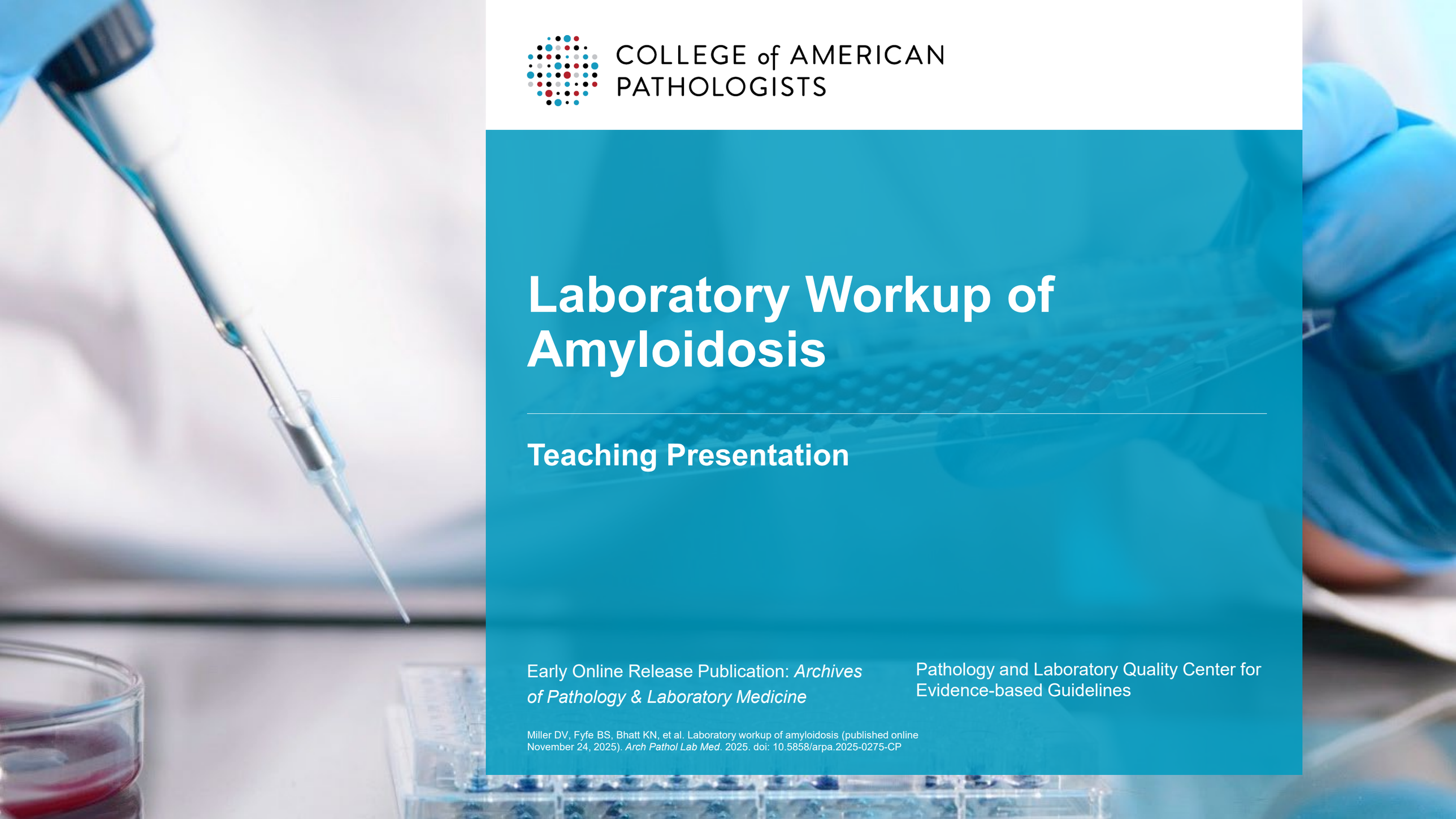


COLLEGE of AMERICAN PATHOLOGISTS

Laboratory Workup of Amyloidosis

Teaching Presentation

Early Online Release Publication: *Archives of Pathology & Laboratory Medicine*

Pathology and Laboratory Quality Center for Evidence-based Guidelines

Miller DV, Fyfe BS, Bhatt KN, et al. Laboratory workup of amyloidosis (published online November 24, 2025). *Arch Pathol Lab Med*. 2025. doi: 10.5858/arpa.2025-0275-CP

