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Cholesterol-adjusted vitamin E serum levels are associated with cardiovascular events in patients with non-valvular atrial fibrillation ☆,☆☆

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ABSTRACT

Background: Non-valvular atrial fibrillation is associated with an increase in thromboembolism, i.e. stroke, and atherosclerotic events, i.e. myocardial infarction. Vitamin E possesses anti-coagulant as well as anti-atherosclerotic properties.

Our aim was to assess whether vitamin E is associated with cardiovascular events in patients with non-valvular atrial fibrillation.

Methods: Serum levels of cholesterol-adjusted vitamin E were measured in 1012 patients with non-valvular atrial fibrillation. Patients were followed for a mean time of 27.0 months, and cardiovascular events, such as cardiovascular death and fatal and nonfatal stroke or myocardial infarction, were recorded.

Results: During the follow-up period, cardiovascular events occurred in 109 (11%) patients (18 fatal and 14 nonfatal myocardial infarction; 13 fatal and 19 nonfatal ischemic strokes; 45 cardiovascular deaths). Lower vitamin E serum levels were found in patients who experienced cardiovascular events compared to those who did not (3.8 ± 1.2 vs. 4.4 ± 1.8 μmol/mmol cholesterol; $p < 0.001$).

Using a Cox proportional hazard model, age, diabetes, history of stroke and myocardial infarction and vitamin E serum levels (HR 0.77; 95% CI: 0.67–0.89; $p = 0.001$) independently predicted cardiovascular events. Patients with vitamin E < 4.2 μmol/mmol cholesterol (median values) had an increased risk of cardiovascular events (HR 1.87; 95% CI: 1.25–2.80: $p = 0.002$).

Conclusions: Low vitamin E serum levels are associated with an increased risk of cardiovascular events in patients with non-valvular atrial fibrillation.

☆ Authorship—R. Cangemi, P. Pignatelli, S. Basili, D. Ferro, R. Carnevale and F. Violi take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation. G.R. Corazza, D. Pastori, and L. Loffredo researched and analyzed the data. Davì, W.R. Hiatt, G. Licata, G.Y.H. Lip, and P.M. Mannucci reviewed and edited the manuscript. A. Farcomeni and A. Vestri analyzed the data and edited the manuscript. The ARA PACIS study group researched the data.

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1. Introduction

Non-valvular atrial fibrillation (NVAF) is the most common cause of cardiac arrhythmia and is known to be associated with clinical events related to thromboembolism, i.e. stroke, or to atherosclerotic disease, i.e. myocardial infarction [1,2].

Strategies for identifying patients at risk for thromboembolism are commonly based on clinical variables, often aggregated in scores (i.e. the $CHADS_2$ [3] and the CHA_2DS_2VASc [4] scores). The role of biochemical markers to provide incremental information on risk evaluation is a matter of debate. Recently high-sensitivity C-reactive protein (hs-CRP) [5,6], cardiac troponin I, N-terminal pro-B-type natriuretic peptide [7], and creatinine clearance [8] have all shown to improve risk stratification in NVAF.

NVAF is a peculiar clinical setting where thromboembolism and athero-thrombosis coexist [9]; thus it may have implications for circulating vitamin E levels, since the latter possesses both anticoagulant (through lowering the activation of vitamin K-dependent clotting factors) [10,11] and anti-atherosclerotic properties (in part related to its antioxidant activity) [12].

At this regard we have recently shown an association between low vitamin E serum levels and atrial fibrillation recurrence in patients undergoing electrical cardioversion [13]. No data exist so far on the relationship between circulating levels of vitamin E and cardiovascular events in NVAF patients. In this prospective study, we tested the hypothesis that circulating levels of vitamin E might predict stroke and myocardial infarction in a "real world" consecutive population of patients with NVAF over a mean follow-up period of 27 months.

2. Methods

2.1. Study design and patient selection

This was a prospective multicenter study which included 1498 consecutive in- or out patients with NVAF who were screened between April 2004 and May 2011.

Six Italian clinical centers participated in this study: 4 from the "Sapienza" University of Rome ("I Clinica Medica - Atherothrombosis Center"; "Secondary Hypertension Unit" and "UOC Medicina Interna F", Department of Internal Medicine and Medical Specialties; and "Department of Cardiovascular, Respiratory, Nephrologic and Geriatric Sciences"); one from the "Catholic University" School of Medicine, Rome ("Institute of Internal Medicine and Geriatrics and Haemostasis Research Centre"); one from the "University of Perugia", Perugia ("Internal Medicine, Angiology and Atherosclerosis", Department of Clinical and Experimental Medicine). The "Atherothrombosis Center" coordinated enrollment and data collection and monitored data quality. The "Atherothrombosis Center" and the "Department of Science of Public Health and Infectious Diseases" at the Sapienza-University of Rome provided statistical support.

