High homocysteine and low folate concentrations in acute aortic dissection

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ABSTRACT

Background: Biomarkers for monitoring progression and prognosis of thoracic aneurysm are of great interest. Homocysteine (Hcy) induces elastolysis in arterial media and may directly affect fibrillin-1 or collagen whereas lipoprotein (Lp) (a) inhibits elastolysis by reducing activation of matrix metallopeptidase-9.

Methods: We studied 31 consecutive patients with acute aortic dissection (AAD) admitted for emergency surgery (group I, 60 ± 13 years old, 25 men), 30 consecutive patients with chronic aneurysms of the ascending aorta (group II, 67 ± 12 years old, 24 men) and 20 healthy controls (group III, 58 ± 15 years old, 14 men). We evaluated Hcy, folate, B12, Lp(a) and methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism at baseline.

Results: Hcy, folate and B12 differed significantly among the 3 studied groups ($P=0.016$, $P=0.004$ and $P=0.001$, respectively). The levels of Hcy and B12 were significantly higher in group I compared to both groups II and III ($P=0.05$ and $P=0.002$, $P<0.001$ and $P=0.017$, respectively) and without significant differences between groups II and III ($P=0.083$ and $P=0.124$). Folate was significantly lower in group I compared to both groups II and III ($P=0.001$ and $P=0.006$, respectively) and without marked difference between groups II and III ($P=0.409$). No significant difference was found in serum levels of Lp (a) ($P=0.074$) or among the frequency of MTHFR C677T genotypes.

Conclusions: Patients with AAD present with higher Hcy and lower folate compared to both chronic aneurysms and controls.

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1. Introduction

Thoracic aortic aneurysm is a potentially lethal disease, mostly clinically silent and, therefore, there is great interest in biomarkers that can detect the disease, monitor its progress and predict its catastrophic complications, such as dissection or rupture [1,2]. Several case-control studies have documented homocysteine (Hcy) elevation in patients with abdominal aortic aneurysms (AAA) [3]. Furthermore, there is correlation between Hcy levels and size of the aneurysm as well as high levels and aneurysm expansion [4,5]. Hyperhomocysteinemia is associated with spontaneous cervical dissection causing stroke in young adults [6]. Hcy is also increased in Marfan patients with severe cardiovascular manifestations, in particular aortic dissection, compared to Marfans with mild cardiovascular disease [7].

Elevated Hcy levels have been related both to genetic or epigenetic factors like vitamin status [8,9]. Deficiency or low availability of folate, vitamins B6 or B12 may result in high Hcy concentrations [8,9]. Specific methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms have shown significant effect on total plasma Hcy, the most common being the cytidine to thymidine at position 677 (C677T), [7]. Hcy has been shown, in vitro, to induce elastolysis in arterial media through the activation of matrix metallopeptidase (MMP)-2 or may directly affect fibrillin-1 or collagen [10–12].

The levels of lipoprotein (Lp)(a) have been found to be an independent risk factor for many forms of vascular diseases, including AAA [13]. Lp(a) inhibits elastolysis by reducing activation of MMP-9 through the impaired generation of plasmin [14].

This is the first study to evaluate Hcy, folate, vitamin B12 and Lp(a) levels and investigate the prevalence of the MTHFR C677T polymorphism in patients with acute aortic dissection (AAD) and chronic thoracic aortic aneurysms.

2. Methods

2.1. Study design and inclusion and exclusion criteria

We studied, prospectively, 31 consecutive patients with AAD (group I), 30 consecutive patients with chronic aneurysms of the ascending aorta scheduled for coronary

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angiography prior to elective surgical repair (group II) and 20 healthy subjects who served as controls (group III). Patients with all types of AAD admitted for emergency surgery were included in the study. The diagnosis of AAD was confirmed in all patients of group I with computed tomography; likewise, all patients in group II underwent computed tomography. Dissection was classified according to the Stanford criteria. The measurement of the diameter of the ascending aorta in patients of groups I and II was based on computed tomography angiograms. Risk factors for atherosclerosis as well as known vascular disease in other beds — coronary, carotid or peripheral arterial disease — were recorded. Exclusion criteria were: significant renal impairment (creatinine > 2 mg/dl), diagnosis of hypothyroidism and use of folate blocker therapy (i.e. methotrexate, carbamazepine).