Outline

- Introduction
- Objective
- Key Questions and Results
- Guideline Recommendations and Good Practice Statements
- Guideline Development Process
- Conclusion

Introduction

- **Amyloidosis: diverse diseases caused by various misfolded proteins that aggregate and form insoluble fibrillar deposits which are toxic to tissues and lead to organ damage**
  - **There are 42 amyloid subtypes in humans and correct fibril identification is critical**

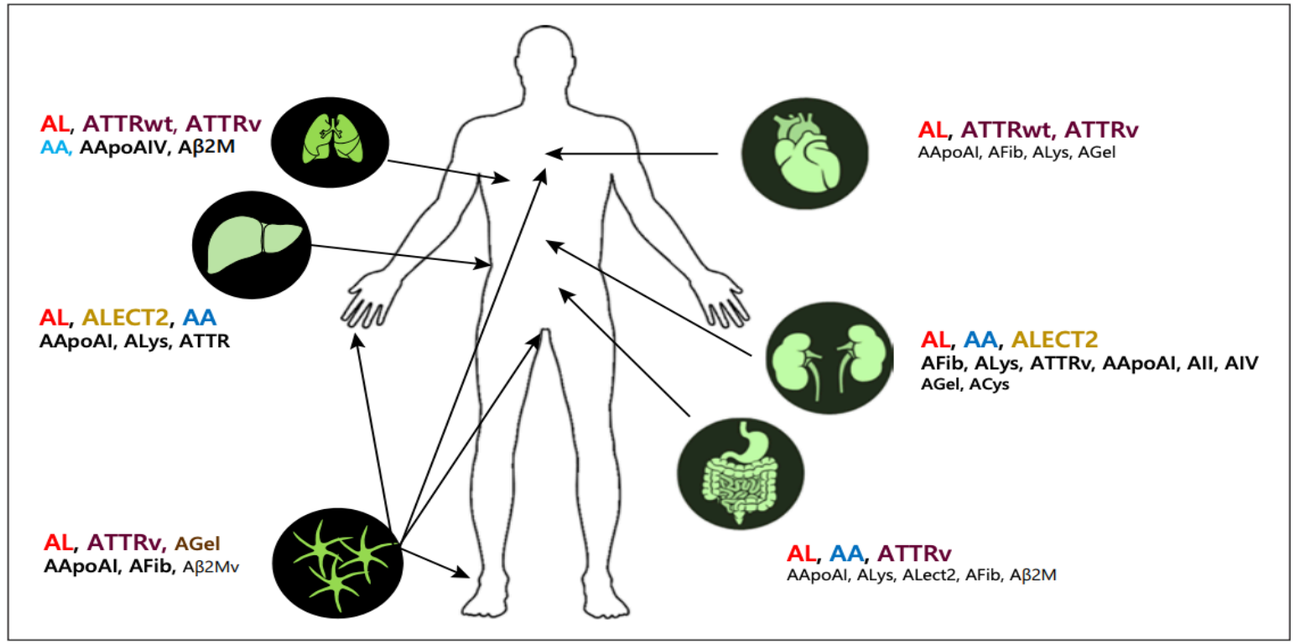
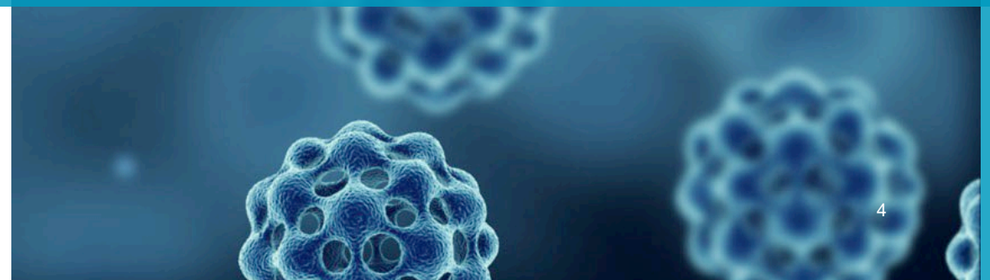


Fig. 1. Systemic amyloidosis types by target organ involvement.

