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ORIGINAL ARTICLE

Artificial intelligence–derived cardiac ageing is associated with cardiac events post-heart transplantation

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Aims An artificial intelligence algorithm detecting age from 12-lead electrocardiogram (ECG) has been suggested to reflect 'physiological age'. An increased physiological age has been associated with a higher risk of cardiac mortality in the non-transplant population. We aimed to investigate the utility of this algorithm in patients who underwent heart transplantation (HTx).

Methods and results A total of 540 patients were studied. The average ECG ages within 1 year before and after HTx were used to represent pre- and post-HTx ECG ages. Major adverse cardiovascular event (MACE) was defined as any coronary revascularization, heart failure hospitalization, re-transplantation, and mortality. Recipient pre-transplant ECG age (mean 63 ± 11 years) correlated significantly with recipient chronological age (mean 49 ± 14 years, $R = 0.63$, $P < 0.0001$), while post-transplant ECG age (mean 54 ± 10 years) correlated with both the donor (mean 32 ± 13 years, $R = 0.45$, $P < 0.0001$) and the recipient ages ($R = 0.38$, $P < 0.0001$). During a median follow-up of 8.8 years, 307 patients experienced MACE. Patients with an increase in ECG age post-transplant showed an increased risk of MACE [hazard ratio (HR): 1.58, 95% confidence interval (CI): (1.24, 2.01), $P = 0.0002$], even after adjusting for potential confounders [HR: 1.58, 95% CI: (1.19, 2.10), $P = 0.002$].

Conclusion Electrocardiogram age-derived cardiac ageing after transplantation is associated with a higher risk of MACE. This study suggests that physiological age change of the heart might be an important determinant of MACE risk post-HTx.

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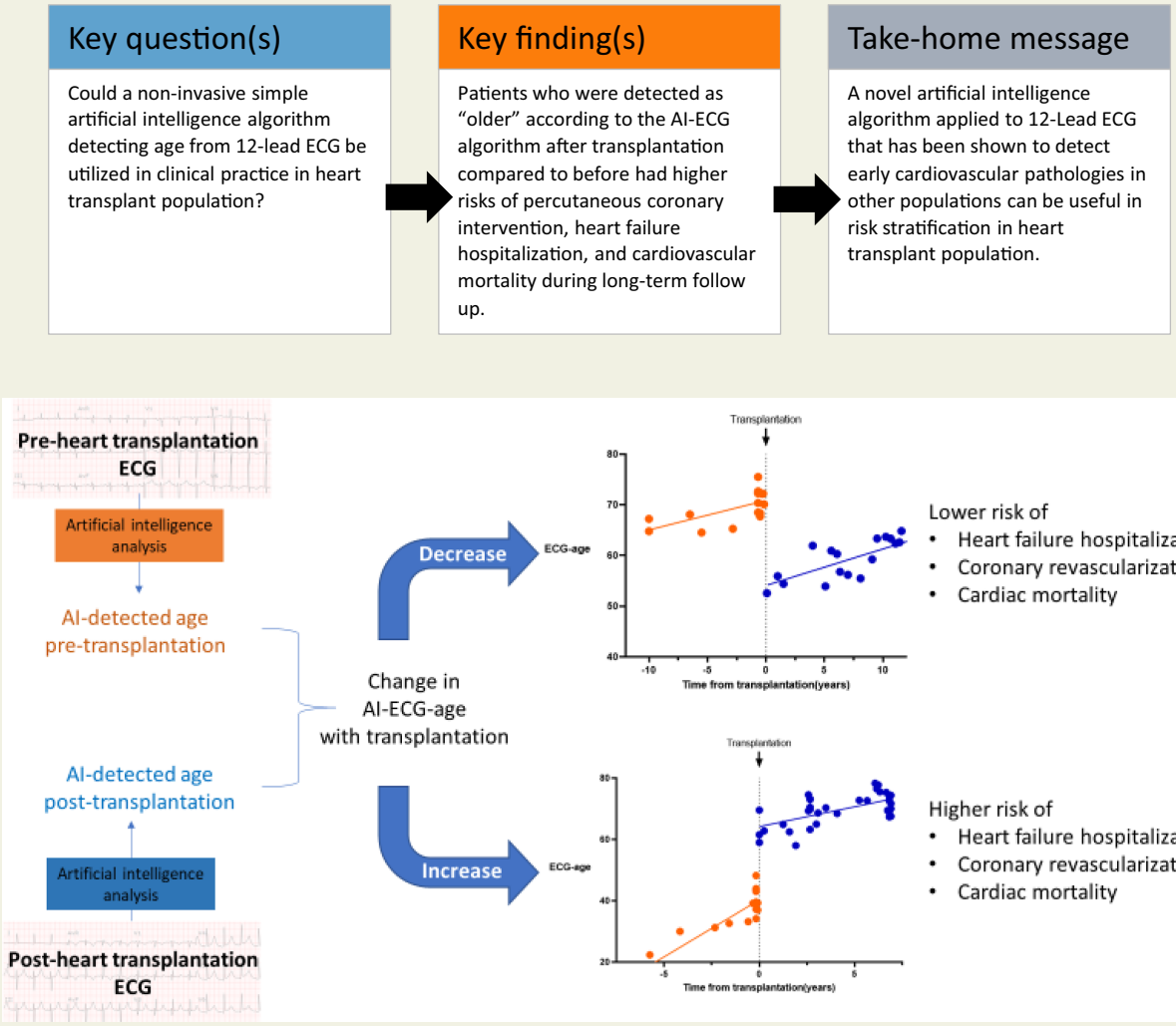
Graphical Abstract