Study procedures were standardized across sites. Data were entered into a centralized, secure, electronic data capture system. Each site received the local Ethical Committees' approval. The study was conducted in accordance with the principles embodied in the Declaration of Helsinki. Written informed consent was obtained from all subjects.

Exclusion criteria were: valvular disease (defined as rheumatic mitral valve disease or prosthetic heart valves [14], or the presence of any "severe" regurgitation or gradient), severe congestive heart failure (New York Heart Association functional class IV), acute myocardial infarction or stroke during the previous 3 months, revascularization procedures performed during the previous 6 months, cerebral hemorrhage, severe involutive cerebral disease, or carotid lesion requiring surgical intervention. Furthermore, subjects were excluded from the study if they had neoplastic diseases, liver insufficiency defined as chronic hepatic disease (e.g., cirrhosis) or biochemical evidence of significant hepatic derangement (e.g., bilirubin $> 2 \times$ upper limit of normal, in association with aspartate aminotransferase/alanine aminotransferase/alkaline phosphatase $> 3 \times$ upper limit), or serious renal disorders (serum creatinine > 2.8 mg/dL). Concomitant use of vitamin supplementation represented an additional exclusion criterion.

Based on the above criteria, 387 patients (26%) were excluded. Moreover, 51 (3%) withdrew and/or moved out from the geographic area and 48 (3%) refused to be enrolled in the follow-up study (Fig. 1). Thus, 1012 NVAF patients participated in the study. Of the 1012 NVAF patients who entered in the study, 618 (61%) had permanent or persistent (lasting >6 months) NVAF, and 394 (39%) had paroxysmal NVAF as previously defined [14].

Patients were treated with oral anticoagulants or aspirin according to $CHADS_2$ score [3].

However, anticoagulant treatment could be judged inappropriate at the discretion of the physician in case of: (*i*) specific risk of bleeding, (*ii*) patients' unwillingness to take oral anticoagulants or patients' inability to comply with monitoring of the international normalized ratio. Patients with $CHADS_2 = 1$ were generally treated with aspirin.

Patient's medical history was recorded at the time of study entry and physical examination was performed. The following diagnostic procedures were also performed: routine blood laboratory tests, including fasting serum total, HDL and LDL-cholesterol and triglycerides, baseline 12-lead ECG, M-mode- and 2-dimensional echocardiography.

Renal creatinine clearance (CrCl) was estimated using the Cockcroft–Gault formula [15]. Arterial hypertension was defined as repeatedly elevated blood pressure exceeding 140/90 mm Hg or taking antihypertensive-drugs [16]. Hypercholesterolemia [17], metabolic syndrome [18], diabetes [19] and heart failure [20] were defined as previously described.

2.2. Follow-up

All patients were followed-up for the entire duration of the study and outcome events were recorded. Patients were regularly seen every 6 months for vital status evaluation: ECG, standardized questionnaire and clinical examination were carried out and compliance with the prescribed drugs was checked. Given that dietary changes could potentially influence anticoagulation rate by warfarin, patients treated with oral anticoagulants, were advised not to modify the diet suggested by their general practitioners. Weight changes and diet style were also checked at each follow-up visit. Each patient was advised to avoid antioxidant supplements during the follow-up. Follow-up data were obtained by review of hospital databases, medical records, death certificates or telephone interviews.

2.3. Outcome events

The combined incidence of the following cardiovascular events was considered the primary outcome of the study: first occurrence of ischemic stroke (nonfatal or fatal) or myocardial infarction (nonfatal or fatal) and cardiovascular death. A diagnosis of myocardial infarction required at least 2 of the following criteria: history of chest discomfort, development of a pathological Q wave on ECG tracings, and elevation of specific cardiac enzymes to values of more than twice the upper normal limit. The occurrence of stroke was determined based on clinical manifestations and confirmed by CT. If a patient died within 4 weeks of stroke or myocardial infarction, this event was recorded as fatal stroke or fatal myocardial infarction. Death was classified as cardiovascular unless an unequivocal non-cardiovascular cause of death was confirmed by the central adjudication committee. Cardiovascular death included sudden death; death due to cardiogenic shock in patients with NYHA-IV heart failure; procedure related death (cardiovascular investigation/procedure/operation); death due to other specified cardiovascular causes; and presumed cardiovascular deaths (i.e. those for which a non-cardiovascular cause had not been clearly established).

Adjudication of cardiovascular events was performed by a committee (FV, DF, PP, DP) who did not participate to the patients' recruitment and follow-up and was unaware of the clinical and laboratory characteristics of any patient.

2.4. Blood collection and laboratory analysis

After overnight fasting and supine rest for at least 10 min, blood was withdrawn from the antecubital vein. Serum was divided into aliquots and stored at −80 °C.

2.5. Vitamin E

Serum levels of vitamin E (α-tocopherol) were measured by HPLC as previously reported [21], using tocopheryl acetate as internal standard (Sigma Chemical, St Louis, MO, USA).

