



## The association between hyperhomocysteinemia and ischemic stroke in patients with non-valvular atrial fibrillation

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**Background and Objectives.** Atrial fibrillation is complicated by a high rate of ischemic stroke. Previous studies have shown that an increased level of circulating total plasma homocysteine (tHcy) is an independent predictor of stroke, but it is unclear whether it is also predictive of stroke in patients with atrial fibrillation. The objective of this study was to evaluate whether increased tHcy is an independent predictor of cardio-embolic stroke in patients with non-valvular atrial fibrillation.

**Design and Methods.** We studied 163 consecutive patients (77 males and 86 females; mean age  $72.3 \pm 8.8$  years) with permanent ( $n=118$ ) or paroxysmal ( $n=45$ ) atrial fibrillation of non-valvular origin hospitalized for cardiac reasons. Ischemic stroke, documented by nuclear magnetic resonance or computerized tomography imaging, had occurred at an average of 2 years before hospitalization in 40 patients (16 males and 24 females, mean age  $74.8 \pm 8.8$  years). Fasting tHcy levels were determined by high performance liquid chromatography.

**Results.** Multivariate analysis adjusting for traditional cardiovascular risk factors, thromboembolic risk factors and predictors of tHcy (glomerular filtration rate, uric acid, gender) and fibrinogen levels (age, alcohol intake) showed that total homocysteine (OR:1.056; for each  $1 \mu\text{mol/L}$  increase, 95% C.I.: 1.00-1.12;  $p=0.042$ ) and fibrinogen (OR:1.008 for each  $1 \text{ mg/dL}$  increase; 95% C.I.: 1.00-1.014;  $p=0.016$ ) were independently associated with ischemic stroke. With respect to patients in the first quartile of the tHcy distribution ( $4.6\text{-}7.5 \mu\text{mol/L}$ ), patients in the fourth quartile of the tHcy distribution ( $18.7\text{-}67.1 \mu\text{mol/L}$ ) had a 2.73-fold increased probability of ischemic stroke

**Interpretations and Conclusions.** In patients with non-valvular atrial fibrillation hospitalized for cardiac reasons, increased fasting tHcy levels are independently associated with a history of ischemic stroke.

**Key words:** homocysteine, fibrinogen, non-valvular atrial fibrillation, ischemic stroke, glomerular filtration rate, cardiovascular risk factors.

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**N**on-valvular atrial fibrillation is a highly prevalent cardiac arrhythmia in the elderly population and carries a 5-fold increase in the risk of stroke.<sup>1</sup> Several clinical characteristics are useful for identifying patients with non-valvular atrial fibrillation at high risk of stroke, but no plasma markers have yet been shown to be predictors of stroke in these patients.<sup>2,3</sup>

Homocysteine is a highly reactive, sulfur-containing amino acid formed as a by-product of the essential amino acid methionine. It is estimated that 5 to 7% of the general population have mild to moderate hyperhomocysteinemia, which increases the risk of developing venous and/or arterial thrombosis.<sup>4,5</sup> Observational data have suggested a link between hyperhomocysteinemia and atherosclerotic disease, including premature coronary

and cerebral artery occlusive disease.<sup>6-8</sup> A relationship between moderate hyperhomocysteinemia and the occurrence of cerebrovascular ischemic events has been suggested by three studies including small series of patients with non-valvular atrial fibrillation.<sup>9-11</sup> However, in a larger study, total plasma homocysteine (tHcy) levels were strongly associated with ischemic stroke caused by large- and small artery atherosclerosis, but not with cardioembolic stroke.<sup>12</sup> More recently, in a large population of patients with non-valvular atrial fibrillation on oral anticoagulant treatment, Marcucci *et al.*<sup>13</sup> retrospectively observed an association of hyperhomocysteinemia with the occurrence of ischemic stroke, transient ischemic attacks (TIA) and peripheral embolism.

tHcy levels increase with increasing age and kidney function plays a major role in

the regulation of tHcy levels. Even a moderate impairment in the glomerular filtration rate, highly prevalent in the elderly population, may lead to hyperhomocysteinemia.<sup>14</sup> The above mentioned studies in non-valvular atrial fibrillation patients did not explore the relationship between hyperhomocysteinemia and kidney function. To assess the independent role of hyperhomocysteinemia in the occurrence of ischemic stroke, we analyzed the relationship between a history of ischemic stroke, fasting total plasma tHcy, kidney function and age in a consecutive series of 163 patients with non-valvular atrial fibrillation requiring hospitalization.

