SUPPLEMENTAL MATERIAL

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Echocardiographic methods

Echocardiographic evaluation was performed using a GE Vivid E9 ultrasound machine equipped with a 5S probe (London, Trieste, Bologna) and a GE Vivid E80 (Florence, Genoa), and measurements performed offline using EchoPAC software (Version 202). At least 3 consecutive beats were recorded for each view, and images were stored for off-line analysis. LV chamber morphology was assessed following the latest American Society of Echocardiography/European Association of Cardiovascular Imaging Guideline[20]: LV mass was calculated using Devereux's formula, relative wall thickness (RWT) was calculated as 2*posterior wall thickness in diastole/Left ventricular end diastolic diameter (2*PWTd/LVEDD), stroke volume (SV) was calculated as SV was calculated as: end diastolic volume (EDV) − end systolic volume (ESV), as previously reported.[36] Left atrial area (LAA) and right atrial area (RAA) were measured in the 4-chamber view. LV ejection fraction (EF) was calculated with the biplane Simpson's method from volumes acquired in both the 4-chamber and the 2-chamber views. Tricuspid annular plane systolic excursion (TAPSE) was assessed with M-mode in the 4-chamber view. LV early (E wave), late (A wave) diastolic filling, its ratio (E/A) were evaluated with pulsed Doppler in the 4-chamber view. Lateral and septal mitral annulus velocity (e' wave) was assessed with tissue Doppler in the 4-chamber view; the ratio between the LV early diastolic filling wave and septal mitral annulus velocity (E/e') was calculated.[37] Pulmonary artery systolic pressure (PASP) was estimated based on the peak tricuspid regurgitation (TR) velocity, as described by the simplified Bernoulli equation, taking into account right atrial pressure , as estimated based on the diameter and respiratory variation in diameter of the inferior vena cava.[20,38,39] Digitally acquired clips were considered suitable for offline 2D Speckle Strain Imaging analysis if at least three cardiac cycles were available, with high frame rates (70 to 100 frame/s) and

without dropout of more than one LV segment or significant foreshortening of the ventricle. The endocardial border was traced at the end-diastolic frame in the apical view. End-diastole was defined by the QRS complex or by the frame just before mitral valve closure. The software tracked speckles along the endocardial and epicardial borders throughout the cardiac cycle, and the width of the region of interest was adjusted to fit the entire myocardium. All strain and strain-derived variables were measured in the apical 4-chamber view. Peak longitudinal strain (LS) was computed automatically, generating regional data from 6 segments (basal, mid, apical interventricular septum and basal, mid, apical lateral wall), to calculate an average value. Strain-derived variables were acquired and calculated according to previous studies: septal longitudinal systolic apex to base (SAB) ratio[40] and relative apical longitudinal strain (RALS) as the average 4-chamber apical segments peak longitudinal strain/average basal and mid 4-chamber peak longitudinal strain.[41]

Supplementary Table 1. Technical characteristics, cameras, bone tracers and acquisition protocols adopted in each participating centre.

	Trieste	Bologna before Oct 2016	Bologna after Oct 2016	Genoa	Florence	London
Acquisition machine	Symbia SPECT/TC T2 Siemens	ECAM 7776, Siemens Medical Systems	Discovery NM 670 GE	Discovery NM 630 GE	Discovery GE, ECAM Siemens	Infinia Hawkeye 4, Discovery NM 670 GE
Tracer molecule	PYP	DPD	DPD	HMDP	HMDP	DPD
Mean dose per exam	370 MBq	740 MBq	740 MBq	740 MBq	740 MBq	700 MBq
Planar images (matrix)	256x256	256x1024	256x256	256x256	256x1024	256x1024
SPECT images (matrix)	64x64	128x128	128x128	64x64	128x128	128x128
Collimator	LEHR	LEHR	LEHR	LEHR	LEHR	LEHR
Acquisition after injection	60 and 80 min	5 and 180 min	5 and 180 min	180 min	150 min	180 min
Elaboration software for planar images	Hermes NM Processing	Xeleris Functional Imaging	Xeleris Functional Imaging	Xeleris Functional Imaging	Xeleris Functional Imaging	Xeleris Functional Imaging
Elaboration software for SPECT images	Hermes Hybrid Reconstruction Cardiology Hermes Hybrid Recon	Volumetrix MI	Volumetrix MI	Myovation Evolution QPS/QGS	Volumetrix MI	Volumetrix MI used for fusing NM with CT (i.e localisation). Myovation used for cardiac reconstruction in positive studies.

