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ORIGINAL ARTICLE

Progression and prognostic significance of electrocardiographic findings in patients with cardiac amyloidosis

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Abstract

Aims This study aimed to evaluate the change of the main electrocardiographic (ECG) characteristics and their prognostic role across the main subtypes of cardiac amyloidosis [light-chain amyloidosis (AL) and hereditary (ATTRv) and wild-type transthyretin amyloidosis (ATTRwt)].

Methods and results This multicentre, retrospective study was performed in six referral centres for cardiac amyloidosis. Clinical and ECG data were collected at the first and last evaluations. Three hundred fifty-six patients were included (AL, $n = 105$; ATTRv, $n = 50$; ATTRwt, $n = 201$). The median age was 76 (67–81) years, and 271 (74%) were men. At baseline, patients with ATTRwt showed a higher prevalence of conduction abnormalities compared with those with AL [first-degree atrioventricular block, $n = 51$ (40%) vs. $n = 13$ (34%), $P < 0.01$; left bundle branch block, $n = 23$ (11%) vs. $n = 2$ (2%), $P < 0.01$], and patients with AL more often had low QRS voltage [$n = 58$ (55%); in ATTRv, $n = 17$ (34%); in ATTRwt, $n = 67$ (33%), P value < 0.01] and T wave inversion compared with those with ATTR [$n = 39$ (37%); in ATTRv, $n = 9$ (18%); in ATTRwt, $n = 37$ (18%)]. After a median follow-up of 15 (8–26) months, the adjusted differences in mean PR, QRS interval, total, peripheral, and precordial QRS scores were similar across subtypes of amyloidosis (P value for linear regression > 0.05). The adjusted odds ratios for the development of right bundle branch block were higher in AL compared with ATTRwt [odds ratio 4.7 (95% confidence interval 1.5–15), $P < 0.05$]. QRS duration at baseline remained independently associated with patient survival in the overall population even after adjustment for relevant clinical variables [hazard ratio 1.78 (95% confidence interval 1.13–2.8), $P < 0.01$].

Conclusions The progression of the ECG abnormalities seems similar across amyloidosis subtypes. QRS duration could be a marker of more advanced disease.

Keywords Cardiac amyloidosis; ECG; Light-chain amyloidosis; Transthyretin amyloidosis

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Introduction

Cardiac amyloidosis (CA) is an infiltrative disease characterized by the extracellular deposition of amyloid fibrils. The most common amyloid precursors are immunoglobulin light chains, originating from a plasmacellular clone, and senescent or mutated transthyretin, that respectively give rise to light-chain amyloidosis (AL) and wild-type (ATTRwt) and hereditary transthyretin amyloidosis (ATTRv). Electrocardiography (ECG) has a fundamental role in the diagnosis of

CA, with low QRS voltage patterns, pseudonecrosis, and conduction abnormalities as useful diagnostic clues.[1] Furthermore, artificial intelligence further increased the diagnostic power of ECG.[2] On the other hand, the prognostic role of ECG is still debated. Previous studies reported no correlation between adverse outcomes and ECG abnormalities,[3,4] while others highlighted the role of fragmented QRS in AL,[5] and low QRS voltages in both ATTR and AL as predictors of adverse prognosis.[6] Moreover, less is known about the progression of ECG features over time among the main subtypes of amyloidosis. In this study, for the first time, we describe the change of the main ECG characteristics over time and evaluate their prognostic role.

Methods

Study design

In this multicentre observational retrospective study, we included 446 patients evaluated between January 2017 and December 2020 in six referral centres for amyloidosis: Padua (Padua University Hospital), Trieste (Cattinara Hospital), Rome (Sant'Andrea Hospital), Florence (Careggi Hospital), Genoa (San Martino Hospital), and Messina (Messina University Hospital). All study sites received Institutional Review Board approval, and each patient provided written informed consent.

In patients with a monoclonal component, the diagnosis of AL was confirmed by biopsy of abdominal fat pad or of an involved organ. All positive biopsies showed typical Congo Red birefringence under polarized light and positivity for anti-k or anti-λ light-chain antibodies at immunohistochemistry or electron microscopy.[7] Cardiac involvement in AL amyloidosis was confirmed according to consensus criteria, and patients with AL have been treated according to current guidelines.[7,8] The diagnosis of ATTR was confirmed by tissue biopsy stained with anti-TTR antibodies at immunohistochemistry or electron microscopy (irrespectively of cardiac uptake at bone-tracer scintigraphy), or according to the non-invasive criteria.[1,9] All patients with ATTR underwent genetic testing. The study population was followed up from the first cardiological evaluation at the referral centre, considered as baseline, until the end of the study period, 31 October 2021. The following clinical data registered within ±1 month from the baseline and last follow-up visit have been recorded: physical examination, ECG, and echocardiography. Methods and study population have been described in previous works from the same group.[6,10]