Source: Picken MM. The Pathology of Amyloidosis in Classification: A Review. *Acta Haematol*. 2020;143(4):322-334. doi:10.1159/000506696.

Objective

- To establish evidence-based recommendations for appropriate laboratory testing to detect amyloid deposition and subsequent testing to identify the specific amyloidogenic protein detected.



Key Questions and Results

Key Questions (KQs)

KQ1. In patients with suspected systemic amyloidosis (ATTR, AA, and AL), does fat pad aspiration/biopsy, salivary gland biopsy, and rectal biopsy all provide adequate diagnostic sensitivity?

KQ2. When screening fat pad biopsies for amyloidosis, can cytology samples be used as an alternative to surgical pathology samples (FFPE blocks)?

KQ3. In patients with suspected amyloidosis, what method most accurately and reproducibly detects amyloid?

KQ4. When using Congo red stain on slides from patients with suspected amyloidosis, does the addition of fluorescence microscopy to polarized light microscopy provide increased sensitivity and reproducibility for the detection of amyloid?

KQ5. When performing protein subtyping on a case with confirmed amyloid deposition in a paraffin block, what is the diagnostic accuracy of immunohistochemistry when compared with mass spectrometry, and is this dependent on amyloidosis type or biopsy site?

KQ6. When performing protein subtyping on a case with a confirmed amyloid deposition in frozen tissue (i.e. renal, cardiac biopsy), what is the diagnostic accuracy of immunofluorescence when compared with mass spectrometry?

Results

The expert panel conducted a systematic review of more than 4,400 titles to address the key questions.

Four conditional recommendations and three good practice statements were established to provide guidance for proper testing and workup for amyloidosis.

Guideline Recommendations and Good Practice Statements

Recommendation Statement 1

- **In patients with suspected systemic amyloidosis, pathologists may screen cytology specimens (conventional smears and/or cell blocks) of aspirated abdominal fat for detection of amyloid.**

  Note: **Best preparation methods should be determined and optimized by individual laboratories and ancillary testing technique should be validated on cytologic material.**

  Note: **If cytologic smears only are prepared in the absence of a cell block, this limits the ability for further testing including subtyping.**
- **Conditional Recommendation**
- **Addresses KQ2**

Rationale/Discussion

Study	Detection Method	Amyloid Type	Surrogate Biopsy Site	Diagnostic Test Characteristics
				Sensitivity
Hansen et al, 2021	Congo red	Systemic	Fat pad biopsy	58.3%
			Skin punch biopsy	20.8%
			Combination	62.5%
Kimmich et al, 2017	Congo red with polarized light	AL Amyloidosis	Fat pad aspirate	96%
			Bone marrow smear	33%
			Bone marrow histology	57%
Quarta et al, 2017	Congo red plus IHC	AL Amyloidosis	Fat pad biopsy	84%
		ATTRv	Fat pad biopsy	45%
		ATTRwt	Fat pad biopsy	15%
Miyazaki et al, 2015	Congo red with polarized light	AL Amyloidosis	Fat pad	78%
			Bone marrow	45%
	Congo red plus IHC	AL Amyloidosis	Fat pad	62.1%
			Bone marrow	52.9%

Abbreviations: AL, immunoglobulin light chain; ATTR, amyloidosis derived from transthyretin; IHC, immunohistochemistry; wt, wild-type; v, variant.

Rationale/Discussion

- Cytology specimens include conventional smears that are stained with Congo red as well as cell blocks and formalin-fixed paraffin embedded tissue.
- Limitations to cytologic evaluation
  - impact on staining due to variable sample cellularity and thickness of cytologic smears
  - effect of blood and other contaminants on evaluation and interpretation
  - overstaining of Congo red
  - weak staining of Congo red
  - weak or focal birefringence
  - birefringence of other specimen components, such as connective tissue elements like collagen, that may appear white, yellow, or green, and interobserver variability in interpretation
- Performance of aspiration smears alone without concurrent cell block preparation limits the ability to perform ancillary testing including amyloid subtyping.

