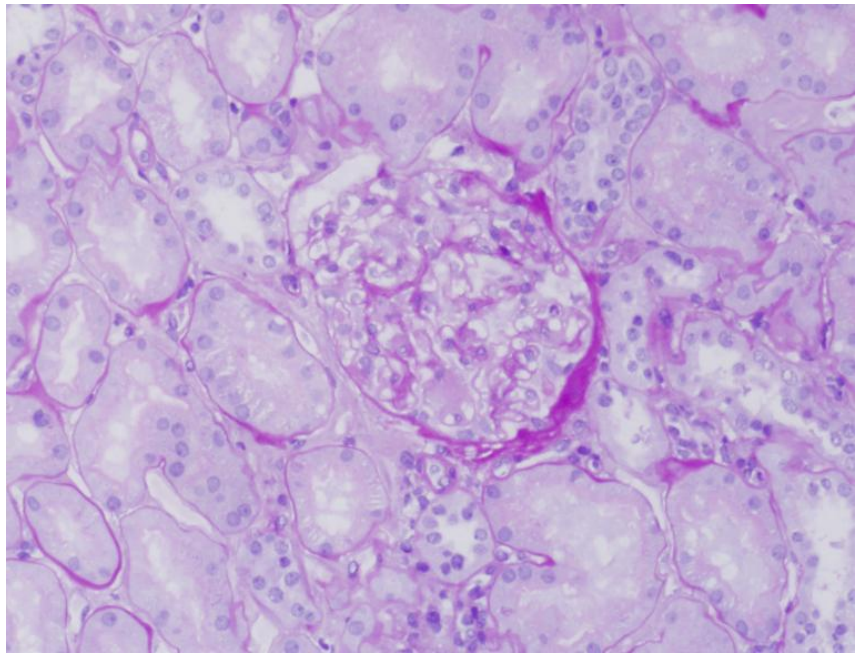
Systemic amyloidosis

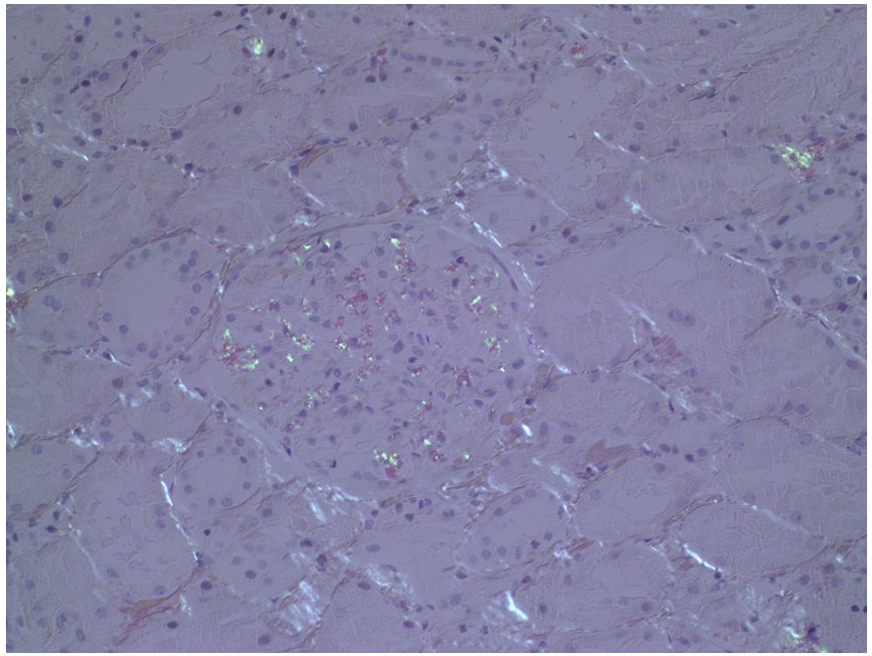
Pathology:

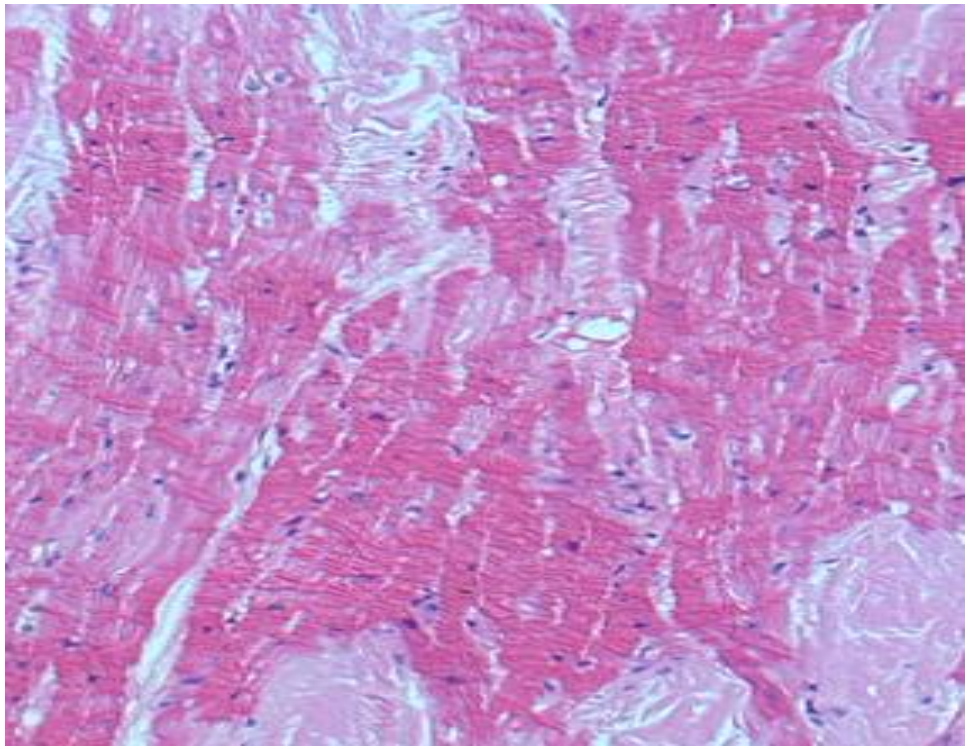
- kidney, cardiac, peripheral nerves, other sites

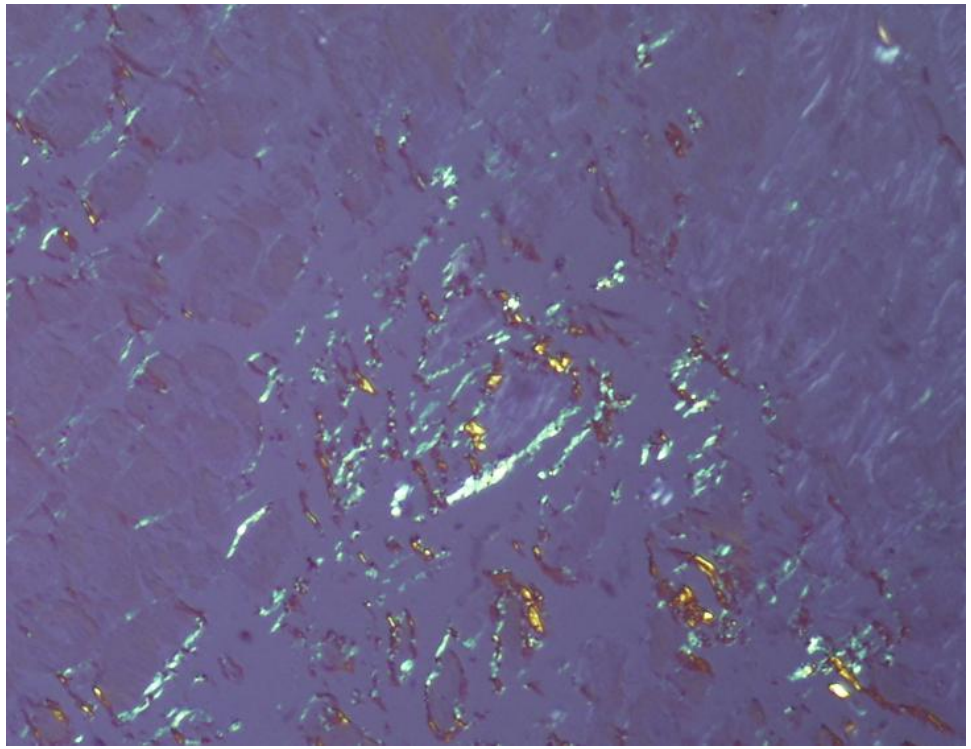
Early amyloidosis may be inconspicuous by routine stain

