A simplified cardiac amyloidosis score is associated with all-cause and cardiovascular disease mortality and morbidity, in the general population: the Ikaria Study.

Abstract

Introduction

Transthyretin amyloid cardiomyopathy (ATTR-CM) is an under-appreciated disease. The aim of this study was to evaluate the T-Amylo score, i.e., ATTR-CM related, in relation to all-cause and cardiovascular disease (CVD) morbidity and mortality, in the general population.

Material and methods

The T-Amylo score (range 0-11) is based on clinical and echocardiographic features (age and gender, IVSd thickness >=16 mm, low QRS interval voltage, and carpal tunnel syndrome) that have been previously introduced in clinical practice.

Results

During 2009, 1,420 middle aged and older inhabitants agreed to enroll into the Ikaria study (678 males aged 67(14) years, and 742 females aged 66(14) years); in 2013, the participants were re-evaluated. Survival analysis revealed that the T-Amylo prediction score was associated with all-cause mortality (Hazard Ratio 1.59, 95%CI 1.40 to 1.81), and the risk of combined CVD events (1.32, 95%CI 1.11 to 1.56), after various adjustments made. ROC analysis revealed that the AUC of T-Amylo score was 0.70, the accuracy was 81.52%, and the net-reclassification indices suggested better reclassification performance than its components. Stratifying by age group, the score predicts all-cause and CVD mortality and morbidity only among >65 years old individuals.

Conclusions

The prognostic value for CVDs of the T-Amylo score observed here seems promising for its use in the general population, too.

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Keywords: transthyretin amyloid cardiomyopathy; risk; score.

1. Introduction

Amyloidosis is a rare and complex group of diseases characterized by the abnormal accumulation of protein deposits known as amyloids in various tissues and organs of the body. There are different types of amyloidosis, and the specific type is determined by the protein that forms the amyloid deposits [1]. ATTR cardiac amyloidosis can lead to heart failure, arrhythmias, and other heart-related outcomes. It is considered as a rare disease, mainly affecting older adults, but the true prevalence of ATTR cardiac amyloidosis it is hard to estimate, as it can vary significantly depending on various factors such as the specific type of ATTR amyloidosis, the region and the population being studied [2-4]. Undoubtedly, cardiac amyloidosis is a serious disorder that requires specialized care. Timely diagnosis and appropriate management are essential to optimize outcomes and improve the patient's prognosis. However, it is an underestimated health condition, whereas the exact paths and mechanisms that affect cardiac system and risk of morbidity and mortality are not fully understood and appreciated.

Although, diagnosis of cardiac amyloidosis typically involves a combination of tests, including Echocardiography, Cardiac Magnetic Resonance Imaging (MRI), Endomyocardial biopsy, as well as blood and urine tests [3], recently a simple ATTR-CM score has been introduced, the T-Amylo score, including six components, i.e., age, sex, and echocardiographic measurements. The score has shown acceptable accuracy in specific heart failure populations (sensitivity 47%, specificity 94% and AUC 0.86, p<0.001). However, the implementation of this score within the general population has never been examined before, especially in relation to morbidity and mortality due to cardiovascular disease (CVD). By employing the T-Amylo score within a general population context offers significant advantages in terms of early detection, public health research, personalized medicine, and health education, ultimately contributing to better health outcomes and disease management strategies. In the realm of personalized medicine, the T-Amylo score may

Thus, the aim of this study was to evaluate the associations between the T-Amylo score with a variety of anthropometric, biochemical, clinical, and echocardiographic indices related to CVD, as well as with CVD morbidity and mortality, in a general population, the Ikaria Island inhabitants.

2. Materials and Methods

2.1. Design

The Ikaria Study is an observational prospective, population-based study that was carried out on the island of Ikaria, northeast Aegean Sea, Greece, from June to October 2009. All inhabitants of the Ikaria Island, aged 40 years old or older, were asked to participate (pop. 2,503 census 2011).

2.2. Sampling procedure

The sampling was on a volunteer, convenience basis. Participants were interviewed by study's trained personnel, i.e., cardiologists, general practitioners, nurses, and other healthcare professionals, who used a standard protocol and validated questionnaires. People living in institutions were excluded from the sampling.

2.3. Sample

Of the individuals asked to participate, 1,420 middle aged and elderly inhabitants agreed to enroll into the study (participation rate 54%). Mean (standard deviation) age of 678 men participants was 67(14) years, and of the 742 females was 66(14) years old. The studied sample was adequate to evaluate two-sided hypothesis for odds ratios equal to 1.2,

at significance level of 0.05, achievement statistical power equal to 79.9% (NCSS and PASS software, Kaysville, Utah, 2004).