Legend: CT. Computed Tomography; DPD. 3,3-Diphosphono-1,2-Propanodicarboxylic acid; HMDP. Hydroxymethylene Diphosphonate; LEHR. Low Energy High Resolution; MBq. Megabecquerel; PYP. Pyrophosphate; QPS/QGS. Quantitative Perfusion and Gated SPECT; SPECT. Single Photon Emission Computed Tomography.

Supplementary Table 2. Inter-operator variability for cardiac scintigraphy with bone tracer – Cohen's kappa

Parameter	Trieste	Genova	Bologna	London
Perugini score (grade 0 to 3)	0.986	0.914	0.965	0.958
Planar BiV uptake (yes vs no)	0.584	0.552	0.566	0.598
RV uptake on SPECT (yes vs no)	0.982	0.963	0.982	0.964

Legend: BiV. Biventricular; N. Number; RV. Right Ventricle; SPECT; Single-Photon Emission Computed Tomography.

Supplementary Table 3. Baseline characteristics of the UK cohort.

	Available data	Study population (n=1298)	Focal RV uptake (n=426)	Diffuse RV uptake (n=872)	p value
Age, years	1298	78 [72-83]	79 [72-83]	77 [72-82]	**0.044**
Male, %	1298	1137 (87.6%)	368 (86.4%)	769 (88.2%)	0.35
ATTRv-CM, %	1298	332 (25.6%)	108 (25.4%)	224 (25.7%)	0.89
V122I TTR	1298	190 (14.6%)	63 (14.8%)	127 (14.6%)	0.91
Ethnicity	1298				0.29
Caucasian		1065 (82.0%)	337 (79.1%)	728 (83.5%)	
Afro-Caribbean		217 (16.7%)	83 (19.5%)	134 (15.4%)	
Asian		11 (0.8%)	4 (0.9%)	7 (0.8%)	
Other		5 (0.4%)	2 (0.5%)	3 (0.3%)	
Hypertension, %	1298	488 (37.6%)	192 (45.1%)	296 (33.9%)	**<0.001**
Diabetes, %	1298	204 (15.7%)	69 (16.2%)	135 (15.5%)	0.74
Stroke/TIA	1298	140 (10.8%)	47 (11%)	93 (10.7%)	0.84
Atrial fibrillation, %	1298	664 (51.2%)	211 (49.5%)	453 (51.9%)	0.41
PPM, %	1298	134 (10.3%)	44 (10.3%)	90 (10.3%)	1.00
LABORATORY					
eGFR	1298	60 [48-75]	62 [51-76]	59 [47-74]	**0.005**
NT-proBNP	1298	2687 [1345-4875]	1885 [946-3880]	2996 [1674-53632	**<0.001**
NAC ATTR Stage	1298				**<0.001**
I		673 (51.8%)	268 (62.9%)	405 (46.4%)	
II		456 (35.1%)	112 (26.3%)	344 (39.4%)	
III		169 (13.0%)	46 (10.8%)	123 (14.1%)	
ECHOCARDIOGRAPHY					
IVS, mm	1298	17 [15-18]	16 [14-17]	17 [16-19]	**<0.001**
PW, mm	1298	16 [15-18]	15 [13-17]	17 [16-18]	**<0.001**
RWT	1276	0.75 [0.63-0.87]	0.68 [0.56-0.81]	0.79 [0.66-0.90]	**<0.001**
LVM index, gr/m2	992	169 [139-208]	159 [129-196]	174 [145-214]	**<0.001**
LVEF, %	1298	50% [42-56]	51% [44-59]	50% [41-55]	**<0.001**
LVEF < 50%	1298	599 (46.7%)	176 (42.1%)	423 (49%)	**0.021**
SV index, ml/m2	1271	20.6 [16.1-26]	21.9 [16.8-27.7]	20.2 [15.9-25.5]	**<0.001**
LV-GLS, %	1256	-10.7 [-13.5 to -8.5]	-11.9 [-15 to -9.4]	-10.4 [-12.8 to -8.2]	**<0.001**
LAA index, cm2/m2	1266	13.1 [11.3-15.4]	12.6 [10.8-15.3]	13.3 [11.5-15.5]	**0.001**
RAA index, cm2/m2	1266	11.9 [9.8-14.3]	11.8 [9.2-14.1]	12.0 [10.1-14.5]	**0.009**
E/e'	1260	15 [11.6-19]	13.9 [10.9-17.7]	15.0 [12-20]	**<0.001**
TAPSE, mm	1218	16 [12-19]	17 [13-20]	15 [12-19]	**<0.001**
S' RV	1088	10 [8-12]	11 [9-13]	10 [8-12]	**<0.001**
sPAP, mmHg	774	37 [24-46]	37 [23-47]	37 [25-45]	0.45
CARDIAC SCINTIGRAPHY					
Perugini grade	1298				**<0.001**
I		55 (4.2%)	53 (12.4%)	2 (0.2%)	
II		1072 (82.6%)	344 (80.8%)	728 (83.5%)	
III		171 (13.2%)	29 (6.8%)	142 (16.3%)	