## Design and Methods

One hundred and sixty-three consecutive patients with non-valvular atrial fibrillation were included in the study. All patients were seen as in-patients because of paroxysmal, persistent or permanent atrial fibrillation (persistent for at least 6 months) with an abrupt increase in heart rate or heart failure. Concomitant treatments included amiodarone in 10.6% of patients, propafenone in 8%, angiotensin-converting enzyme inhibitors in 70%, calcium antagonists in 35%,  $\beta$ -blockers in 30%, digitalis in 25%, diuretics in 40%, and doxazosin in 5%. No patient was on vitamin treatment.

Exclusion criteria were mitral stenosis, mitral valve prosthesis or severe mitral regurgitation, significant stenosis (>70%) of the carotid arteries, lone atrial fibrillation, thyrotoxicosis, cancer, liver or kidney diseases, acute or chronic inflammatory disorders, TIA, lacunar infarction, hemorrhagic stroke or ischemic stroke in the 3 months prior to hospitalization.

All patients underwent electrocardiography, two-dimensional echocardiography, and carotid artery ultrasound. Occurrence of ischemic stroke was determined by reviewing medical records documenting a history of focal neurological symptoms of sudden onset persisting for more than 24 hours and supported by nuclear magnetic resonance or computerized tomography imaging. Cardiovascular risk factors and characteristics associated with ischemic complications were evaluated. Hypertension was defined according to the criteria of the Sixth Joint National Committee Report on the Detection, Evaluation and Treatment of Hypertension or assumed in subjects currently taking anti-hypertensive medication.<sup>15</sup> Diabetes mellitus was defined according to the American Diabetic Association's new criteria and by the use of insulin or oral hypoglycemic drugs.<sup>16</sup> Coronary heart disease was defined from medical records on the basis of a history of myocardial infarction, or stable or unstable angina. Dyslipidemia was

defined according to the criteria of the Third Report of the National Cholesterol Education Program or assumed in subjects who were currently taking lipid-lowering drugs.<sup>17</sup> Current smokers were defined as patients smoking at least five cigarettes a day. Body mass index was calculated as weight/height<sup>2</sup>.

Glucose, insulin, lipids, creatinine and uric acid concentrations were determined on fasting serum samples with an automated analyzer. Fasting venous blood samples were obtained from an antecubital vein and collected in vacutainer tubes (BD Vacutainer System, Plymouth, UK) containing 0.129 M sodium citrate (9:1, v/v) and then immediately centrifuged for 20 min at 2000  $\times$ g at  $-4^{\circ}$  C. Plasma samples were stored at  $-80^{\circ}$  C until assay. Plasma fibrinogen was evaluated according to the Clauss method (Instrumentation Laboratory, Milan, Italy) employing an Electra 1400C coagulometer (Instrumentation Laboratory, Milan, Italy).<sup>18</sup> Intra- and inter-assay coefficients of variation were 6% and 7%, respectively. In healthy subjects the mean $\pm$ SD was 233 $\pm$ 54 mg/dL (range 138-361 mg/dL). Patients with a plasma fibrinogen level over 450 mg/dL were excluded from our study. tHcy was measured by high performance liquid chromatographic analysis after cleavage and reduction with sodium borohydride followed by derivatization with ammonium-7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate (SBD-F<sup>19</sup>). Reagents, chemicals and solvent were purchased from Fluka (Buchs, Switzerland), Wako Chemicals (Neuss, Germany) and BDH (Poole, UK). Intra- and inter-assay coefficients of variation determined on two plasma samples with low (6.9  $\mu$ mol/L) and high (27.5  $\mu$ mol/L) tHcy concentrations ranged from 1.9% to 3.8% and from 4.0% to 4.7%, respectively. In a control population of 40 women and 40 men aged 33.0 $\pm$ 10 years fasting tHcy was 7.7 $\pm$ 2.4  $\mu$ mol/L in women and 8.9 $\pm$ 2.1  $\mu$ mol/L in men (range 4.2-16.2  $\mu$ mol/L). In an unselected population of 181 women and 206 men aged 60 or more years (mean age 69.2 $\pm$ 6.5 years) referred to the Coagulation Service & Thrombosis Research Unit (IRCCS H.S. Raffaele) for evaluation, fasting tHcy levels after removal of outliers ( $n = 23$ ) were 9.1 $\pm$ 3.0  $\mu$ mol/L and 10.8 $\pm$ 3.2  $\mu$ mol/L, respectively. Corresponding 95<sup>th</sup> percentiles of the tHcy distribution were 15.0  $\mu$ mol/L in women and 16.7  $\mu$ mol/L in men.