Levels were expressed as ratio (μmol/mmol) between serum α-tocopherol concentration (μmol/L) and serum total cholesterol concentration (mmol/L), which better express the circulating (absolute) levels of vitamin E [22].

All samples were analyzed within one month of storage. We test three different samples (baseline, 7 and 30 days) from 50 patients to analyze the influence of storage time on vitamin E concentrations. The results showed no significant changes of vitamin E concentrations in the three different samplings (not shown).

2.6. C-reactive protein

High sensitivity C-reactive protein (hs-CRP) was measured by a commercially available immunoassay (Tema Ricerca, Italy). Intra-assay and inter-assay coefficients of variation were 9.5% and 9.0%, respectively.

2.7. Urinary 8-iso-prostaglandin F2α

The levels of 8-iso-prostaglandin F2α were measured with a commercial immunoassay (Assay the Designs, Ann Arbor, MI) as previously described [23]. Intra- and inter-assay coefficients of variation were 5.7% and 5.8%, respectively.

The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology.

2.8. Statistical methods

2.8.1. Sample size determination

A Cox regression of the log hazard ratio on a covariate with a standard deviation of 1.5 based on a sample of 796 observations achieves 95% power at a 0.05 significance level to detect a regression coefficient equal to −0.22. The sample size was adjusted

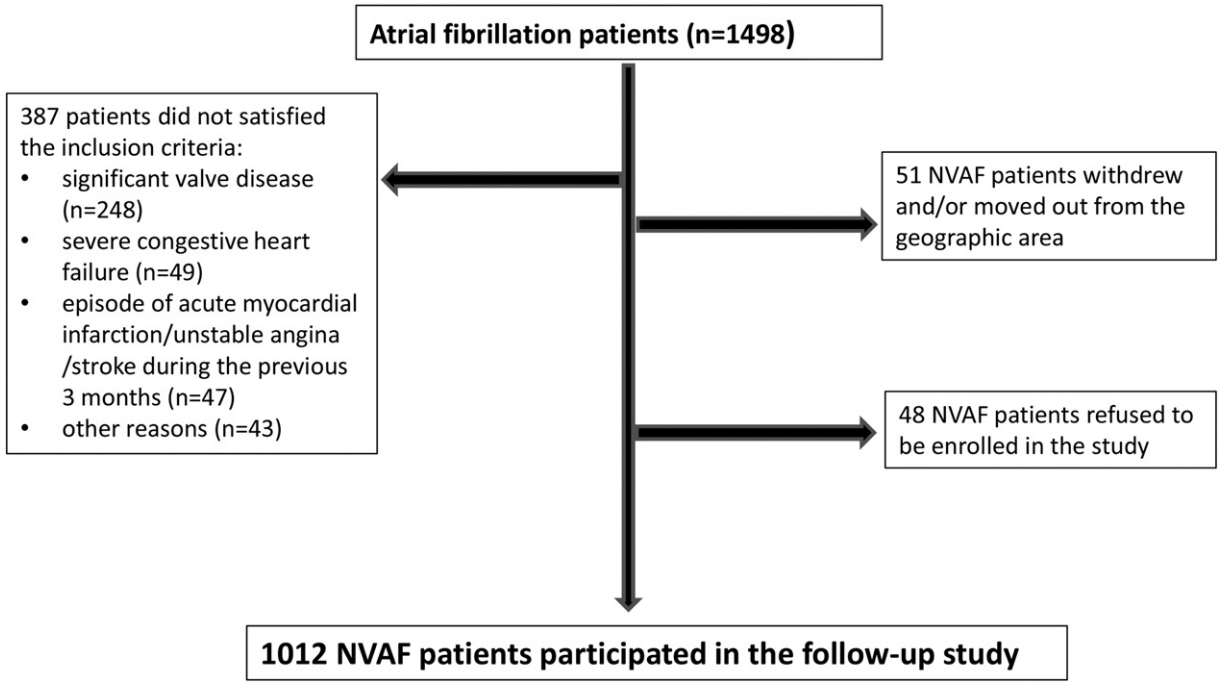

Fig. 1. Patient flow diagram of the study. NVAF = nonvalvular atrial fibrillation.

for an anticipated event rate of 0.15. The expected hazard ratio, the event rate and the standard deviation have been conservatively obtained from analyzing data from the previous studies [2,24,25].

2.8.2. Statistical analysis

Cox proportional hazards analysis was used to calculate the adjusted relative hazards of outcome events by each clinical variable. In addition to vitamin E serum levels the following variables, whereby the $CHADS_2$, and Framingham Heart Study [17] (assessed at the baseline evaluation) were considered as potential predictors of cardiovascular events: age, sex, hypertension, diabetes, previous cerebral ischemia, previous coronary heart disease, heart failure, lipid profile (total cholesterol, LDL and HDL cholesterol, triglycerides), glycemia and smoking habit. Creatinine clearance, hs-CRP, left atrium diameter [26], statins [27], oral anticoagulant and antiplatelet treatment were also considered in this model. Stochastic level of entry into the model was set at a p-value = 0.10, and interaction terms were explored for all the variables in the final model.