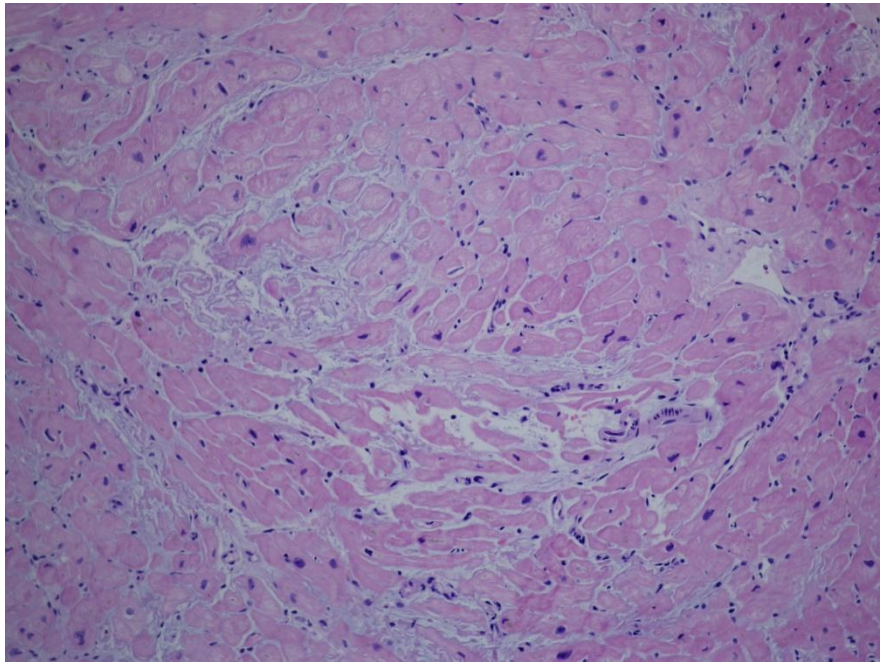
Congo red stain to *rule out* amyloid and not just to confirm suspicion of amyloid based on routine H&E stain

