

## Original Article

# Abdominal aortic aneurysm and the association with serum levels of Homocysteine, vitamins B6, B12 and Folate

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Received September 4, 2012; accepted October 5, 2012; Epub October 25, 2012; Published November 15, 2012

**Abstract:** Previous investigations have shown hyperhomocysteinemia in patients with abdominal aortic aneurysm (AAA). In the present study we evaluated the circulating level of homocysteine (Hcy) in relation to renal function, vitamins B6, B12 and folate status in AAA patients with special regard to aneurysm size, and rupture. Hcy, Creatinine, B6, B12 and folate were measured in 119 patients with AAA and 36 controls without aneurysm matched by age, gender and smoking habit. As expected there was a weak correlation between Hcy and vitamins B6, B12 or folate. We found similar levels of Hcy, B6 and folic acid in patients with nonruptured AAA compared to the control group matched by age, gender and smoking habit. There was no correlation between maximum diameter of the nonruptured AAA (n=78) and Hcy, B6 or folate. However, the present study shows a significant inverse correlation between maximum diameter of the nonruptured AAA (n=78) and B12 ( $r = -0.304$ ,  $p=0.007$ ) with significant higher levels in small AAA compared to large AAA. In conclusion, Hcy does not seem to be a useful biomarker in AAA disease. The unexpected finding of B12 levels correlating to aneurysm diameter warrants urgent further investigation of B12 supplement to prevent progression of small AAA.

**Keywords:** Homocysteine, C-reactive protein, vitamin B6, vitamin B12, folate, aneurysm, aorta

## Introduction

In recent years, a relationship between milder degrees of hyperhomocysteinemia and vascular disease has emerged and has been the subject of intense research [1-4]. Hyperhomocysteinemia can be caused by genetic defects of the enzymes involved in homocysteine (Hcy) metabolism and/or deficiencies of their cofactors: folate (former vitamin B9), vitamin B12 and vitamin B6 [5]. Renal impairment and inflammation are other possible causes of elevated plasma Hcy [4]. Moreover, cigarette smoking increases Hcy, which is strongly correlated with cotinuria and plasma thiocyanates [5]. Furthermore, smokers have a tendency to develop hypofolatemia, hypovitamin B12 and hypovitamin B6, particularly when the duration of smoking habit exceeded 20 years [5, 6]. In a review 2007 Moroz et al concluded that there is evidence for an association between Hcy and abdominal aortic aneurysm (AAA), however, not

a strong enough association to conclude that it plays a causal role in the pathogenesis of AAA [7]. Moreover, further research was suggested, given the potential benefit that simple vitamin supplementation may have for patients with AAA [7]. Previous studies concerning the relationship between AAA and Hcy vary greatly in terms of population, definition of hyperhomocysteinemia, criteria for defining AAA, size of AAA, and adjustment for confounding variables such as age, gender and smoking habit [7-9]. This makes them prone to bias. Hence, based on the above discussion, further investigation is justified to assess the role of Hcy as a potential circulating biomarker that is associated with aneurysm enlargement or predictive of rupture.

The main purpose of the present study was to investigate whether elevated levels of Hcy exist in patients with AAA compared to controls without aneurysm matched by age, sex and smoking habit. A second purpose was to find out whether

AAA are associated with decreased levels of vitamin B6, vitamin B12 and/or folate to assess its possible role in pathogenesis or progression of AAA.

### Material and methods

One hundred nineteen patients with infrarenal AAA treated at Sundsvall County Hospital were studied prospectively. The study was performed in accordance with the principles of the Declaration of Helsinki and was approved by the regional ethics committee. Patients and control subjects gave their approval by written informed consent. The case-control cohort has been described in detail elsewhere [10].

#### *Ruptured AAA patients (n=41)*

Forty-one patients with ruptured AAA were included. All patients had a retroperitoneal hematoma confirmed by operation.

#### *Nonruptured AAA patients (n=78)*

Thirty-eight patients with elective surgery for nonruptured AAA, with an aneurysm diameter of at least 5.0 cm (large AAA), were included. Forty patients with asymptomatic AAA on surveillance with an aneurysm diameter smaller than 5.0 cm (small AAA) were also included.

#### *Controls (n=36)*

Several studies have established male gender, age, smoking habit and a family history of AAA as independent risk factors for AAA [11]. Furthermore, serum levels of Hcy, vitamins B6, B12 and folic acid are also dependent on gender, age and smoking habit (5-6). The control group was selected in accordance with the guidelines given by Grimes and Schulz [12]. A control group of thirty-six volunteers with normal infrarenal aortic diameter were matched to the AAA patients according to age, gender and smoking habit. Smoking was defined as having a smoking habit at the time of inclusion.