We checked for interactions by building interaction terms from the main effects in the final model, and then using the same model selection approach used to build the multivariate model with only main effects, with a level to entry p = 0.1. No interaction was included, and none was indeed significant.

A minimum "events-to-variable" ratio of 10 was maintained in multivariate modeling to avoid over fitting, and Schoenfeld's test was performed to check the validity of proportional hazard assumption.

A COX analyses were also performed to verify if vitamin E has addictive value to predict cardiovascular events in addition to the $CHADS_2$ and the CHA_2DS_2VASc score, used as a linear categorical covariates, rather than including separately the above mentioned risk factors. The net reclassification index [28] was calculated to assess improvement in cardiovascular risk categorization compared with the $CHADS_2$ and the CHA_2DS_2VASc scores for low-, intermediate-, and high-risk patients by combining values so the overall event rates would be similar for each risk category across the 3 models.

Two-sided *t* tests or Wilcoxon rank sum test, depending on the shape of the distribution curve, were used to compare means. Pearson chi-square test was used to compare proportions.

Bivariate analysis was performed with Pearson's linear correlation.

To better define the relationship between serum vitamin E serum levels and outcome events, the entire NVAF population was categorized on the basis of the median value of vitamin E serum levels observed in the overall population: above (or equal) and below the median vitamin E level (4.2 μmol/mmol cholesterol). The cumulative risk of cardiovascular events (nonfatal/fatal ischemic stroke and nonfatal/fatal myocardial infarction) within each group was estimated through the Kaplan–Meier method. The survival curves of the 2 groups were then formally compared using the log-rank test. The validity of constant incidence ratios over the follow-up was checked using Nelson–Aalen cumulative hazard estimates.

Data are presented as mean (±SD) or as median and interquartile range (IQR; 25th, 75th percentile). Only p values lower than 0.05 were regarded as statistically significant. All tests were two-tailed and analyses were performed using computer software packages (SPSS-19.0, SPSS Inc. or R version 2.15.2, R Development Core Team, Vienna, Austria).

3. Results

Clinical characteristics of AF population are reported in Table 1. Vitamin E serum levels in the overall population were 4.3 ± 1.8 [Median (IQR): 4.2 (3.2–5.2)] μmol/mmol cholesterol.

In order to analyze the variability of vitamin E serum levels during the follow-up, the analysis was repeated after 1-year follow-up in a randomly selected subgroup of patients, age and sex frequency matched with the entire follow-up population (n = 45, 24 males, 21 females, age: 72.6 ± 8.7 years). For each subject difference between serum vitamin E at one year and at baseline was on average 0.0031 ± 0.389 μmol/mmol cholesterol. The paired *t*-test did not reject the null hypothesis (p = 0.957). Changes of the mean values over time were also not significant (4.3 ± 1.6 vs. 4.3 ± 1.5 μmol/mmol cholesterol; p = 0.963).

To analyze the association between vitamin E and oxidative stress, we analyzed the relationship between urinary excretion of isoprostanes (8-iso-prostaglandin $F_{2\alpha}$), index of oxidative stress in vivo [29], and cholesterol-adjusted vitamin E levels in a randomly selected subgroup of NVAF patients (n = 162), age and sex frequency matched with the entire follow-up population (males = 82; females = 80, age: 72.9 ± 7.4 years). We found a weak inverse correlation between isoprostanes and vitamin E serum levels (r = −0.2, p = 0.028).

Sex, hypertension, smoking habit and metabolic syndrome were not significantly discriminated by vitamin E serum levels (not shown); conversely, patients with diabetes had lower values compared with non-diabetic ones (4.1 ± 1.6 vs. 4.4 ± 1.8 μmol/mmol cholesterol; p = 0.030).

3.1. Survival analysis in the overall population

All patients were followed for a mean time of 27 months (minimum follow-up: 6 months; maximum: 96 months) yielding a total of 2291 person-years of observation.

During the entire study period, INR values of patients on oral anticoagulation therapy fell in the therapeutic range (2.0–3.0) 64% of the time; while INR values of these patients were below or above this range 23% and 13% of the time, respectively. One-hundred-nine patients (11%) experienced a primary outcome (fatal and nonfatal myocardial

Table 1
Baseline characteristics of patients in relation to cardiovascular events development during the follow-up.