The study was approved by the local review committee and patients gave their written informed consent to take part in the study.

## Statistical analysis

For descriptive purposes, results are expressed as mean value $\pm$ standard deviation (SD for continuous variables and as counts (percentages) for categorical variables. Categorical variables are reported as counts

**Table 1.** Demographic and clinical characteristics of patients with non-valvular atrial fibrillation according to the history of ischemic stroke.

	Stroke + (n = 40)	Stroke - (n = 123)	Univariate analysis OR (95% C.I.)#	p
Age (years)	74.8±8.3	69.2±11.5	1.057 (1.02-1.10)	0.008
Male gender	16/24 (40%)	61/62 (49.6%)	0.68 (0.31-1.48)	0.38
Body mass index (kg/m <sup>2</sup> )	26.7±4.3	27.1±5.0	0.977 (0.90-1.06)	0.69
Paroxysmal atrial fibrillation (yes/no)	7/33 (17.5%)	38/85 (30.9%)	0.46 (0.17-1.29)	0.14
Hypertension (yes/no)	34/6 (85.0%)	88/35 (71.5%)	2.16 (0.72-6.46)	0.17
Diabetes (yes/no)	3/37 (7.5%)	16/107 (13.0%)	0.65 (0.19-2.19)	0.53
Coronary heart disease (yes/no)	8/32 (20.0%)	33/90 (26.8%)	0.72 (0.21-2.46)	0.60
Dyslipidemia (yes/no)	10/30 (25.0%)	36/87 (29.3%)	0.77 (0.23-2.56)	0.67
Smoking (current/previous /never)*	2/2/36 (10.0%)	17/6/100 (18.7%)	0.62 (0.11-3.35)	0.45
Alcohol intake (yes/no)	6/34 (15.0%)	32/91 (26.0%)	0.56 (0.16-1.96)	0.37
Left ventricular ejection fraction (%)	55.9±21.0	56.8±32.3	1.002 (0.78-1.22)	0.66
Left atrium diameter (mm)	46.5±19.0	45.0±17.7	1.024 (0.89-1.19)	0.46
On oral anticoagulants (yes/no)	28/12 (70.0%)	78/45 (63.4%)	1.36 (0.49-3.80)	0.55
On aspirin (yes/no)	12/28 (30.0%)	28/95 (22.8%)	1.43 (0.49-4.15)	0.51
On statins (yes/no)	4/36 (10.0%)	12/111 (9.8%)	1.04 (0.76-1.41)	0.81

# $\chi^2$  or Mantel-Haenszel statistics adjusted for gender for categorical variables and logistic regression analysis adjusted for gender for continuous variables; \*analysis comparing current and previous vs never.

(percentages) and continuous variables as means±SD. With the exception of age, body mass index and glomerular filtration rate, continuous variables were log-transformed to approximate normal distributions. Univariate analyses were performed using one-way analysis of variance and the Student's t test for continuous variables and  $\chi^2$  analysis for categorical variables. Odds ratios for stroke adjusted for gender were estimated with the Mantel-Haenszel statistics (categorical variables) or logistic regression analysis adjusted for gender. In the latter analysis tHcy levels were included either as a continuous variable or as

**Table 2.** Laboratory variables of patients with non-valvular atrial fibrillation according to the history of ischemic stroke.