Keywords Artificial intelligence • Electrocardiogram • Heart transplantation • Cardiac allograft vasculopathy

Introduction

Electrocardiogram (ECG) is a simple non-invasive assessment that can provide important information regarding a patient's cardiovascular status. The application of recently developed artificial intelligence (AI) algorithms to standard 12-lead ECG allowed early detection and prediction of several cardiovascular pathologies that could not be detected with the human eye, from left ventricular systolic dysfunction[1] and aortic stenosis[2] to determining the risk of atrial fibrillation in patients with microvascular dysfunction.[3] Similarly, an algorithm predicting patient's age from their ECG (ECG age) has been suggested to represent physiological age based on the fact that the difference between ECG age and chronological age (age gap) is the greatest in patients with cardiovascular comorbidities, providing prognostic information beyond what is provided by chronological age.[4] In fact, patients with a greater age gap are under an increased risk of cardiovascular and all-cause mortality.[5] We also demonstrated that vascular ageing may potentially accelerate the physiological ageing gauged by the age gap, leading to future cardiovascular events.[6]

Orthotopic heart transplantation (HTx) is considered an ultimate treatment option for patients with end-stage heart failure. Survival after HTx has improved significantly with the recent advances in immunosuppression and management, yet there are no current effective stratification strategies for long-term complications, including cardiac allograft vasculopathy (CAV) and recurrent heart failure. Given that heart transplant patients constitute a special population with discrepant donor heart and recipient ages, it is currently unknown if AI-detected ageing might be helpful in risk stratification of this population as the non-transplant one.

We therefore aimed to assess the utility of the ECG-derived physiological age in heart transplant patients and determine the

association between the ECG-detected physiological ageing, patient and donor characteristics, and future cardiovascular events.

Methods

Study population

This retrospective, single-centre, cohort study was performed at Mayo Clinic in Rochester, MN, using our prospective HTx database. The study conformed to the principles outlined in the Declaration of Helsinki. Study protocol was approved by our institutional review board, and all participants provided written informed consent.

We included a total of 564 consecutive cardiac transplant patients who underwent HTx in our institution between 1990 and 2018.

All blood tests used in the analyses were performed during routine clinical examinations at the time of transplantation. Patient information, including the clinical data and laboratory results were collected by a review of medical records documented around the time of transplantation, by an investigator unaware of ECG-derived data.

To assess cardiac rejection, the first cardiac biopsy after transplantation, maximum cellular rejection grade and any antibody-mediated rejection within the first-year post-HTx were used. All biopsies were graded according to the International Society for Heart and Lung Transplantation (ISHLT) 2004 grading system.[7]

Coronary angiogram was performed according to the standard protocol and CAV was categorized on the first-year angiogram using the ISHLT guidelines as ISHLT CAV 0 (not significant), ISHLT CAV 1 (mild), ISHLT CAV 2 (moderate), and ISHLT CAV 3 (severe).[8]

Assessment of electrocardiogram age from 12-lead electrocardiogram

An AI-ECG algorithm that was previously developed and validated in the non-transplant population was used without additional training to calculate the estimations of age for the current study population.[4,5] The design of the network has been extensively described previously and the algorithm network architecture is available on request.[4] In brief, the convolutional neural network model was developed using Keras with a TensorFlow (Google, Mountain View, CA, USA) and Python backend. A total of 774 783 subjects with ECG were used to develop the neural network. Of all patients, 399 750 unique patients consisted of the training set, 99 977 in the internal validation set, and 275 056 ECGs in the holdout testing set. The convolutional neural network was trained by inputting raw 12-lead ECGs and the patients' chronological age at the time of the ECG during the training process, and the weights of the convolutional filters were adjusted to extract meaningful and relevant features of the inputs with respect to the patients' chronological age. The network had a single output (age) as a continuous number.

The ECG age used for the study was calculated as the average of the ECG ages that were obtained within 1 year before and 1 year after transplantation, to represent the ECG ages pre- and post-transplantation, respectively. The ECGs that were used in the training and validation sets were excluded. In total, 4422 pre-transplantation ECGs for 544 patients and 4516 post-transplantation ECGs for 561 patients were used to calculate the ECG ages. The median time from transplantation of the pre- and post-transplantation ECGs that were used were 88 (22, 195) and 45 (11, 142) days, respectively.

Assessment of outcome events

All clinical data were collected by a detailed review of medical records to determine the adverse events during follow-up. Information was collected to detect the following events: coronary revascularization, heart failure hospitalization, re-transplantation, all-cause, and cardiovascular death. Cause of death was determined by reviewing death certificates, autopsy reports, and phone interviews; when available. Major adverse cardiac events (MACEs) were defined as any incident of coronary revascularization, heart failure hospitalization, re-transplantation, and all-cause mortality.

