# ATTR Diagnostics and Staging

MED-US-DZSTATE-2400021

# ATTR Disease State Slide Deck

- This resource provides information about ATTR.
- This resource is intended to be viewed in its entirety to support scientific exchange and is not intended as recommendations for clinical practice.
- This resource may contain hyperlinks that are not functional in this format.
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# Diagnostics and Staging

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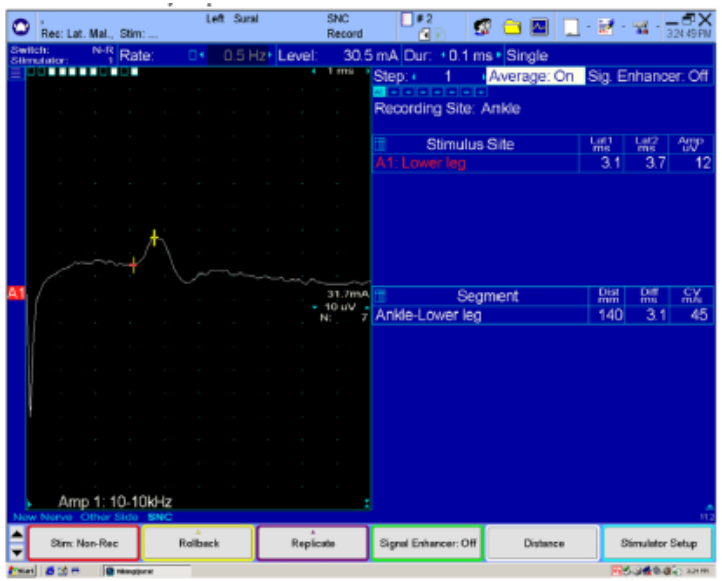
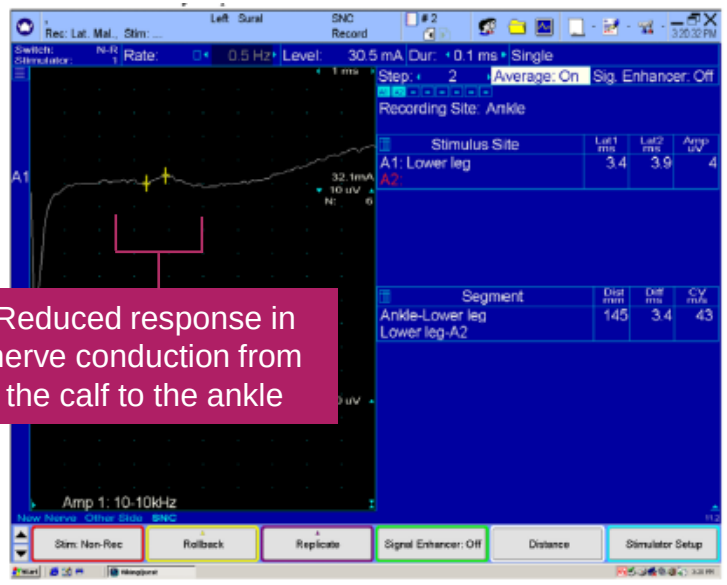
# Nerve Conduction Studies (NCS)

- NCS use an electrode to measure the speed at which electrical impulses move through the nerves and can detect abnormalities along the length of mixed, motor, or sensory nerves[1,2]

## NCS-measured sensory responses from the calf to the ankle[3]




Normal sensory response.




Reduced response in nerve conduction from the calf to the ankle

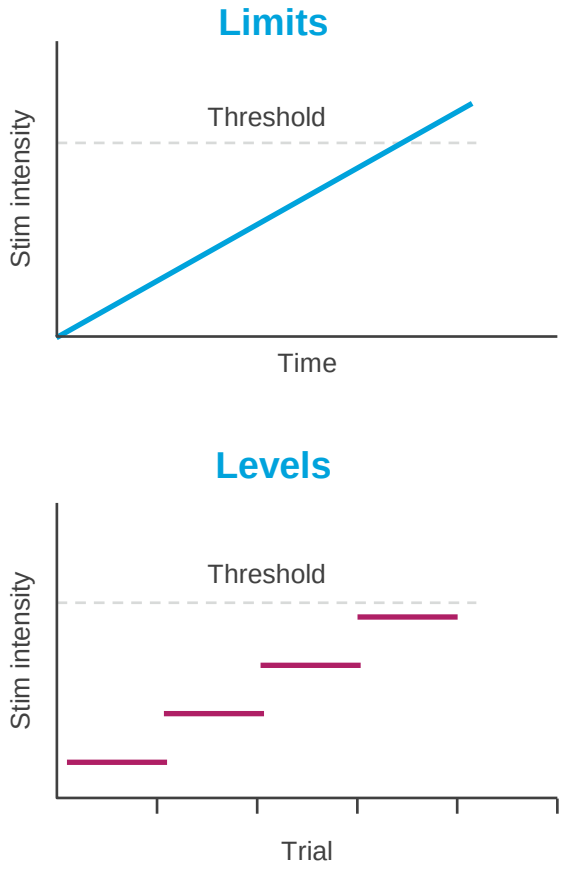
Abnormal sensory response, as seen in many neuropathies, including due to amyloid.

hATTR, hereditary transthyretin amyloidosis; NCS, nerve conduction studies.

1. Johns Hopkins Medicine. Nerve conduction studies. Accessed October 20, 2023. https://www.hopkinsmedicine.org/health/treatment-tests-and-therapies/nerve-conduction-studies. 2. Mallik et al. *J Neurol Neurosurg Psychiatry.* 2005;76(Suppl II):ii23–31; 3. Shin et al. *Mt Sinai J Med.* 2012;79(6):733–48.

# Quantitative Sensory Test (QST)

- QSTs measure the detection threshold of vibratory, thermal or pain stimuli and are used to detect small fiber neuropathy present in early disease manifestation of hATTR-PN[1,2]
- QST alone cannot confirm an hATTR-PN diagnosis[1]

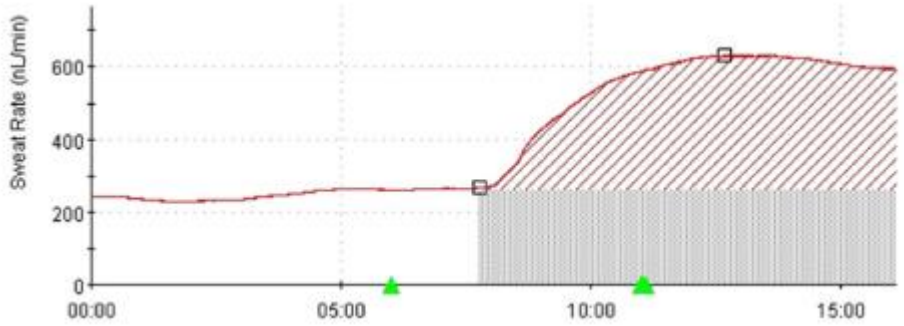



ATTR, transthyretin amyloidosis; hATTR, hereditary ATTR; QST, quantitative sensory test.
1. Shy et al. *Neurology.* 2003;60(6):898–904. 2. Cakar et al. *Noro Psikiyatr Ars.* 2019;56(2):150–6.