Electrocardiography

Twelve-lead ECGs were acquired at standard speed (25 mm/s) and amplification (10 mm/mV) and retrospectively reviewed for heart rhythm, atrioventricular (AV), and intraventricular conduction abnormalities [right bundle branch block (RBBB), left bundle branch block (LBBB), and left anterior fascicular block (LAFB)]. LAFB, RBBB, and LBBB were identified as previously defined.[11,12] The pattern including negative T waves of $\geq$0.1 mV in depth in $\geq$2 contiguous leads was defined as T wave inversion (TWI) in absence of R/LBBB. Low QRS voltages were defined in case of total QRS amplitude $<$ 5 mm in all peripheral leads, or when the total QRS amplitude was $<$10 mm in all precordial leads.[13] Pseudonecrosis was defined in presence of Q wave duration $>$ 40 ms and Q wave amplitude $>$ 25% of the total QRS amplitude in at least two contiguous leads in the absence of ischaemic heart disease and LBBB.[14] Peripheral and precordial QRS scores were calculated through the sum of Q, R, and S heights, each taken as absolute value in millimetres (1 mm = 0.1 mV), respectively, in peripheral and precordial leads.[15]

Patients with a pacemaker (PM) at the first or last evaluation have been excluded from the present analysis due to the impossibility to obtain reliable ECG measurements. Patients with R/LBBB have been excluded from the QRS score measurements.

Echocardiography

Echocardiographic data were systematically reviewed for the present study. Echocardiography was performed according to the American Society of Echocardiography recommendations.[16] Left ventricular ejection fraction was assessed using biplane Simpson's equation method. Tricuspid annular plane systolic excursion (TAPSE) was used as a measure of right ventricular systolic function. The E-to-e' ratio was calculated as the ratio between early diastolic trans-mitral flow velocity (E) and the average of tissue Doppler-derived early diastolic peak velocity (e') at the lateral and medial mitral annuli.

Biomarkers

Brain natriuretic peptide (BNP) and N-terminal pro-BNP (NT-proBNP) were measured according to local laboratories. High BNP levels were defined as NT-proBNP $>$ 332 ng/L or BNP $>$ 81 ng/L for AL[17] and NT-proBNP $>$ 3000 ng/L or BNP $>$ 250 ng/L for ATTR.[18,19] Patients with ATTR were staged according to Gillmore *et al.*[18] in stages from I (NT-proBNP and eGFR of $\leq$3000 ng/L and 45 mL/min/1.73 m^2) to III (both NT-proBNP and eGFR above the cut-off point).

Statistical analysis

Continuous data are expressed as medians and interquartile ranges, while categorical data are expressed as numbers

and percentages. The non-parametric Kruskal–Wallis test was used to compare continuous variables among the three subgroups. When a significant result arose, the Dunn test was used on pairwise comparisons to establish which groups were different, accounting for multiplicity. Categorical variables were analysed with the χ^2 test followed by pairwise *z*-tests with Bonferroni's correction for multiple comparisons. The Pearson linear correlation coefficient between echocardiography and ECG variables was calculated followed by a significance test for correlation coefficient different from 0.

For each continuous variable, the mean differences between groups (AL, ATTRwt, and ATTRv) at the last visit were estimated through linear regression, adjusting for baseline value, age, and duration of the follow-up. Similarly, for each binary variable, the adjusted odds ratios of comparison between groups at the last visit were estimated through logistic regression, adjusting for the same confounders.

Setting the follow-up time as time axis, the overall survival curves were obtained for the entire cohort and by QRS level (QRS $\leq$ 120 ms or QRS $>$ 120 ms) through the Kaplan–Meier estimator. The two curves were compared via the log-rank test. Then the association between patients' characteristics and mortality has been explored following a two-step procedure. First, the variables were included one at a time in a univariate Cox regression model. Second, the variables that were important predictors at the first step ($P < 0.05$) were included in a multivariable Cox regression model to determine which of them were independently associated with mortality. Co-linearity of variables was tested. The follow-up duration was 15 (8–26) months in the whole population, and 15 (8–26), 22 (13–29), and 14 (9–24) months for AL, ATTRv, and ATTRwt, respectively. Receiver operating characteristic (ROC) curves were used to identify the optimal QRS duration cut-off for the considered outcome. A *P* value < 0.05 was considered statistically significant. Analyses were performed with STATA 17 (StataCorp, College Station, TX, USA) and SPSS 20 Version IBM Package, with *P* values < 0.05 considered statistically significant.

Results

Baseline characteristics

There were 455 patients in the whole study cohort, and 9 patients did not have ECG data at follow-up, generating a cohort of 446 patients (121 patients with AL, 63 with ATTRv, and 262 with ATTRwt). The most frequent TTR mutations in the ATTRv-CA cohort were Glu89Gln (*n* = 18, 28%), Ile68Leu (*n* = 11, 17%), and Val30Met (*n* = 6, 10%). Patients with a PM at the first evaluation (*n* = 49, 7 patients with AL, 10 with ATTRv, and 32 with ATTRwt) and patients who implanted a PM during follow-up (*n* = 41, 9 patients with AL, 3 with ATTRv, and 29 with ATTRwt) were excluded from the analysis (see Supporting Information, *Figure S1* for the study flow chart).