	Stroke + (n=40)	Stroke - (n=123)	Univariate analysis OR (95% C.I.)#	p
Creatinine (mg/dL)	1.16±0.24	1.08±0.29	3.84 (1.01-14.6)	0.031
Glomerular filtration rate (mL/min)	56.7±13.7	65.2 ± 16.4	0.965 (0.94-0.99)	0.005
Uric acid (mg/dL)	5.49±1.78	5.41±1.61	1.042 (0.83-1.30)	0.77
tHcy (μmol/L)	18.1±9.0	15.4±9.3	1.031 (0.99-1.07)	0.042
Fibrinogen (mg/dL)	363±106	314±78	1.006 (1.00-1.01)	0.008

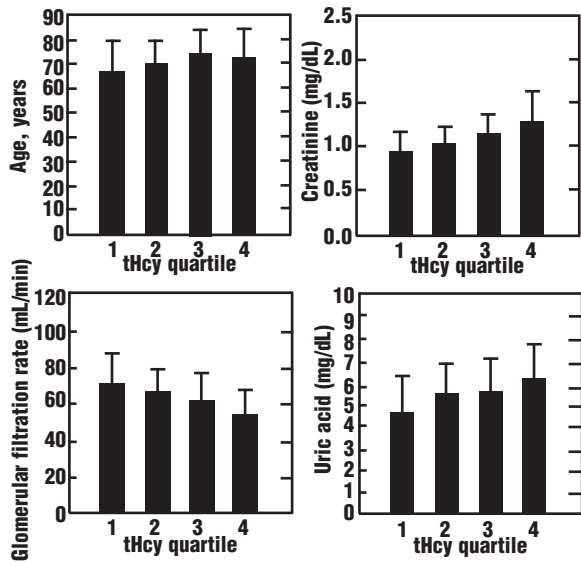
\*Logistic regression analysis adjusted for gender.

quartiles of the distribution. The generalized linear model was used to assess independent predictors of fasting tHcy and fibrinogen levels. Multiple logistic regression analysis was used to estimate the adjusted odds ratio and their 95% CI for ischemic stroke. A p value <0.05 was considered statistically significant. Statistical analyses were carried out with the SPSS software package, version 11.0.

## Results

Eighty-six women (mean age: 71.6±11.0 years) and 77 men (mean age: 69.4±11.1 years) were enrolled in the study. One hundred and eighteen patients had permanent and 45 paroxysmal atrial fibrillation. Hypertension was detected in 122 patients (74.8%), diabetes in 19 (11.7%), and current or previous smoking in 27 (16.6%). Forty-one patients had suffered from coronary heart disease. Ischemic stroke had occurred in 16 of the 77 men (22.2%) and 24 of the 86 women (27.9%), an average of 2 years before the current admission to hospital. At that time, 28 patients were receiving treatment with oral anticoagulants and 12 with aspirin. Patients with permanent atrial fibrillation at admission to hospital already suffered from the disease at the time of their ischemic stroke.

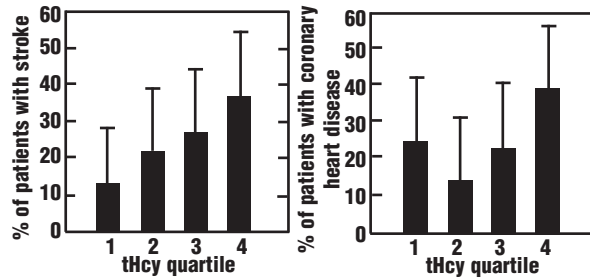
Demographic and clinical characteristics of patients with or without ischemic stroke, laboratory variables and corresponding odds ratios adjusted for gender are reported in Tables 1 and 2. At univariate analysis, age, serum creatinine, glomerular filtration rate, tHcy and fibrinogen were significantly associated with an increased risk of ischemic stroke (Tables 1 and 2).



**Figure 1.** Relationship of age, creatinine levels, glomerular filtration rate, and uric acid levels (mean $\pm$ SD) with quartiles of the tHcy distribution (quartile 1: 4.6-7.5  $\mu$ mol/L; quartile 2: 9.7-14.1  $\mu$ mol/L; quartile 3: 14.3-18.6  $\mu$ mol/L; quartile 4: 18.7-67.1  $\mu$ mol/L). Patients in the third quartile of the tHcy distribution were older than patients in the first quartile ( $p=0.034$ , left upper panel). Glomerular filtration rate (left lower panel) was lower in patients from the third and fourth quartiles of the tHcy distribution than in patients from the remaining quartiles ( $p=0.035$  and  $p<0.0001$ ). Creatinine (right upper panel) and uric acid levels (right lower panel) were higher in patients from the third and fourth quartiles of the tHcy distribution than in patients from the lower quartiles ( $p\leq 0.001$ ).