#### *Imaging*

The aortic size was confirmed in all patients and controls by ultrasonography and the largest aortic diameter was recorded. Normal diameter was defined as maximum infrarenal aortic diameter < 3.0 cm.

#### *Blood sampling and assays*

Peripheral venous blood samples were taken from controls and each patient preoperatively and before any blood product transfusions. Serum samples were frozen in plastic tubes and stored at -70°C until analysis. Four commercially available assays were used according to manufacturers' instructions for levels of: high sensitive C-reactive protein (hsCRP), vitamin B6 (B6), vitamin B12 (B12) and folate. Immunonephelometry was performed for quantitative determination of hsCRP (*CardioPhase® hsCRP*, Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany). Folic acid and vitamin B12 were determined using an immunoenzymatic method (Folate III reagent kit and Vitamin B12 reagent kit, Roche Diagnostics GmbH, Mannheim, Germany). Pyridoxal 5-phosphate is the biologically active form of vitamin B6 and is the ideal analyte to assess the B6 status. However, the total amount of vitamin B6 (pyridoxine, pyridoxal, pyridoxamine) was measured using a microbiological assay (ID-vit® Vitamin B6, Immundiagnostik AG, Bensheim, Germany) due to its higher reliability in stored samples compared to, Pyridoxal 5-phosphate, the instable biologically active form of vitamin B6 [13]. The intraassay coefficients of variation (CV) were 2.2 – 5.8% for hsCRP, 4.7 – 6.3% for folic acid, 3.6 – 5.6% for B12, and 9.8 – 16.2% for B6. Total homocysteine (Hcy) was measured by using liquid chromatography-tandem mass spectrometry with an intraassay CV of 6%. Furthermore, hematocrit and creatinine levels were analyzed by routine clinical methods with intraassay CV <2.5% in both assays.

#### *Statistical analysis*

All analyses were carried out using SPSS® statistical software 16.0 for Windows™ (SPSS, Chicago, Illinois, USA). Median (interquartile range) values were calculated for continuous variables and categorical data was expressed as absolute numbers with percentages. Differences in findings between study groups were assessed by Chi-square tests (two-tailed without Yates correction) for categorical variables and by Mann-Whitney tests for continuous variables. Correlation was assessed by Spearman's method between each biomarker and the maximum diameter of the AAA. Results were considered statistically significant when *p*-values were < 0.05.

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**Table 1.** Demographics of controls and patients with AAA

	Controls (n=36)	Nonruptured AAA (n=78)		Ruptured AAA (n=41)	p-values ruptured compared to nonruptured AAA
		Small (n=40)	Large (n=38)		
Age, years	72(67-78)	70(66-77) <sup>NS</sup>	73(63-79) <sup>NS</sup>	73(70-76) <sup>NS</sup>	0.509
Sex, male, no(%)	30(83%)	30(75%) <sup>NS</sup>	32(84%) <sup>NS</sup>	33(80%) <sup>NS</sup>	0.665
Current smoking, no(%)	15(42%)	17(43%) <sup>NS</sup>	17(45%) <sup>NS</sup>	17(41%) <sup>NS</sup>	0.769
Aneurysm diameter, cm	no aneurysm	4.0(3.5-4.3)	5.9(5.2-7.0)	7.0(6.0-8.0)	<0.001

The figures indicate median (interquartile range) or the number (percentage) of patients/controls. <sup>NS</sup> Non-significant, \*  $p < 0.001$  compared with control group value.

**Table 2.** Laboratory results of controls and patients with AAA

Assay, reference interval	Controls (n=36)	Nonruptured AAA (n=78)		Ruptured AAA (n=41)	p-values ruptured compared to nonruptured AAA
		Small (n=40)	Large (n=38)		
Hcy, <15.0 $\mu\text{mol/L}$	14.1 (10.8-17.4)	13.9 (12.4-18.9) <sup>NS</sup>	13.8 (10.9-18.5) <sup>NS</sup>	11.0 (7.6-14.7)*	<0.001
B12, $\geq 141$ pmol/L	264 (194-321)	365 (247-459)*	276 (220-333) <sup>NS</sup>	197 (147-265)*	<0.001
Folat, $\geq 10$ nmol/L	12.5 (10.5-15.5)	11.5 (8.9-14.2) <sup>NS</sup>	12.0 (9.0-15.6) <sup>NS</sup>	10.4 (8.2-12.6)*	0.022
B6, 4.8-17.7 $\mu\text{g/L}$	4.7 (3.5-6.4)	3.9 (3.0-6.5) <sup>NS</sup>	3.6 (2.7-5.8) <sup>NS</sup>	2.7 (2.0-3.6)**	<0.001
Creatinine, 50-110 $\mu\text{mol/L}$	93 (83-100)	98 (83-110) <sup>NS</sup>	94 (80-108) <sup>NS</sup>	110 (91-133)**	0.007
hsCRP, <2.9 mg/L	1.5 (0.8-3.1)	2.2 (1.1-7.4) <sup>NS</sup>	3.5 (1.9-6.1)*	6.6 (2.2-28.5)**	0.005
Hematocrit, %	41 (39-43)	42 (40-44) <sup>NS</sup>	41 (39-43) <sup>NS</sup>	36 (30-40)**	<0.001

Median (interquartile ranges). Mann-Whitney  $U$  test. <sup>NS</sup> Non-significant, \*  $p < 0.01$ , \*\*  $p < 0.001$  compared with control group value.