A total of 356 patients were included in this analysis (AL, *n* = 105; ATTRv, *n* = 50; ATTRwt, *n* = 201). The clinical characteristics of the patients at the first evaluation and at follow-up are summarized in *Table 1* and Supporting Information, *Table S1*, respectively. The median age was 66 (58–73) years and 65% (*n* = 69) were men among patients with AL; the median age was 69 (56–75) years and 76% (*n* = 38) were men in patients with ATTRv; and the median age was 81 (76–83) years and 82% (*n* = 164) were men among patients with ATTRwt.

Baseline electrocardiographic parameters

The ECG parameters at baseline are displayed in *Table 2*. Patients with ATTRwt had the longest PR interval and a higher proportion of patients with first-degree AV block compared with those with AL. QRS duration was similar across the subtypes of CA. The highest total and precordial QRS scores were found in patients with ATTRwt. Patients with ATTRwt more frequently showed LBBB compared with those with AL. Patients with AL more frequently showed low QRS voltage compared with those with ATTRv and ATTRwt. Anterior pseudo-infarction pattern was more common in AL compared with ATTRwt. Patients with AL more commonly presented with TWI compared with those with ATTR.

Electrocardiographic parameters at follow-up

After a follow-up of 15 (8–26) months, patients with ATTRwt continued to show the longest PR interval and more commonly presented first-degree AV block compared with those with AL (Supporting Information, *Table S2*). QRS duration was longer in patients with ATTRwt compared with those with AL and ATTRv. The greatest total and peripheral QRS scores were seen in patients with ATTRwt. Patients with ATTRwt presented LBBB more commonly compared with those with AL. Patients with AL showed low QRS voltage and TWI more frequently compared with those with ATTRwt.

Changes over time of electrocardiographic features

The adjusted differences in mean PR interval, QRS interval, total, peripheral, and precordial QRS scores were similar across subtypes of amyloidosis (*Table 3*). No differences in the adjusted odds ratios for the development of LBBB, LAFB, low QRS voltage, pseudo-infarction pattern, and TWI were seen. The odds ratios for the development of RBBB were higher in AL compared with ATTRwt [odds ratio 4.7 (95% confidence interval 1.5–15)] (*Table 4*).

Table 1 Clinical and echocardiographic features among the three subtypes of amyloidosis at the first evaluation

	AL (n = 105)		ATTRv (n = 50)		ATTRwt (n = 201)		P value
BMI	**24 (22–27)**	**103**	**24 (22–27)**	**50**	**25 (23–28)**	**200**	**<0.01****
Age, years	**66 (58–73)**	**105**	**69 (56–75)**	**50**	**81 (76–83)**	**201**	**<0.01*,**,***
Male sex, n (%)	**69/36 (65%)**	**105**	**38/12 (76%)**	**50**	**164/62 (82%)**	**201**	**<0.01**
NYHA class		**105**		**50**		**201**	**<0.01**
I	**19 (18%)**		**10 (20%)**		**29 (14%)**		
II	**48 (45%)**		**34 (68%)**		**123 (61%)**		
III	**33 (31%)**		**6 (12%)**		**48 (24%)**		
IV	**5 (5%)**		**0 (0%)**		**1 (0.5%)**		
NAC stage				**35**		**124**	**<0.01**
I	**NA**	**NA**	**25 (71)**		**46 (37)**		
II			**9 (26)**		**65 (52)**		
III			**1 (3)**		**13 (10)**		
IVS thickness, mm	**15 (13–18)**	**105**	**16 (13–19)**	**50**	**17 (16–20)**	**200**	**<0.01**,****
LA diameter, mm	**44 (38–48)**	**101**	**43 (35–46)**	**49**	**46 (42–51)**	**199**	**<0.01**,****
EF, %	**55 (50–60)**	**104**	**60 (55–65)**	**50**	**55 (47–61)**	**199**	**0.02*****
E/e'	**17 (11–22)**	**84**	**11 (9–17)**	**46**	**17 (13–21)**	**175**	**<0.01*,****
TAPSE, mm	19 (16–22)	87	19 (16–22)	46	**19 (15–21)**	**182**	0.7
PAPs, mmHg	**32 (25–42)**	**80**	**30 (25–35)**	**46**	**38 (30–45)**	**173**	**<0.01**,****
Creatinine, mg/dL	**1.19 (0.83–1.6)**	**75**	**0.9 (0.72–1.1)**	**42**	**1.1 (0.92–1.3)**	**156**	**<0.01*,****
BNP, pg/mL	**658 (245–1130)**	**44**	**97 (45–169)**	**12**	**361 (157–6)**	**38**	**<0.01*,**,***
NT-proBNP, ng/L	**2694 (990–7829)**	**49**	**981 (322–4133)**	**31**	**3236 (1288–6266)**	**110**	**<0.01*,****
NT-proBNP > 3000 or BNP > 250, n (%)			**12/28 (30)**	**40**	**96/62 (61)**	**158**	**<0.01**
NT-proBNP > 332 or BNP > 81, n (%)	**73 (90%)**	**81**					