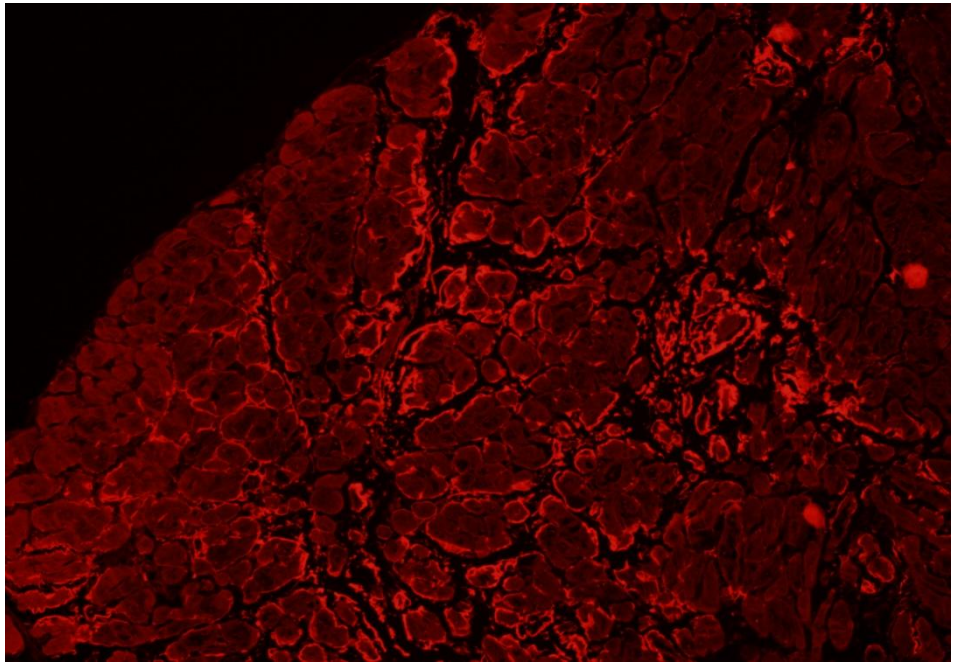


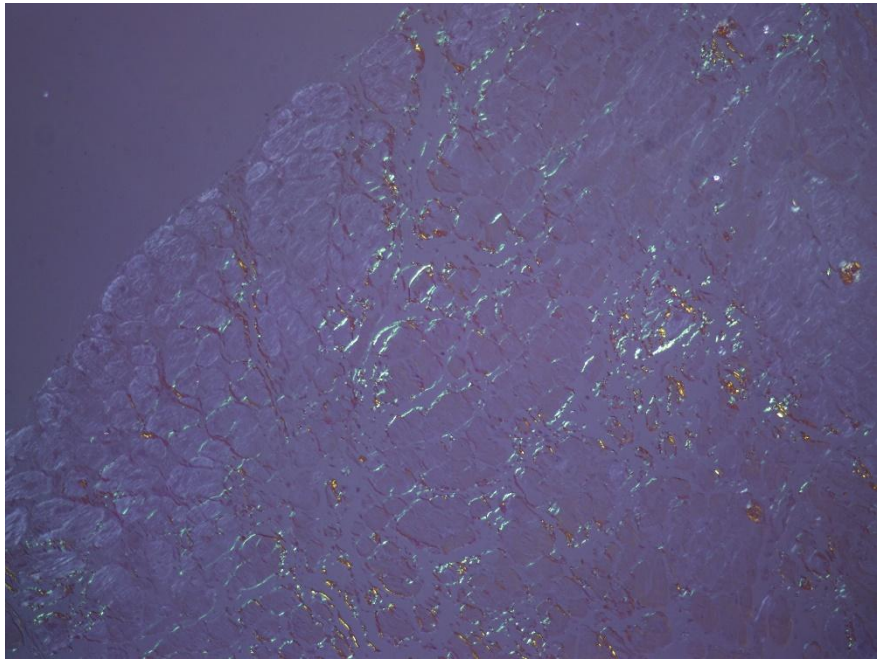


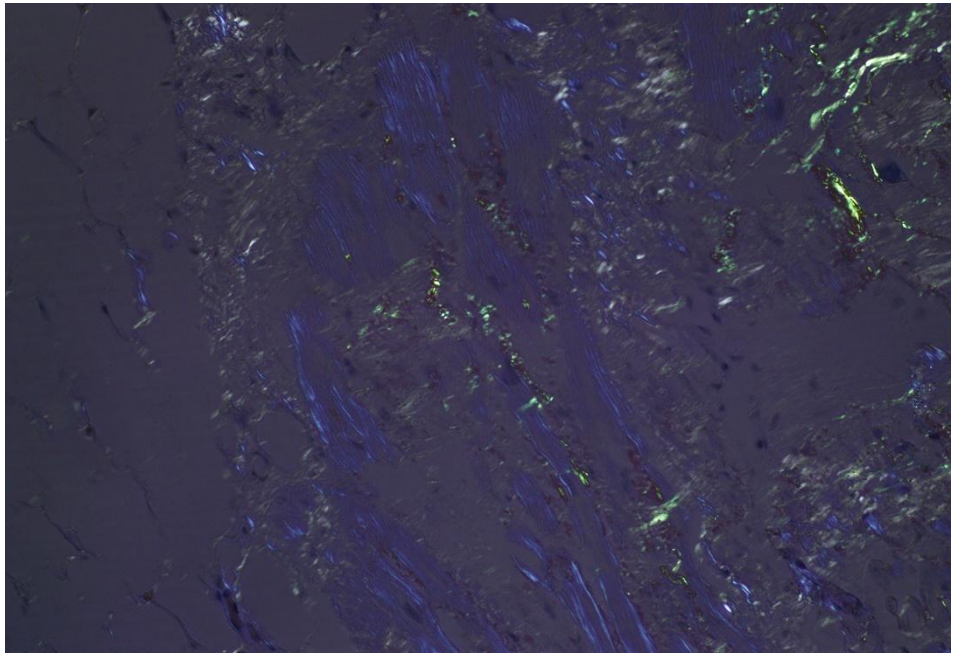


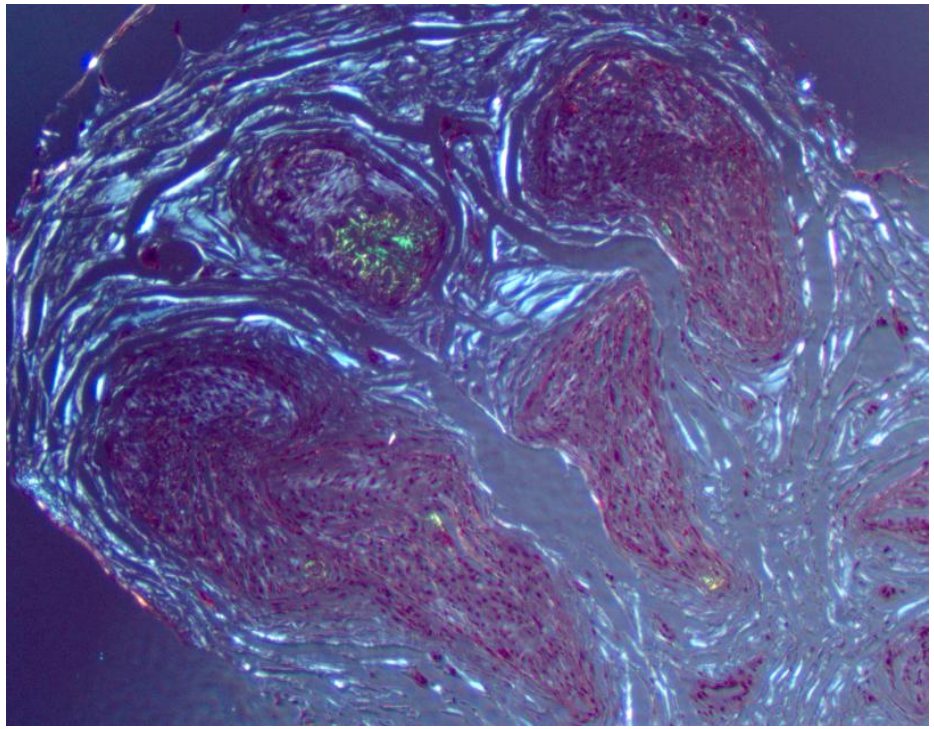


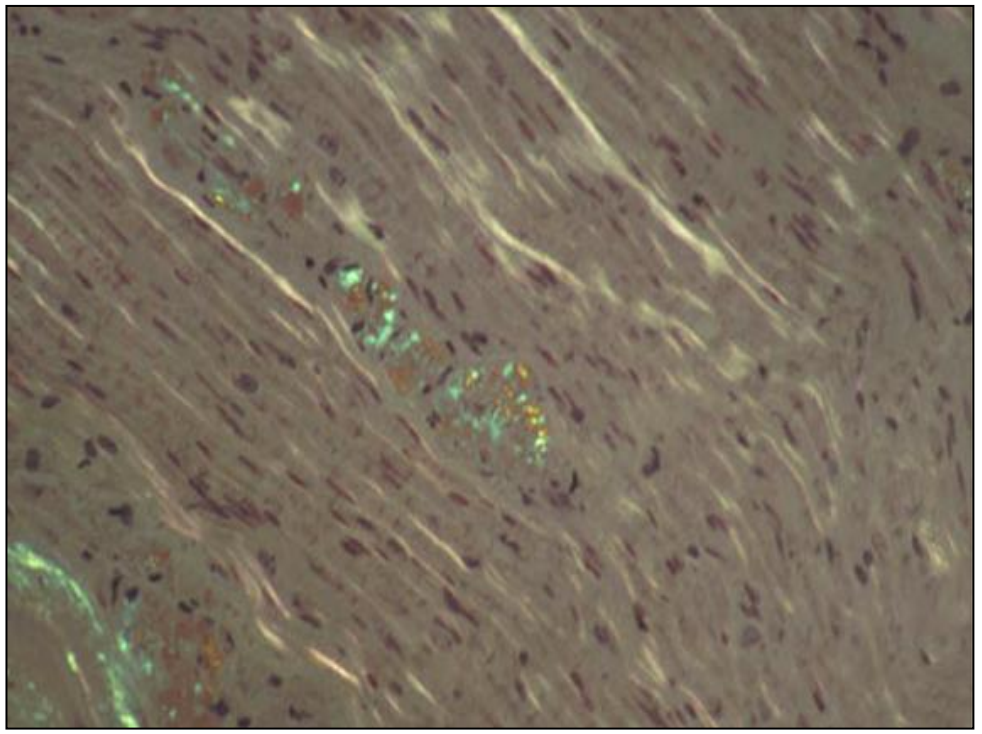






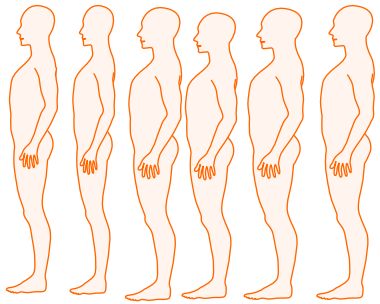






Fibril protein	Precursor protein	Systemic &/or loc	Acquired or hereditary	Target organs
AL, AH	Immunoglobulin light or heavy chain	S, L	A (H)	All, except CNS
AA	(Apo) Serum Amyloid A	S	A	All, except CNS
ATTR	**Transthyretin, wild type variants**	**S S**	**A H**	**Cardiac, PNS, ANS, heart, eye, leptomeninges**
AApoAI, AII, C-III AIV	**Apolipoprotein AI, AII, C-III, wild type (AIV)**	**S S**	**H A**	**Heart, liver, kidney, PNS… kidney**
AFib	**Fibrinogen α, variants**	**S**	**H**	**Kidney primarily**
ALECT2	**Leukocyte chemotactic factor-2**	**S**	**A**	**Kidney primarily, liver**
Aβ2M	β2Microglobulin, wild type variant	L S	A H	Musculoskeletal ANS
Cerebral: Aβ, ABri, ACys, APrP	Wild Variants, Wild	L	A H	CNS
Endocrine	ACal (Pro)calcitonin, Islet amyloid polypeptide (Amylin), Atrial natriuretic factor, Prolactin	L	A	Thyroid (C-cell), Islets of Langerhans, atria, pituitary
Iatrogenic	AIns (insulin), AEnf (Enfurvitide)	L	A	Site of injection
other				Lung, skin, aorta, cornea…

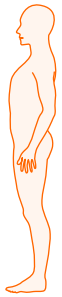
Renal Amyloidoses – protein types and treatments



AL: ~85%

- derived from immunoglobulin light chain
- clonal plasma cells proliferation
- **treatment: anti-plasma cell therapies…**

Non-AL: ~15%



AA:
- derived from SAA (serum amyloid-associated)
- chronic inflammation, sporadic or familial
- **treatment: anti-inflammatory**

ALect2: leukocyte chemotactic factor 2
- pathogenesis?
- **no specific therapy**

hereditary: avoid misdiagnosis as AL!
- derived from various mutant proteins; transthyretin, fibrinogen, other
- **liver transplantation**
- **clinical trials (transthyretin amyloidosis)**
- **genetic testing**

Differential diagnosis of
of proteinuria/nephrotic syndrome in adults:

1. Focal and Segmental Glomerular Sclerosis/Minimal change disease
2. Membranous nephropathy
3. Diabetes
4. **Amyloidosis!!!**

Cardiac amyloidosis – heart failure, arrhythmia, long list of differential
Polyneuropathy – sensory and autonomic disturbances, long list of differential
Amyloid deposits are unevenly distributed in tissues

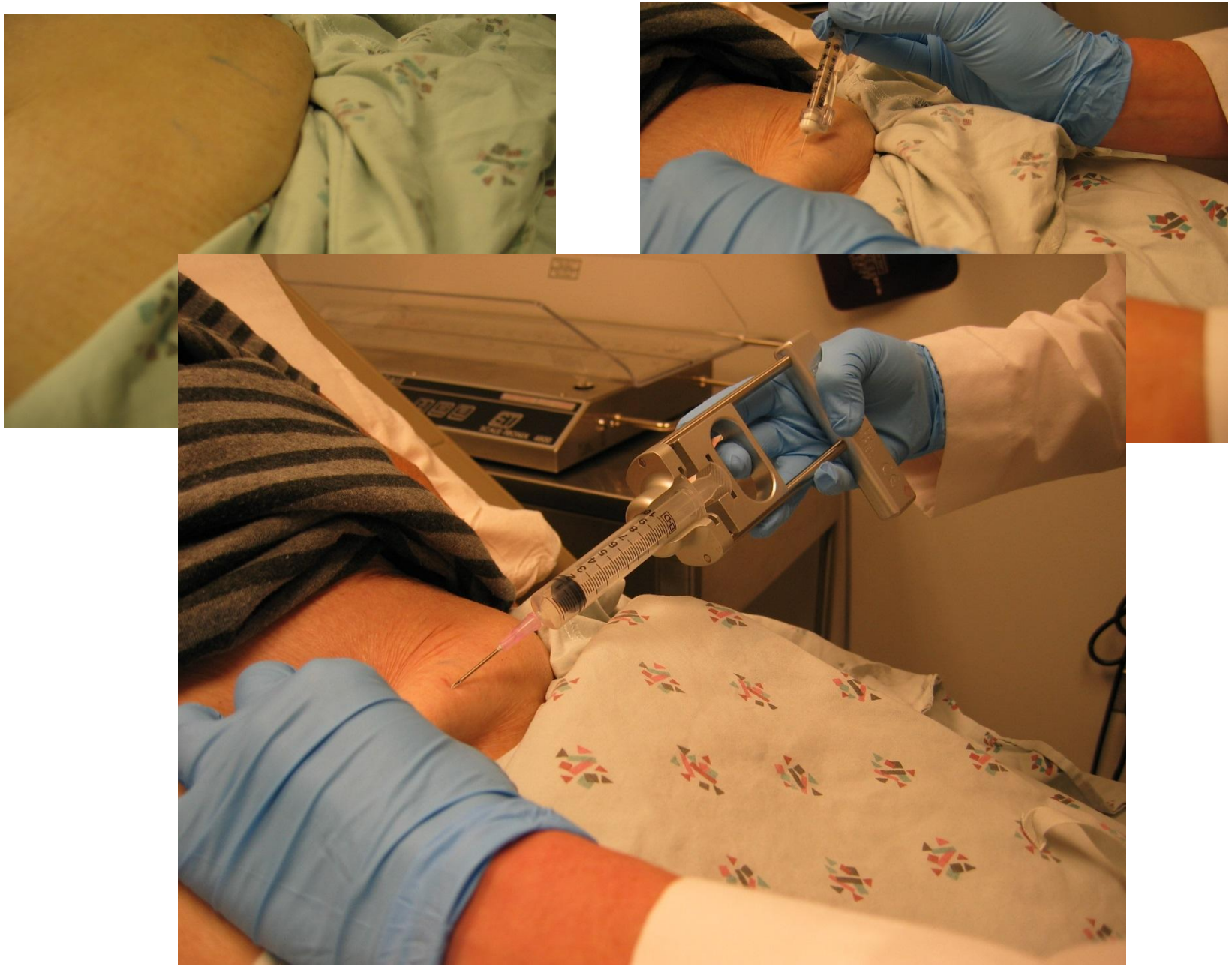
Congo red stain should be examined routinely on these biopsies!