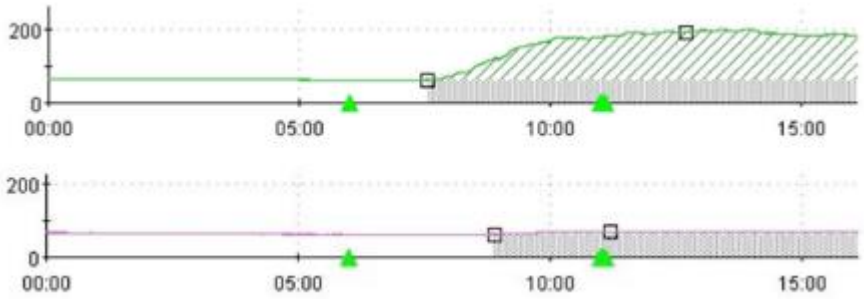
# Quantitative Sudomotor Axon Reflex Testing (QSART)

- QSART determines the volume of sweat produced by this stimulation over time and demonstrates small-fiber involvement and autonomic dysfunction, which typically occurs early in the hATTR disease course[1,2]

## Sweat rates measured by QSART[2]




Normal sweat response




Diminished (top) or near absent (bottom), abnormal sweat response

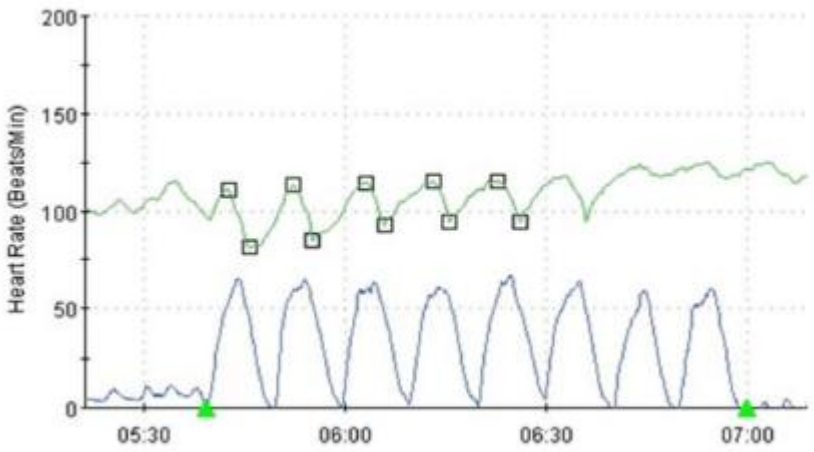
ATTR, transthyretin amyloidosis; hATTR, hereditary ATTR; QSART, quantitative sudomotor axon reflex testing.

1. Illigens and Gibbons. *Clin Auton Res.* 2009;19:79–87; 2. Shin et al. *Mt Sinai J Med.* 2012;79:733–48.

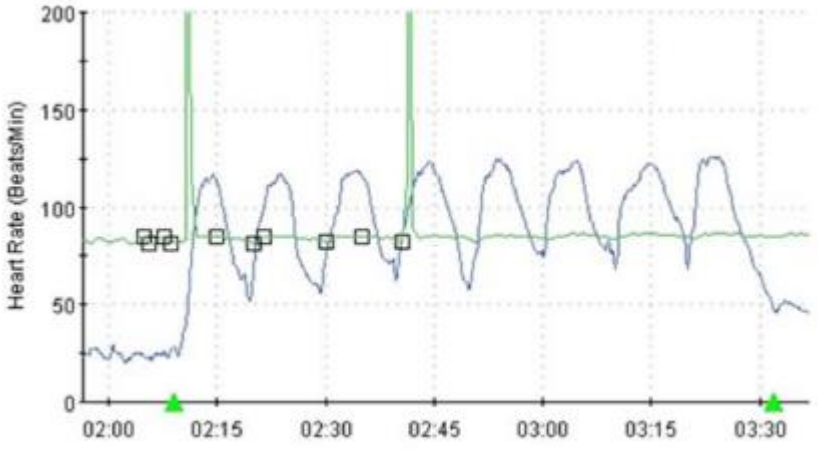
# Heart Rate Variability With Deep Breathing (HRdb)

- HRV and HRdb are sensitive measures of cardiovagal or parasympathetic cardiac function — which are often affected before sympathetic function — that can detect autonomic neuropathy, an early disease manifestation of hATTR-PN[1,2]

## Heart rate variability in response to deep breathing[3]




Normal heart rate (green trace) increases and decreases with paced deep breathing (blue trace).




Near absent heart rate variability (green trace) during paced deep breathing (blue trace) in a patient **with** autonomic neuropathy.

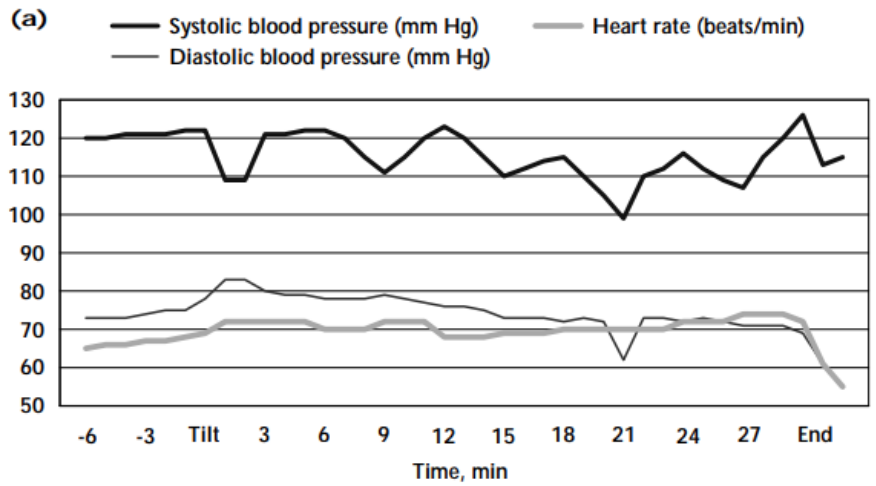
hATTR, hereditary ATTR; HRdb, heart rate variability with deep breathing; HRV, heart rate variability.

1. Gonzalez-Duarte. *Clin Auton Res.* 2019;29(2):245-51; 2. Shields. *Cleve ClinJ Med.* 2009;76(Suppl. 2):S37–40; 3. Shin, Robinson-Papp. *Mt Sinai J Med.* 2012;79:733–48.