2.4. Bioethics

Participants were informed about the aims and procedures of the Ikaria Study and gave written informed consent to participate. The study was approved by the Ethics Committee of our Institution and was carried out in accordance with the principles of the Declaration of Helsinki (1989).

2.5. Measurements

2.5.1. Calculation of the T-Amyl score

The T-Amylo score which has been previously proposed and validated by Arana-Achaga et al., for the diagnosis of ATTR-CM, was calculated in this study for all participants [9]. In brief, Arana-Achaga et al., applied univariable logit models with a variety of clinical and biochemical parameters as covariates and the status of ATTR-CM as the outcome variable, using information retrieved from a multi-center study of heart failure patients. Continuous variables were dichotomized using clinically relevant thresholds, or by calculating its AUC and the relevant cut-off through ROC analysis. The regression coefficients of the final model became the weights of each parameter entered in the T-Amylo score. Each point was obtained considering the lowest beta coefficient; each weight was allocated based on the obtained coefficient rounded. Thus, a simplified score (i.e., T-Amylo score) was obtained assigning points as follows:

- 1 point for age >=80 years,

- 2 points for IVSd thickness >=16 mm,

- 2 points for low QRS interval voltage,

- 3 points for male gender, and

- 3 points for carpal tunnel syndrome.

The total score ranges from 0 to 11. In addition, a T-Amylo score cut-off point (i.e., score > 6) was used to classify patients as being at high risk for ATTR-CM (thereinafter high score), as proposed by Arana-Achaga et al. This cut-off was derived based on a sensitivity and specificity analysis performed [9].

2.5.2. Lifestyle characteristics

Current smokers were defined as those who smoked at least one cigarette per day during the past year; former smokers were defined as those who had stopped smoking for at least one year. The rest of the participants were defined as non-current smokers.

Physical activity was evaluated using the shortened version of the self-reported International Physical Activity Questionnaire (IPAQ) [10], which has been validated for the Greek population. Frequency (times per week), duration (minutes per time) and intensity of physical activity during sports, occupation and/or free-time activities were assessed. Participants who did not report any physical activities or reported very low (i.e., < 600 MET/min/week) were defined as physically inactive, while the rest were defined as at least, minimally active.

2.5.3. Anthropometric measurements

Weight and height were measured following standard procedures and body mass index (BMI) was calculated in kg/m^2. Obesity was defined as a BMI >29.9 kg/m^2. Waist circumference was measured at the midpoint between the bottom of the rib cage and the top of iliac crest from patients at minimal respiration. Body surface area (BSA), in m2, was calculated according to Mosteller equation ($0.20247 \times weight^{0.425} \times height^{0.725}$) [11].

2.5.4. Biochemical measurements

For this work several biochemical markers were retrieved from the bio-database of the Ikaria study and examined in relation to amyloid disease status. In particular, levels of high-sensitivity C-reactive protein (hs-CRP) was assayed by particle-enhanced immune-nephelometry (N Latex, Date-Behring Marburg GmbH, Marburg, Germany), Interleukin

- 6 (IL-6) was measured with high sensitivity enzyme linked immunoassay (R & D Systems Europe Ltd, Abingdon, UK), serum uric acid were determined using an enzymatic colorimetric test through the uricase-peroxidase method (UA plus, Roche Diagnostics, Manheim, Germany). Serum creatinine and urea were measured using a colorimetric method (BioAssay Systems, Hayward, CA, USA). Kidney function was evaluated by the creatinine clearance (CCr) rate, which is the volume of blood plasma that is cleared of creatinine per unit time. In particular, the CCr was calculated using the Cockcroft-Gault (CG) formula [16]: CCr= [[(140 - age) x weight] / (72 x serum creatinine)] for males, while for females, the result of the above equation was multiplied by 0.85. Moreover, thyroid stimulating hormone (TSH) was also measured in mIU/mL to evaluate thyroid function.

2.5.5. Clinical measurements

Resting arterial blood pressure was measured three times in the right arm, at the end of the physical examination with subject in sitting position. Participants whose average blood pressure levels were greater or equal to 140/90 mmHg or were under anti-hypertensive medication were classified as having hypertension. Hypercholesterolemia was defined as total serum cholesterol levels higher than 200 mg/dL or the use of lipid lowering agents. Diabetes mellitus type 2 was determined by fasting plasma glucose tests and was analyzed in accordance with the American Diabetes Association diagnostic criteria (i.e., fasting blood glucose levels >125 mg/dl or use of special medication, indicated the presence of diabetes). History of CVD (ICD-10 I25.0), i.e., acute myocardial infarction or angina, stroke (ICD-10 I63.9), heart failure (ICD-10 I50.9), atrial fibrillation (ICD-10 I48.91), were evaluated by the cardiologists of the study who also accessed participants medical files. Moreover, Peripheral Artery Disease (ICD-10 I73.9), Chronic Obstructive Pulmonary Disease (ICD-10 J44.9) and Thyroid disease (ICD-10 E07.9), were also evaluated through participants medical files.