Statistical analysis

Continuous normally distributed parameters were presented as mean ± standard deviation (SD) and compared by Student's *t*-test. Non-normally distributed data were presented by median and first and third quartiles and compared by non-parametric Wilcoxon rank-sum test. Categorical data were presented as numbers and relative frequencies and compared between groups with the χ^2 test or Fisher's exact test. Correlation between two variables was assessed using Pearson's correlation test. Cox proportional hazard models were used to estimate hazard ratios (HRs) for composite MACE in univariable and multivariable analyses. The Fine and Gray method was used to perform competing risk analyses for the outcome of MACE excluding non-cardiovascular mortality. Only the first event was used for the MACE analyses in patients with multiple events. For the multivariable analyses, potential confounders that might be associated with future events in the transplant population; recipient age, donor age, recipient sex, history of hypertension, history of diabetes mellitus, ischaemic cardiomyopathy, ISHLT CAV grade on the first post-transplantation angiogram, HDL-C levels, triglyceride levels, sirolimus conversion within 12 months were used. Kaplan–Meier methods were used to assess event-free survival rates, and the difference between the groups was analysed using the log-rank test. A two-tailed *P*-value of <0.05 was considered statistically significant. Analyses were conducted using JMP software (SAS Institute, Inc., Cary, NC, USA) and R (R Foundation for Statistical Computing, Vienna, Austria).

Results

Baseline characteristics

A total of 540 patients with available pre- and post-transplantation ECG data were included in the analyses.

Baseline patients' characteristics are summarized in *Table 1*. The mean age of donors was significantly younger than that of the recipients (32 ± 13 vs. 49 ± 14 years, $P < 0.0001$). The aetiology of transplantation was mostly non-ischaemic [$n = 407$ (75%)]. A total of 119 (22%) patients had a history of diabetes mellitus and 247 (46%) had a history of hypertension.

Within the first year of transplantation, the primary immunosuppression was initiated with tacrolimus in 252 (46%) patients, while 288 (54%) were on cyclosporine. Primary immunosuppression was converted to sirolimus within the first-year post-transplantation in 172 (32%) patients. Of these, 110 (64%) were initially on tacrolimus. The time to first-year angiogram was a median 368 IQR (356, 381) days. International Society for Heart and Lung Transplantation CAV grade at 1-year post-transplant was assessed in 482 patients, of which 339 (70%) had Grade 0, 136 (28%) had Grade 1, and 7 (2%) had Grade 2 or 3. A total of 93 patients (17%) required a pacemaker, of which 75 (81%) were within the first 3 months post-transplantation.

Electrocardiogram age pre- and post-heart transplantation

The mean pre-HTx ECG age was 63 ± 11 years, and the mean age gap between real age and ECG age pre-transplantation was 13.3 ± 11 years.

Table 1 Patient characteristics around the time of transplantation

	Total ($n = 540$)	Change in ECG age with transplantation		*P*-value
		Decrease ($\Delta < 0$, $n = 405$)	Increase ($\Delta \geq 0$, $n = 135$)	
ECG age pre-HTx, mean (SD), years	63 (11)	66 (7)	52 (12)	<0.0001
ECG age post-HTx, mean (SD), years	54 (10)	52 (10)	60 (10)	<0.0001
Recipient age, mean (SD), years	49 (14)	51 (13)	43 (16)	<0.0001
Donor age, mean (SD), years	32 (13)	31 (13)	35 (14)	0.005
Recipient sex, male, *n* (%)	366 (68)	281 (69)	85 (62)	0.15
Donor sex, male, *n* (%)	361 (67)	276 (69)	85 (63)	0.26
History of hypertension, *n* (%)	247 (46)	193 (48)	54 (40)	0.12
History of diabetes mellitus, *n* (%)	119 (22)	93 (23)	26 (19)	0.37
Ischaemic cardiomyopathy, *n* (%)	133 (25)	104 (26)	29 (22)	0.33
LDL cholesterol, mean (SD), mg/dL	107 (46)	106 (46)	108 (47)	0.67
HDL cholesterol, mean (SD), mg/dL	58 (21)	59 (21)	55 (19)	0.04
Triglycerides, mg/dL	136 (100, 202)	133 (98, 193)	148 (100, 220)	0.09
Ischaemic time, mean (SD), min ($n = 498$)	173 (56)	171 (56)	179 (55)	0.21
Primary immunosuppression, *n* (%)				0.11
Tacrolimus	252 (46)	197 (48)	55 (41)	
Cyclosporine	288 (54)	208 (52)	80 (59)	
Secondary immunosuppression, *n* (%)				0.11
Mycophenolate mofetil	264 (49)	206 (51)	58 (43)	
Azathioprine	272 (51)	196 (49)	76 (58)	
Sirolimus conversion within 1-year post-HTx	172 (32)	138 (34)	34 (25)	0.05
First-year acute cellular rejection ≥ 2R, *n* (%)	138 (26)	95 (24)	43 (32)	0.05
First-year antibody-mediated rejection ≥1 ($n = 370$)	45 (12)	39 (13)	6 (8)	0.27
Cellular rejection grade on first biopsy post-HTx ($n = 459$)				0.52
Early pacemaker implantation[a]	75 (14%)	50 (12%)	25 (19%)	0.08
Late pacemaker implantation	18 (3%)	14 (3%)	4 (3%)	0.78
Grade 0R	224 (49)	173 (49)	51 (49)	
Grade 1R	202 (44)	158 (45)	44 (42)	
Grade 2–3R	33 (7)	23 (7)	10 (9)	
ISHLT CAV grade at first angiogram ($n = 482$)				0.29
Grade 0	339 (70)	264 (72)	75 (66)	
Grade 1	136 (28)	100 (27)	36 (31)	
Grade 2–3	7 (2)	4 (1)	3 (3)	

CAV, cardiac allograft vasculopathy; HTx, heart transplantation; ISHLT, International Society for Heart and Lung Transplantation.
[a]Pacemaker implantation <3 months post-transplantation.