All patients gave informed consent and the study protocol was approved by the Ethics Committee of our institution. The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology.

2.2. Biochemical analyses

In patients with AAD sampling was performed as soon as the patient came to the hospital; in patients with chronic aortic aneurysm admitted for coronary angiography, sampling was performed the morning of the admission and prior to catheterization.

2.2.1. Measurement of Hcy, folate, B12 and Lp(a) in serum

Total serum Hcy, folate and B12 were quantitatively evaluated with a chemiluminescent microparticle immunoassay on the ARCHITECT analyzer i1000R (Abbot Diagnostics, Wiesbaden, Germany). Blood samples were collected into evacuated tubes containing EDTA, centrifuged within 10 min, and stored at or below −20 °C until analyzed. The measurement ranges were 0–50 μmol/L, 1.5–20 ng/mL and 0–2000 pg/ml, respectively. The within-assay coefficient of variation (CV) for all assays was less than 10%. Lp(a) was determined by nephelometry, which is based on the latex-enhanced particle agglutination technology (Nephelometer Analyzer 100, Behring, Germany). The lower detection limit of this assay was 30 mg/1.

2.2.2. Determination of D-dimers, C-reactive protein, N-terminal pro-B-type natriuretic peptide and fibrinogen

Plasma fibrin D-dimers were assessed with a two-step enzyme immunoassay sandwich method with a final fluorescent detection (Vidas D-dimer, Biomerieux, France). The measurement range was 45–10,000 ng/mL. The mean inter-assay CV, was quoted by the manufacturer as being lower than 5%. C-reactive protein (CRP) was evaluated with a particle-enhanced immunoturbidimetric assay (Cobas Integra, Roche, Mannheim, Germany) with limits of detection > 0.025 mg/dl and normal values < 0.5 mg/dl. N-terminal pro-B-type natriuretic peptide (NT-proBNP) was measured with immunoassay working according to the electrochemiluminescence sandwich immunoassay principle (Elecsys proBNP, Roche, Mannheim, Germany). The measuring range was 5–35000 pg/ml. The NT-proBNP assay has an intra-assay precision between 1.2% and 1.5% and an inter-assay precision between 4.4% and 5.0%. Fibrinogen was determined with thrombin clotting time on a BCS Siemens coagulation analyzer (Siemens Healthcare Diagnostics, Marburg, Germany) according to Clauss method; in the presence of an excess of thrombin, fibrinogen is transformed into fibrin and clot formation time is inversely proportional to the concentration of fibrinogen in the sample plasma. The intra-assay CV was 2% and the inter-assay CV was 3.5%.

2.2.3. Measurement of white blood count, hemoglobin, creatinine and cardiac enzymes

White blood count (WBC) and hemoglobin (Hb) were measured by an automated cell counter (Coulter LH780, Hematology Analyzer, Beckman, Miami, USA). Serum creatinine was determined by the Jaffe reaction using a Cobas 600 analyzer (Roche diagnostics, Mannheim, Germany). Creatine kinase (CPK) was measured on a Cobas Integra 800 analyzer (Roche, Bazel, Switzerland) and creatine kinase-MB (CPK-MB) and troponin I (Tn-I) were measured by immunoassay on the Dimension system (Dade Behring, Deerfield Il, USA). Reference normal values are as follows: CPK < 190 mU/ml, CPK-MB < 3.6 ng/ml and Tn-I < 0.1 ng/ml. This is the 95th percentile of the reference control group for CPK and CPK-MB and the 99th percentile of the reference control group for Tn-I in our institution.