Bold values indicate statistical significance.
AL, light-chain amyloidosis; ATTRv, hereditary (genetically abnormal) transthyretin amyloidosis; ATTRwt, wild-type transthyretin amyloidosis; BMI, body mass index; BNP, brain natriuretic peptide; EF, ejection fraction; IVS, interventricular septum; LA, left atrial; NAC, National Amyloidosis Centre; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association; PAPs, systolic pulmonary artery pressure; TAPSE, tricuspid annular plane systolic excursion.
*$P < 0.05$ for AL vs. ATTRv.
**$P < 0.05$ for AL vs. ATTRwt.
***$P < 0.05$ for ATTRv vs. ATTRwt.

Table 2 Electrocardiographic features among the three subtypes of amyloidosis at the first evaluation

Variables	AL (n = 105)		ATTRv (n = 50)		ATTRwt (n = 201)		P
HR, b.p.m.	**79 (70–90)**	**105**	**74 (68–78)**	**50**	**72 (64–80)**	**201**	**<0.01*,****
Sinus rhythm, n (%)	**91 (86%)**	**105**	**43 (92%)**	**50**	**128 (63%)**	**201**	**<0.01**,****
PR, ms	**160 (154–190)**	**91**	**178 (160–200)**	**43**	**200 (180–237)**	**128**	**<0.01**,****
First-degree AV block, n (%)	**13 (34)**	**91**	**10 (23)**	**43**	**51 (40)**	**128**	**<0.01****
QRS, ms	**96 (86–110)**	**103**	**90 (82–110)**	**50**	**100 (88–122)**	**197**	**0.06**
Total QRS score	**91 (83–116)**	**85**	**92 (75–106)**	**38**	**108 (91–132)**	**133**	**<0.01**,****
Peripheral QRS score	**27 (20–35)**	**87**	**29 (24–39)**	**38**	**30 (23–40)**	**135**	**0.34**
Precordial QRS score	**69 (56–86)**	**85**	**59 (52–74)**	**38**	**76 (64–91)**	**133**	**<0.01*,**,***
LBBB, n (%)	**2 (2)**	**105**	**4 (8)**	**50**	**23 (11)**	**201**	**<0.01****
RBBB, n (%)	11 (10)	105	7 (14)	50	39 (19)	201	0.11
LAFB, n (%)	**42 (40)**	**105**	**10 (20)**	**50**	**75 (37)**	**201**	**0.01***
Low QRS voltage, n (%)	**58 (55)**	**105**	**17 (34)**	**50**	**67 (33)**	**201**	**<0.01*,****
Pseudonecrosis pattern, n (%)	46 (43)	105	17 (34)	50	66 (33)	201	0.15
Anterior pseudonecrosis, n (%)	**43 (41)**	**105**	**15 (30)**	**50**	**50 (25)**	**201**	**0.01****
Inferior pseudonecrosis, n (%)	11 (11)	105	6 (12)	50	24 (12)	201	0.9
Lateral pseudo-necrosis, n (%)	24 (23)	105	17 (34)	50	44 (22)	201	0.19
High-lateral pseudonecrosis, n (%)	11 (11)	105	6 (12)	50	18 (9)	201	0.78
TWI, n (%)	**39 (37)**	**105**	**9 (18)**	**50**	**37 (18)**	**201**	**<0.01*,****

Bold values indicate statistical significance.
AL, light-chain amyloidosis; ATTRv, hereditary (genetically abnormal) transthyretin amyloidosis; ATTRwt, wild-type transthyretin amyloidosis; AV, atrioventricular; HR, heart rate; LAFB, left anterior fascicular block; LBBB, left bundle branch block; RBBB, right bundle branch block; TWI, T wave inversion.
*$P < 0.05$ for AL vs. ATTRv.
**$P < 0.05$ for AL vs. ATTRwt.
***$P < 0.05$ for ATTRv vs. ATTRwt.

Thirty-three (31%) patients with AL, 4 (8%) with ATTRv, and 26 (13%) with ATTRwt died. The relationship among clinical, echocardiographic, and ECG parameters with survival was explored in the univariate Cox regression analysis (*Table 5*). Changes at follow-up from baseline in the QRS, PR duration, and QRS score (Δ) were also included in the analysis. QRS duration at baseline remained independently associated with patient survival in the overall population also after adjust-

Table 3 Means of electrocardiographic characteristics by group and adjusted mean differences from linear regressions (adjustment for baseline value, age, and follow-up duration)