The mean post-HTx ECG age was significantly younger with 54 ± 10 years ($P < 0.0001$). The figures representing three individuals and the change in their respective ECG ages are presented in Supplementary material online, *Figure S1*. The ECG age changed according to the donor age in most patients; however, this was not always the case. In some patients, ECG age was increased even though they had undergone transplantation from a younger donor, and in others, it would decrease even though they had an older donor.

The mean change in ECG age after transplantation was −8.2 ± 12.9 years. The correlations of pre- and post-HTx ECG ages with donor and recipient chronological ages are presented in *Figure 1*. Pre-heart transplantation ECG age significantly correlated with the recipient chronological age at the time ($R = 0.63$, $P < 0.0001$) (*Figure 1A*). Interestingly, post-HTx ECG age demonstrated a significant correlation with both the recipient ($R = 0.38$, $P < 0.0001$) (*Figure 1B*) and donor chronological ages ($R = 0.45$, $P < 0.0001$) (*Figure 1C*).

Electrocardiogram-derived cardiac ageing

Next, we divided patients into two groups; patients who had an increase in ECG age post-HTx (ΔECG age ≥0) and those who had a decrease in ECG age post-HTx (ΔECG age <0). (*Table 1*) In total, 135 patients (25%) had an increase in ECG age after transplantation compared with before (ΔECG age ≥0). Patients with ΔECG age ≥0 were younger, whereas their donors were older. They had lower HDL-C levels and tended to have higher triglyceride levels. Patients that underwent pacemaker implantation within the first 3 months tended be more likely to have ΔECG age ≥0 [25 (19%) vs. 50 (12%), $P = 0.08$].

There was no difference regarding cellular rejection on the first biopsy post-transplantation. However, patients with ΔECG age ≥0 tended to experience greater than grade 2R cellular rejection, and