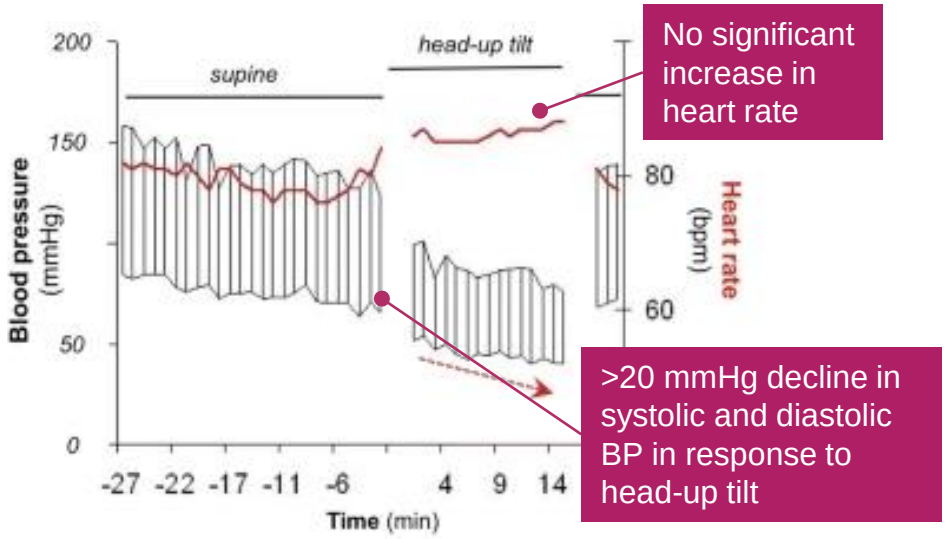
# Heart Rate Variability–Tilt Table Test

- A tilt table test measures heart rate and BP changes in response to postural changes, and can detect autonomic neuropathy (eg, orthostatic hypotension), an early disease manifestation of hATTR-PN[1,2]

## Heart rate and blood pressure response to upright tilt[3,4]




Normal heart rate and blood pressure response to upright tilt in a patient **without** ATTR




Substantial and sustained reduction in systolic and diastolic blood pressure without significant increase in heart rate. In response to upright tilt, in a patient **with** acquired hATTR-PN after domino liver transplant.

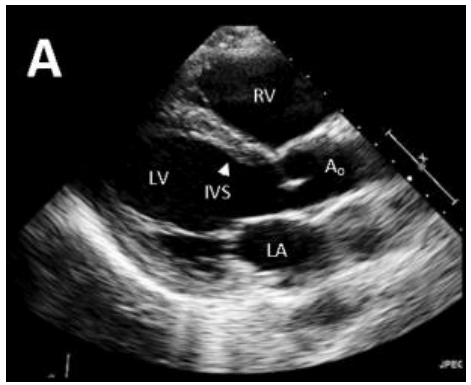
ATTR, transthyretin amyloidosis; BP, blood pressure; hATTR, hereditary ATTR.

1. Gonzalez-Duarte. *Clin Auton Res.* 2019;29(2):245-51; 2. Zysko D, Jamil RT, Anilkumar AC. In: StatPearls [Internet]. Updated October 25, 2022. Accessed October 20, 2023. https://www.ncbi.nlm.nih.gov/books/NBK482320; 3. Lamarre-Cliché et al. *CMAJ.* 2001;164(3):372–6; 4. Palma et al. *Clin Auto. Res.* 2019;29(Suppl1):S33–S44.

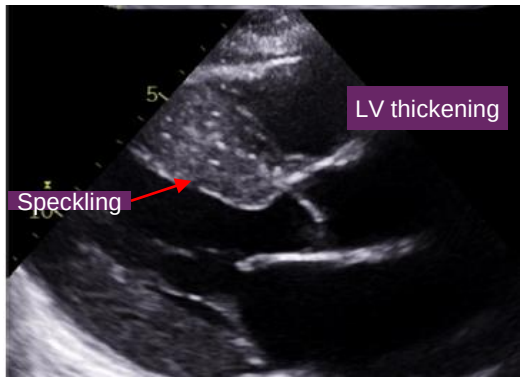
# Echocardiogram

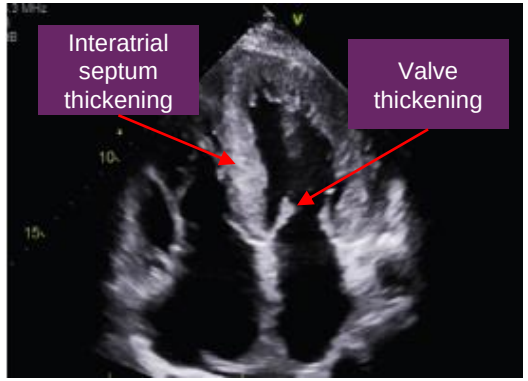
- Echocardiography uses ultrasonic waves to assess cardiac structure and function[1]
- Echocardiographic findings have low diagnostic accuracy due to low sensitivity, and echocardiography cannot be used to diagnose cardiac amyloidosis on its own[2]

## Echocardiogram imaging[2-4]



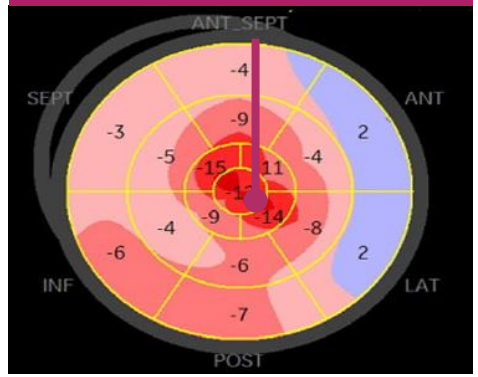

Echocardiogram of a patient with ATTR-CM **without** cardiac disease.




Echocardiograms showing increased septal thickening and speckling of the heart of patients **with** ATTR-CM

2D speckle tracking plot showing characteristic basal to apical gradient of impaired longitudinal function (bull's-eye pattern)




Images taken from Porcari et al. 2022,[2] ASNC. 2023,[3] and Dhamarajan et al. 2012[4]

ATTR, transthyretin amyloidosis; LV, left ventricle.

1 . Johns Hopkins Medicine. Echocardiogram. Accessed October 20, 2023. https://www.hopkinsmedicine.org/health/treatment-tests-and-therapies/echocardiogram; 2. Porcari et al. *Cardiovasc Res.* 2022;cvac119. doi:10.1093/cvr/cvac119;
*3.* ASNC. Think Amyloid Transthyretin cardiac amyloidosis (ATTR-CA). Accessed November 8, 2023. https://www.asnc.org/files/Think_Amyloid_Echocardiography_Laboratory.pdf 4. Dharmarajan et al. *J Am Geriatr Soc.* 2012;60:765–74.