Recommendation Statement 2

- **When evaluating specimens for the presence of amyloid, pathologists should use Congo red staining method.**

  Note: **Laboratories may use other methods but should validate against Congo red or electron microscopy and must show equivalency.**

- **Conditional Recommendation**
- **Addresses KQ3**

Rationale/Discussion

Study	Amyloid Type	Detection Method	Diagnostic Test Characteristics			
			Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Fernandez de Larrea, et al 2015	Systemic	Congo red with polarized light	79.0% (74.7-82.7)	79.7% (74.4-84.2)	71.6%; (66.2-76.4)	85.4%; (81.4-88.7)
		Electron microscopy	76.1% (71.7-78.0)	100% (98.4-100)	74.0% (69.2-78.2)	100% (98.4-100)
	AL Amyloidosis	Congo red with polarized light	80.9% (76.1-85.0)	Not reported	Not reported	Not reported
		Electron microscopy	79.4% (74.4-83.6)	Not reported	Not reported	Not reported

Study	Amyloid Type	Biopsy Type	Detection Method	Positivity Rate
Yamamoto et al, 2023	Cardiac amyloid	Endomyocardial	Congo red	94.4% (n=17/18)
			Electron microscopy	100% (n=18/18)

Rationale/Discussion

- **Congo red staining is challenging to perform and interpret. Couple of suggestions to consider:**
  - **Staining protocols should be optimized and confirmed using on-slide control tissue each time it is performed**
  - **Preferable thickness of the tissue section, 8-10 um, should be included in the protocol**
  - **Difficult cases should prompt consultation with experienced colleagues**
  - **Birefringence under plane polarized light microscopy should be performed as a routine part of every Congo red interpretation using a sufficiently bright light source**
  - **Other amyloid stains (Sulfated Alcian blue, crystal violet, Thioflavins, etc) may be used if validated**

Recommendation Statement 3

- **When assessing Congo red histochemistry, pathologists may add fluorescence microscopy with the tetramethylrhodamine isothiocyanate/Texas red filter to increase sensitivity for amyloid detection, if available.**
- **Conditional Recommendation**

Rationale/Discussion

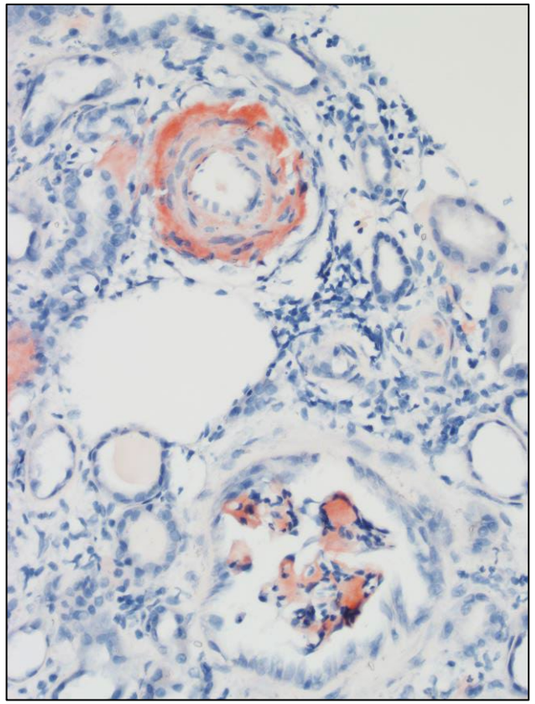
Study	Reference Standard	Microscopy	Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)
Lee et al, 2021	Electron microscopy	Fluorescence	100% (85.8-100)	99.2% (97.6-99.8)	88.9% (72.2-96.1)	100%
		Polarized light	95.8% (78.9-99.9)	99.4% (98.0-99.9)	92.0% (74.2-97.9)	99.7% (98.1-100)
Cazzaniga et al, 2023	Polarized light Congo red	Fluorescence / TRITC	100% (91-100)	99% (95-100)	98% (87-100)	100% (97-100)
Pinedo Pichilingue et al, 2023	Polarized light Congo red	Fluorescence / TRITC	100% (98.4-100)	97.6% (96.0-99.3)	73.1% (68.2-78.0)	100% (99.8-100)

Abbreviations: TRITC, tetramethylrhodamine isothiocyanate.