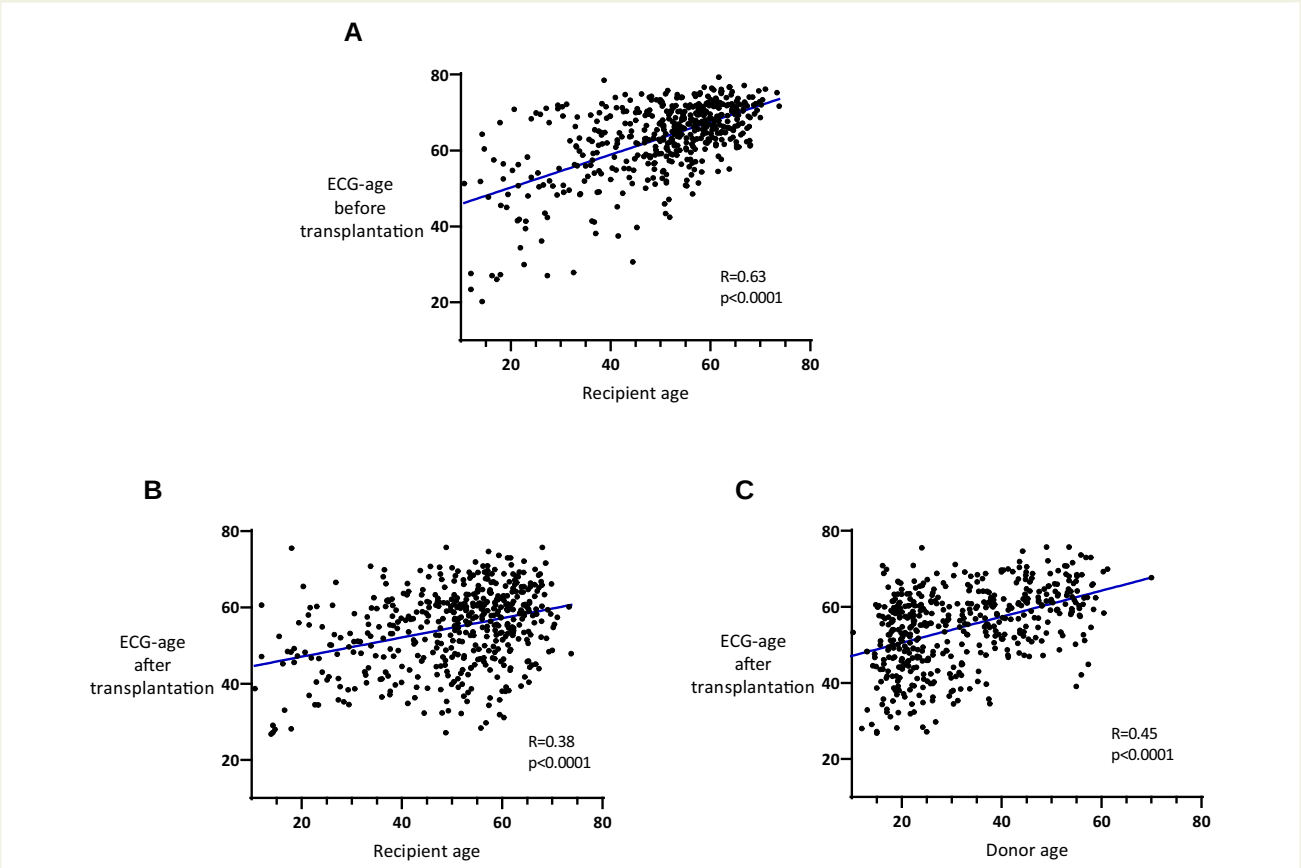


Figure 1 The correlations between recipient and donor chronological ages with electrocardiogram age pre- and post-transplantation. Before transplantation, pre-transplant electrocardiogram age significantly correlated with recipient chronological age. (*A*) After transplantation, post-transplant electrocardiogram age and recipient chronological age remained to correlate significantly. (*B*) Post-transplant electrocardiogram age also demonstrated a significant correlation with donor chronological age (*C*).

were less likely to undergo conversion to sirolimus within the first-year post-HTx.

Electrocardiogram-derived cardiac ageing and future adverse events

During a median 8.8 (4.9, 14.3) year follow-up, 307 patients had an adverse cardiovascular event. This included 57 patients who underwent coronary revascularization, 109 patients with heart failure hospitalization, 9 who had re-transplantation, and 241 that deceased. The aetiology of heart failure was documented or suspected episodes of rejection in 49 patients (45%), whereas it was due to CAV-related reasons in 21 patients (19%). Other reasons included valvular disease (12%) and episodes of arrhythmia (5%). The cause of death was determined as cardiovascular in 68 patients (28%), malignancy in 45 patients (19%), respiratory failure in 19 patients (8%), and infection in 17 patients (7%), while a definitive cause could not be determined in 43 patients (18%).

The potential predictors of composite MACE are shown in *Table 2*. In the univariable analyses; increased ECG age post-HTx (ΔECG age ≥0), recipient age, donor age, recipient history of diabetes mellitus, history of hypertension, transplant aetiology of ischaemic cardiomyopathy, triglyceride levels, and CAV ISHLT grade on the first angiogram were all significantly associated with an increased risk of MACE. However, factors such as transplantation from an older donor compared with the recipient [HR: 1.20, 95% confidence interval (CI): (0.89, 1.61), $P = 0.23$], or the chronological age difference between donor and the recipient [HR: 1.00, 95% CI: (0.99, 1.01), $P = 0.69$] were not.

In the multivariable model, only ΔECG age ≥0 [HR: 1.58, 95% CI: (1.18, 2.10), $P = 0.002$], triglyceride levels [HR 1.01, 95% CI: (1.01, 1.01), $P = 0.039$], and CAV ISHLT Grade ≥1 at the first angiogram [HR: 1.38, 95% CI: (1.05, 1.80), $P = 0.019$] remained significantly associated with MACE. (*Table 2*, *Figure 2A*) The association was also significant in both univariable and multivariable competing risk regression analyses using the same parameters, for the outcome of MACE excluding non-cardiovascular mortality [HR: 1.81, 95% CI: (1.41, 2.33), $P = 0.001$ for univariable; HR: 1.59, 95% CI: (1.19, 2.110, $P = 0.007$ for multivariable].

ΔECG age as a continuous variable was also associated with composite MACE in both univariable and multivariable Cox proportional hazard analyses [HR: 1.02, 95% CI: (1.00, 1.03), $P = 0.008$ for univariable; HR: 1.01, 95% CI: (1.00, 1.02), $P = 0.035$ for multivariable] (*Figure 3*).

Table 2 Univariable and multivariable Cox proportional hazard models for composite major adverse cardiovascular events

	Univariable		Multivariable	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
ΔECG age ≥0 post-transplantation	1.58 (1.24, 2.01)	**0.0002**	1.58 (1.18, 2.10)	**0.002**
Recipient age, per year	1.01 (1.00, 1.02)	**0.032**	1.01 (0.99, 1.02)	0.244
Donor age, per year	1.01 (1.00, 1.02)	**0.004**	1.01 (0.99, 1.02)	0.236
Recipient sex, male	0.85 (0.67, 1.08)	0.181	0.75 (0.55, 1.01)	0.059
Diabetes mellitus	1.33 (1.01, 1.77)	**0.046**	1.24 (0.91, 1.70)	0.175
Hypertension	1.27 (1.01, 1.60)	**0.044**	1.12 (0.86, 1.48)	0.397
Ischaemic cardiomyopathy	1.47 (1.15, 1.87)	**0.002**	1.33 (0.98, 1.80)	0.067
CAV ISHLT grade[a]	1.54 (1.19, 1.96)	**0.001**	1.38 (1.05, 1.80)	**0.019**
HDL-C, per 1 mg/dL	0.99 (0.99, 1.00)	0.053	0.99 (0.98, 1.00)	0.254
Triglyceride, per 1 mg/dL	1.01 (1.01, 1.02)	**0.027**	1.01 (1.01, 1.01)	**0.039**
Sirolimus conversion within 12 months	0.76 (0.58, 1.02)	0.064	0.90 (0.67, 1.21)	0.493

CAV, cardiac allograft vasculopathy; HDL-C, HDL cholesterol; HR, hazard ratio; ISHLT, International Society for Heart and Lung Transplantation;
[a]First-year angiographic CAV ISHLT Grade ≥1