# Cardiac MRI (CMRI)

- CMRI detects structural changes to the heart as a result of amyloid deposition in cardiac amyloidosis, such as[1]:
  - Thickened ventricular walls
  - Diffuse subendocardial LGE in the myocardial interstitial space
- CMRI can raise suspicion but cannot directly diagnose cardiac amyloidosis[1]

## CMRI imaging[2]

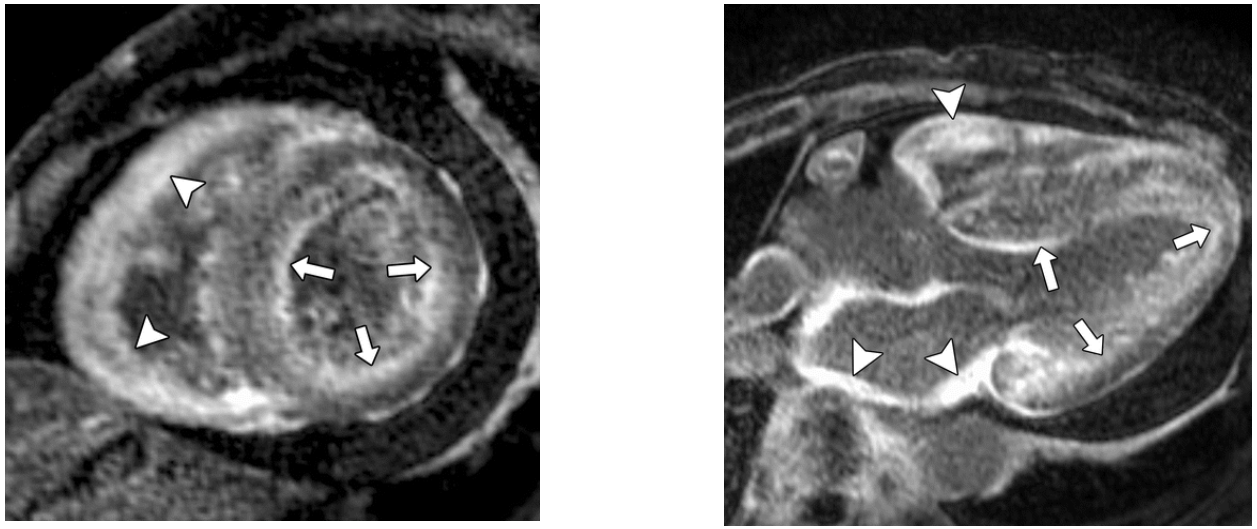


Image taken from Oda et al. 2020.[2]

MRI with LGE from a patient with ATTR-CM: heart mid-LV shot-axis view (left) and three-chamber LV outflow tract view (right) showing LV subendocardial LGE (arrows) and a dark blood pool, characteristic of cardiac amyloidosis.



ATTR, transthyretin amyloidosis; CMRI, cardiac magnetic resonance imaging; LGE, late gadolinium enhancement; LV, left ventricle; MRI, magnetic resonance imaging.

1. Dharmarajan et al. *J Am Geriatr Soc.* 2012;60:765–74; 2. Oda et al. *Radiographics.* 2020;40:961–81.

# Biopsy

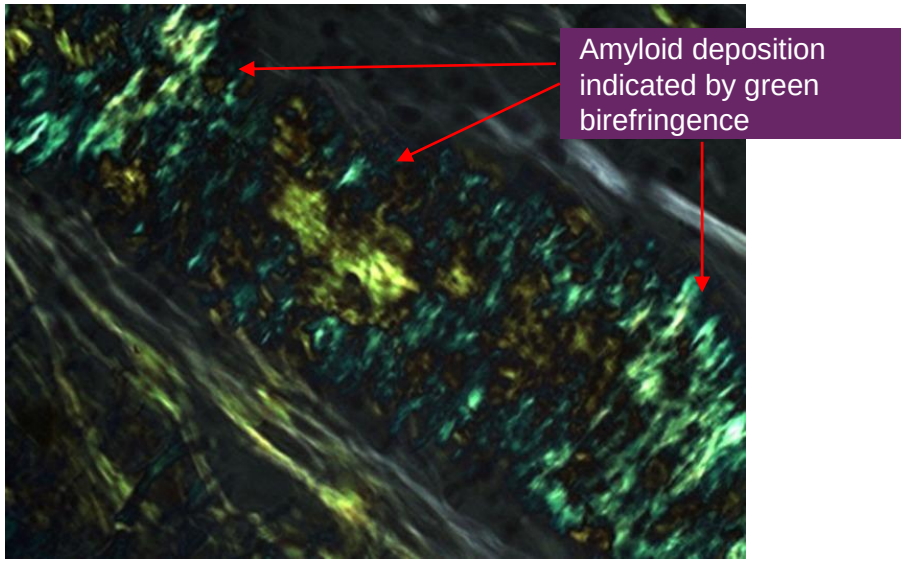
- Confirmation of amyloidosis can be obtained with a biopsy of a clinically affected organ, with Congo red histology demonstrating pathognomonic green birefringence indicative of amyloid deposition[1]

## Biopsy site sensitivity

| Biopsy sites | Sensitivity in wtATTR | Sensitivity in hATTR | Sensitivity in ATTR |
|---|---|---|---|
| **Heart**[2] | 99% | 100% | 99% |
| **SC adipose tissue** | 15%[3]–25%[2] | 45%[3]–91%[4] | 12%[5]–41.7%[6] |
| **GI** | 57%[2] | 74%[2] | 44.6%[7]–61%[2] |

As sensitivity varies depending on the biopsy site, negative biopsy results should not rule out an ATTR diagnosis[8]

## Congo-red positive tissue specimen[9]




Congo-red positive tissue specimen viewed under green birefringence (right)

ATTR, transthyretin amyloidosis; GI, gastrointestinal; hATTR, hereditary transthyretin ATTR; wtATTR, wild-type ATTR; SC, subcutaneous.

1. Gillmore et al. *Circulation.* 2016;133(24):2404–12; 2. Nishi et al *Circulation J.* 2022;86(7):1113–20; 3. Quarta et al. *Eur Heart J.* 2017;38(24)-1905–8; 4. Rokke et al. *Orphanet J Rare Dis.* 2020;15:278; 5. Garcia et al. *Hum Pathol.* 2018;72:71–9; 6. Hansen et al. *Molecules.* 2021;26(12):3649; 7. Cohen et al. *Eur J Haematol.* 2020;105(3):352–6; 8. Ando et al. *Orphanet J Rare Dis.* 2013;8:31; 9. Howie. *Int J Exp Path.* 2019;100:208–21.

# Genetic Testing

- Genetic testing should be considered in all patients suspected of ATTR, especially those presenting with “red flag” neurological signs (eg, in cases of idiopathic polyneuropathy with other symptoms or family history) and those with a positive scintigraphy imaging scan[1]
  - Genetic testing can distinguish between a wtATTR or hATTR diagnosis

| Genetic testing | Description |
|---|---|
| **Single gene testing** | Sequencing of the *TTR* gene alone for intragenic deletions/insertions and missense, nonsense, and splice site variants.[2] |
| **Multigene panel testing** | Includes analysis of any cardiomyopathy, neuropathy, or amyloidosis panel with *TTR*.[1] Identifies genetic cause of condition. Limits identification of variants of uncertain significance and pathogenic variants in genes unrelated to the underlying phenotype.[2]<br><br>Genes included in the panel and diagnostic sensitivity may vary by laboratory. Methods include sequence analysis, deletion/duplication analysis, and/or non-sequencing-based tests.[2] |



ATTR, transthyretin amyloidosis; hATTR, hereditary ATTR; wtATTR, wild-type ATTR; TTR, transthyretin.

1. Gillmore et al. *Adv Ther*. 2022;39:2292–301; 2. Sekijima. *Gene Rev*. Last updated June 2021. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1194/?report=classic (accessed October 5, 2022).