Characteristics	Whole cohort	Patients without cardiovascular events	Patients with cardiovascular events	P value
n	1012	903	109	/
Age (years)[a]	73.0 ± 8.4	72.6 ± 8.4	75.6 ± 7.3	<0.001
Male sex n. (%)	55	55	51	0.441
Body mass index (kg/m^2)[a]	27.2 ± 4.0	27.3 ± 4.1	26.9 ± 3.6	0.329
Total cholesterol (mmol/L)[a]	4.62 ± 1.06	4.60 ± 1.04	4.81 ± 1.12	0.066
LDL cholesterol (mmol/L)[a]	2.83 ± 1.02	2.81 ± 1.01	3.01 ± 1.00	0.063
HDL cholesterol (mmol/L)[a]	1.16 ± 0.38	1.16 ± 0.38	1.14 ± 0.40	0.754
Glycemia (mmol/L)[a]	5.57 ± 1.61	5.57 ± 1.59	5.58 ± 1.73	0.952
CrCl (ml/min)[a]	64.8 ± 19.2	65.4 ± 19.4	59.6 ± 17.1	0.003
hs-CRP (mg/ml)[b]	0.84 (0.40–1.73)	0.79 (0.40–1.72)	1.2 (0.46–1.77)	0.008
Systolic blood pressure (mm Hg)[a]	131 ± 14	131 ± 14	132 ± 14	0.783
Diastolic blood pressure (mm Hg)[a]	80 ± 9	80 ± 9	80 ± 7	0.729
LAd (mm)[a]	44.5 ± 6.6	44.2 ± 6.4	47.0 ± 7.3	<0.001
TTR (%)	63.9 ± 17.2	64.2 ± 16.9	57.7 ± 24.8	0.168
Hypertension (%)	87	87	87	0.918
Diabetes mellitus (%)	19	17	28	0.009
Current smoking (%)	10	10	13	0.472
Age ≥ 75 years (%)	45	43	57	0.005
Heart failure (%)	18	16	30	0.001
Metabolic syndrome (%)	35	35	36	0.834
History of stroke (%)	12	9	31	<0.001
History of myocardial infarction (%)	19	16	41	<0.001
$CHADS_2$[b]	2 (1–3)	2 (1–2)	3 (2–4)	<0.001
CHA_2DS_2VASc[b]	3 (2–4)	3 (2–4)	4 (3–5)	<0.001
Baseline medications				
Oral anticoagulants (%)	81	82	72	0.008
Anti-platelets (%)	19	18	23	0.204
ACE inhibitor/ ARBs (%)	67	67	65	0.656
Statins (%)	32	32	29	0.480
Antiarrhythmic drugs (%)	33	34	27	0.186

LAd: left atrium diameter; TTR: percent time in therapeutic INR range.
[a] Data are expressed as mean ± SD.
[b] Data are expressed as median (interquartile range).

infarction; fatal and nonfatal ischemic strokes; cardiovascular deaths) during follow-up. Forty-seven people were censored from the analysis (3 non-fatal hemorrhagic stroke, 1 fatal hemorrhagic stroke, and 43 deaths for non-cardiovascular causes).

Clinical characteristics of patients with and without primary outcomes are summarized in Table 1. Age, diabetes, previous history of myocardial infarction and stroke and use of oral anticoagulants was significantly different between the two groups (Table 1). Lower vitamin E levels (adjusted for serum cholesterol) were found in patients who experienced cardiovascular events compared to those who did not (3.8 ± 1.2 vs. 4.4 ± 1.8 μmol/mmol cholesterol; $p < 0.001$); this difference was not significant when unadjusted absolute vitamin E levels were compared, which was 18.0 ± 7.2 μmol/L and 19.8 ± 8.6 μmol/L ($p = 0.053$) in patients who had or not cardiovascular events, respectively.

A Cox proportional hazard model demonstrated that age, diabetes mellitus, history of stroke and myocardial infarction and cholesterol-adjusted vitamin E levels independently predicted cardiovascular events (Table 2).

Table 2
Adjusted hazard ratios (HR), based on a Cox proportional hazards model, of cardiovascular events according to selected variables.

Variable	HR	P	CI 95%	
			Lower	Upper
Vitamin E serum levels[a]	0.773	0.001	0.668	0.894
History of stroke	2.010	0.001	1.323	3.054
History of myocardial infarction	2.677	<0.001	1.814	3.952
Age ≥ 75 years	1.936	0.001	1.319	2.843
Diabetes	1.680	0.017	1.099	2.570

After adjusting for heart failure, total and LDL cholesterol, oral anticoagulants, CrCl, hs-CRP, and left atrium diameter (none of these variables was significant).
[a] HR for an increasing unit change in the independent factor.

3.2. Vitamin E and cholesterol in predicting cardiovascular events

To clarify to what extent this finding was related to serum cholesterol alone, we built different COX regression analyses. The univariate Cox model, in which total cholesterol levels were included, gave not significant HR ($p = 0.546$); similar findings were found with HDL ($p = 0.410$) and LDL cholesterol ($p = 0.323$). Also, we built a Cox model in which we used cholesterol-adjusted vitamin E (vitamin E/cholesterol ratio) and total serum cholesterol levels as predictors. The model confirmed that cholesterol-adjusted vitamin E independently predicted cardiovascular events (HR = 0.769, $p < 0.001$), while serum cholesterol did not (HR = 1.000, $p = 0.82$). There were no collinearity issues, the smallest eigenvalue of the correlation matrix was 0.81 and the correlation was −0.19 ($p < 0.001$).