Creatinine levels and glomerular filtration rate were higher in men than in women ( $1.22\pm 0.28$  mg/dL vs  $1.03\pm 0.24$  mg/dL,  $p<0.006$  and  $66.4\pm 15.7$  mL/min vs  $60.2\pm 16.1$  mL/min,  $p=0.04$ ), but there was no significant stroke-gender interaction for any of the variables evaluated.

The relationship of age, creatinine, glomerular filtration rate and uric acid levels with the tHcy distribution is shown in Figure 1. Patients in the third quartile of the tHcy distribution were older than patients in the first quartile ( $p=0.034$ ). Creatinine and uric acid levels were higher in patients from the third and fourth quartiles of the tHcy distribution than from the lower quartiles ( $p<0.0001$  and  $p=0.001$ , respectively), while the opposite was true for glomerular filtration rate values ( $p<0.0001$ ). In a generalized linear model, 24.3% of the variation in plasma tHcy was independently predicted by glomerular filtration rate values ( $r$  partial  $-0.36$ ,  $p<0.0001$ ), uric acid levels ( $r$  partial  $0.33$ ,  $p<0.0001$ ), and by the interaction of gender with creatinine levels ( $p=0.024$ ), but not by the interaction of gender with glomerular filtration rate or by any of the other variables reported in Tables 1 and 2. When excluding the 28 patients with serum creatinine concentrations



**Figure 2.** Relationship of history of ischemic stroke (% of patients and 95% C.I., left panel) and coronary heart disease (% of patients and 95% C.I., right panel) with quartiles of the tHcy distribution (quartile 1: 4.6-7.5  $\mu$ mol/L; quartile 2: 9.7-14.1  $\mu$ mol/L; quartile 3: 14.3-18.6  $\mu$ mol/L; quartile 4: 18.7-67.1  $\mu$ mol/L). Trends were significant for an increasing percentage of patients with stroke across the quartiles of the tHcy distribution ( $p=0.011$ , left panel), but not for the percentage of patients with coronary heart disease ( $p=0.09$ , right panel).

higher than 1.3 mg/dL, 12.5% of the variation in plasma tHcy was independently predicted by glomerular filtration rate values ( $r$  partial  $-0.23$ ,  $p=0.011$ ), uric acid levels ( $r$  partial  $=0.24$ ,  $p=0.009$ ) and by the interaction of gender with creatinine levels ( $p=0.038$ ). No significant effect on tHcy levels was observed according to treatment with drugs (vitamin K antagonists, aspirin, beta-blockers, angiotensin-converting enzyme inhibitors, calcium antagonists, diuretics, statins) possibly affecting these levels (*data not shown*).

Alcohol intake ( $r$  partial  $-0.21$ ,  $p=0.024$ ) and age ( $r$  partial  $0.19$ ,  $p=0.04$ ) explained 7.8% of the variation in fibrinogen levels. There was no significant correlation of fibrinogen with tHcy levels ( $r=0.12$ ,  $p=0.18$ ).

There was a significant trend for an increasing percentage of patients with stroke across the quartiles of the tHcy distribution (from 12.5%, 5 of 40 patients in the lower quartile to 36.6%, 15 of 41 patients in the highest quartile, Cochran's linear trend,  $\chi^2=6.51$ ,  $p=0.011$ , Figure 2). A similar trend across the quartiles of the tHcy distribution for the percentage of patients with coronary heart disease did not reach statistical significance (from 25.0%, 10 of 40 patients in the lower quartile to 39.0%, 26 of 41 patients in the highest quartile, Cochran's linear trend,  $\chi^2=2.91$ ,  $p=0.09$ , Figure 2).

Multivariate logistic regression analysis including as independent variables fibrinogen, tHcy levels and their predictors in addition to gender, traditional cardiovascular risk factors, type of fibrillation, and thromboembolic risk factors (left ventricular ejection fraction, left atrium diameter and treatment with oral anticoagulants or aspirin) identified fibrinogen ( $p=0.016$ ) and tHcy ( $p=0.042$ ) levels, but no longer glomerular filtration rate and age as independent pre-

**Table 3.** Risk factors for ischemic stroke in patients with non-valvular atrial fibrillation: univariate and multivariate analyses.