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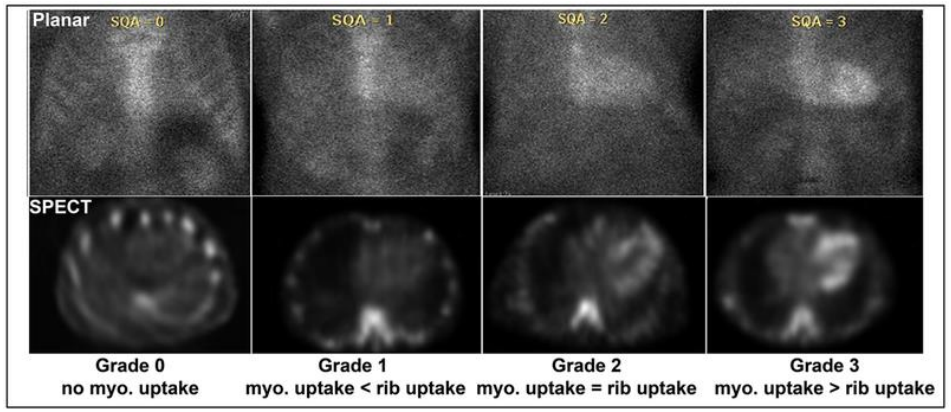
# Scintigraphy

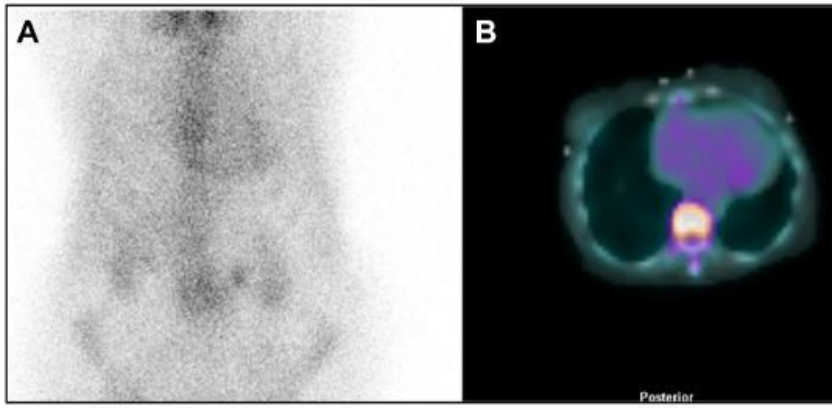
- Scintigraphy uses bone-avid ($^{99m}$Tc) radiotracers and is the only non-invasive confirmatory diagnostic tool for ATTR[1]
- Ratio of radiotracer uptake can be represented visually (visual grading of nuclear tracer uptake) or with semiquantitative metrics (H/CL and H/WB ratios)[2]

## Visual grading of $^{99m}$Tc-PYP planar and SPECT scans[2]




Images taken from Singh et al. 2019[2]

## Planar $^{99m}$Tc-PYP and SPECT/CT scans[2]



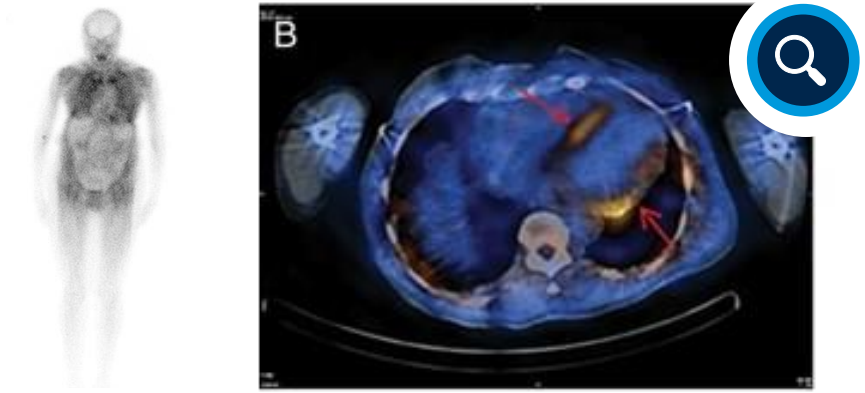

$^{99m}$Tc-PYP with Grade 2 cardiac uptake on the planar chest image (left), which was confirmed as blood pool activity on SPECT/CT fusion image (right), indicative of ATTR

ATTR, transthyretin amyloidosis; H/CL, heart-to-contralateral lung; H/WB, heart-to-whole body; SPECT, single-photon emission computed tomography; SPECT/CT, single-photon emission computed tomography/computed tomography; Tc-PYP, technetium pyrophosphate.

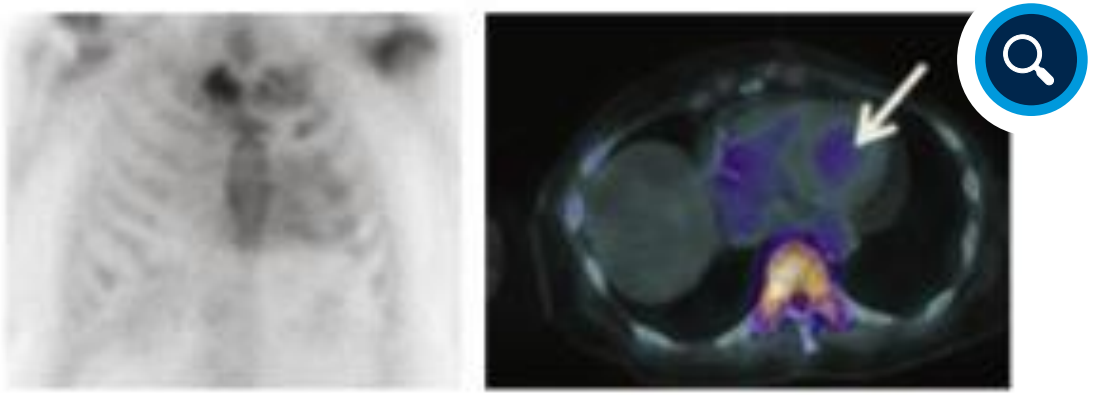
1. Ruberg et al. *J Am Coll Cardiol.* 2019;73(22):2872–91; 2. Singh et al. *J Nucl Cardiol.* 2019;26(1):158-173.

# Limitations of Scintigraphy

- Soft-tissue uptake may obscure visualization of tracer uptake in the heart on planar imaging[1]
- Overlying rib radiotracer uptake in the rib (eg, due to rib fracture) may give rise to a **false-positive** planar imaging[2]
- Blood pool uptake may cause **false-positive** planar imaging[3]




(A) False-negative soft-tissue uptake of $^{99m}$Tc-DPD with obscured cardiac uptake on planar imaging which is apparent on (B) SPECT-CT (arrows).



**Positive $^{99m}$Tc-PYP = blood pool uptake, no amyloid**
Misdiagnosis due to LV dysfunction with a false-positive planar imaging due to blood pool.