## Results

The matching procedure gave similar age, gender and current smoking habit in the controls and AAA patients (Table 1). Furthermore, there were no significant differences between patients with ruptured AAA and nonruptured AAA according to age, gender and current smoking habit. The laboratory results are shown in Table 2. The correlation analysis of the nonruptured AAA (n=78) showed a significant inverse correlation between hsCRP and B6 ( $r = -0.280$ ,  $p = 0.013$ ) but not between hsCRP and Hcy, B12 or folate. As expected inverse correlations were shown between Hcy and B6 ( $r = -0.257$ ,  $p = 0.023$ ), B12 ( $r = -0.321$ ,  $p = 0.004$ ) and folate ( $r = -0.464$ ,  $p < 0.001$ ) but not between Hcy and Creatinine ( $r = 0.177$ ,  $p = 0.121$ ). There was a significant inverse correlation between B12 ( $r = -0.304$ ,  $p = 0.007$ ) and maximum diameter of the nonruptured AAA but not between Hcy, B6 or

folate and maximum diameter of the nonruptured AAA.

## Discussion

In recent years, the impact of circulating levels of Hcy in the pathogenesis and progression of AAA have been the subject of investigation [7-9]. However, these studies concerning the relationship between AAA and Hcy vary greatly in terms of population, definition of hyperhomocysteinemia, criteria for defining AAA, size of AAA, and adjustment for confounding variables such as age, gender and smoking [7-9]. This makes them prone to bias. Even the role of the Hcy associated vitamin B6, B12 and folate has been explored in relation to AAA disease [14, 15]. In the present study we investigated the impact of Hcy in patients with abdominal aortic aneurysm in relation to hsCRP, vitamins B6, B12 and folate status, the relationship to aneurysm size

and association with rupture. Since male gender, increasing age and smoking habit are the dominant risk factors for AAA [1] we used a control group matched by age, gender and smoking habit to the AAA patient group to eliminate possible bias in accordance with the guidelines given by Grimes and Schulz [12]. The results of this study support earlier studies suggesting a state of activated inflammatory response in patients with nonruptured infrarenal aortic aneurysm as expressed by elevated hsCRP compared with the control group [16]. Our data also verifies earlier studies that rupture of an AAA further activates the inflammatory system as we find significantly higher levels of hsCRP in patients with ruptured AAA compared to nonruptured AAA [16]. Since Hcy levels have been suggested to be associated with inflammation [4] it was surprising to find that Hcy levels were similar in controls and nonruptured AAA. Furthermore, there was no correlation between levels of hsCRP and Hcy; a finding in agreement with a recent report [17]. Even vitamin B6 and folate showed similar levels in controls and nonruptured AAA. It is well-known that concentration of blood cells and plasma proteins are decreased by massive bleeding due to hemodilution. This explains the decreased levels of hematocrit, Hcy, vitamins B6, B12 and folate in ruptured compared to nonruptured AAA.

We found elevated vitamin B12 levels in small AAA compared both to controls and large AAA. Furthermore, there was a significant inverse correlation between B12 levels and aneurysm diameter in patients with nonruptured AAA. This finding suggests that high B12 levels might protect against AAA progression. Recent reports show decreasing AAA incidence which is explained by smoking cessation in the general population [18]. Furthermore, a current meta-analysis stated that growth and rupture rate of small AAA was increased in smokers compared to former/never smokers [19]. B12 status might also contribute since B12 levels are lower in smokers compared with non-smokers [5]. The present finding must be confirmed by future animal experimental studies and in AAA surveillance follow-up studies to investigate whether vitamin B12 supplement protects against AAA progression.

In summary, Hcy does not seem to be a useful biomarker in the AAA disease. Instead, the unexpected finding of B12 levels correlating to

aneurysm diameter warrants urgent further investigation of the possible benefit of B12 supplement to prevent progression of small AAA.

### Acknowledgements

The authors are grateful to Carola Berg and Madeleine Lindqvist for excellent technical assistance and also thank Nikki Stephensen Nyberg for helpful linguistic comments on this manuscript.

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