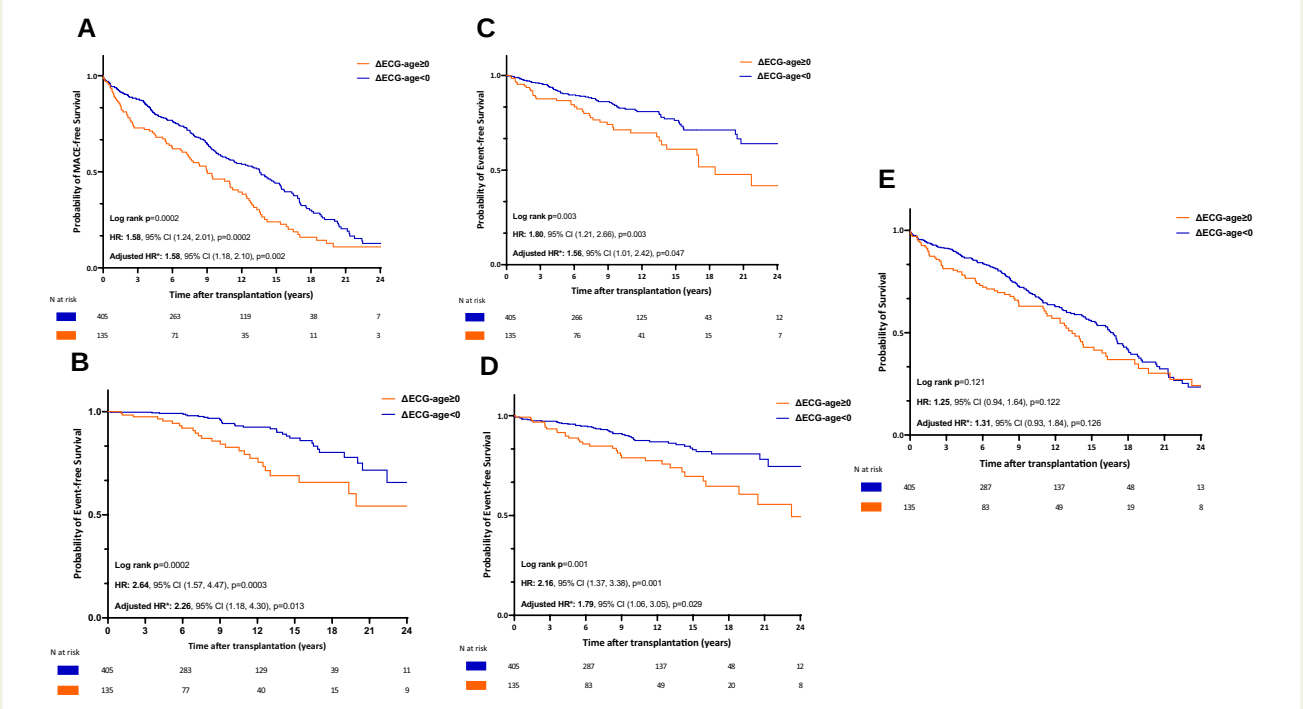


Figure 2 Association of electrocardiogram age with individual adverse cardiac events. Patients who had an increase in electrocardiogram age post-transplant compared with pre-transplant (ΔECG age ≥ 0) were under increased risk for composite major adverse cardiovascular events (any incidence of coronary revascularization, heart failure hospitalization, re-transplantation, and all-cause mortality) (*A*), as well as coronary revascularization (*B*), heart failure hospitalization (*C*), cardiovascular death or re-transplantation (*D*) but not all-cause mortality (*E*). Results of the univariable and multivariable (adjusted for recipient age, donor age, recipient sex, history of hypertension, history of diabetes mellitus, ischaemic cardiomyopathy, International Society for Heart and Lung Transplantation cardiac allograft vasculopathy grade on the first post-transplantation angiogram, HDL-C levels, triglyceride levels, sirolimus conversion within 12 months) Cox proportional hazard analyses are presented below their respective figures. CAV, coronary allograft vasculopathy; CI, confidence interval; HR, hazard ratio; ISHLT, International Society for Heart and Lung Transplantation.