2.5.6. Electrocardiographic measurements

Participants underwent a standard 12-lead ECG recording and a complete echocardio-graphic assessment; 195 subjects were excluded from the data analyses due to poor-quality ECG tracings (n=88) or poor left ventricular M-mode echocardiographic tracings (n=107). A resting 12-lead ECG was recorded during quiet respiration for each participant (duration 10s) using SE-1010 PC ECG (EDAN instruments. Inc., Nanshan Shenzhen, China). Smart ECG Measurement and Interpretation Programs (SEMIP version 1.5), which is part of EDAN SE series electrocardiograph and PC ECG, was used for the auto-mated measurement and interpretation of amplitudes and duration of ECG waves in each of the 12 leads. Adjustment of automatically designated amplitudes and duration of ECG waves were performed by two blinded physicians of the study. From these measurements, seven ECG criteria in consideration of both their general acceptance and recognized per-formance, five "pure voltage" criteria based on wave amplitude measurements, and two "time-voltage" criteria, were used, i.e., Sokolow-Lyon voltage (sum of the amplitudes of S wave on V_1 and R wave on V_5 or $V_6 \geq 3.5$ mV) [15], sex-specific Cornell voltage (sum of the amplitudes of S wave on V_3 and R wave on aVL >2.0 mV in females and >2.8 mV in men [16], Gubner-Ungerleider voltage (sum of the amplitudes of R wave on lead I and S wave on lead III ≥ 2.5 mV) [17], Lewis voltage (sum of the amplitudes of R wave on lead I and S wave on lead III, minus the amplitudes of S wave on lead I and R wave on lead III, ≥ 1.7 mV) [18], Framingham criterion (coexistence of a definite strain pattern and at least one of the following voltage criteria: sum of the amplitudes of the R wave on lead I and the S wave on lead III ≥ 2.5 mV, sum of the amplitudes of the S wave on lead V_1 or V_2 and the R wave on lead V_5 or $V_6 \geq 3.5$ mV, the S wave on the right precordial lead ≥ 2.5 mV and the R wave on the left precordial lead ≥ 2.5 mV) [19], Sokolow-Lyon product ($SV_1 + RV_5$ or V_6 x QRS duration ≥ 3000 mm.ms for females and ≥ 4000 mm.ms for

males) [20], and Cornell product [(RaVL + SV$_3$) + 8 mV for females] x QRS duration $\geq$ 2440 mm.ms) [20].

2.5.7. Cardiac ultrasonography

Standard transthoracic echocardiographic examination was carried out by study's physician in a dimly light room using a Vivid e cardiovascular ultrasound system (General Electric, Milwaukee, Wisconsin, USA) equipped with a 2.0 to 3.6 MHz (harmonics) phased-array transducer. The two-dimensional guided M-mode echocardiographic study of the left ventricle (LV) was performed at the parasternal long-axis view, and LV end-systolic and end-diastolic dimensions, as well as posterior wall and septal thicknesses, were measured as the mean from five consecutive cardiac cycles [21]. Reliability of the echocardiographic measurement of LV mass has been demonstrated in previous studies [22]. LV mass was calculated with the method of Devereux et al.: LV mass = 0.8 x 1.04 x [(LVID+VST+PWT)^3 - LVID^3] + 0.6, where LVID is left ventricular internal diameter, VST is ventricular septal thickness, and PWT is posterior wall thickness [23]. LV mass was indexed both for body surface area (BSA) and for height$^{2.7}$. LVH was defined as LV mass indexed for BSA $\geq$125g/m^2 in males and $\geq$110g/m^2 in females [24] or LV mass indexed for height$^{2.7}$ $\geq$49g/m$^{2.7}$ in males and $\geq$45g/m$^{2.7}$ in females [25].

Details about the assessment of biochemical, clinical and lifestyle characteristics of the Ikaria study's participants have been extensively published elsewhere [12-14]. Further details about echocardiographic and ultrasonography methods applied in the Ikaria study participants may be found elsewhere [26].

2.6. Follow-up evaluation

Four years after first enrolment (in 2013), the study's investigators performed a follow-up examination of the participants. Participants were appointed through telephone calls and then had face-to-face interviews with the study's investigators.

The investigators performed a detailed evaluation regarding their:

(a) vital status (death from any cause or due to CVD),

(b) development of myocardial infarction, angina pectoris, other identified forms of ischemia (WHO-ICD coding 410-414.9, 427.2, 427.6-, heart failure of different types, and chronic arrhythmias -WHO-ICD coding 400.0-404.9, 427.0 -427.5, 427.9-), and

(c) development of stroke (WHO-ICD coding 430-438).