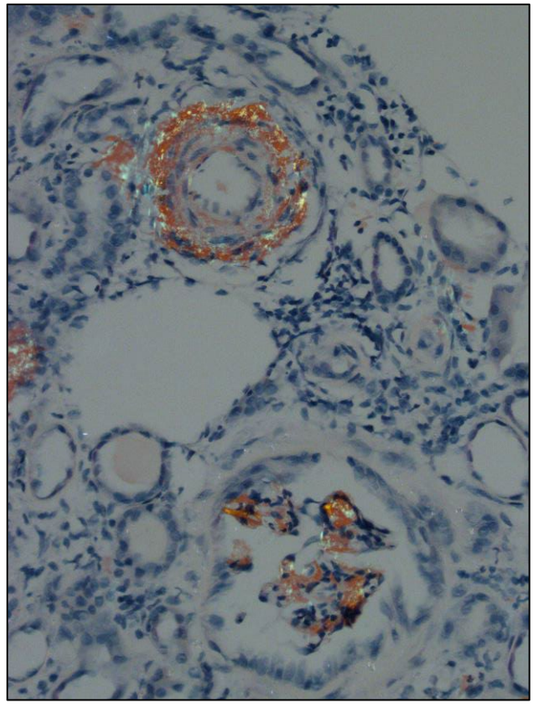
Rationale/Discussion

- Fluorescence microscopy of Congo red improves the signal-to-noise ratio
- Nonspecific congophilic staining may also fluoresce, cautious interpretation

Congo red - brightfield



Congo red - polarized



Congo red – TRITC Fluorescence

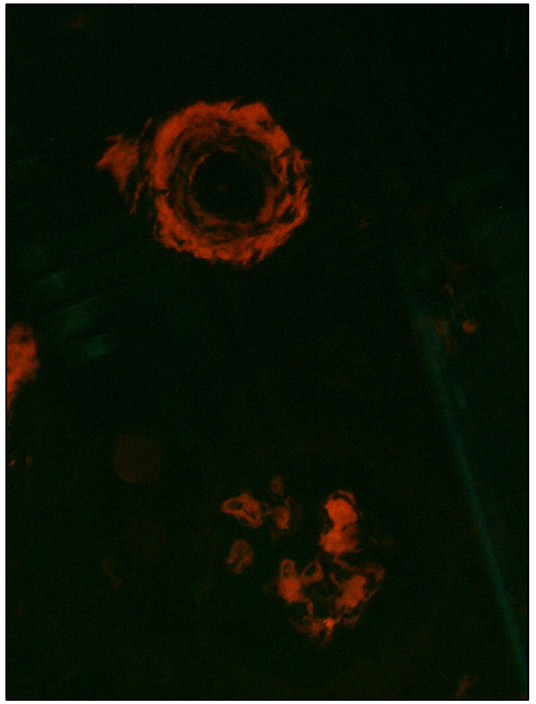


Image courtesy of Dylan V. Miller, MD from Intermountain Central Laboratory

Recommendation Statement 4

- **In patients with amyloidosis being considered for therapy to optimize diagnostic yield and tissue utilization, pathologists should use mass spectrometry to identify the fibril protein type.**

  Note: **In renal amyloidosis, amyloid fibril typing may often be successfully accomplished by immunofluorescence, although reflex to mass spectrometry-based proteomics should be performed in difficult or equivocal cases.**