3.3. Vitamin E, $CHADS_2$ and CHA_2DS_2VASc as predictors of cardiovascular events

To verify if vitamin E predicted cardiovascular events in addition to the traditional risk score for NAVF, we built a model with two predictors: the $CHADS_2$ score and cholesterol-adjusted-vitamin E. Both predictors resulted significant: $CHADS_2$ score (HR 1.57; 95% CI: 1.36–1.81; $p < 0.001$) and cholesterol-adjusted vitamin E (HR 0.77; 95% CI: 0.66–0.88; $p < 0.001$). The net reclassification improvement when cholesterol-adjusted vitamin E was added to the $CHADS_2$ score was 5% (95% CI: 3.7–6.4).

A similar situation was found modeling the CHA_2DS_2VASc score and cholesterol-adjusted-vitamin E: CHA_2DS_2VASc score (HR 1.54; 95% CI: 1.36–1.73; $p < 0.001$) and cholesterol-adjusted vitamin E (HR 0.77; 95% CI: 0.66–0.89; $p < 0.001$). The net reclassification improvement when normalized vitamin E was added to CHA_2DS_2VASc score was 15% (95% CI: 12.8, 17.2).

3.4. Vitamin E as a predictor of cardiovascular events in patients without history of vascular events

Since history of stroke or myocardial infarction strongly predicted cardiovascular events during the follow-up (Table 2) we reanalyzed the data after excluding NVAF patients with history of myocardial infarction and stroke to see if vitamin E was a predictor of new cardiovascular events also in this population.

The population consisted of 742 NVAF patients followed-up for a mean time of 27 months. A multivariate Cox proportional hazard model confirmed that cholesterol-adjusted vitamin E (HR = 0.73; 95% CI: 0.59–0.91; $p = 0.006$) and age ≥ 75 years (HR 2.36; 95% CI: 1.27–4.37; $p = 0.006$) predicted cardiovascular events.

3.5. NVAF patients with low vs. high vitamin E levels

Clinical characteristics of patients categorized on the basis of the median value of vitamin E were equally distributed between the two groups

Table 3
Baseline characteristics of patients with atrial fibrillation according to the value of serum vitamin E.

Characteristics	Low vitamin E serum levels (<4.2 μmol/mmol cholesterol)	High vitamin E serum levels (≥4.2 μmol/mmol cholesterol)	P value
n	506	506	/
Age (years)[a]	73.0 ± 8.5	73.0 ± 8.3	0.957
Male sex n. (%)	53	56	0.283
Body mass index (kg/m^2)[a]	27.2 ± 4.3	27.3 ± 3.9	0.638
Total cholesterol (mg/dl)[a]	4.78 ± 1.10	4.46 ± 0.98	<0.001
LDL cholesterol (mmol/L)[a]	2.96 ± 1.05	2.70 ± 0.97	<0.001
HDL cholesterol (mmol/L)[a]	1.18 ± 0.38	1.13 ± 0.38	0.065
Glycemia (mmol/L)[a]	5.67 ± 1.68	5.47 ± 1.52	0.050
CrCl (ml/min)[a]	64.1 ± 19.7	65.4 ± 18.9	0.273
hs-CRP (mg/ml)[b]	0.83 (0.40–1.75)	0.84 (0.42–1.72)	0.950
Systolic blood pressure (mm Hg)[a]	131 ± 13	131 ± 14	0.945
Diastolic blood pressure (mm Hg)[a]	80 ± 9	80 ± 9	0.497
LAd (mm)[a]	44.6 ± 6.7	44.4 ± 6.4	0.669
TTR (%)[a]	64.6 ± 16.5	63.5 ± 16.4	0.760
Hypertension (%)	87	87	0.971
Diabetes mellitus (%)	21	16	0.039
Current smoking (%)	9	12	0.142
Age ≥ 75 years (%)	45	44	0.777
Heart failure (%)	19	17	0.390
Metabolic syndrome (%)	36	35	0.795
History of stroke (%)	13	11	0.318
History of myocardial infarction (%)	20	16	0.099
$CHADS_2$[b]	2 (1–3)	2 (1–2)	0.336
CHA_2DS_2VASc[b]	3 (2–4)	3 (2–4)	0.730
Baseline medications			
Oral anticoagulants (%)	79	83	0.138
Anti-platelets (%)	19	18	0.808
ACE inhibitor/ARBs (%)	67	67	0.889
Statins (%)	32	31	0.766
Antiarrhythmic drugs (%)	33	32	0.731

[a] Data are expressed as mean ± SD.
[b] Data are expressed as median (interquartile range).