2.3. Genetic analysis

Genomic DNA was extracted from 200 μl of peripheral blood by QIAamp DNA mini blood kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. A 233 bp fragment of the MTHFR gene was amplified with oligonucleotide primers obtained from TIB (TIB MOLBIOL, Berlin, Germany) in a LightCycler 1.5 instrument at conditions described by the manufacturer. Amplicon detection was based on dual fluorescence energy transfer probes (TIB MOLBIOL, Berlin, Germany) hybridized to internal sequences of the amplified fragment during the annealing phase. Each test run included a reagent blank, in which template DNA was replaced with PCR-grade water. Genotyping was achieved by melting curve analysis. Wild type MTHFR C677 DNA exhibits a melting point of 63.0 ± 2.5 °C while mutant 677 T exhibits a melting point of 54.5 ± 2.5 °C. All analyses were performed with color compensation using reagents from Roche Diagnostics.

2.4. Statistical analysis

Differences in frequencies of qualitative variables (hypertension, diabetes, dyslipidemia, smoking) among the three groups were assessed using chi-square or Fishers' exact test, when appropriate. Continuous variables are expressed as mean ± standard deviation and investigation for differences among the three groups was performed using one-way analysis of variance (ANOVA) or Kruskal–Wallis test when normality assumption was violated. Post-hoc tests between groups, using Bonferroni correction, were performed after a significant overall test. Furthermore, linear regression models were fitted to investigate differences among groups, adjusting for potential confounders (age, sex, hypertension, dyslipidemia, diabetes mellitus and cigarette smoking). All tests were two-sided and a P-value < 0.05 was considered as statistically significant.

3. Results

3.1. Characteristics of study groups

There were 25 men and 6 women, 60 ± 13 years old in group I, 24 men and 6 women, 67 ± 12 years old in group II and 14 men and 6 women, 58 ± 15 years old in group III (Table 1). There were significant differences in age and the prevalence of hypertension among the 3 study groups ($P = 0.026$ and $P = 0.006$, respectively) (Table 1). In detail, age was significantly different between groups I and II ($P = 0.013$) and between groups I and III ($P = 0.008$). The prevalence of hypertension was significantly higher in groups I and II as compared with group III ($P = 0.035$ and $P = 0.004$, respectively). The diameter of the ascending aorta did not differ between groups I and II nor did the ejection fraction; the ejection fraction was preserved in both groups. There were 26 type A and 5 type B dissections in group I. Eight patients had aortic regurgitation. Of 31 patients with AAD, the dissection involved only the thoracic aorta in 16, both the thoracic and the abdominal aorta in 8 and the thoraco-abdominal aorta extending up to the level of the common iliac arteries in 7. Time from symptom onset was 13 ± 11 hours (range 6–52). Five patients (all type B) were managed conservatively and did well; the remaining was operated and all but 3 survived.

3.2. Levels of Hcy, folate, B12 and Lp(a)

Hcy differed significantly among the 3 study groups ($P = 0.016$). Multiple comparisons showed significant higher levels of Hcy in group I compared to both groups II and III ($P = 0.05$ and $P = 0.002$, respectively) and no significant difference between groups II and III ($P = 0.083$) (Table 2). Eighteen patients in group I had moderate hyperhomocysteinemia (15–30 μmol/L), 4 intermediate (30–100 μmol/L) and none severe (> 100 μmol/L). Folate differed significantly between study groups ($P = 0.004$), with group I having lower values compared to both groups II and III ($P = 0.001$ and $P = 0.006$, respectively) and without difference between groups II and III ($P = 0.409$). Similarly, vitamin B12 differed significantly between the groups ($P =$

Table 1
Baseline characteristics of patients with acute aortic dissection (group I), chronic aortic aneurysms (group II) and normal controls (group III).

	Group I (n = 31)	Group II (n = 30)	Group III (n = 20)	*P value
Age, y	60 ± 13	67 ± 12	58 ± 15	0.026
Male/female	25/6	24/6	14/6	0.627
Hypertension	74%	83%	40%	0.006
Diabetes	6%	17%	15%	0.437
Dyslipidemia	39%	63%	60%	0.130
Smoking	42%	27%	30%	0.455
Aortic diameter (mm)	56 ± 16	54 ± 8		0.531
Ejection Fraction (%)	55 ± 6	56 ± 5		0.735

Results are expressed as mean ± standard deviation, absolute values or percentages (%).
* $P < 0.05$ significantly different mean values among the 3 study groups by ANOVA.