- **Conditional Recommendation**

Rationale/Discussion

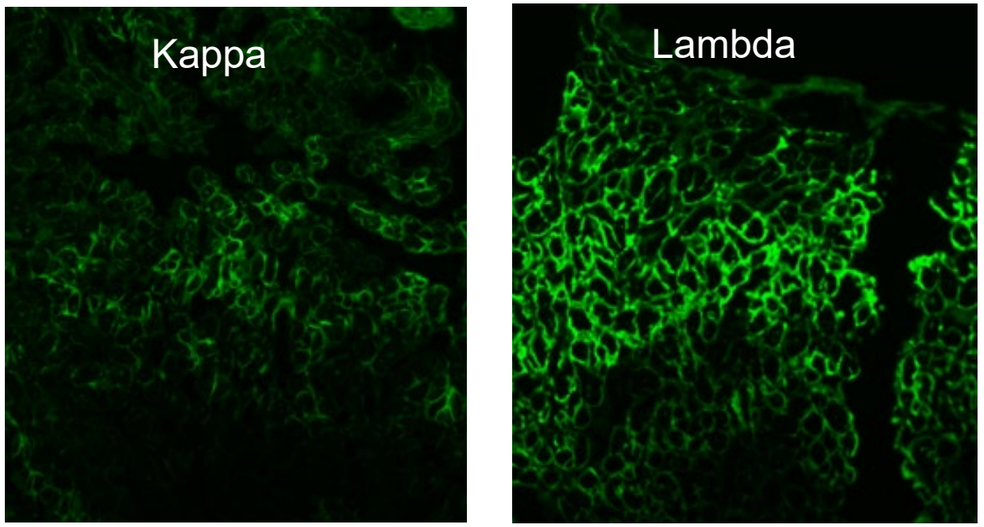
Study	Immunohistochemistry	Mass Spectrometry	Sample Size (n)	Amyloid Detection Frequency	Assay Concordance
Gibertson et al, 2015	AL and ATTR panels	Laser microdissection plus mass spectrometry	142	ATTR n=22	100%
				AL n=71	100%
Rezk et al, 2019	AL, ATTR, AA, and 11 antibody panels	Laser microdissection plus mass spectrometry	700	Systemic, renal biopsy n=139	82%
				Systemic, cardiac biopsy n=103	88%
				Systemic, bone marrow n=30	75%
Mollee et al, 2016	No details reported	No details reported	39	ATTRwt n=19	81.8%
				AA n=5	100%
				AL n=11	94.7%
Abe et al, 2021	ATTR, AA, AL panels	No details reported	36	AL n=5	80%

Abbreviations: AA, amyloid A; AL, immunoglobulin light chain; ATTR, amyloidosis derived from transthyretin; wt, wild-type.

Rationale/Discussion

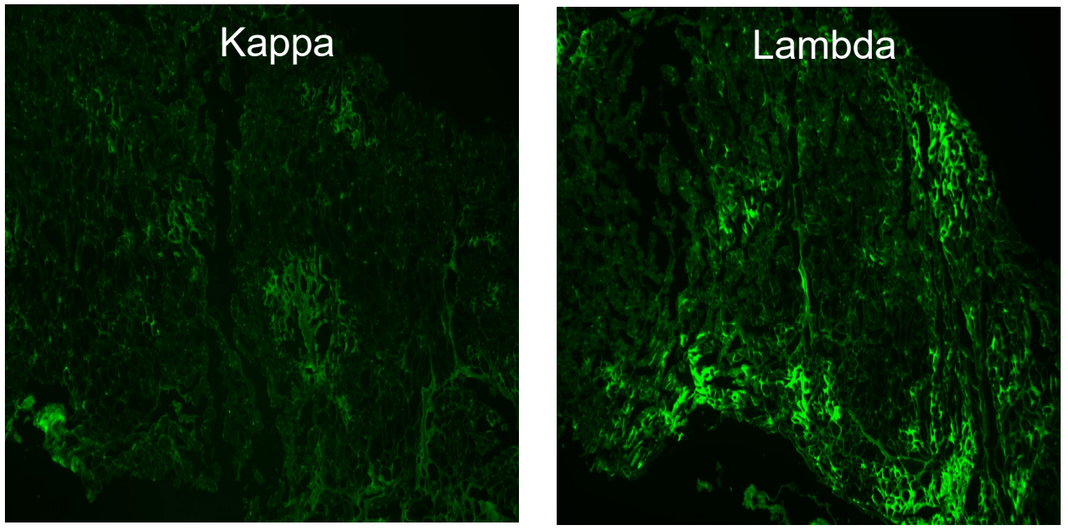
Endomyocardial biopsy – Immunofluorescence

CASE 1



Mass Spectrometry Fibril Typing – AL (Lambda)

CASE 2



Mass Spectrometry Fibril Typing – ATTR

Image courtesy of Billie S. Fyfe, MD from Rutgers Robert Wood Johnson Medical School.