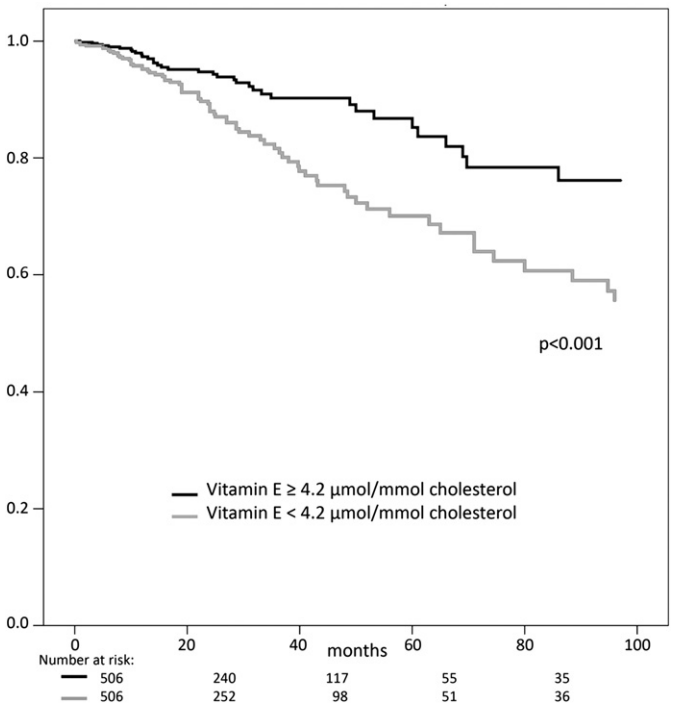

Fig. 2. Kaplan–Meier estimates of time to composite outcome events (first occurrence of myocardial infarction, ischemic stroke or cardiovascular death) by values of serum vitamin E below and above the median (4.2 μmol/mmol cholesterol).

with the exception of higher prevalence of diabetes and higher serum cholesterol levels in patients with vitamin E <4.2 μmol/mmol cholesterol (Table 3). Primary outcome events occurred in 74 (13 fatal and 9 nonfatal myocardial infarction; 9 fatal and 14 nonfatal ischemic strokes; 29 cardiovascular deaths) out of 506 patients with low (15%) and in 35 (5 fatal and 5 nonfatal myocardial infarction; 4 fatal and 5 nonfatal ischemic stroke; 16 cardiovascular deaths) out of 506 patients with high (7%) values of vitamin E serum levels.

Serum vitamin E below the median was significantly associated to outcome events (log-rank test: $p < 0.001$; Fig. 2). Using a Cox proportional hazards model, patients with serum vitamin E below the median were more likely to experience primary outcome events (HR 1.87, 95% CI: 1.25 to 2.80; $p = 0.002$) than patients with vitamin E above the median, after controlling for the other clinical variables (Fig. 3).

4. Discussion

In this study we have shown for the first time that in patients with NVAF, low cholesterol-adjusted vitamin E serum levels are predictive of poor vascular outcome including myocardial infarction and stroke and cardiovascular death.

NVAF is associated with high risk of stroke in case of coexistence of risk factors such as age, hypertension, diabetes or heart failure. The cohort screened encompassed patients with these characteristics who were currently treated with warfarin. During follow-up this population experienced 32 ischemic strokes (approximately 1.4% per year). This is consistent with previous reports showing that in NVAF patients, treated with adjusted-dosed warfarin, stroke incidence ranged from 1.1% to 4% per year [1]. Moreover, NVAF patients often show atherosclerosis risk factors that account for the coexistence of systemic atherosclerosis and related clinical events such as myocardial infarction. The published rate of myocardial infarction and cardiovascular deaths in our cohort of patients was 3.4% per year, consistent with an Italian study in which high-risk NVAF patients reported an annual rate of 3.7% [25].

The occurrence of an inverse relationship between vitamin E serum levels and coronary or carotid atherosclerosis or a history of cardiovascular events has been previously hypothesized [30]. However, the data obtained from observational studies were often contradictory probably due to the cross-sectional nature of these studies, which often reported only the intake of vitamin E supplements and not its serum concentrations. Moreover, assessment of vitamin E status is not easy, given that its circulating levels not only depend upon the nutrient concentration, but also on the concentrations of the circulating lipids [22,31]. Indeed, the use of cholesterol-adjusted vitamin E levels has seldom been taken into account, which might have led to inconsistent results [24].

After adjusting vitamin E levels for serum cholesterol [22,31], we demonstrated that NVAF patients with low levels of vitamin E are at high risk of cardiovascular events independently from other recognized risk factors.