All study's participants were found at follow-up examination.

2.7. Statistical analysis

Continuous variables that followed a normal distribution are presented as mean (standard deviation (SD)), while T-Amylo score is presented as Median (IQR) due to its skewed distribution. Categorical variables are presented as absolute and relative frequencies. The independent samples t-test and the non-parametric Mann-Whitney U-test were used for comparisons between means of normally or non-normally distributed continuous variables, respectively. Associations between categorical variables were tested by forming contingency tables and performing chi-square tests. Pearson or Spearman correlation coefficients were used, as appropriate, to test for correlations between continuous variables. Cox proportional hazards models were estimated to evaluate the association between T-Amyloid score, all-cause mortality, and CVD related outcomes, after various adjustments made. Results are presented as hazard ratios (HR), along with their corresponding 95% confidence intervals. Log-rank test was used to compare survival curves during the follow-up. Furthermore, ROC analysis and the calculation of the Area Under the Curve (AUC) was applied to evaluate the discriminating ability of the T-Amyloid score in predicting combined CVD outcomes. To further evaluate the additive discrimination and reclassification ability of T-Amylo score on CVD outcomes in relation to its components, the Net-Reclassification Improvement (NRI) and the Integrated Discrimination Improve-

ment (IDI) were also calculated (higher values suggest better performance of the predictive model). All reported p-values were based on two-sided hypotheses. All statistical calculations were performed using Stata statistical software (version 18.0; Texas, USA).

3. Results

3.1. Distribution of T-Amylo score

Median (IQR) T-Amylo score was 3.0(3.0) and mean was 2.72 ± 2.04, and it was significantly higher in males as compared to females (median (IQR) , 4.0.(3.0) vs. 2.0(3.0), p<0.001). When focused on participants aged 65 years or older the mean T-Amylo score was 4.02 ± 1.59 versus 1.36 ± 1.50 for the younger (p<0.001). In addition, T-Amylo score was highly correlated with age, irrespective of gender of the participants (rho = 0.741, p<0.001). The prevalence of participants with high risk (score > 6) in the entire sample was 9.6%; specifically, 120 (18.8%) males and 10 (1.4%) females had T-Amylo score greater than 6 (*p* for gender differences <0.001). This prevalence raised up to 18.5% for both sexes when focusing on older than 65 years old participants. The actual prevalence of amyloidosis in the referent population was estimated to be 17% for males and 7% for females.

3.2. Univariate and multivariate associations between participants' characteristics and high T-Amylo score

In *Tables 1* and *2*, participants' characteristics are presented according to the T-Amylo score levels. As it can be seen, participants with high score were older, males, had higher waist circumference and waist-to-hip ratio; however, differences in anthropometric indices became insignificant when age and gender were considered (p-values > 0.770). Moreover, participants with high T-Amylo score were more likely to be ever smokers (p<0.001), but this was explained (i.e., became insignificant, p=0.554) when sex of the participants was considered, as males were 14-times more likely to be smokers compared to females (p<0.001) (Table 1).

Moreover, participants with a history of a CVD event were 2-times more likely to have high T-Amylo score as compared to those without history of CVD (95%CI 1.23 to 3.32, p=0.005), irrespective of age, and gender. Higher prevalence of all CVD manifestations studied was observed among those at high T-Amylo score as compared to those at lower recorded. Similarly, participants with high T-Amylo score were more likely to have history of COPD but were less likely to have thyroid disease. (Table 2)

Regarding biochemical markers, participants with high T-Amylo score had higher inflammation markers levels, as well as uric acid and creatinine levels, but they had lower level of creatinine clearance (Table 2). These associations of biochemical markers with high T-Amylo score remained significant even after adjustments were made considering age, gender, smoking, and physical activity status, as well as BMI of the participants (p-values < 0.05).

3.3. All-cause and CVD mortality and morbidity during the follow-up

During the follow-up, 53 deaths were observed, and thus, the mortality rate was 373 deaths per 10,000 inhabitants. Causes of death were myocardial infarction (21% of the cases), stroke (15% of the cases), cancer (21% of the cases), infection (10% of the cases), renal failure (4% of the cases), respiratory (3% of the cases) and the rest 26% from other causes (e.g., accidents, etc.). Stratifying the analysis by T-Amylo score group the distribution of cause of death was: *low*, 18% myocardial infarction, 12% stroke, 20% cancer, 11% infection, 4% renal failure, 3% respiratory and 25% from other causes and for *high*, 22% myocardial infarction, 17% stroke, 21% cancer, 9% infection, 4% renal failure, 3% respiratory and 27% from other causes. No significant differences were observed in the distribution of causes of death between T-Amylo score groups (all p-values >0.20). Moreover, 134 participants (72 males, 22.6%, p for gender comparisons=0.006) had a non-fatal CVD event (i.e., 45% acute myocardial infarction, 23% heart failure, 22% stroke, and 10% other manifestations of CVD) during the follow-up period (p for gender difference

= 0.20). Stratifying the analysis by T-Amylo score group the distribution of non-fatal CVD events was: *low*, 44% myocardial infarction, 17% heart failure, 21% stroke, 18% other CVD and for *high,* 47% myocardial infarction, 27% heart failure, 22% stroke, and 4% from other CVDs.