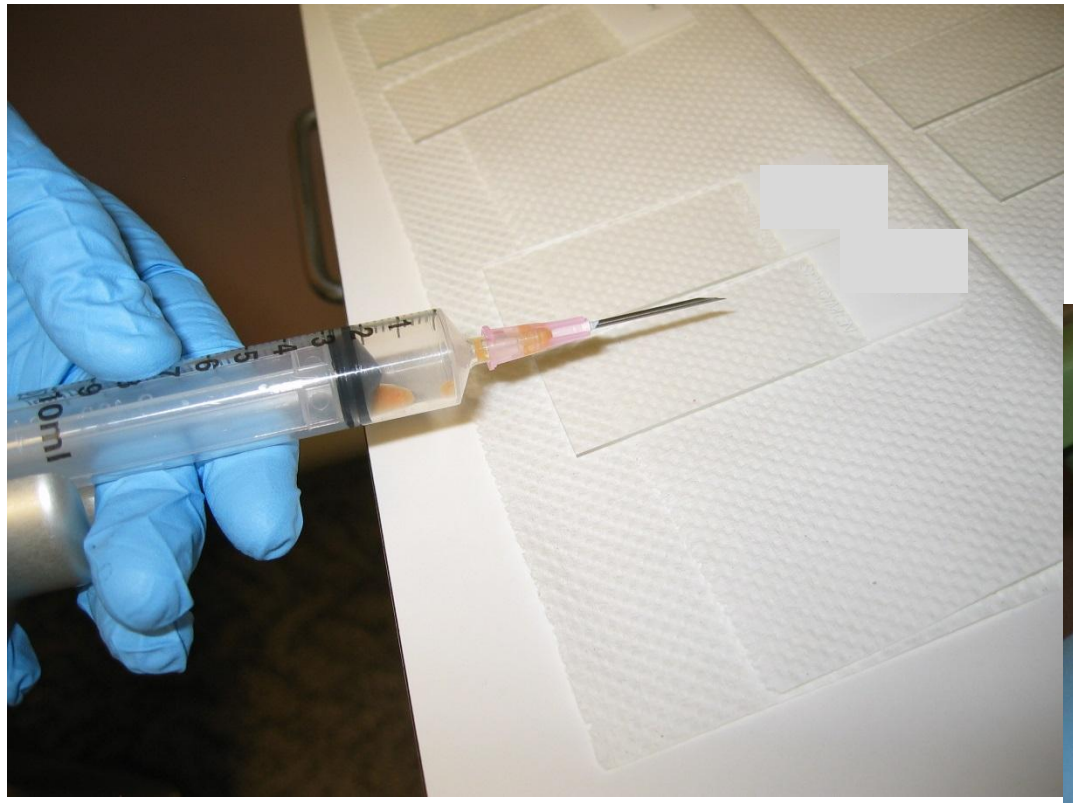
FAT STORY

Schilder (1909): amyloid frequently present in subcutaneous fat tissue in patients with amyloid A (AA) amyloidosis

P. Westermark and Stenkvist B (1971): diagnosis of secondary (AA) generalized amyloidosis by fine needle biopsy of the skin

Libbey, Skinner, Cohen, 1983, high yield of detection (88%) in AL, ATTR

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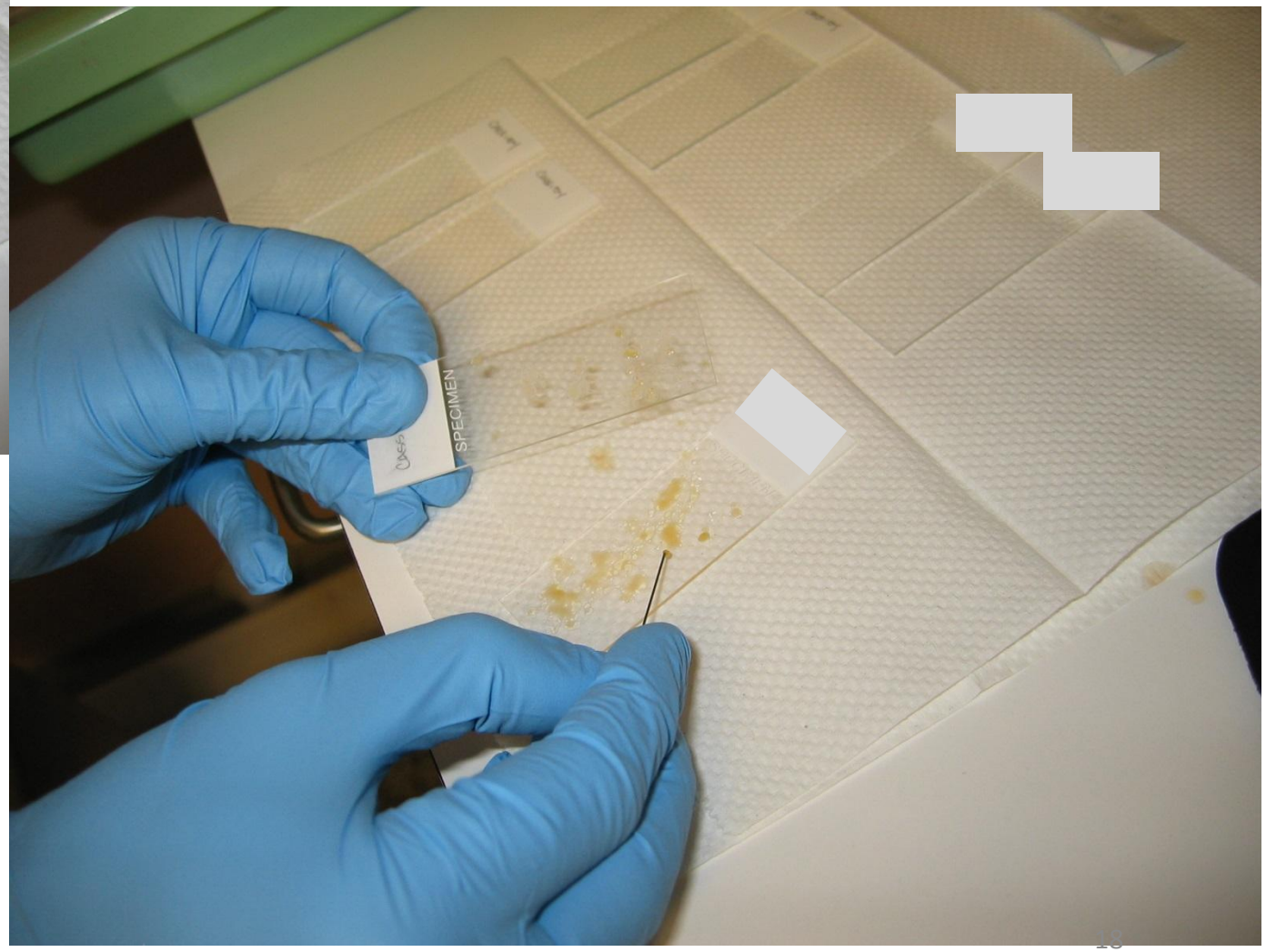
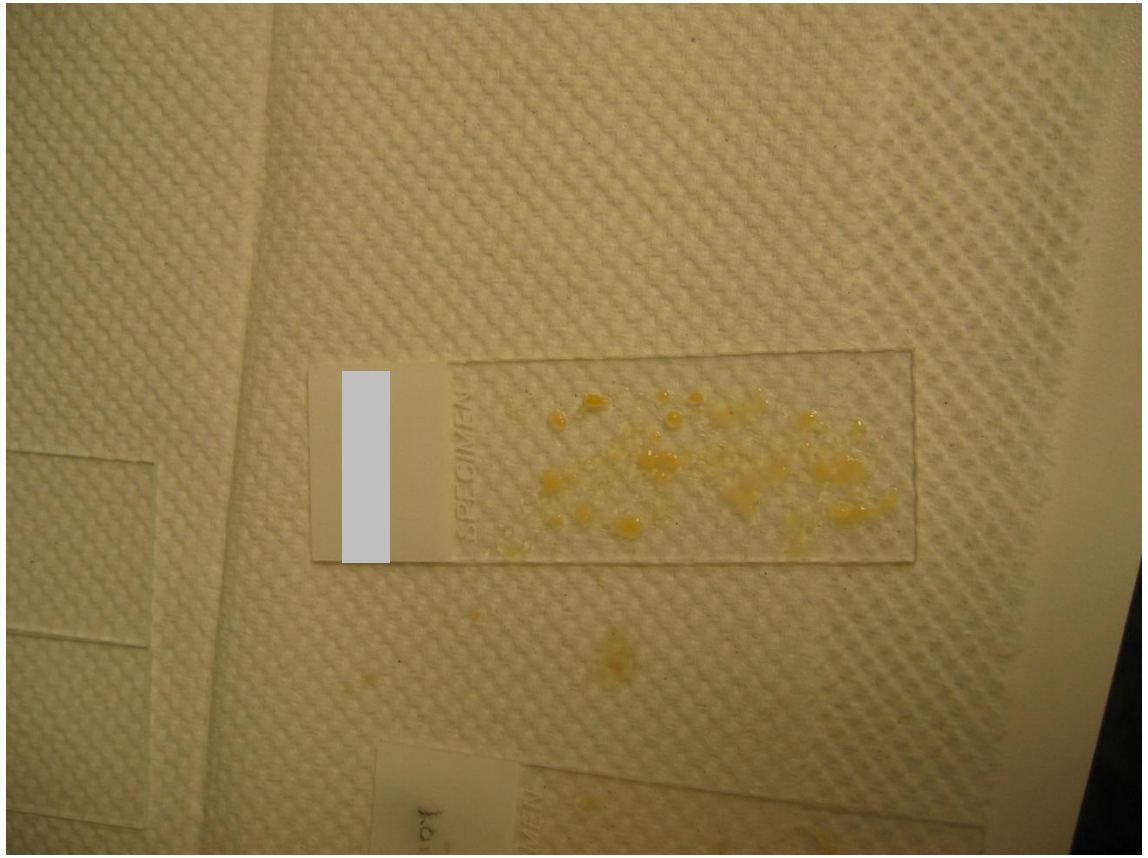