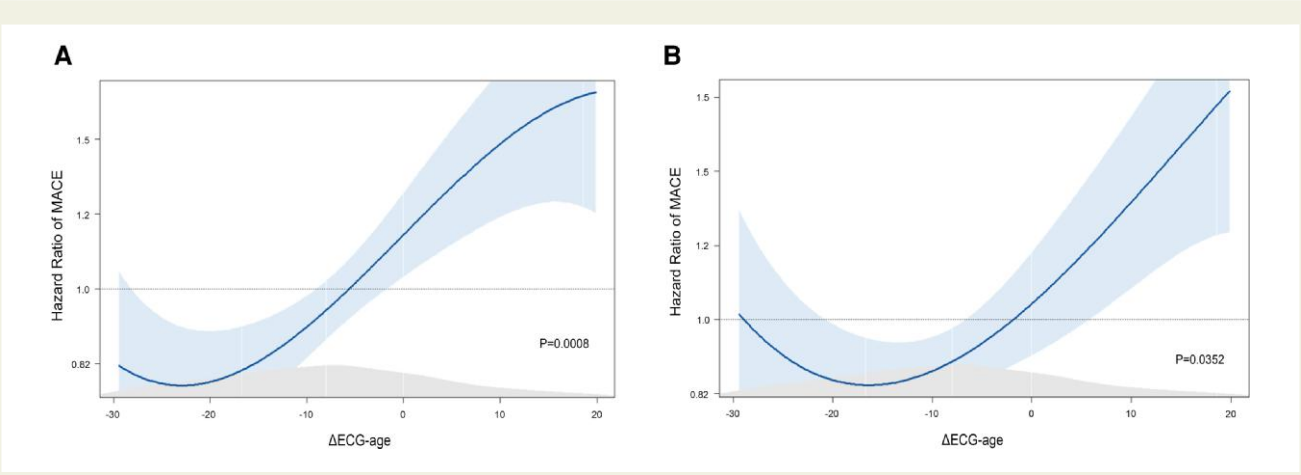


Figure 3 Univariable and multivariable proportional hazard ratio plots for major adverse cardiovascular events by ΔECG age. ΔECG age as a continuous parameter was associated with major adverse cardiovascular events (any incidence of coronary revascularization, heart failure hospitalization, re-transplantation, and all-cause mortality) in univariable (*A*) and multivariable (*B*) Cox proportional hazard analyses.

To assess the clinical relevance in a more recent period of transplantation, we performed the Cox proportional hazard analyses for those who underwent transplantation within the last 10-year of our study period. This subgroup included a total of 229 patients, and of these, 75 had composite MACE during a follow-up of 5.9 IQR (3.7, 8.2) years. ΔECG age ≥0 was significantly associated with future events in both univariable and multivariable analyses [univariable: HR: 1.70, 95% CI: (1.01, 2.89), $P = 0.04$; multivariable: HR: 1.95, 95% CI: (1.07, 3.57), $P = 0.03$].

Patients with an increase in ECG age post-transplantation ($\Delta \geq 0$) had a greater rate of individual adverse events, including coronary revascularization, heart failure hospitalization, cardiac death or re-transplantation but not all-cause mortality (*Figure 2B–E*). These associations remained significant in multivariable models with the potential confounders of recipient age, donor age, recipient sex, ischaemic cardiomyopathy, history of diabetes mellitus and hypertension, triglyceride levels, HDL-C levels, ISHLT CAV Grade ≥1 on the first post-transplantation angiogram, sirolimus conversion within the first-year post-HTx [coronary revascularization: HR: 2.26, 95% CI: (1.18, 4.30), $P = 0.013$; heart failure hospitalization: HR: 1.56, 95% CI: (1.01, 2.42), $P = 0.047$; cardiac death/re-transplantation: HR: 1.79, 95% CI: (1.06, 3.05), $P = 0.029$].

Furthermore, in patients with an angiographic ISHLT Grade 0 ($n = 337$), ΔECG age ≥0 with transplantation was associated with an increased risk of future revascularization [HR: 3.55, 95% CI: (1.24, 10.17), $P = 0.018$], as well as MACE [HR: 1.56, 95% CI: (1.09, 2.21), $P = 0.014$].

Discussion

In the current study, we demonstrate the utility of our AI-derived algorithm predicting age from 12-lead ECG in heart transplant population. The patients who become 'physiologically older' according to the algorithm after transplantation were found to be under higher risk of adverse cardiovascular events during follow-up even after adjustment for other covariates. This study suggests that AI-derived ECG algorithm may serve as a non-invasive tool to identify high risk patients following cardiac transplantation.

The application of an ECG-derived algorithm predicting the age of individuals might represent the physiologic age of the cardiovascular system.[4] It was reported that patients with a higher AI-detected age than their chronological age were more likely to have cardiovascular comorbidities.[4] Further, vascular ageing reflected in peripheral endothelial dysfunction was associated with increased ECG age.[6] The current study is in line with these previous findings, as we have observed a mean age gap of more than 10 years in our population pre-transplantation, which is high compared with the previous studies where the mean difference is around 1 year.[6,5] Interestingly, after transplantation, ECG age began to correlate with the donor's age, whereas the remaining correlated with the recipient age. This finding could be due to recipient-related factors such as connective tissue and conductivity changes with ageing which might cause subclinical alterations in the electrical signal, or due to the vascular health of the recipient. Pacemaker implantation could be one of the reasons for the change in electrical signal alternations, as in the current we have observed a tendency to be more likely to have ΔECG age ≥0 in those with early pacemaker implantation.

Older ECG age has been associated with an increased risk of future cardiovascular mortality in a population of over 25 000 subjects without cardiovascular disease, supporting ECG age as a representative of cardiovascular fitness.[5] Here, we have further demonstrated that patients who became 'physiologically older' according to the algorithm after transplantation were under increased risk of future events. Recipients with post-HTx ΔECG age ≥0 were younger than those with ΔECG age <0, whereas their donors were older than those with ΔECG age <0. Donor age has been shown as an important factor in CAV in various studies,[9] whereas recipient age was not,[10,11] thus, the results of our study might be attributed to the differences in the donor ages between the two groups. However, we

demonstrate that the association between 'AI-detected ageing' and future risk of revascularization is independent of both the donor and recipient ages, as well as the initial angiographic CAV grade. Furthermore, we did not observe a difference in outcomes in patients who underwent transplantation from a chronologically older donor compared with a younger one, consistent with a previous study.[12] Most probably, a change in ECG age represents the cardiovascular improvement/deterioration in the recipient. Along with immune factors, recipient environment, such as metabolic factors,[13,14] or ischaemic cardiomyopathy[15] may play important roles in the prognosis post-transplantation. An index of these factors, peripheral endothelial dysfunction, which is associated with vascular ageing in the non-transplant population,[6] was shown to precipitate plaque progression and adverse events in transplant patients.[16] In fact, peripheral endothelial function can be ameliorated post-HTx through improvement in cardiac function,[17] and those showing improvement are less likely to develop future CAV.[18] Coronary endothelial dysfunction precipitates transplant vasculopathy[19] and is associated with worse prognosis in heart transplant patients.[20] Hence, the ECG age post-transplantation might show evidence of vascular ageing that may contribute to coronary transplant vasculopathy, combined with the subsequent change of the recipient endothelial function.