Legend: ATTRv-CM, variant Transthyretin Amyloid Cardiomyopathy; eGFR, Estimated Glomerular Filtration Rate; IVS, Interventricular Septum; LAA, Left Atrial Area; LV-GLS, Left Ventricular Global Longitudinal Strain; LVEF, Left Ventricular Ejection Fraction; LVM, Left Ventricular Mass; MR, Mitral Regurgitation; NAC, National Amyloidosis Centre; NT-proBNP, N-Terminal pro Brain Natriuretic Peptide; PPM, Permanent Pacemaker; PW, Posterior Wall; RV, Right Ventricle; RWT, Relative Wall Thickness; sPAP; Systolic Pulmonary Arterial Pressure; TAPSE. Tricuspid Annular Plane Systolic Excursion; TIA, Transient Ischemic Attack; TTR. Transthyretin; TR, Tricuspid Regurgitation.

Supplementary Table 4. Univariable and multivariable Cox regression analysis for all-cause mortality in the UK cohort.

Variables	Missing data	Univariable model		Multivariable model		
		HR (95% CI)	p value	Variables	HR (95% CI)	p value
Age, year	0	1.04 (1.02 - 1.05)	**<0.001**	Age, year	1.03 (1.02 - 1.04)	**0.001**
Male sex	0	1.06 (0.80 – 1.40)	0.69			
ATTRv-CM	0	1.15 (0.94 – 1.41)	0.17			
V122I TTR	0	1.47 (1.16 – 1.85)	**0.001**	V122I TTR	1.42 (1.20 – 1.81)	**0.004**
Diabetes Mellitus	0	1.32 (1.05 – 1.67)	**0.018**	Diabetes Mellitus	1.17 (0.92 - 1.50)	0.20
Hypertension	0	0.99 (0.83 – 1.20)	0.96			
AF	0	1.18 (0.99 – 1.42)	0.07			
Stroke/TIA	0	0.92 (0.69 – 1.24)	0.61			
Pacemaker	0	1.13 (0.86 – 1.50)	0.38			
eGFR, ml/min	0	0.98 (0.97 - 0.98)	**<0.001**			
NT-proBNP per each 400 ng/L	0	1.03 (1.03 - 1.04)	**<0.001**			
NAC ATTR Stage	0	Reference	**<0.001**	NAC Stage	Reference	**<0.001**
II		2.59 (2.10 – 3.20)	**<0.001**	II	1.71 (1.35-2.16)	**<0.001**
III		3.38 (2.62 – 4.37)	**<0.001**	III	2.10 (1.58-2.80)	**<0.001**
IVS, mm	0	1.11 (1.07 - 1.15)	**<0.001**			
RWT	22	5.27 (3.24 –8.56)	**<0.001**			
LVM index, gr/m2	306	0.99 (0.99 – 1.01)	0.13			
LVEF, per %	0	0.97 (0.96 – 0.97)	**<0.001**			
LVEF<50%	0	2.00 (1.65 – 2.40)	**<0.001**			
SV index, ml/m2	27	0.96 (0.95 – 0.97)	**<0.001**	SV index, ml/m2	0.99 (0.97 – 0.99)	**0.043**
E/e'	38	1.03 (1.02 – 1.05)	**<0.001**	E/e'	1.02 (1.007 - 1.03)	**0.004**
LAA index, cm2/m2	32	1.08 (1.05 – 1.10)	**<0.001**			
RAA index, cm2/m2	32	1.08 (1.06 – 1.11)	**<0.001**	RAA index, cm2/m2	1.05 (1.02 - 1.08)	**0.001**
TAPSE, mm	80	0.94 (0.92 – 0.96)	**<0.001**			
LV-GLS, %	42	1.13 (1.10 – 1.16)	**<0.001**	LV-GLS, %	1.06 (1.03 - 1.09)	**<0.001**
Perugini grade	0	Reference	**0.006**	Perugini grade	Reference	0.57
II		2.82 (1.40-5.67)	**0.004**	II	1.31 (0.57-3.02)	0.52
III		3.30 (1.58-6.86)	**0.001**	III	1.15 (0.48 – 2.78)	0.75
Diffuse RV Uptake	0	2.08 (1.67 – 2.60)	**<0.001**	Diffuse RV Uptake	1.60 (1.26 - 2.04)	**<0.001**