3.4. Assessment of T-Amylo score in relation to all-cause and CVD mortality and morbidity

The T-Amylo score was highly associated with all-cause mortality (Hazard Ratio 1.59, 95%CI 1.40 to 1.81), after adjusting for age, gender, physical activity and smoking status, BMI, history of previous CVD, as well as medical history of diabetes, hypertension, and hypercholesterolemia. This was evident in older participants (>65 years), but not among younger participants (Hazard Ratio 1.44, 95%CI 1.22 to 1.70, and 1.16, 95%CI 0.72 to 1.86, respectively).

When focusing on combined CVD outcomes, participants with high T-Amylo score had lower survival rates compared to those with low score (p for log-rank test =0.03) (Figure 1). In addition, T-Amylo score was positively associated with the risk of CVD events (fatal or non-fatal); in particular, one-unit increase (1/11) in the score was associated with 32% higher risk for CVD (Hazard Ratio 1.32, 95%CI 1.11 to 1.56), after adjusting for age, gender, physical activity and smoking status, BMI, history of previous CVD, as well as medical history of diabetes, hypertension, and hypercholesterolemia. Then the analysis was repeated stratifying the sample into those < 65 and >65 years old; it was observed that the T-Amylo score was not associated with younger participants (Hazard Ratio 1.21, 95%CI 0.96 to 1.52), whereas it was highly associated with CVD events among older adults, as one-unit increase was associated with 41% higher risk for CVD (Hazard Ratio 1.41, 95%CI 1.16 to 1.71), after the aforementioned adjustments were made.

Furthermore, to evaluate the classification ability of the T-Amylo score, ROC analysis was applied and revealed that the AUC was 0.70, and the accuracy of the score was

81.52%. The NRI and the IDI indices were 0.330 and 0.011 (p=0.03), respectively, suggesting that the inclusion of T-Amylo score in the model that included the rest of parameters adds significantly in the correct classification of the model.

Significant interactions were observed between T-Amylo score, age, and sex of the participants (all p-values < 0.001). Thus, the analysis was stratified to participants below and above 65 years of age; it was observed that the AUC was 0.61, and the accuracy was 73.39%, for the younger and the AUC was 0.83, and the accuracy was 92.25%, for the older ones. Moreover, we have assessed the predictive value of the T-Amyloid score stratifying by gender, and found that the AUC was 0.67, and the accuracy of the score was 77.78% in males and the AUC was 0.77, and the accuracy of the score was 85.97% in females.

However, some of the parameters that are included in the T-Amylo score are well-known to have impact on CVD mortality and morbidity. Thus, we further explored the additive predictive value of the suggested score on CVD outcomes, as compared to its components. It was observed that compared to age, gender, IVSd thickness, QRS interval voltage, and carpal tunnel syndrome, the T-Amylo score improved the correct classification ability of the models by 4%, 7%, 8%, 5% and 5%, respectively (IDI , all p-values <0.001).

4. Discussion

The aim of this study was to evaluate the associations between a cardiac amyloidosis score, the T-Amylo score, with CVD morbidity and mortality and its related predictors, in a population known for longevity. Transthyretin amyloid cardiomyopathy is often found in patients with heart failure with preserved ejection fraction (HFpEF). Previous studies have demonstrated the clinical value of the T-Amylo score for ATTR-CM in selected populations, i.e., patients with diagnosed heart failure [27]. The data analysis of

approximately 1,400 apparently healthy, middle-aged individuals from the general population, revealed that the implementation of an ATTR-CM related score, i.e., the T-Amylo score, seemed to act as a good discriminator in predicting all-cause mortality and CVD events. The predictive value of the score was irrelevant of the presence of obesity, smoking status, history of diabetes mellitus, arterial hypertension, chronic obstructive pulmonary disease, and thyroid disease, but it was moderated by older age and female sex of the participants. Moreover, the T-Amylo score improved the correct classification ability of the models that included solely its components, i.e., age, gender, IVSd thickness, QRS interval voltage, and carpal tunnel syndrome, by 4% to 8%; suggesting that a considerable number of CVD patients could have been identified by using this score.