We took into consideration that the majority of patients included in the trial were on anticoagulant treatment (81%), and optimal therapeutic range could vary between patients with vitamin E above or below median values and bias the results. In NVAF trials on treatment with oral anticoagulants, patients had optimal INR values 58.1–69.5% of the time; below 14–27.7% and above therapeutic range 10.4–26.8% of the

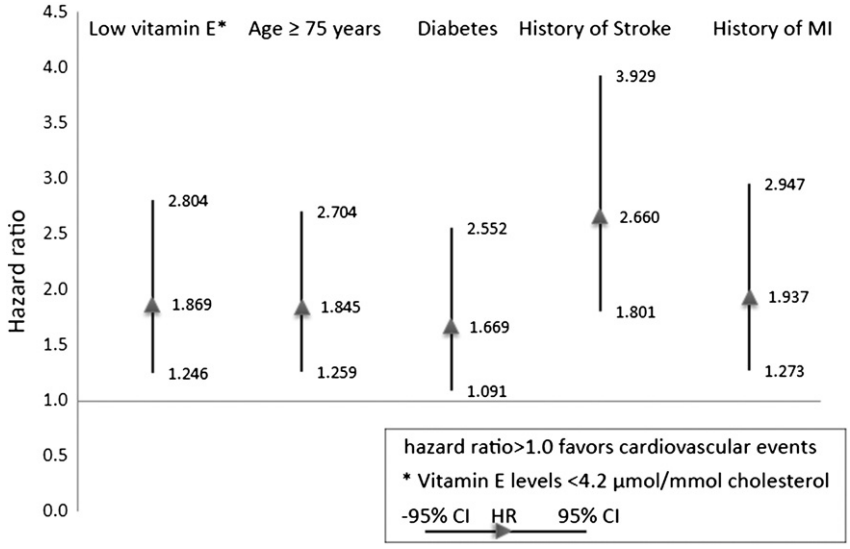

Fig. 3. Adjusted hazard ratios (HR), based on a Cox proportional hazards model, of cardiovascular events according to selected variables. CI: confidence interval.

time [32,33]. Similar data were observed in our study with no significant difference between patients with vitamin E above or below the median values.

The inverse association between serum vitamin E and cardiovascular events, such as stroke and myocardial infarction, suggests a potential role of vitamin E in the thromboembolic and atherosclerotic process. Such a hypothesis is biologically plausible for several reasons. Firstly, vitamin E possesses anticoagulant properties as it is capable of reducing vitamin K-dependent clotting factor activation [10,11] and monocyte expression of tissue factor (a glycoprotein that converts factor X to Xa) [34]. These properties are likely to account for the reduced risk of thromboembolism observed in healthy women treated with vitamin E [35,36]. Secondly, experimental studies performed in animals showed that vitamin E possesses anti-atherosclerotic properties [37–39], which have been attributed to its antioxidant activity [39]. However, we found a weak inverse correlation between serum vitamin E and urinary excretion of isoprostanes suggesting that other anti-atherosclerotic properties of vitamin E, not closely dependent on its antioxidant property, could account for such association. Finally, vitamin E may interfere with several processes related to atherosclerosis initiation and progression such as inhibition of smooth cell proliferation, preservation of endothelial integrity, inhibition of monocyte-endothelium adhesion, inhibition of cytokine release from monocytes, anticoagulant and platelet activation via antioxidant-independent mechanisms [12].

The study has implication and limitation. The association between serum levels of vitamin E and cardiovascular events may suggest a cause–effect relationship between vitamin E levels and cardiovascular events but this cannot be established at the moment because of the observational nature of the study. Even if we have no elements to establish the reason for low vitamin E in our population, diet may have some influence. Thus, NVAF patients are often advice to exclude from diet vegetables, which are rich in vitamin E; it is, therefore, possible that low vitamin E levels are merely expression of changes in individual dietary habit. Thus, a low adherence to the Mediterranean diet, which is rich in vegetables and vitamin E and is associated with low risk of cardiovascular events [40], could be responsible for the inverse association between vitamin E and cardiovascular events in our population. However, prospective study should be done to see if adherence to the Mediterranean diet affects vitamin E levels and ultimately reduces cardiovascular events also in NVAF patients.

Another limitation of the study is that the predictive values of serum vitamin E can be applied only to NVAF patients at the moment. Other studies should be done to assess if serum vitamin E is predictive of stroke or myocardial infarction in other clinical setting of atherosclerosis and thrombo-embolism.

The extension to which this finding can be extrapolated to other population or clinical settings cannot be specified at the moment and for this reason our report should be considered preliminary. Further study including larger population of different races should be done to substantiate our results. Finally the predictive values of cholesterol-adjusted vitamin E versus other validated biomarker such as N-terminal pro-brain natriuretic peptide and mid-regional pro-atrial natriuretic peptide should be investigated [7,41].

In conclusion, our study provides evidence on an inverse relationship between cholesterol-adjusted vitamin E serum levels and cardiovascular events and suggests the need of monitoring dietary habit in NVAF patients.

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