SPECIMEN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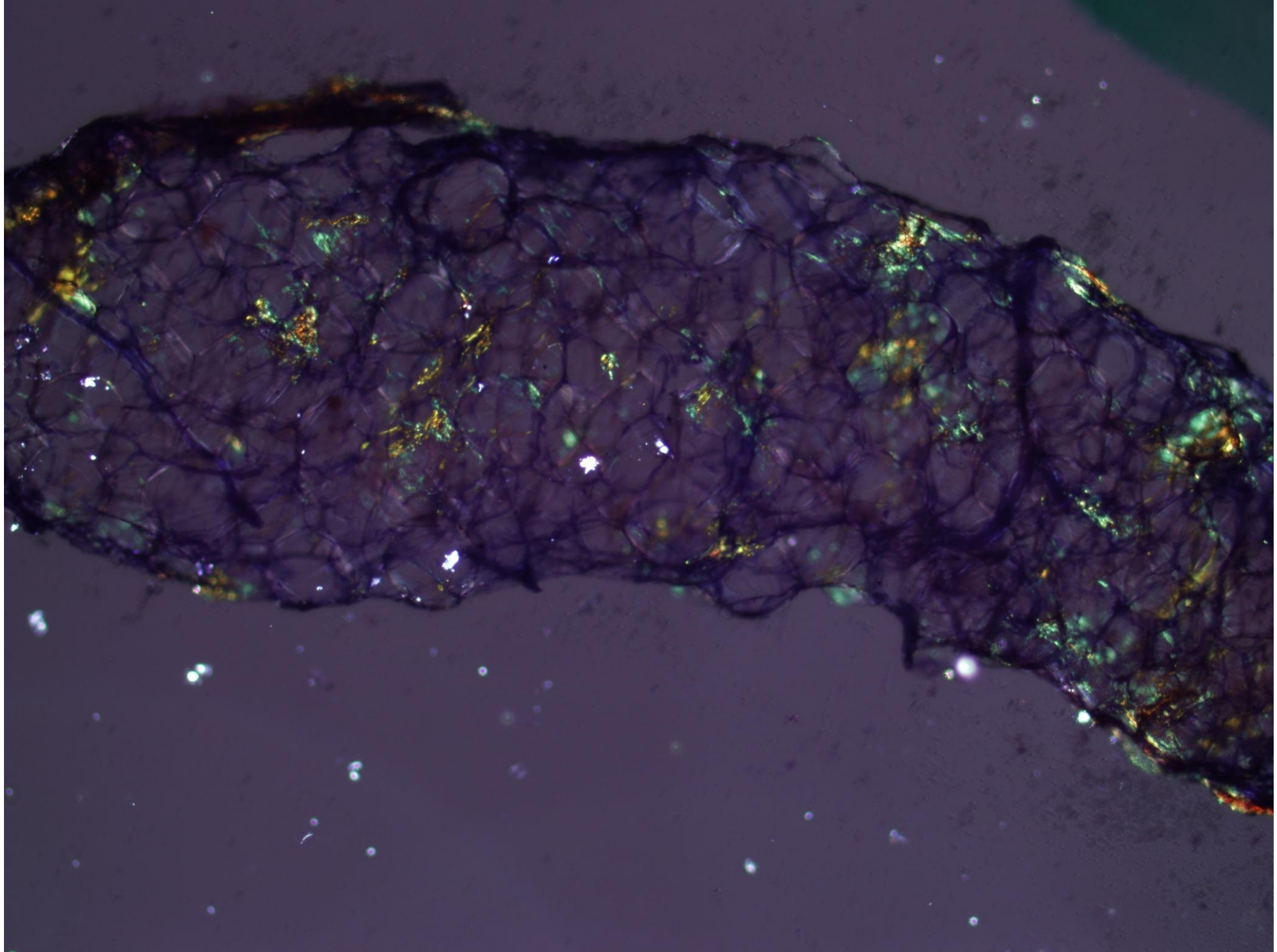
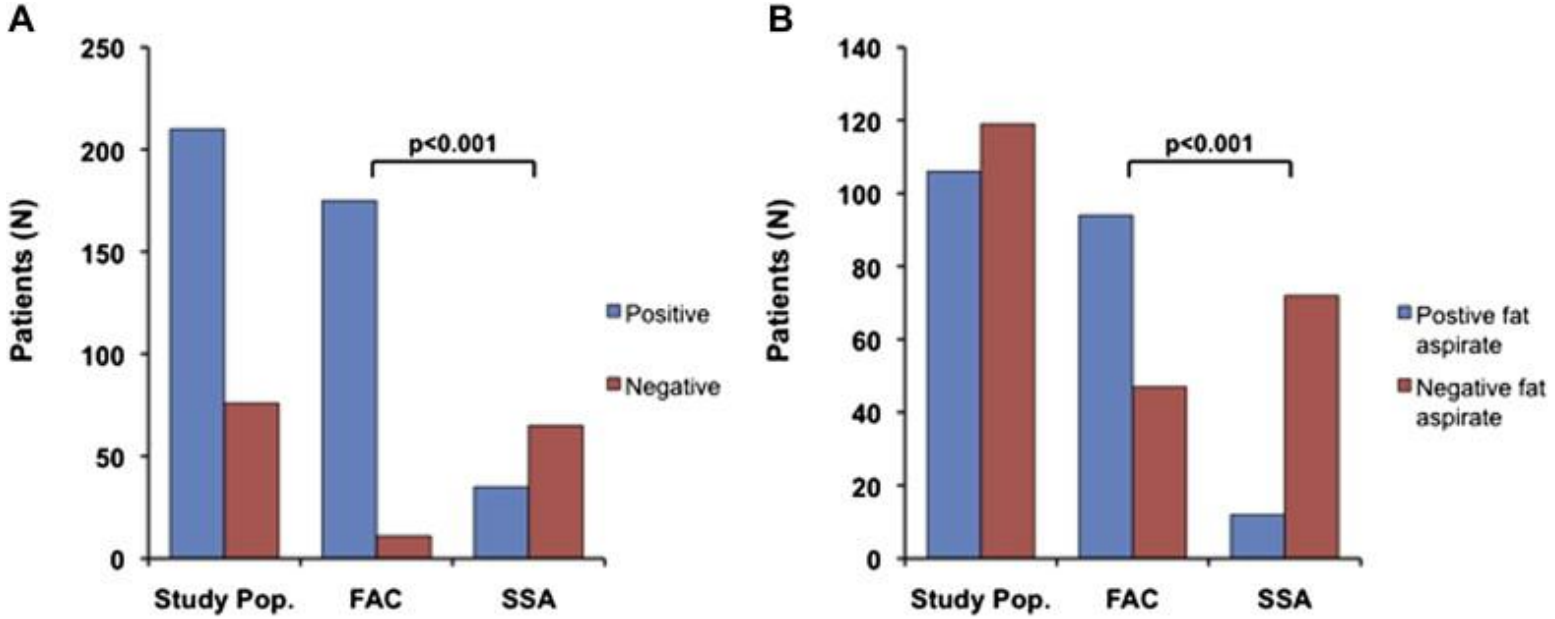


Amyloid detection in fat – AA, AL, ATTR:

Sensitivity highly variable 54-93%
Specificity: 93-100%

Affected organ – best yield
Other options?