In line with the findings of a previous study who applied the T-Amylo score in HFpEF patients, [9] in this study individuals with low T-Amylo score (i.e., score <6) compared to those with high , were older, male, and had lower body mass index, left ventricular ejection fraction, and estimated glomerular filtration rate. In addition, in other relevant studies in patients with clinically diagnosed HFpEF that have also incorporated ATTR-CM scores, like the Mayo ATTR-CM score, subjects with high score had lower prevalence of hypertension, but a higher prevalence of atrial fibrillation, and coronary artery disease. Additionally, have also shown that the ATTR-CM scores were associated with increased risks of CVD outcomes, as well as all-cause mortality, and hospitalization due to heart failure [27, 29]. In this study, we expanded the previous findings to the general population by showing that the T-Amylo score for ATTR-CM is significantly associated with all-cause and CVD mortality and morbidity. However, it should be noted that our findings were more prominent among older adults than in younger ones.

However, a question arises, why the score is not as effective in individuals younger than 65? It may be that the factors weighted heavily in the score are less prevalent or significant in a younger individuals. Moreover, age, septal thickness, and gender, all components of the T-Amylo score, are significant predictors in older populations but may not capture the nuanced risk factors relevant to younger individuals. Additionally, many subjects with increased septal thickness might have hypertension, a condition that is more common and impactful in older adults.

As patients with ATTR-CM experience a severe, progressive disease, α greater understanding of the presentation and progression of ATTR-CM can guide physicians in earlier diagnosis and treatment of patients with ATTR-CM. Even, beyond screening for ATTR-CM, higher values of ATTR-score are significantly correlated with the existence of CVD, chronic obstructive pulmonary disease, thyroid disease, lower creatinine clearance, higher IL6 levels, male sex, and lower body weight. This was also evident in the TOPCAT study's sub-analyses, where compared with patients with low ATTR-CM score, those with high ATTR-CM score were, older, male, and had lower body mass index, blood pressure, LVEF and estimated glomerular filtration rate. In addition, patients with low ATTR-CM score had lower prevalence of hypertension, but a higher prevalence of atrial fibrillation, and coronary artery disease [27].

Furthermore, higher score levels were related with presence of LVH, while independently of those relationships higher ATTR-CM score was associated with increased risks of clinical outcomes. This comes in line with the findings of this study and illustrates the difficulty to detect ATTR-CM in individuals with many comorbidities; in whom even symptom may be undetected. Thus, the clinical implication of simplified scores has significant

prognostic role, as it seems that even when applied them in general population without known heart failure diagnosis, are significant related with cardiovascular events.

The T-Amylo score has been designed and tested in selected populations and not in a general population, as in the present study; although this might be a limitation, this is also a novelty of this study. The diagnosis of amyloidosis requires a multidisciplinary approach, combining clinical assessment, laboratory tests, imaging studies, and tissue biopsies. By implementing the T-Amylo score in the general population, early detection and intervention efforts are enhanced, as laboratory testing, imaging and genetic studies, can be difficult to implemented in daily clinical practice in identifying cardiac ATTR amyloidosis. Recognizing individuals at an elevated risk for amyloid-related conditions, allows for timely preventive measures, potentially slowing disease progression or even preventing onset of the disease. In addition, the T-Amylo score provides a standardized, objective measure that can be broadly applied, making it a valuable tool for public health initiatives. Its use in population-wide screening programs can facilitate large-scale epidemiological studies, yielding insights into the prevalence and distribution of amyloid-related diseases. The general population's use of the T-Amylo score also supports personalized medicine approaches. By integrating individual risk profiles into healthcare planning, clinicians can tailor recommendations for lifestyle modifications, monitoring, and treatment plans, thereby optimizing patient outcomes. Moreover, the widespread application of the T-Amylo score can contribute to raising public awareness about amyloid-related diseases. As individuals become more informed about their health risks, they may be more proactive in seeking medical advice and adopting healthier behaviors.

Limitations

Sampling procedure may hide potential selection bias and healthy volunteer effect, as people living in institutions were excluded from the Ikaria study, and the general participation rate was 54%. For evaluating all-cause and CVD mortality and morbidity in a general population, the sample of 1420 subjects is not adequate to be considered as representative of the general population, taking also into account that the follow-up was only of 4 years. The M-mode was used for the LV measurements according to the former ESC guidelines (2005); however, the current guidelines recommend 2D measurements for LV dimensions. In the clinical diagnosis of ATTR-CM several echocardiographic indices are also used, like longitudinal left ventricular strain, and other multiparametric scores that incorporate more advanced echocardiographic features, to increase the diagnostic accuracy, which there were no available in this study. Thus, to avoid confusion, and due to the lack of relevant validation of the T-Amylo score in the general population, no diagnosis of ATTR-CM is presented here.