Rationale/Discussion

- **Mass spectrometry is recommended over immunohistochemistry (IHC) for several reasons:**
  - **subset of cases remain untyped even if using a broad panel of antibodies**
  - **many cases yield inconclusive results by IHC**
  - **small tissues or small deposits of amyloid may be exhausted by performing multiple sections for IHC**

Good Practice Statements

- When specimens are received for detection of systemic amyloidosis, pathologists should evaluate for the presence of amyloid using validated method(s), and the validated method(s) used should be identified in the pathology report.
- If a clinical concern for amyloidosis persists after a negative biopsy from a surrogate site but potentially affected organ(s) was (were) not sampled, then suggesting biopsy of the potentially affected organ(s) and/or recommending suitable archived specimen(s) to evaluate is appropriate.
- In patients with amyloidosis being considered for therapy, pathologists should determine the fibril protein type.

Screening for Amyloid: non-targeted tissues

Certain clinical specimens have “higher yield” for potential early diagnosis of cardiac amyloidosis

- Carpal Tunnel Syndrome (CTS) specimens
  - 15-60% of patients with ATTR WT have CTS
  - Among 98 pts – 10% amyloid - ATTRwt (5), ATTRv (2), AL (2), untyped (1)
- Lumbar Stenosis Specimens
  - 33-44% of lumbar stenosis specimens

Localized Amyloidosis

- **Typical sites**
  - **Larynx, skin, bowel (in areas of injury), urinary tract (rare)**
  - **Pulmonary nodular deposits of amyloid**
  - **Insulin injection sites**

Guideline Development Process

Panel Composition

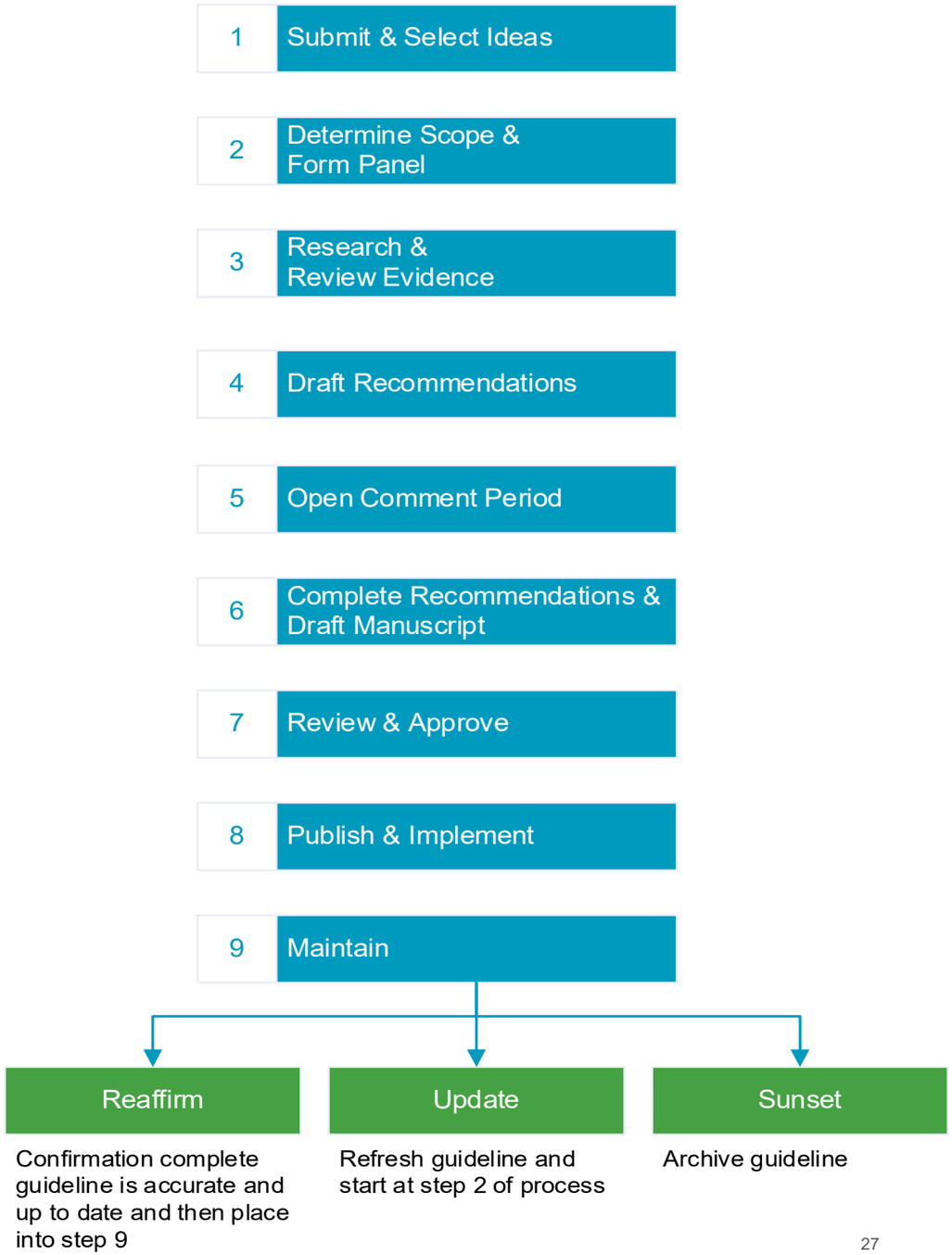
Expert Panel
Billie Fyfe, MD, FCAP
Dylan Miller, MD, FCAP
Kunal Nikhil Bhatt, MD
Anthony Chang, MD
Pallavi Gopal, MD, PhD
Gregary Bocsi, DO
Ellen D. McPhail, MD
Megan O. Nakashima, MD
Vanda F. Torous, MD
Allison Jackson Zemek, MD