	Univariate analysis OR (95% C.I.)	p	Multivariate analysis OR (95% C.I.) <sup>a</sup>	p
Age (years)	1.057 (1.02-1.10)	0.008	1.049 (0.98-1.12)	0.15
Glomerular filtration rate (mL/min)	0.965 (0.94-0.99)	0.005	0.962 (0.77-1.21)	0.75
tHcy (μmol/L)	1.031 (0.99-1.07)	0.042	1.056 (1.00-1.12)	0.042
Fibrinogen (mg/dL)	1.006 (1.00-1.01)	0.008	1.008 (1.00-1.01)	0.016

<sup>a</sup>Logistic regression analysis adjusted for gender, hypertension, diabetes mellitus, dyslipidemia, smoking habits, history of coronary heart disease, left ventricular ejection fraction, left atrium diameter, oral anticoagulants or aspirin treatment, and predictors of tHcy and fibrinogen levels.

dictors of ischemic stroke. Corresponding odds ratios for unitary increments in fibrinogen (mg/dL), and tHcy (μmol/L), as obtained from untransformed data, are shown in Table 3. Relative to patients in the first quartiles of the tHcy distribution, patients in the second, third and fourth quartiles had adjusted odds ratio for stroke of 0.75 (0.31-1.82), 1.30 (0.55-3.07) and 2.73 (1.23-6.08), respectively. Odds ratios were virtually unchanged when the 28 patients who had creatinine levels higher than 1.3 mg/dL (9 of whom had suffered from ischemic stroke) were excluded from the analysis.

## Discussion

Blood stasis and thrombophilia are major determinants of thrombus formation in the venous system and they may also play a major role in the occurrence of ischemic events in patients with atrial fibrillation.<sup>20</sup> Moderate hyperhomocysteinemia is a prothrombotic condition that may favor the occurrence of both arterial and venous thrombosis.<sup>4</sup> Genetic abnormalities of the enzymes involved in methionine metabolism, nutrient status, kidney function, life-style habits and advancing age are all involved in the hyperhomocysteinemic phenotype.<sup>14,21</sup> Four reports have underlined an association of hyperhomocysteinemia with thrombotic events occurring in patients with atrial fibrillation. Ay *et al.*<sup>10</sup> studied the relationship between tHcy and left atrial thrombi in 42 patients with non-valvular atrial fibrillation and acute stroke, observing higher tHcy levels in 20 patients with left atrial thrombus than in the remaining patients, and suggesting that hyperhomocysteinemia may favor clot formation in the left atrium of these patients.

Friedman<sup>9</sup> measured tHcy levels in 40 patients with atrial fibrillation; because patients with a history of stroke had higher levels of tHcy and were older than the remaining patients, he suggested that the increased homocysteine levels observed in elderly persons may provide an explanation for the sharp increase in stroke in patients with atrial fibrillation after 65 years of age.<sup>9</sup> Similar results were obtained by Cingozbay *et al.*<sup>11</sup> in a series of 38 patients with non-valvular atrial fibrillation. More recently Marcucci *et al.*<sup>13</sup> examined 310 patients with non-valvular atrial fibrillation on oral anticoagulant treatment. One hundred and sixty-eight patients in this series had suffered from vascular events, including ischemic stroke, TIA or peripheral embolism. Patients in the fourth quartile of the tHcy distribution had a 2.7-fold higher probability of vascular events than did patients in the first quartile of the tHcy distribution. tHcy levels were dependent on folic acid levels and methylenetetrahydrofolate reductase status, but the relationship of tHcy levels with kidney function was not evaluated in that study.<sup>13</sup>

In this study, we analyzed the association of hyperhomocysteinemia with a history of ischemic stroke in a consecutive series of 163 patients with non-valvular atrial fibrillation, adjusting for glomerular filtration and uric acid levels. The clinical history of our patients was complicated by stroke in about 25% of cases, a figure similar to that reported in a large study of patients with non-valvular atrial fibrillation.<sup>22</sup> At univariate analysis, the 40 patients with a history of stroke were older and had higher tHcy levels and lower glomerular filtration rates than the remaining patients; in line with a previous report,<sup>23</sup> increased fibrinogen levels were also associated with a history of stroke. The percentage of patients with a history of stroke increased from 12.5% in the first quartile of the tHcy distribution to 36.6% in the fourth quartile (Figure 2). Variations in plasma tHcy levels were partially explained by glomerular filtration rate and uric acid levels, and by the interaction of gender with creatinine levels, but not by gender, age, blood pressure, drugs, or any of the other metabolites evaluated, indicating that impaired kidney function is a major determinant of hyperhomocysteinemia in patients with non-valvular atrial fibrillation.