Images taken from Hutt et al. 2014[1] and Hanna et al. 2020[3]

**A negative $^{99m}$Tc-PYP scan does not exclude cardiac amyloidosis.[2] SPECT imaging should be used to verify cardiac uptake in the myocardium[3]**



LV, left ventricle; Tc-DPD, technetium 3,3-diphosphono-1,2-propanodicarboxylic acid; Tc-PYP, technetium pyrophosphate; SPECT, single-photon emission computed tomography.
1. Hutt et al. *Eur Heart J Cardiovasc Imaging.* 2014;15(11):1289–98; 2. Singh et al. *J Nucl Cardiol.* 2019;26(1):158-173; 3. Hanna et al. *J Am Coll Cardiol.* 2020;75(22):2851–62.

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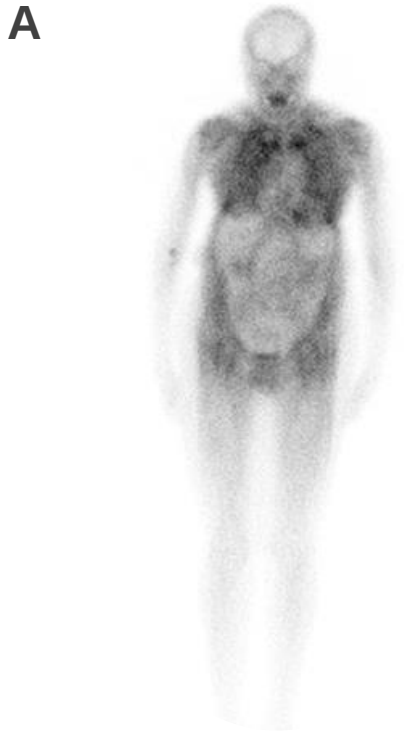
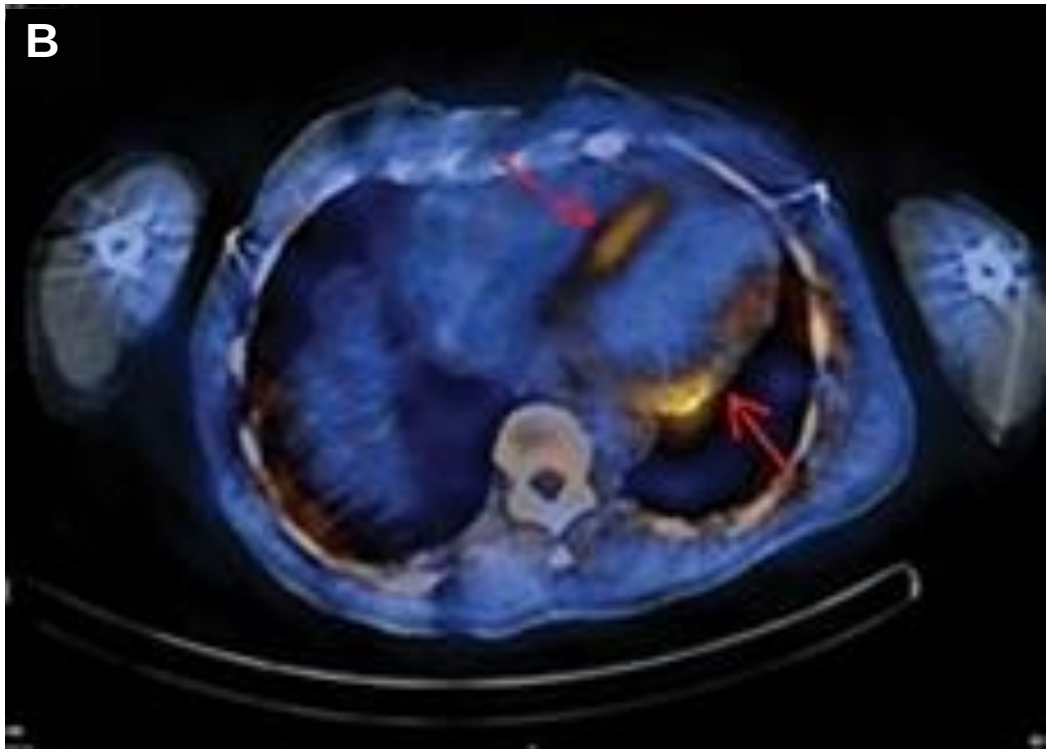
# Diagnostic Tools for ATTR : Scintigraphy (1/2)

## Limitations of scintigraphy

(A) Soft-tissue uptake of $^{99m}$Tc-DPD with obscured cardiac uptake on planar imaging which is apparent on (B) SPECT-CT (arrows).



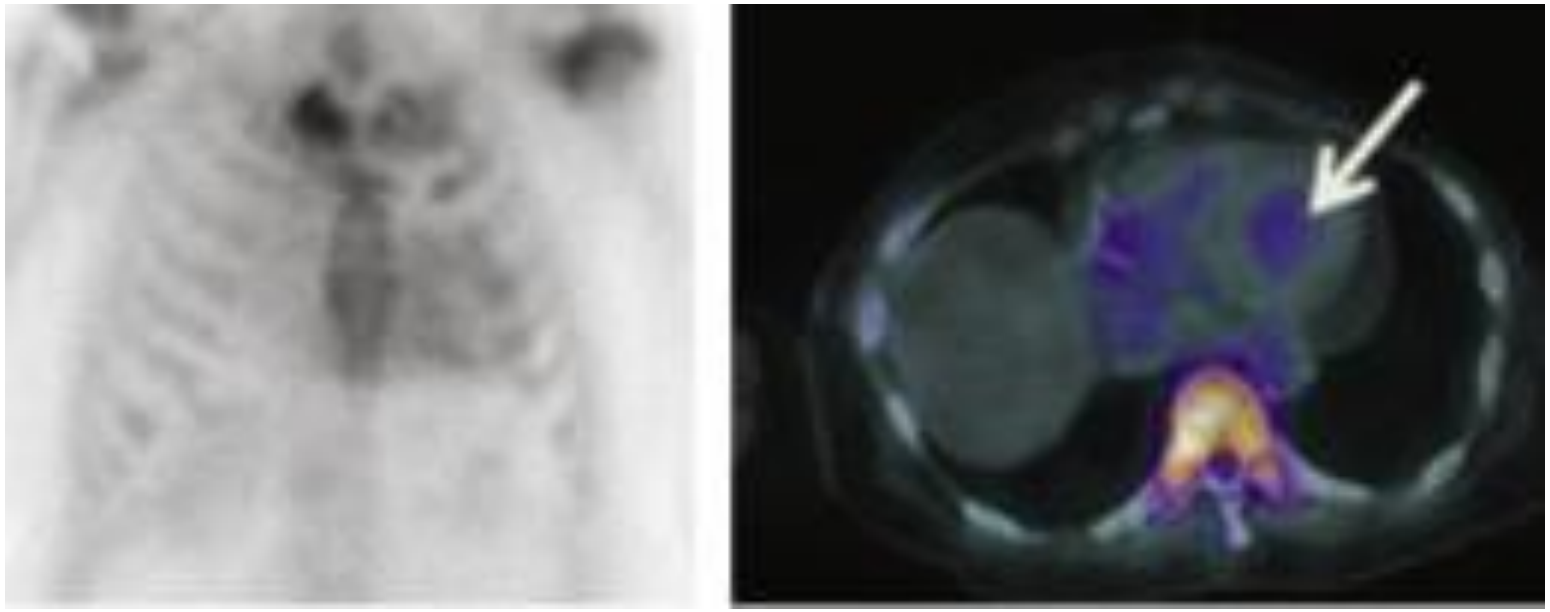

Tc-DPD, technetium 3,3-diphosphono-1,2-propanodicarboyxylic acid; SPECT-CT, single-photon emission computed tomography.