Rejection is an important cause of recurrent heart failure hospitalizations, as well as CAV progression.[21] In the current study, patients with an increase in ECG age with transplantation tended to experience high-grade cellular rejection within the first-year post-transplantation; thus, ΔECG age ≥0 may be reflecting the ongoing rejection in the heart, being one of the mechanisms linking 'ECG ageing' with future events. However, this association might also be due to the differences in immunosuppressive strategies between groups. Although initial primary immunosuppression did not differ between the groups, patients with ΔECG age ≥0 tended to be less likely to transition to sirolimus within the first year of transplantation. Sirolimus has been associated with reduced rates of acute rejection,[22] thus could be confounding the results. Sirolimus could also improve cardiac hypertrophy[23] or diastolic dysfunction,[24] which are possible parameters of ageing gauged by the AI algorithm.

In the current study, the donor ECG age was not assessed due to the limited availability of data. Although we have suggested recipient-related factors and the donor-recipient interaction as the underpinning of ECG-ageing post-HTx, our observations could also be due to the immediate change in the 'physiological age' of the heart itself. Indeed, although recipient-related factors play a prominent role in prognosis post-HTx, donor factors also have an influence.[9] Whereas some of these factors have been consistently shown to be associated with survival post-HTx, there is no established stratification strategy for donor selection. Yet, the increase in the number of end- stage heart transplant patients make strategies for expanding the donor eligibility criteria is a necessity.[25] Prospective studies determining the influence of donor ECG age on 'ageing' with transplantation could show whether this algorithm could prove useful in pre-transplantation management and expansion of the donor pool.

Autonomic nervous system plays an important role in cardiovascular homeostasis. Heart transplantation results in the complete denervation of the donor heart, causing pathologic processes such as impaired vasoregulatory responses, increased resting heart rate, and loss of sinus arrythmia.[26] All these factors might further be detected by the algorithm as older age, thus could represent one of the reasons that the post-HTx ECG age is detected older than the donor's chronological age. Future studies looking at ECG ages in a longitudinal manner and comparing these with reinnervation of the heart could prove whether the algorithm could be useful in assessing cardiac reinnervation.

Cardiac allograft vasculopathy remains the major limiting factor of long-term survival in heart transplant recipients. As a disease that is inherently asymptomatic due to denervation,[27] CAV requires continuous surveillance via invasive methods, and currently, there is no effective method for risk stratification. Furthermore, the value of coronary angiography in CAV remains limited compared with traditional atherosclerotic lesions due to the concentric and longitudinal characteristics of the disease.[28] Other imaging modalities such as intravascular ultrasound and optical coherence tomography are more sensitive but not readily available in many institutions. Our study suggests that patients with ΔECG age ≥0 post-HTx are under increased combined risk of future coronary revascularization, re-transplantation, and cardiac death, even in those with no overt sign of angiographic disease on the first examination after transplantation. Thus, early stratification of these patients and referring them to intravascular imaging could be of benefit.

Our study has some limitations worth mentioning. As a retrospective cohort study, the study is subject to limitations inherent to its design. Parameters such as diabetes mellitus and hypertension were assessed at the time of transplantation, and it is difficult to assess whether post-transplantation dysregulation of the metabolic state affected outcomes. Future studies might elucidate whether these changes, as well as immunosuppressive strategies, affect ageing after transplantation. In addition, although our multivariable analyses included most variables previously shown as potential confounders, they remain limited, since some parameters such as donor-specific antibodies were not evaluated. Finally, although we have suggested potential mechanisms for the interpretation of our results, the factors behind the AI-ECG algorithm detecting age remain speculative and require further investigation. Importantly, we believe that ECG age should not be thought of as a static value representing the age of the patient, and rather, should be seen as a dynamic parameter showing the physiological fitness of the heart. It should further be emphasized that the comparison between ECG ages before and after transplantation is not the comparison of ages between the donor and the recipient but rather represents the cardiovascular improvement/deterioration of the individual with transplantation. As mentioned, although the difference between donor and recipient ages did not have any effect on post-transplantation event-free survival, the difference between AI-detected 'physiological ages' did.

Conclusions

We demonstrate that AI-detected ageing with transplantation is associated with a higher risk of adverse events after HTx, suggesting that the change in ECG age represents an improvement/deterioration of cardiovascular health. The biological underpinning of AI-detected ageing in this population remains to be investigated,

and further research is warranted to guide adequate screening and treatment strategies for heart transplant patients using this algorithm. Nevertheless, our results suggest that this simple non-invasive assessment using 12-lead ECG may aid in non-invasive risk stratification in this population.

Supplementary material

Supplementary material is available at *European Heart Journal – Digital Health* online.

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Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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