ATTR cardiac amyloidosis



(A) any type of noncardiac tissue

(B) abdominal subcutaneous fat aspiration

Figure 1. Prevalence of amyloid protein deposition among patients with ATTR cardiac amyloidosis for the study population and for patients with FAC and SSA for (A) any type of noncardiac tissue sampling including noncardiac biopsy or abdominal subcutaneous fat aspiration and *(B)* only abdominal subcutaneous fat aspiration. Positive = positive for amyloid protein deposition, Negative = negative or equivocal for amyloid protein deposition.

Nowell M. Fine, Adelaide M. Arruda-Olson, Angela Dispenzieri, Steven R. Zeldenrust, Morie A. Gertz, Robert A. Kyle, Paul L. Swiecicki, Christopher G. Scott, Martha Grogan

Yield of Noncardiac Biopsy for the Diagnosis of Transthyretin Cardiac Amyloidosis

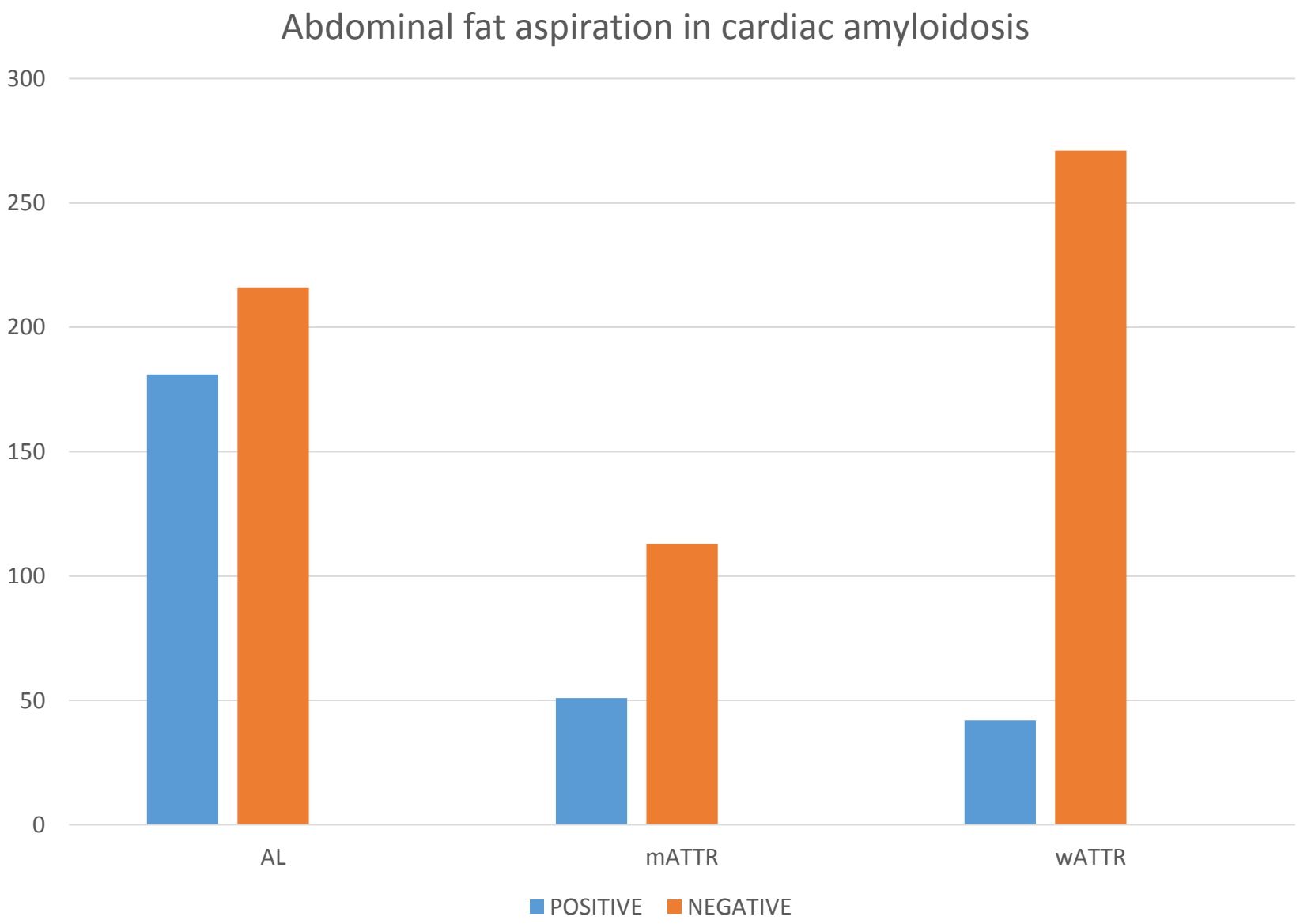
The American Journal of Cardiology, Volume 113, Issue 10, 2014, 1723–1727

http://dx.doi.org/10.1016/j.amjcard.2014.02.030

Fine et al, 2014:
ATTR, cardiac versus non-cardiac tissue sampling:

biopsy	all	Familial ATTR	Wild type senile ATTR
Fat aspirate	225/106+ 47%	141/94+ **67%**	84/12+ **14%**
Bone marrow	164/60+ 37%	100/41+ 41%	64/19+ 30%
heart	131/131+ **100%**	42/42+ **100%**	89/89+ **100%**
Sural nerve	54/45+ 83%	54/45+ 83%	0

Fat aspiration was the most commonly performed followed by bone marrow biopsy. Other: rectum, kidney, carpal ligament, liver, small intestine, sural nerve



Diagnostic sensitivity of abdominal fat aspiration in cardiac amyloidosis

Amyloid detected on Congo red staining in: 84% cardiac AL, 45% mATTR, 15% wtATTR

Quarta et al, European Heart Journal, Vol. 38, Issue 24, 21 June 2017, Pages 1905–1908,

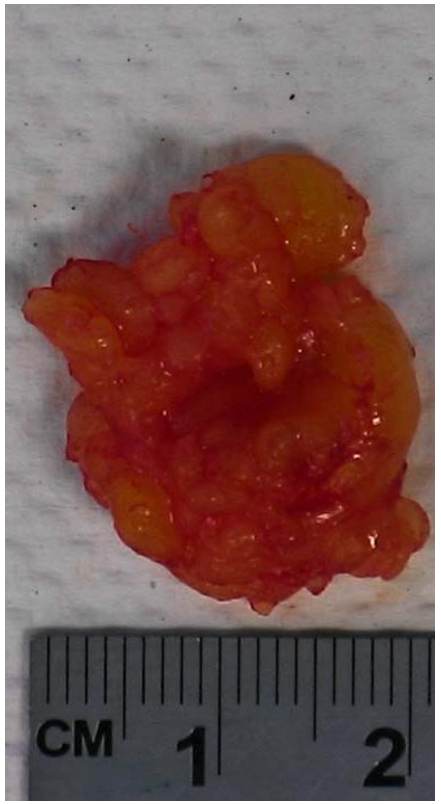
Coelho et al in FAP:
Labial salivary gland: 89%
Abdominal fat: sensitivity 50-70%
Nerve biopsy: 75-90%

Fat aspirate in wild-type (senile) ATTR amyloid cardiomyopathy

Fine et al 2014, 84 patients, sensitivity of 14%

Ikeda et al 2011, sensitivity increased to 73% (8 of 11 patients), deep layer of **surgical fat biopsy**, patchy distribution