1. Hutt et al. *Eur Heart J Cardiovasc Imaging.* 2014;15(11):1289–98.

# Diagnostic Tools for ATTR: Scintigraphy (2/2)

## Limitations of scintigraphy

Misdiagnosis due to LV dysfunction with positive planar imaging due to blood pool.



**Positive PYP = blood pool uptake, no amyloid**

- **Always perform SPECT**

ATTR, transthyretin amyloidosis; LV, left ventricle; PYP, pyrophosphate; SPECT, single-photon emission computed tomography.

1. Hanna et al. *JACC.* 2020;75(22):2851–62.

# FAP/PND Score hATTR-PN Staging System

## Disease staging in patients with hATTR-PN is commonly based on FAP stages/PND scores[1,2]

### FAP Stage 1 (PND I, II)
**5.6** ± 2.8 years

- **Bilateral neuropathy** typically starting in the feet and legs
- **Motility disorders** and **sexual dysfunction** may also manifest early in disease
- **Unassisted walking**

### FAP Stage 2 (PND IIIa, IIIb)
**4.8** ± 3.6 years

- **Motor weakness** in the limbs
- **Loss of touch** sensation
- Mobility is declining, **crutches or a stick are needed for walking**
- **Pain and temperature sensation is lacking** apart from in the head and neck

### FAP Stage 3 (PND IV)
**2.3** ± 3.1 years

- **Generalized weakness**, cachexia, and incontinence
- Patient is **wheelchair-bound or bedridden**

## End-stage (death)

**Median survival from disease diagnosis is**

**4.7 years[3]**

Common causes of death include **cachexia, cardiac insufficiency, and infections**[2,4]

Progression through FAP/PND stages can lead to severe symptoms, disability, and mortality



hATTR, hereditary ATTR; FAP, familial amyloidotic polyneuropathy; PND, polyneuropathy disability.

1. Coutinho et al. *Amyloid and Amyloidosis. Excerpta Medica* 1980;88–98; 2. Adams. *Ther Adv Neurol Disord.* 2013;6(2):129–39; 3. Swiecicki et al. *Amyloid.* 2015;22:123–31; 4. Castaño et al. *Heart Fail Rev.* 2015;20:163–78.

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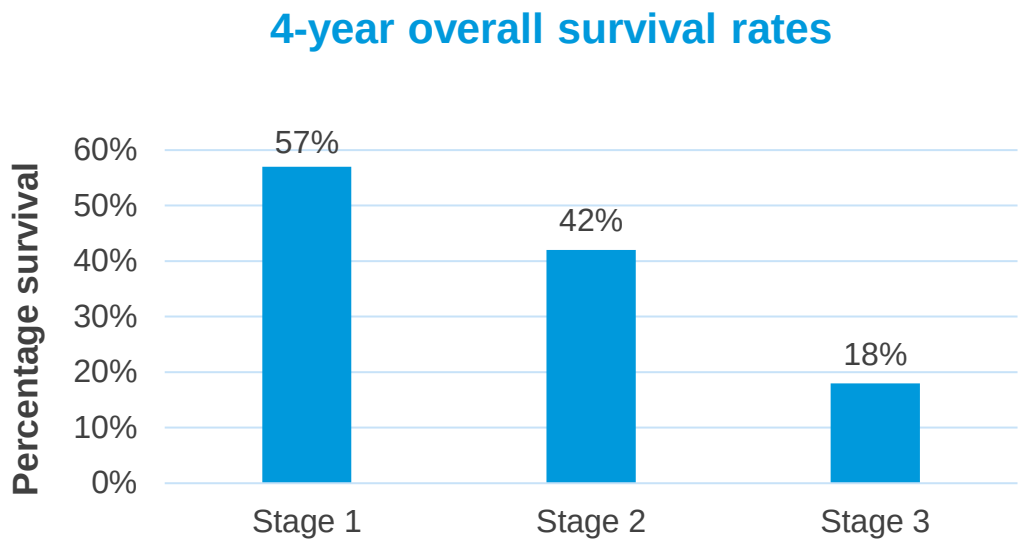
# Mayo Clinic ATTR-CM Prognostic Staging System

## Retrospective analysis of patients diagnosed with wtATTR-CM seen at the Mayo Clinic from 1965-2013[1]

- Cardiac biomarkers hold predictive power among patients with AL amyloidosis; thus, thresholds of **troponin T and NT-proBNP** were explored as prognosis for wtATTR. Cross-validation, in combination with the method of Contal and O'Quigley, established a cutpoint of **0.05 µg/L for troponin T** and **3,000 ng/L for NT-proBNP** to be associated with death
- Univariate predictors of survival included age, ejection fraction, mitral deceleration time, estimated right atrial and pulmonary artery systolic pressures, cardiac index, stroke volume index, serum uric acid, and the presence of a pericardial effusion
- Multivariate predictors of survival include age, ejection fraction, NT-proBNP, and troponin T




| Stage Criteria | Troponin T (µg/L) | NT-proBNP (ng/L) | Troponin T (µg/L) | NT-proBNP (ng/L) | Troponin T (µg/L) | NT-proBNP (ng/L) |
|---|---|---|---|---|---|---|
| | <0.05 | <3,000 | <0.05 | >3,000 | >0.05 | >3,000 |
| | | | >0.05 | <3,000 | | |

AL, primary/amyloid light chain; wtATTR, wild-type ATTR; NT-proBNP, N-terminal pro–B-type natriuretic peptide.

1. Grogan et al. *JACC*. 2016;68(10):1014–20.



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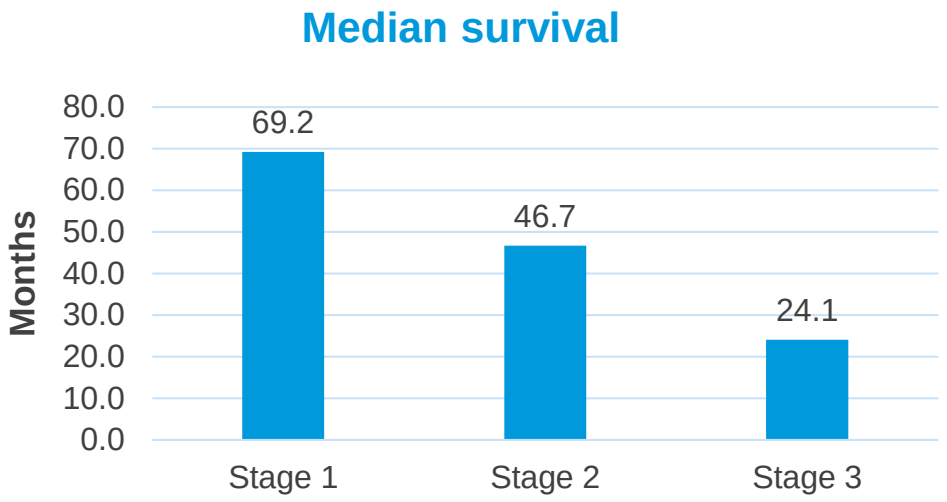
# UK ATTR-CM Prognostic Staging System