5. Conclusions

As the general population is getting older, and the predisposing factors for CVD in older individuals are not well understood and appreciated, the application of markers for the prognosis of CVD morbidity and mortality through uncovering other predisposing health states, is of major importance. In this study the prognostic value of a ATTR-CM score, the T-Amylo score, was evaluated and found promising for prognosis of CVDs in the general population, too.

Supplementary Materials: None.

Valatsou, D Vasiliou, G Vogiatzi, S Vogiatzoglou, M Xynogala, M Zaromytidou, C Zisimos, V Zoulia.

Conflicts of Interest: The authors declare no conflicts of interest.

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Table 1. Demographic, and lifestyle, characteristics of the study's participants, according to T-Amylo score of cardiac amyloidosis.

Parameter	T-Amylo score		p-value
	Low (n=1290)	**High** (n=130)	
Age (years)	65 ± 13	82 ± 7	<0.001
Sex (males) (%)	42%	92%	<0.001
Lifestyle characteristics			
Current smokers (%)	28%	24%	0.145
Ever smokers (%)	56%	80%	<0.001
Physically inactive (%)	34%	47%	0.384

Participants' characteristics are presented as mean (Standard Deviation) and relative frequencies. P-values derived using independent samples t-test, or Pearson's chi-square test.

Abbreviations: ATTR-CM: Transthyretin amyloid cardiomyopathy.

Table 2. Clinical, electrocardiographic, and echocardiographic characteristics of the study's participants, according to T-Amylo score of cardiac amyloidosis.

Parameter	T-Amylo score		p-value
	Low (n=1290)	High (n=130)	
Anthropometric indices			
Body mass index (kg/m^2)	28.6 ± 5.1	27.2 ± 4.6	0.001
Waist (cm)	101.7 ± 13.6	104.0 ± 11.4	0.044
Waist-to-Hip ratio	0.94 ± 0.09	0.99 ± 0.10	<0.001
BSA (m^2)	1.90 ± 0.22	1.87 ± 0.17	0.175
Medical history			
Hypertension (%)	49%	49%	0.999
Diabetes (%)	17%	22%	0.082
Dyslipidemia (%)	42%	37%	0.322
Cardiovascular disease (%)	11%	37%	<0.001
Acute Myocardial Infarction (%)	3%	13%	<0.001
Stroke (%)	2%	9%	<0.001
Heart failure (%)	2%	7%	0.001
Atrial fibrillation (%)	5%	19%	<0.001
Peripheral Artery Disease (%)	3%	8%	0.060
Chronic Obstructive Pulmonary Disease (%)	6%	21%	<0.001
Thyroid disease (%)	26%	5%	<0.001
Biochemical markers			
hs-CRP (mg/L)	2.85 ± 3.7	3.56 ± 6.9	0.128
IL-6 (mg/L)	3.31 ± 7.5	9.20 ± 6.9	<0.001
Uric acid (mg/dL)	5.5 ± 1.5	6.2 ± 1.7	<0.001
Creatinine (mg/dL)	0.91 ± 0.24	1.07 ± 0.30	<0.001
Creatinine clearance	83 ± 33	62 ± 19	0.018
TSH (mIU/mL)	1.93 ± 3.2	2.05 ± 2.7	0.672
Echocardiographic and electrocardiographic indices			
Ejection fraction (%)	60.5 ± 4.2	53.7 ± 7.2	<0.001
LVMI^{BSA} (g/m^2)	93 ± 23	125 ± 28	<0.001
IVSd, cm	0.99 ± 0.1	1.11 ± 0.2	<0.001
LVEDV, ml/m^2	45.2 ± 4.4	47.1 ± 4.9	<0.001
PWT, cm	0.96 ± 0.1	1.11 ± 0.2	<0.001
QRS duration (ms)	95 ± 17	110 ± 35	<0.001

ECG-LVH (SL-voltage) (%)	2.9%	6.5%	0.024
ECG-LVH (SL-product) (%)	2.9%	6.5%	0.023
ECG-LVH (Cornell-voltage) (%)	3.9%	7.3%	0.049
ECG-LVH (Cornell-product) (%)	9.6%	14.7%	0.047
ECG-LVH (Gubner) (%)	2.4%	2.7%	0.326
ECG-LVH (Lewis) (%)	11%	10.9%	0.960
ECG-LVH (Framingham) (%)	7.9%	18.7%	<0.001

Participants' characteristics are presented as mean (Standard Deviation) and relative frequencies. P-values derived using independent samples t-test, or Mann-Whitney U-test (for not normally distributed variables), or Pearson's chi-square test. Abbreviations: ATTR-CM: Transthyretin amyloid cardiomyopathy, hs-CRP: high-sensitivity C-reactive protein, ECG-LVH: Electrocardiography left ventricular hypertrophy, IL-6: interleukin 6, LVMI: Left ventricular mass index, TSH: Thyroid stimulating hormone, IVSd: Interventricular septal end diastole, LVEDd = Leftventricular enddiastolic volume, PWT: posterior wall thickness.