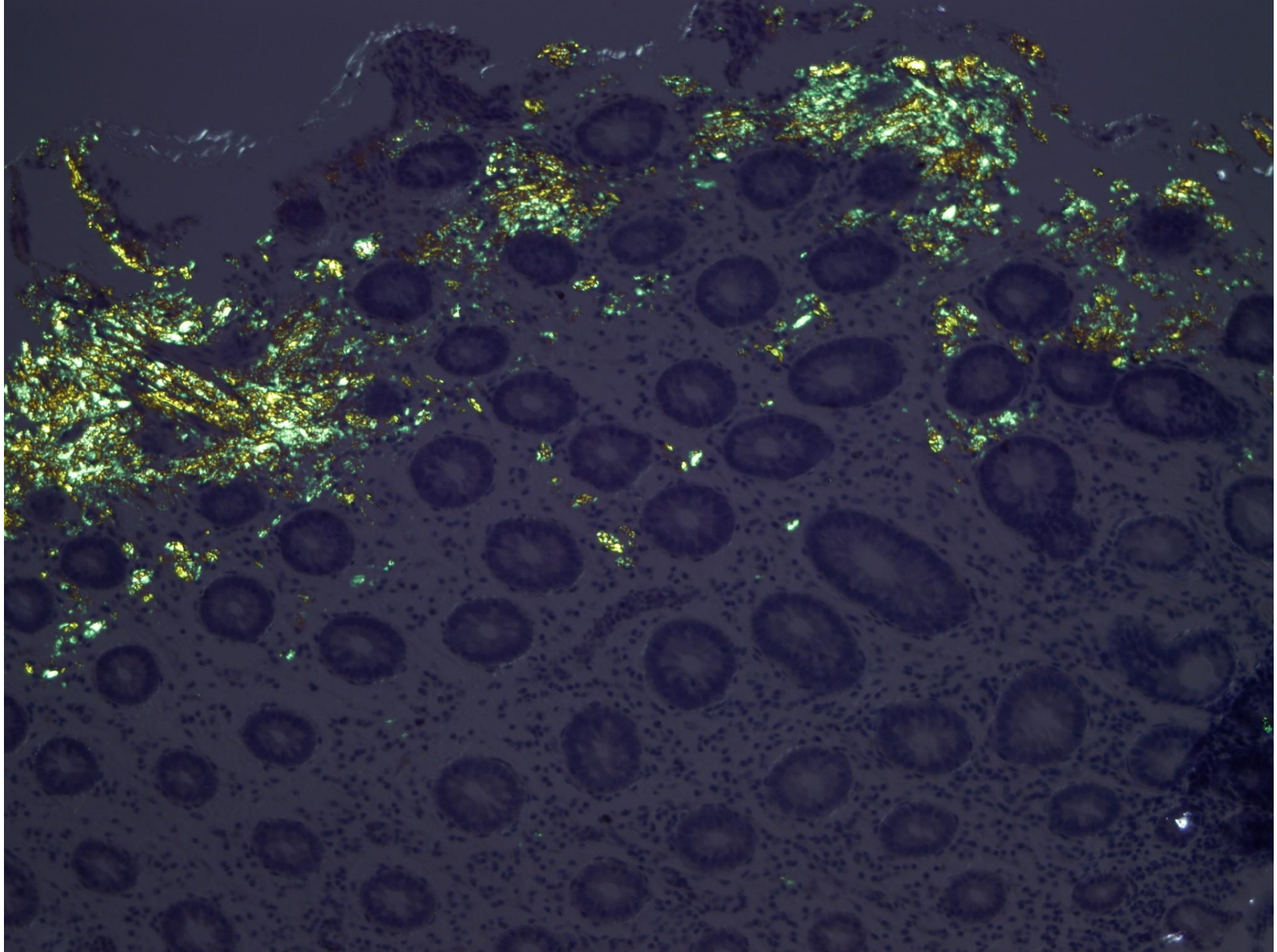
Takashio et al 2012: amyloid in blood vessels of fat
AL > ATTR cardiomyopathy (14 patients)

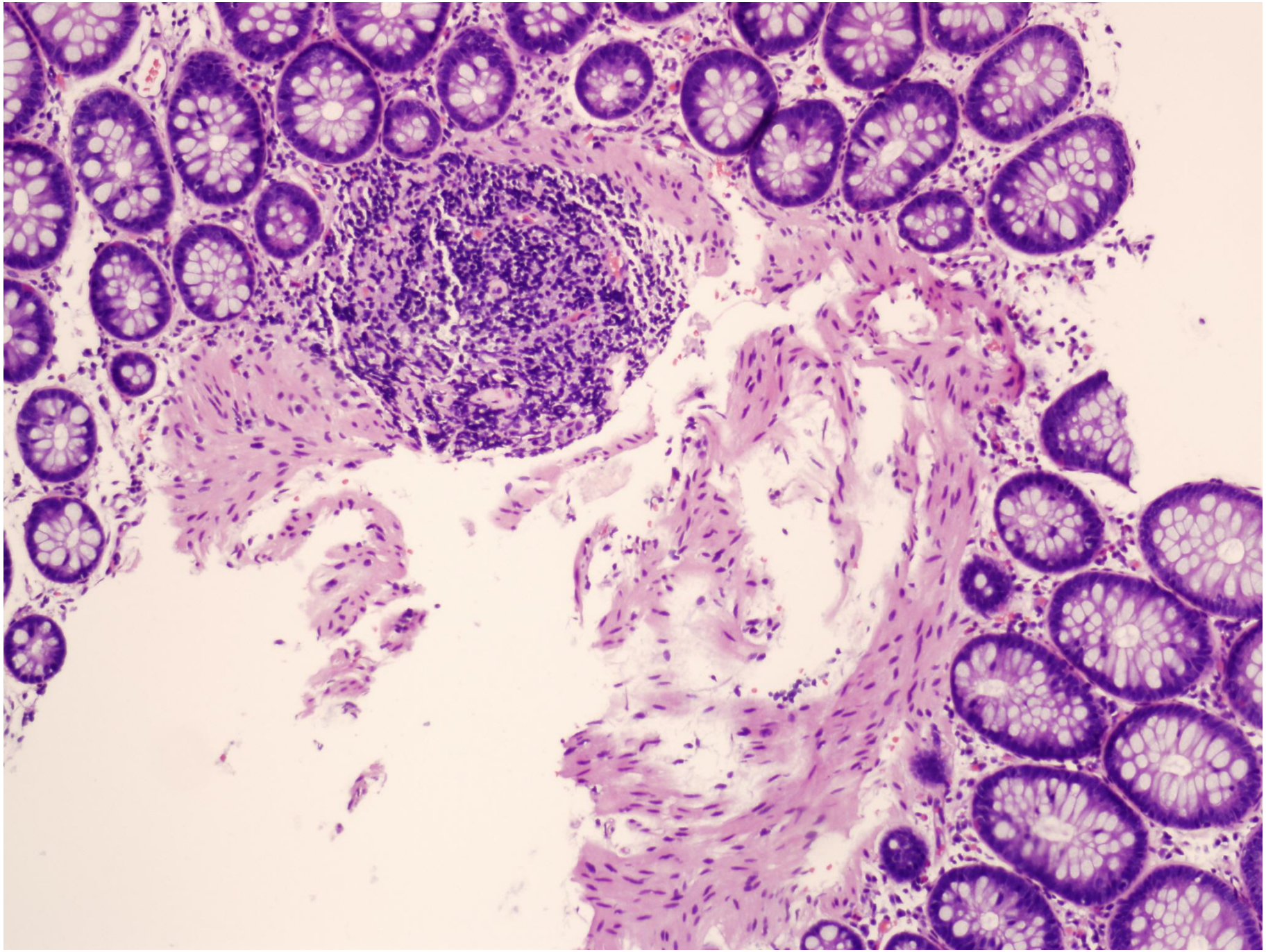

CM
1
2

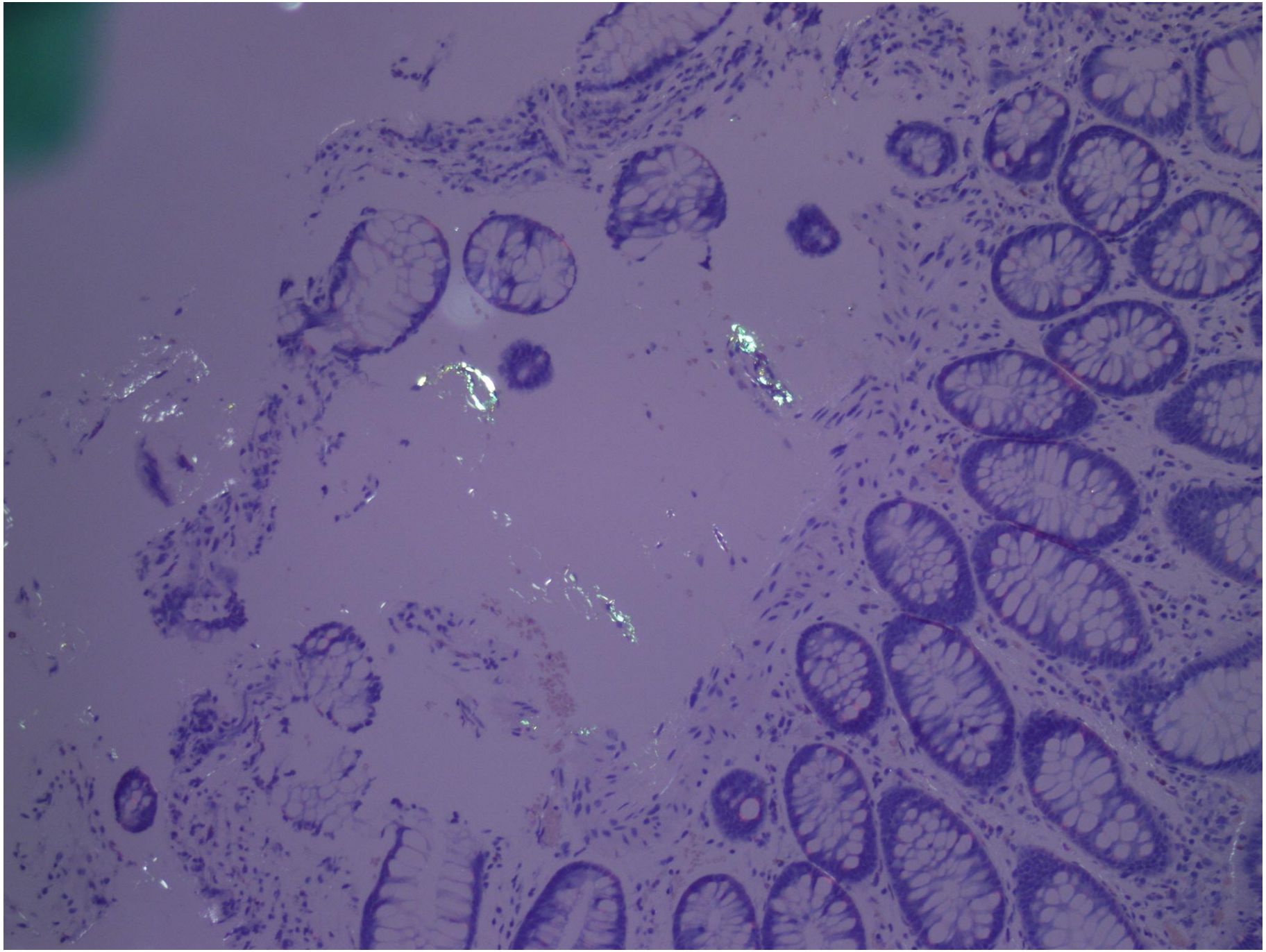
Pathology of Familial amyloidoses:

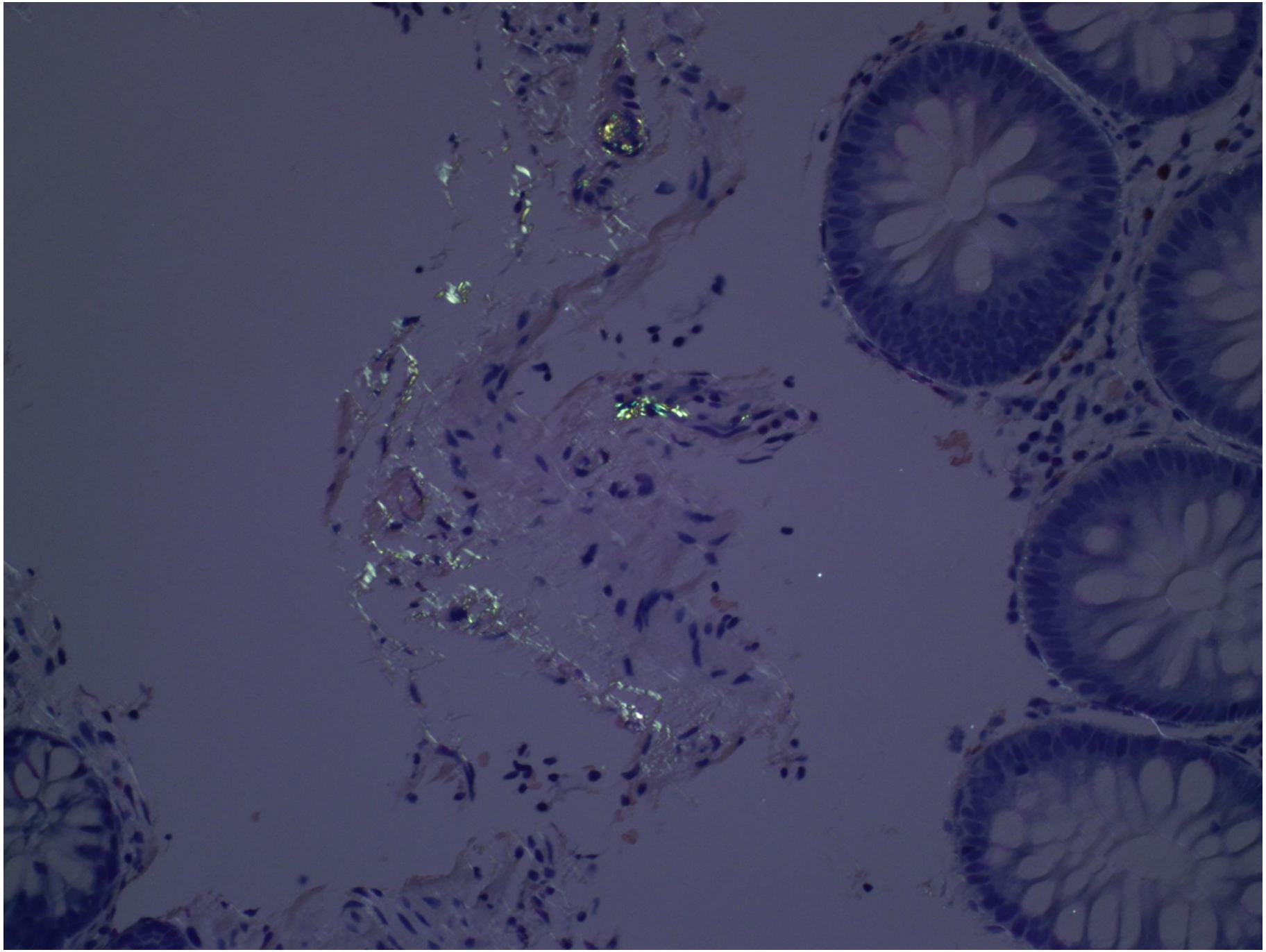
1. Detection of amyloid in the index patient
   - lack of a family history
   - new mutation
2. Examination of family members/known carriers

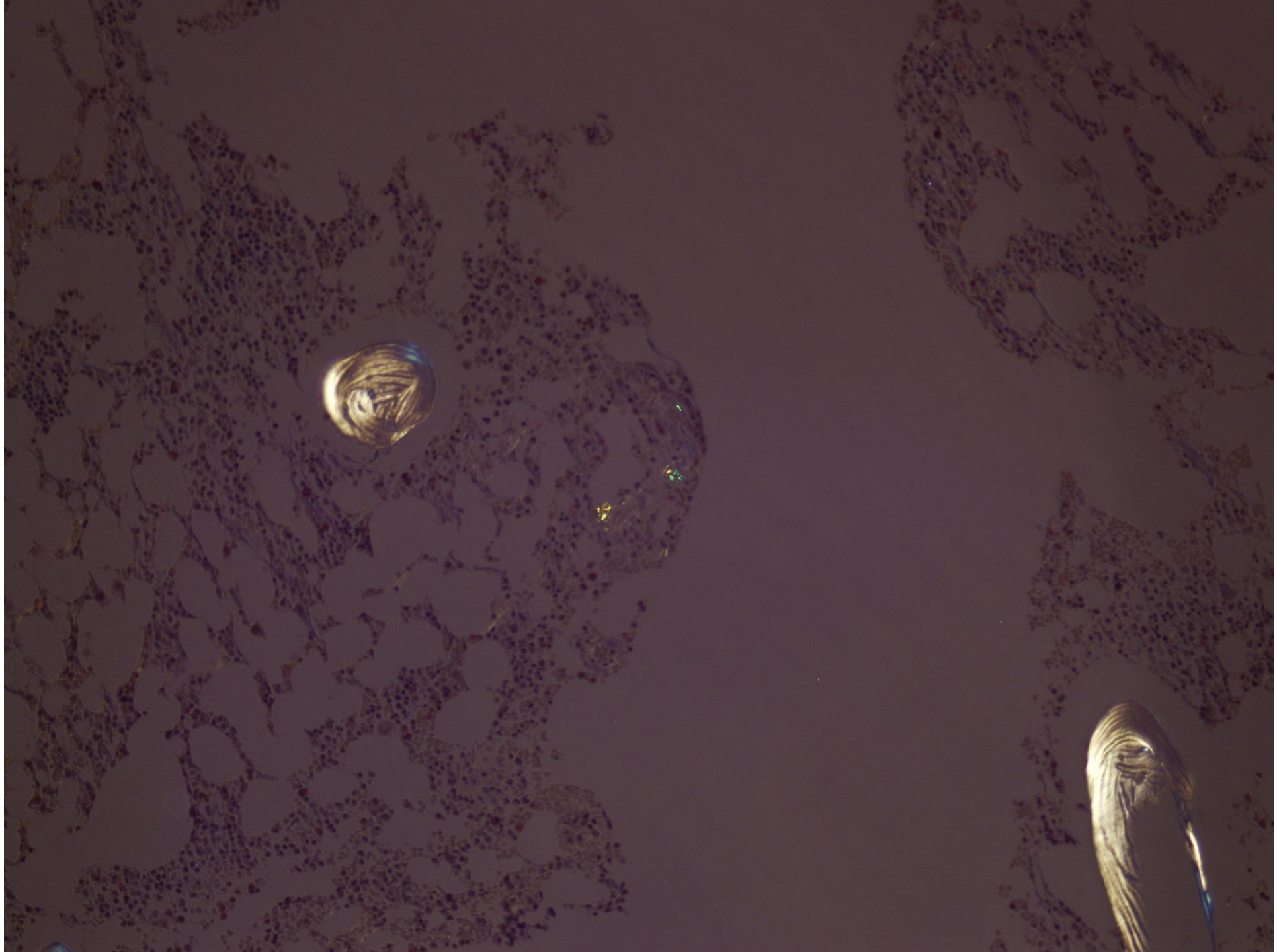
3. Staging, organ involvement











Screening?

↑ awareness

Suspicion → 2nd opinion

Questions?

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