Advisory Panel
Michael Joseph Cascio, MD
Valerie Cortright, HTL
Ahmet Dogan, MD, PhD
Patrick Fitzgibbons, MD
Morie Gertz, MD
Carmela D Tan, MD
Maria M. Picken MD, PhD
Guillermo Herrera, MD

Patient Advocates:
Walter Feigenson, MBA
Sheldon Pinsker

Guideline Development Process

- The Center follows the standards endorsed by the National Academy of Medicine for developing Clinical Practice Guidelines.
- Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach was utilized in updating the guideline.
- A detailed description of the guideline development process can be found online [Evidence-based Guidelines Development Methodology Manual]



Literature Search

- Search was conducted in Ovid MEDLINE, Embase, Cochrane Library.
- Initial literature search ran on: February 17, 2023
  - 4480 studies from January 1, 2013 to February 17, 2023
- Literature refresh ran on: March 11, 2024
  - 464 studies from February 17, 2023 to March 11, 2024

Systematic Review of the Literature

- Each level of systematic review (title-abstract screening, full-text review, and data extraction) was performed in duplicate by two members of the expert panel.

Quality Assessment

- Systematic Reviews (SRs) and Meta-analyses questions were assessed as per the Assessing the Methodological Quality of SRs (AMSTAR) tool
- Non-randomized studies were assessed using the Risk of Bias in Non-Randomized Studies – of Intervention (ROBINS-I) tool.
- Diagnostic studies were assessed using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) tool.

Open Comment Period

- Open Comment Period held from March 13, 2024 to April 3, 2024
  - More than 200 comments were received for the seven statements

Review and Approval

- The AP reviewed and provided feedback on the draft recommendations and manuscript.
- The EP approved the final recommendations and good practice statements with a formal vote.
- The independent review panel (IRP) representing the Council on Scientific Affairs reviewed and approved the guideline for the CAP.
  - IRP members were masked to the expert panel and vetted through the conflicts of interest (COI) process

Conclusion

Conclusion

- Importance of typing – as critical as predictive markers in cancer
- Systemic amyloid types include:
  - AL, ATTR(wt/v), AA, ALECT2, (etc.)
- Fat pad utility, processing (cell block for fibril typing), recommendations to include in report when negative
- Congo red - polarize and fluorescence microscopy (starts with H&E!)
- Other amyloid stains are appropriate but should be validated
- Amyloid fibril typing assays - strengths and weaknesses
  - Mass spectrometry is recommended as optimal

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