Variables	Group	Mean (95% CI)	Adj. coefficient (95% CI)	*P* value of the test on the single regression coefficient	Overall *P* value
PR, ms	AL	39 (6 to 74)	−1.12 (−13 to +10)	0.85	0.89
	ATTRv	42 (8 to 77)	1.76 (−12 to +16)	0.80	
	ATTRwt	41 (0.7 to 81)	Ref	—	
QRS, ms	AL	12 (−2 to +27)	1.6 (−4 to +7)	0.57	0.55
	ATTRv	11 (−4 to +26)	Ref	—	
	ATTRwt	12 (−4 to +30)	1.3 (−4 to +7)	0.66	
Total QRS score	AL	12 (−10 to +34)	−6 (−15 to +2.4)	0.15	0.18
	ATTRv	18 (−4 to +41)	Ref	—	
	ATTRwt	18 (−8 to +45)	−0.1 (−9 to +9)	0.98	
Peripheral QRS score	AL	11 (2 to +20)	0.6 (−2 to +4)	0.70	0.83
	ATTRv	11 (2 to +20)	1.1 (−3 to +5)	0.55	
	ATTRwt	10 (−0.2 to +21)	Ref	—	
Precordial QRS score	AL	−0.5 (−18 to +17)	−6 (−13 to −0.4)	0.03	0.07
	ATTRv	5 (−12 to +23)	−1 (−8 to +6)	0.8	
	ATTRwt	6 (−14 to +27)	Ref	—	

95% CI, 95% confidence interval; AL, light-chain amyloidosis; ATTRv, hereditary (genetically abnormal) transthyretin amyloidosis; ATTRwt, wild-type transthyretin amyloidosis; Ref, reference.

Table 4 Crude odds ratios for group comparison and adjusted odds ratios from logistic regressions (adjustment for baseline value, age, and follow-up duration) for electrocardiographic characteristics

Variables	Group	Odds ratio (95% CI)	*P* value of the test on the single regression coefficient	Overall *P* value
LBBB	AL	1.5 (0.4–6)	0.52	0.52
	ATTRv	2 (0.4–9.1)	0.32	
	ATTRwt	Ref	—	
RBBB	AL	**4.7 (1.5–15)**	**0.008**	**0.008**
	ATTRv	1.02 (0.15–6.7)	0.98	
	ATTRwt	Ref	—	
LAFB	AL	1.2 (0.5–3)	0.56	0.56
	ATTRv	1.6 (0.6–4.6)	0.31	
	ATTRwt	Ref	—	
Low QRS voltage	AL	1.6 (0.6–4.2)	0.32	0.32
	ATTRv	1.5 (0.4–4.9)	0.52	
	ATTRwt	Ref	—	
Anterior pseudonecrosis	AL	1.4 (0.62–3.2)	0.40	0.40
	ATTRv	1.7 (0.64–4.8)	0.27	
	ATTRwt	Ref	—	
Inferior pseudonecrosis	AL	0.29 (0.08–1.03)	0.05	0.05
	ATTRv	0.84 (0.21–3.2)	0.8	
	ATTRwt	Ref	—	
TWI	AL	1.8 (0.8–4.12)	0.15	0.15
	ATTRv	0.9 (0.3–2.7)	0.86	

Bold values indicate statistical significance.
95% CI, 95% confidence interval; AL, light-chain amyloidosis; ATTRv, hereditary (genetically abnormal) transthyretin amyloidosis; ATTRwt, wild-type transthyretin amyloidosis; LAFB, left anterior fascicular block; LBBB, left bundle branch block; RBBB, right bundle branch block; Ref, reference; TWI, T wave inversion.

ment for New York Heart Association (NYHA) class, NT-proBNP, age, and creatinine value (*Table 5*). A QRS duration $>$ 120 ms is a generally accepted threshold for prolonged QRS.[20] Thirty-nine (15%) patients with QRS $<$ 120 ms and 24 (38%) with QRS $>$ 120 ms died. There was a statistically significant difference in survival between patients with and without prolonged QRS $>$ 120 ms in the whole population (*Figure 1A*, $P < 0.01$) and across amyloidosis types (Supporting Information, *Figure S2*).

At ROC analysis, QRS duration of 105 ms was the optimal threshold to discriminate the outcome of all-cause death (sensitivity: 57%; specificity: 66%; area under the curve: 0.62; confidence interval: 0.54–0.69) (Supporting Information, *Figure S3*). Thirty-seven patients with QRS $\geq$ 105 ms and 26 with QRS $<$ 105 died, with a statistically significant difference in survival between the two groups, in the whole population and across amyloidosis types (*Figure 1B* and Supporting Information, *Figures S4–S6*).