Kaplan–Meier survival estimates
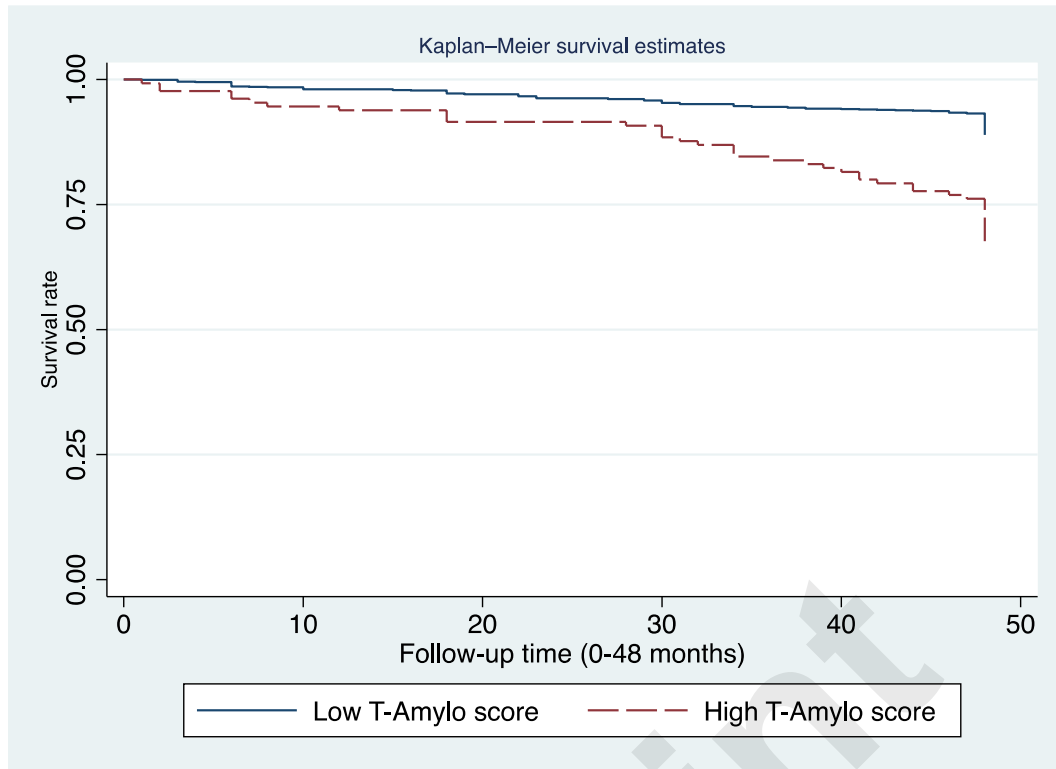
Time (mo)	0-10	10-20	20-30	30-40	40-50
No. at risk	1420	979	957	939	908
CVD Events	27	22	18	31	89

Figure 1. Kaplan-Meier survival plot of T-Amylo score (high, >=6/11 vs. low, <6/11) for combined CVD events (fatal or non-fatal) during the 4-year follow-up (48 months) of n=1,420 middle aged and older participants of the Ikaria Study.

A simplified cardiac amyloidosis score is associated with all-cause and cardiovascular disease mortality and morbidity, in the general population: the Ikaria Study.

Amyloidosis is a rare and complex group of diseases characterized by the abnormal accumulation of protein deposits known as amyloids in various tissues and organs .

There are different types of amyloidosis, and the specific type is determined by the protein that forms the amyloid deposits
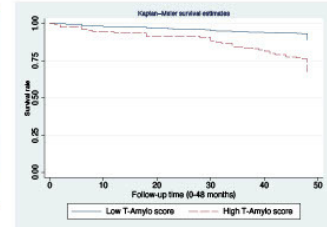
During 2009, 1,420 middle aged and older individuals enrolled in the Ikaria study (678 males 67(14) years, and 742 females 66(14) years); in 2013, the participants were re-evaluated.
- A simple ATTR-CM score, the T-Amylo score, including, age, sex, and echocardiographic measurements, was used.

ATTR cardiac amyloidosis can lead to heart failure, arrhythmias, and other heart-related outcomes.

T-Amylo score was associated with higher all-cause mortality (Hazard Ratio 1.59, 95%CI 1.40 to 1.81), and risk of CVD events (1.32, 95%CI 1.11 to 1.56). AUC of T-Amylo score was 0.70, accuracy was 81.52%.

Kaplan–Meier survival estimates

By employing the T-Amylo score within a general population context offers significant advantages in terms of early detection, public health research, personalized medicine, and health education, ultimately contributing to better health outcomes and disease management strategies.