## Retrospective analysis of patients diagnosed with hATTR and wtATTR seen at the UK National Amyloidosis Centre[1]

- At the time of development, no validated staging system for ATTR-CM existed
  - Mayo Clinic's staging system does not include patients with hATTR-CM and is confounded by various troponin assays created by numerous manufacturers, with different favoring the use of different assays
- **NT-proBNP and eGFR**, along with age, were identified as independent prognostic factors for ATTR-CM and used to establish a novel staging system
- Optimal cut points for relevant variables were chosen based on a receiver operating characteristic curve, followed by the Youden method
  - Cut point for **NT-proBNP was 3000 ng/L** (67% sensitivity, 57% specificity)
  - Cut point for **eGFR was 45 ml/min/1.73 m²** (84% sensitivity, 32% specificity)




| Stage criteria | NT-proBNP (ng/L) | eGFR (ml/min/1.73m²) | NT-proBNP (ng/L) | eGFR (ml/min/1.73m²) | NT-proBNP (ng/L) | eGFR (ml/min/1.73m²) |
|---|---|---|---|---|---|---|
| | ≤3000 | ≥45 | ≤3000 | <45 | >3000 | <45 |
| | | | >3000 | ≥45 | | |

ATTR, transthyretin amyloidosis hATTR, hereditary ATTR; wtATTR, wild-type ATTR; eGFR, estimated glomerular filtration rate; NT-proBNP, N-terminal pro–B-type natriuretic peptide; UK, United Kingdom.

1. Gillmore et al. *Eur Heart J.* 2018;39:2799–806.



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# Diuretic Dose and NYHA Functional Class Addition to Established Risk Scores

## Model adjustment to Mayo Clinic and UK ATTR amyloidosis with cardiomyopathy staging systems[1]

- Previous risk models for ATTR-CM do not incorporate well-established and easily-obtained predictors of outcomes in heart failure
- **Diuretic dose** and **NYHA functional class**, added incremental value to existing risk models of ATTR cardiac amyloidosis all-cause mortality
  - Risk models using ROC curves with estimation of time-dependent AUC at the 2-year timepoint were used to predict mortality
- Daily diuretic dosing categorized into furosemide equivalents and assigned a point system
  - 0 mg/kg = 0 points
  - >0 to 0.5 mg/kg = 1 point
  - >0.5 to 1 mg/kg = 2 points
  - >1mg/kg = 3 points
- NYHA functional class was obtained and assigned 1 point per NYHA functional class, ranging from 1 to 4 points

| Model adjustment | | All-cause mortality AUC (95% CI) | Gain from reference | *P* value[a] |
|---|---|---|---|---|
| **Mayo model** | Mayo model | 0.693 (0.609–0.777) | Reference | Reference |
| | Diuretic dose only | 0.713 (0.627–0.799) | 0.020 | 0.784 |
| | Mayo + diuretic dose | 0.767 (0.692–0.843) | 0.074 | 0.046 |
| | Mayo + diuretic dose + NYHA functional class | 0.798 (0.729–0.868) | 0.105 | 0.006 |
| **UK model** | UK model | 0.711 (0.630–0.792) | Reference | Reference |
| | Diuretic dose only | 0.713 (0.627–0.799) | 0.002 | 0.918 |
| | UK + diuretic dose | 0.787 (0.717–0.856) | 0.076 | 0.059 |
| | UK + diuretic dose + NYHA functional class | 0.816 (0.749–0.883) | 0.105 | 0.009 |

[a]2-sided *P* value <0.05 was considered significant.
ATTR, transthyretin amyloidosis; AUC, area under the curve; CI, confidence interval; NYHA, New York Heart Association; ROC, receiver-operating characteristic; UK, United Kingdom.
1. Cheng et al. *J Am Coll Cardiol.* 2020; 2(3): 414–25.

# Summary

- ATTR is a multisystemic, rapidly progressive, debilitating, and fatal disease caused by misfolded TTR accumulating as amyloid deposits in multiple organs and tissues including nerves, heart, and GI tract [1-4]
  - Patients diagnosed with hATTR and wtATTR amyloidosis have a median survival of 4.7[5] and 2.5-5.5 years,[6-8] respectively
- ATTR remains underdiagnosed or misdiagnosed[4,9,10]
- Patients with ATTR experience substantial burden, including reduced QoL[11-14] and functional impairment[6,15]

## There remains a need for health care professionals to:

**1** Recognize the constellation of red-flag symptoms of ATTR[16,17]

**2** Collaborate with a multidisciplinary team for a potential diagnosis[16,17]

**3** Employ the diagnostic algorithm and confirmatory diagnostic tools to verify diagnosis[17-19]

**4** Assess progression of disease following treatment and provide patient with holistic care (mental, physical, and social support)[20,21]

ATTR, transthyretin amyloidosis; hATTR, hereditary ATTR; wtATTR, wild-type ATTR; GI, gastrointestinal; QoL, quality of life; TTR, transthyretin.

1. Hanna. *Curr Heart Fail Rep.* 2014;11:50–7; 2. Mohty et al. *Arch Cardiovasc Dis.* 2013;106:528–40; 3. Adams et al. *Neurology.* 2015;85:675–82; 4. Maurer et al. *Circ Heart Fail.* 2019;12:e006075; 5. Swiecicki et al. *Amyloid.* 2015;22:123–31; 6. Lane et al. *Circulation.* 2019;140:16–26; 7. Aus dem Siepen et al. *Clin Res Cardiol.* 2018;107(2):158–69; 8. Givens et al. *Aging health.* 2013;9(2):229–35; 9. Hawkins et al. *Ann Med.* 2015;47:625–38; 10. Castano et al. *Heart Fail Rev.* 2015;20:163–78; 11. Coehlo et al. *Muscle Nerve.* 2017;55:323–32; 12. Vinik et al. *J Peripher Nerv Syst.* 2014;19:104–14; 13. Ines et al. *ISPOR Congress* 2015. Poster N21; 14. Obici et al. *Amyloid.* 2020;27:153–62; 15. Bolte et al. *Orphanet J Rare Dis* 2020;15:287; 16. Nativi-Nicolau et al. *Heart Fail Rev*. 2022;27(3):785–93; 17. Kittleson et al. *JACC.* 2023; 81(11):1076–176; 18. Namiranian and Geisler. *Am J Med.* 2022;135 Suppl 1:S13–19; 19. Ando et al. *Orphanet J Rare Dis.* 2013;8:31; 20. Adams et al. *Orphanet J Rare Dis.* 2021;16:411; 21. Obici et al. *BMJ Open.* 2023;13:e073130.



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