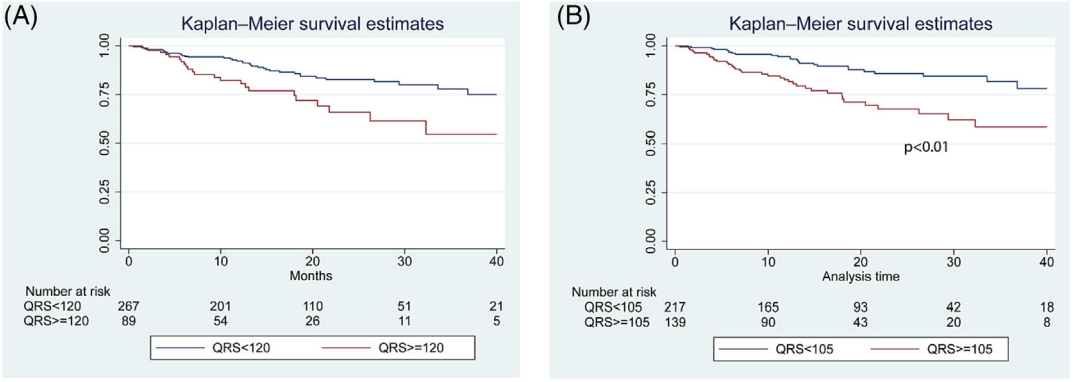
Additionally, interventricular septum thickness values displayed a positive correlation with the QRS duration ($r = 0.25$, $P < 0.01$) (*Figure 2*). Interventricular septum was also positively correlated with PR duration ($r = 0.26$, $P < 0.01$) and

Table 5 Results of univariate and multivariate Cox regression models: associations between hazard of mortality from all causes and key clinical, echocardiographic, and electrocardiographic characteristics

	Univariate Cox regression		Multivariate Cox regression	
Variables	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age	1.00 (0.99–1.00)	0.09	0.99 (0.99–1.00)	0.81
NYHA class	**3.2 (2.2–4.6)**	**<0.01**	**4 (2.2–6.9)**	**<0.01**
NT-proBNP > 3000 or BNP > 250	**5.29 (2.3–11.9)**	**<0.01**	**5.3 (1.7–15.9)**	**0.003**
Creatinine	**1.37 (1.04–1.8)**	**0.02**	**1.5 (1.08–2.23)**	**0.016**
IVS thickness	1.06 (0.99–1.14)	0.06		
EF	**0.97 (0.95–0.99)**	**0.02**	1.01 (0.96–1.05)	0.6
TAPSE	**0.92 (0.87–0.99)**	**0.02**	0.99 (0.87–1.11)	0.8
PR	0.99 (0.99–1.00)	0.7		
Δ PR	0.2 (0.98–1.00)	0.2		
QRS[a]	**1.55 (1.12–2. 13)**	**0.007**	**1.78 (1.13–2.8)**	**0.01**
Δ QRS	0.99 (0.97–1.00)	0.19		
Total QRS score	0.99 (0.99–1.01)	0.91		
Δ Total QRS score	0.98 (0.97–1.00)	0.15		
Peripheral QRS score	0.98 (0.95–1.024)	0.14		
Δ Peripheral QRS score	0.98 (0.95–1.05)	0.62		
Precordial QRS score	1.00 (0.99–1.01)	0.36		
Δ Precordial QRS score	0.98 (0.96–1.02)	0.10		

Bold values indicate statistical significance.
95% CI, 95% confidence interval; BNP, brain natriuretic peptide; EF, ejection fraction; HR, hazard ratio; IVS, interventricular septum; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association; TAPSE, tricuspid annular plane systolic excursion.
[a]HR for QRS is per 40 ms increase.

Figure 1 Estimated survival curves for all-cause mortality for the whole population by QRS using the 120 ms cut-off (Panel A, $P < 0.01$) and the optimal cut-off of 105 ms (Panel B, $P < 0.01$).



precordial QRS score ($r = 0.25$, $P < 0.01$). Left atrial diameter was positively correlated with PR duration ($r = 0.16$, $P = 0.009$) and QRS duration ($r = 0.17$, $P = 0.002$). TAPSE was inversely correlated with PR duration ($r = -0.16$, $P = 0.02$) and QRS duration ($r = -0.22$, $P = 0.002$) and directly correlated with peripheral QRS score ($r = 0.24$, $P = 0.0002$). Ejection fraction was inversely correlated with QRS duration ($r = -036$, $P < 0.01$).