At multivariate analysis, fibrinogen and tHcy levels, but not age, glomerular filtration rate, uric acid, diabetes, hypertension, left atrium diameter or ventricular systolic function, were independent predictors of history of ischemic stroke. With respect to patients in the first quartile of the tHcy distribution (4.6-7.5 μmol/L), patients in the fourth quartile of the tHcy distribution (18.7-67.1 μmol/L) had a 2.73-fold increased probability of ischemic stroke, a figure similar to that reported for overall vascular events by

Marcucci *et al.*<sup>13</sup> These findings suggest that advancing age and renal insufficiency may exert a prothrombotic effect through an increase in tHcy levels. Up to 60 years of age, men have higher tHcy levels than women, a difference which tends to disappear in later decades of life.<sup>14</sup> Female gender and age over 75 years are associated with an additional risk of stroke in non-valvular atrial fibrillation.<sup>24,25</sup> The observation in our series of similar tHcy levels in elderly men and women with non-valvular atrial fibrillation is consistent with these associations because hyperhomocysteinemia may represent an important factor favoring thromboembolism especially in women suffering from atrial fibrillation.

Our case-control study has some limitations. tHcy plasma levels were examined on average 2 years after the occurrence of stroke and we have no information regarding these levels prior to the event. Evidence from the literature does, however, suggest a relative stability over years in tHcy levels,<sup>14</sup> and no patient in our series was on vitamin treatment at the time of blood sampling. Cardio-embolism is recognized as the main, but not the only cause of ischemic stroke in patients with non-valvular atrial fibrillation. Although patients with significant stenosis of the carotid arteries and with evidence of lacunar cerebral infarcts were not included in our study, we cannot rule out the contribution of atherosclerosis of the aortic plaques and cerebral arteries in the ischemic events recorded.<sup>12</sup> Increased tHcy levels have been reported in patients with coronary heart disease,<sup>67</sup> an association potentially confounding the relationship between hyperhomocysteinemia and ischemic stroke in patients with non-valvular atrial fibrillation requiring hospitalization. However, a history of coronary heart disease and of ischemic stroke were not associated in our series. In addition, although 39% of our patients in the fourth quartile of the tHcy distribution also suffered from coronary heart disease, the association of tHcy levels with coronary heart disease did not reach statistical significance.

Atrial fibrillation in western countries represents an important social problem. Chronic oral anticoagulation has an inherent risk of causing bleeding complications, which is greater in elderly patients, who are at higher risk of ischemic stroke.<sup>26</sup> Our findings of

high fibrinogen and tHcy as independent risk factors for ischemic stroke in patients with non-valvular atrial fibrillation suggest the possibility of interventions aimed at reducing their levels. In men with lower limb arterial disease, reductions in fibrinogen levels, as obtained by treatment with bezafibrate, led to a non-significant reduction in major coronary events but also to a non-significant increase in stroke.<sup>27</sup> The unfavorable results obtained with bezafibrate treatment have been explained by a 4.5  $\mu\text{mol}$  rise in homocysteine levels observed in men taking bezafibrate.<sup>28</sup> At variance, moderate to intermediate hyperhomocysteinemia can successfully be treated with vitamin supplementation, even in patients with impaired kidney function.<sup>14</sup> There is evidence of a three-fold acceleration in the decline of stroke-associated mortality in the US, which is temporally related to fortification of flour with folic acid, is associated with a 14% reduction in tHcy levels and cannot be explained by changes in other major risk factors, such as cigarette smoking, hypertension, diabetes and total serum cholesterol levels, many of which did not improve or worsened during the period studied.<sup>29</sup> Identification of hyperhomocysteinemic patients with non-valvular atrial fibrillation, and normalization of their tHcy levels by multivitamin treatment may reduce their risk of ischemic stroke.

In conclusion, this study shows that in hospitalized patients with non-valvular atrial fibrillation high levels of tHcy are independently associated with a history of ischemic stroke. The role of increased tHcy levels and their normalization by vitamin supplementation should be further evaluated in prospective studies.

*All persons designated as authors qualified for authorship by contributing to the design and development of the study as well as the interpretation of data. All of them approved the final version of the manuscript.*

*The authors reported no potential conflict of interest.*

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