Legend: ATTRv-CM, variant Transthyretin Amyloid Cardiomyopathy; CI, Confidence Interval; eGFR, Estimated Glomerular Filtration Rate; IVS, Interventricular Septum; LAA, Left Atrial Area; LV-GLS, Left Ventricular Global Longitudinal Strain; LVEF, Left Ventricular Ejection Fraction; LVM, Left Ventricular Mass; MR, Mitral Regurgitation; NAC, National Amyloidosis Centre; NT-proBNP, N-Terminal pro Brain Natriuretic Peptide; PPM, Permanent Pacemaker; PW, Posterior Wall; RV, Right Ventricle; RWT, Relative Wall Thickness; sPAP; Systolic Pulmonary Arterial Pressure; TAPSE. Tricuspid Annular Plane Systolic Excursion; TIA, Transient Ischemic Attack; TTR. Transthyretin; TR, Tricuspid Regurgitation.

Supplementary Table 5. Extent of LV and RV uptake on SPECT/CT according to Perugini grade in the whole study population.

|	Perugini grade 1 (n=62)	Perugini grade 2 (n=1133)	Perugini grade 3 (n=227)
LV assessment on SPECT			
LV focal	51.6% (32)	0% (0)	0% (0)
LV diffuse	48.4% (30)	100% (1133)	100% (227)
RV assessment on SPECT			
RV focal	96.8% (60)	33.6% (381)	19.8% (45)
RV diffuse	3.2% (2)	66.4% (752)	80.2% (182)

Legend: LV, Left Ventricle, RV, Right Ventricle.

Supplementary Table 6. Association of diffuse RV uptake with outcome according to TTR genotype

Outcomes	Wild-Type ATTR-CM (n=1076)		Variant ATTR-CM (n=346)		*P*-interaction
	Focal RV uptake	Diffuse RV uptake	Focal RV uptake	Diffuse RV uptake	
All-cause mortality					
N. of events	77	291	32	94	0.18
HR (95% CI)	2.21 (1.72 to 2.85)		1.58 (1.06 to 2.37)		

Legend: ATTR-CM, Transthyretin Amyloid Cardiomyopathy, CI, Confidence Interval, HR, Hazard Ratio, N, Number, RV, Right Ventricle, TTR, Transthyretin.

Supplementary Figure 1.
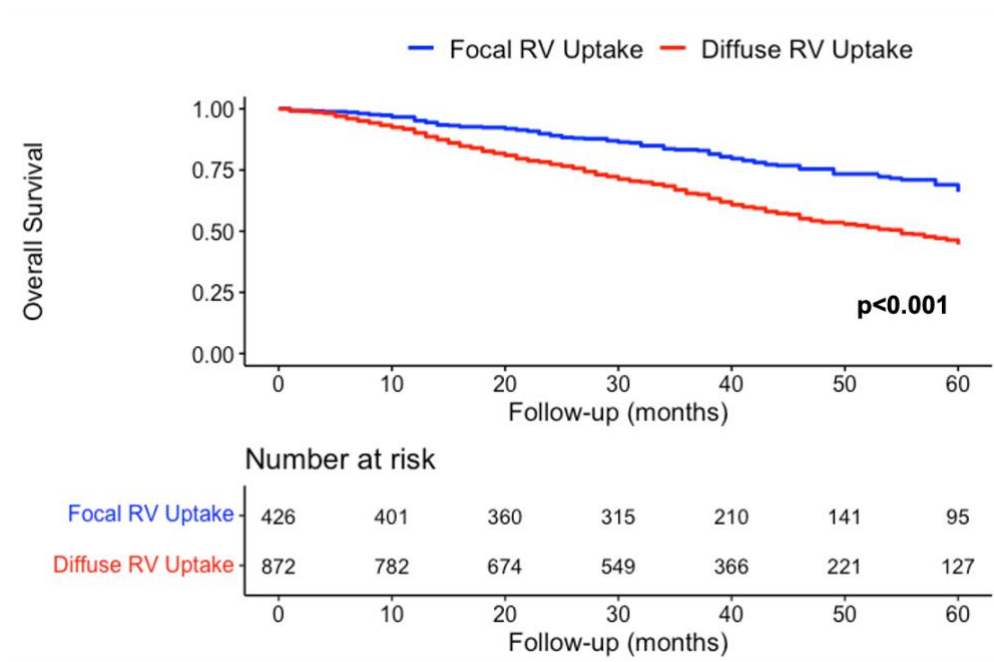
Supplemental Figure Legends

Supplementary Figure 1. Kaplan-Meier survival in the UK cohort stratified by extent of RV uptake on SPECT imaging. Legend: RV, Right Ventricular.