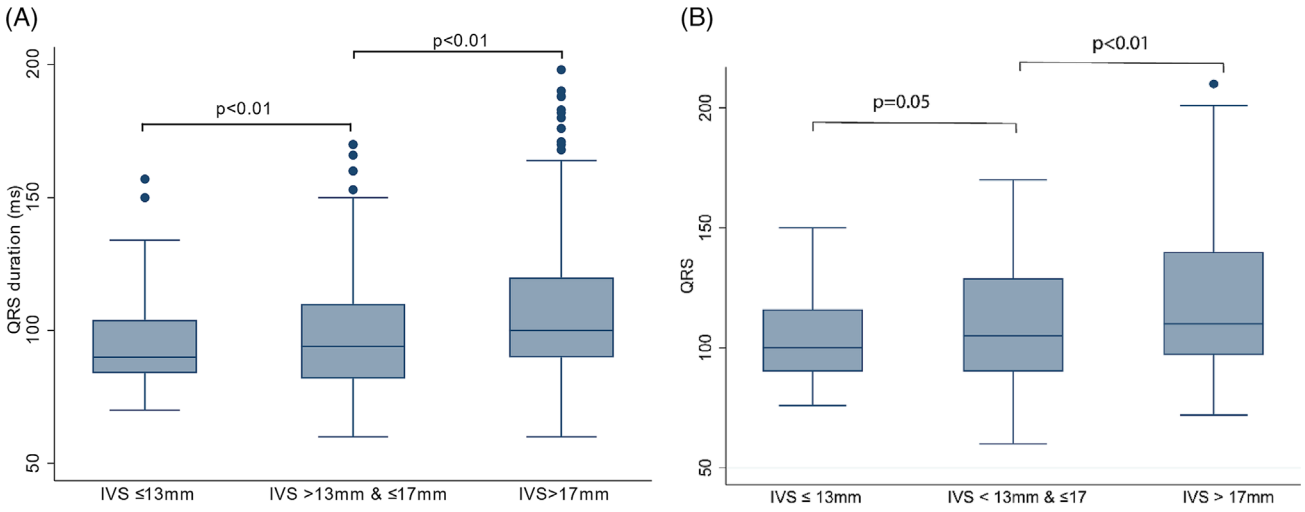
Discussion

In this study, for the first time, we compared the ECG characteristics at baseline and after a median follow-up of 15 (8–26) months among the most frequent subtypes of CA (AL, ATTRv, and ATTRwt).

The main findings of this study include the following: (i) baseline ECG differs across subtypes of amyloidosis: patients with ATTRwt show a more blunted AV and intraventricular conduction compared with those with AL, while patients with AL more frequently present low QRS voltage and TWI compared with those with ATTRv and ATTRwt; (ii) after correction for baseline value, age, and follow-up duration, the progression of ECG abnormalities is similar across the subtypes of amyloidosis with the exception of greater odds to develop RBBB in patients with AL compared with those with ATTRwt; and (iii) QRS duration at baseline is the only ECG parameter that is an independent predictor of adverse outcomes.

Data on ECG in CA are rather scarce. In the pivotal work from Rapezzi *et al.*,[21] patients with ATTRwt at baseline showed more frequently LBBB compared with those with

Figure 2 Box plots showing the increasing QRS duration values according to the interventricular septum (IVS) thickness at baseline (Panel A, $P < 0.01$) and at follow-up (Panel B, $P < 0.01$).



AL and ATTRv, and patients with AL had the lowest total QRS score compared with those with ATTRv and ATTRwt and showed more commonly low QRS voltage compared with those with ATTRv. No differences have been detected in the frequency of pseudo-infarction pattern and TWI among subtypes of amyloidosis. In a more recent work by Cappelli *et al.*,[22] patients with ATTRwt showed first-degree AV block more frequently compared with those with AL, intraventricular delay was more common in ATTR compared with AL, low QRS voltage was more frequent in AL compared with ATTRwt and ATTRv, while pseudo-infarction pattern showed the same distribution across subtypes of amyloidosis. Our work resembles the previous findings; in fact, AV conduction was more blunted in ATTRwt compared with AL and ATTRv, LBBB was more common in ATTRwt compared with AL, and patients with AL showed more frequently low QRS voltage and TWI compared with those with ATTRv and ATTRwt.

The differences at baseline ECG reflect the differences in pathophysiology between AL and ATTR. The first is a rapidly progressive disease in which the direct toxic effect of light chains plays a pivotal role, whereas the latter has a chronic progression, and the gradual amyloid accumulation is strictly correlated with worsening clinical manifestations and outcomes. Thus, in AL, the light-chain-mediated cytotoxicity may cause myocyte loss and oedema that may lead to low QRS voltage and TWI.[23] In ATTR, beyond amyloid fibril deposition, compensatory myocyte hypertrophy may concur with left ventricular hypertrophy,[23] also justifying the greater QRS score in ATTRwt compared with AL.

However, in the time interval evaluated in this study, the ECG characteristics slightly modify with similar adjusted mean differences of ECG features and similar odds ratios for the development of ECG abnormalities across subtypes of amyloidosis. Only patients with AL show greater odds for the development of RBBB compared with those with ATTRwt [odds ratio 4.7 (95% confidence interval 1.5–15)]. This phenomenon may be due to the greater vulnerability of the slender right bundle branch compared with the left bundle branch that may be altered even for the limited burden of amyloid infiltration of AL amyloidosis or by oedema.[24,25]

The lack of difference among groups in ECG pattern evolution could be influenced by the specific therapy for AL that nowadays can lead to a complete/very good haematological response in a significant group of patients, while for ATTRwt, at the time of the study, no targeted therapies were commercially available. During the follow-up time, a significant haematological response could lead to reduction of oedema, while amyloid disruption has been shown in a minority of patients and expected in a larger amount of time.[26]

In this study, QRS complex duration was an independent predictor of all-cause mortality in the whole cohort of patients with CA and, considering the correlation with the interventricular septum thickness, may be considered a sign of more advanced disease.

In a recent study by Guo *et al.*,[3] including patients with AL, more severe late gadolinium enhancement patterns were associated with lower QRS voltage and longer QRS duration. In univariate Cox regression analysis, QRS duration, together with fractionated QRS, Q waves, Sokolow index, and limb lead voltages, was associated with all-cause mortality; however, in the multivariate analysis, including NT-proBNP and extracellular volume, none of the ECG data was independently associated with adverse outcomes. In the study by Perlini *et al.*,[5] including only patients with AL, the presence of fragmented QRS was independently associated with worse prognosis after adjustment for cardiac biomarkers and systolic function, while QRS duration had no prognostic significance. In the study by Murtagh *et al.*,[4] which evaluated ECGs of patients with cardiac

amyloid infiltration confirmed by endomyocardial biopsy, none of the ECG parameters correlated with survival. However, in a very recent study including 1140 patients with both AL and ATTR, electrical dissincrony, defined as a QRS duration > 130 ms, was associated with a more advanced disease stage with a lower ejection fraction, greater diastolic dysfunction, and higher National Amyloidosis Centre stage and NYHA class.[27] Furthermore, patients with electrical dissincrony showed a higher risk for all-cause mortality compared with those without, in accordance with our study.

Eventually, the prognostic value of QRS duration has been proven in different settings, including the general population,[28] patients with left ventricular hypertrophy,[29] those with dilated cardiomyopathy,[30] and those with low-flow, low-gradient aortic stenosis.[31] Further studies are needed to confirm our findings, and the evaluation of the correlation between QRS duration and the burden of amyloid infiltration evaluated through extracellular volume could be of great interest.

The study has several limitations. First of all, the time interval between the first and last ECGs was variable among patients. As it is plausible that patients with a shorter follow-up were in a more severe condition than those with a longer follow-up, analyses that do not account for follow-up time could bring to biased comparisons to the extent that the distribution of severity was different among groups. We have dealt with this limitation, including the follow-up time in the regression models, but this adjustment might not be sufficient, and the use of alternative approaches might be worthwhile. Additionally, it is worth noting that it is impossible to obtain from these data an unbiased estimate of the evolution of the ECG measurements over time. Appropriate weights aimed at balancing the sample with respect to the length of the follow-up, thus indirectly to the severity of the health conditions, could be used to construct a pseudo population on which to calculate the patients' variable profiles over time. As an alternative, studies should be planned where the progression of the ECG is evaluated on all the enrolled subjects at definite time intervals. Also, extending the length of the follow-up could be of great interest. A second limitation of the study is related to the exclusion of patients to whom a PM is implanted during the study period. For these patients, it is not possible to obtain reliable ECG measurements; however, their exclusion from the analysis can induce a bias in the comparison among groups.

We have no information about the therapy and the response to therapy for patients with AL. It could be of extreme interest to observe if patients with a complete/very good haematological response also show ECG modifications. The same considerations apply to patients with ATTR. We also did not include information about standard cardiovascular therapy. Data regarding biomarkers such as troponin were not included, because of the lack of standardization among centres.

Conclusions

ECG differs at diagnosis among AL, ATTRv, and ATTRwt; however, the progression of the ECG alterations seems similar across the subtypes of CA. QRS duration could be a marker of more advanced disease, yet further studies are needed to evaluate the correlation between this parameter and amyloid burden.

Conflict of interest

The authors declare that there is no conflict of interest.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Study flow chart.
Figure S2. Estimated survival curves for all-cause mortality by QRS duration with the cut-off of 120 ms for AL (Panel A), hereditary transthyretin cardiomyopathy (Panel B), wild type transthyretin cardiomyopathy (Panel C).
Figure S3. Receiver-operating characteristic (ROC) curve that demonstrates QRS duration as predictor of mortality (ROC derived cut-off 105 ms, sensitivity: 57%; specificity: 66%; AUC: 0.62, CI: 0.54–0.69).
Figure S4. Estimated survival curves for all-cause mortality by QRS duration with the optimal cut-off of 105 ms in patients with AL ($P < 0.01$).
Figure S5. Estimated survival curves for all-cause mortality by QRS duration with the optimal cut-off of 105 ms in patients with hereditary transthyretin amyloidosis ($P < 0.01$).
Figure S6. Estimated survival curves for all-cause mortality by QRS duration with the optimal cut-off of 105 ms in patients with genetic wild type transthyretin amyloidosis ($P = 0.03$).
Table S1. Clinical and echocardiographic features among the three subtypes of amyloidosis at follow-up. *$P < 0.05$ for AL vs. ATTRv **$P < 0.05$ for AL vs. ATTRwt ***$P < 0.05$ for ATTRv vs. ATTRwt.
Table S2. Electrocardiographic features among the three subtypes of amyloidosis at follow-up. *$P < 0.05$ for AL vs. ATTRv **$P < 0.05$ for AL vs. ATTRwt ***$P < 0.05$ for ATTRv vs. ATTRwt.

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