Homocysteine levels in polymyalgia rheumatica and giant cell arteritis: influence of corticosteroid therapy

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**Objectives.** It has been suggested that patients with giant cell arteritis (GCA) may share a common pathway with atherosclerosis. Furthermore, patients with GCA and polymyalgia rheumatica (PMR), in addition to advanced age, are treated for prolonged periods of time with corticosteroids, a factor that can also accelerate atherosclerosis. Hyperhomocysteinaemia is considered an independent risk factor for atherosclerosis, and might play a role in ischaemic manifestations that occur with a variable frequency during the course of GCA. The purposes of the present study were: (i) to analyse the plasma levels of homocysteine in patients with GCA and PMR, (ii) to determine the influence of corticosteroid therapy on the homocysteine levels and (iii) to analyse if the levels of homocysteine may predict the development of ischaemic complications in patients with GCA.

**Methods.** Plasma homocysteine concentration was measured in 56 patients with active PMR/GCA (17 GCA and 39 isolated PMR) before steroid treatment and 23 healthy age-matched volunteers were used as controls. The total plasma homocysteine level was quantified using a fluorescent polarization immunoassay.

**Results.** Homocysteine concentrations were higher in PMR and GCA patients than age-matched controls ($P < 0.05$). Patients with GCA had slightly higher levels of plasma homocysteine than those with isolated PMR ($13.6 \pm 4.3$ vs $12.7 \pm 3.1$ μmol/l, $P = 0.6$). In 30 of these patients (12 GCA and 18 PMR) a second measurement of homocysteine concentration was done when they were in clinical remission with steroid treatment. The post-treatment levels of homocysteine were significantly increased in GCA rather than in PMR patients. In 13 patients with homocysteine levels above the normal upper limit of our laboratory, therapy with folic acid and or vitamin B12 was started. After 3 months of vitamin supplements, the homocysteine concentration significantly decreased from $19.2 \pm 3.1$ to $13.6 \pm 3.2$ μmol/l ($P = 0.001$). Such decrease was less marked in the PMR than in GCA patients. Ten out of the 17 patients with GCA had ischaemic manifestations of the disease. The levels of homocysteine were slightly higher in GCA patients with ischaemia than in those without ischaemic manifestations, although the difference did not reach statistical significance ($15 \pm 4.9$ vs $11.6 \pm 1.9$ μmol/l, $P = 0.46$).

**Conclusions.** Patients with active PMR and GCA had elevated plasma concentrations of homocysteine. Corticosteroid therapy significantly increased...
such levels, especially in GCA patients. Treatment with supplements of folic acid and or vitamin B₁₂ reduced the homocysteine concentrations. These data support the hypothesis that patients with GCA (and to a lesser extend PMR patients) may share a common pathway with atherosclerosis and suggest a new atherogenic mechanism of corticosteroids.

**Key words:** Polymyalgia rheumatica, Giant cell arteritis, Homocysteine, Corticosteroids, Folic acid, Vitamin B₁₂.

Giant cell arteritis (GCA) is a granulomatous vasculitis affecting medium and large-size arteries in elderly people [1, 2]. Around 50% of patients with GCA have polymyalgia rheumatica (PMR), a clinical syndrome characterized by pain and stiffness in the neck, shoulder and pelvic girdles [3]. Although PMR and GCA occur frequently in the same individual, the relationship between these entities is not fully understood. The aetiopathogenesis of these two disorders remains unknown although genetic, autoimmune and environmental factors have been implicated [4, 5]. Nevertheless, one of the most striking features of these two conditions is the development of the disease in an almost exclusive manner in people older than 50 yr of age. Furthermore, the possibility of GCA in patients with PMR increases with age [6]. Despite this close association with age, the pathogenic mechanisms that could explain this age-related predisposition are unknown. Ageing is associated with abnormalities in several systems including the neuroendocrine, immune and cardiovascular systems. These abnormalities lead to an increased frequency of infections, autoimmune diseases, neoplastic disorders and atherosclerosis. Since the beginning of the 1980s, it has been suggested that patients with GCA may share a common pathway with atherosclerosis [7, 8]. These previous observations have been confirmed recently in a prospective multicentre study [9]. In this study, smoking and previous arterial disease were independently associated with GCA in women [9].

Although there are many controversies about the relationship between PMR and GCA, there are several lines of evidence supporting that a significant number of patients with PMR have subclinical vascular inflammation. First of all, around 15% of the patients with biopsy-proven GCA have, as a unique manifestation, a polymyalic syndrome [1, 2]. As shown by Weyand et al. [10], patients with isolated PMR have mRNA of macrophage-derived cytokines and T cell-derived cytokines (mainly IL-2) in temporal artery samples without histological evidence of vasculitis. Finally, recent studies with fluorodeoxyglucose (FDG)-positron emission tomography (PET) in patients with PMR and GCA have shown an increased FDG uptake in their thoracic and upper leg vessels, compared with controls with other inflammatory conditions, suggesting that at least some patients with PMR have a subclinical form of vasculitis [11, 12].

Homocysteine is an intermediary amino acid formed during the conversion of methionine to cysteine. Hyperhomocysteinaemia may occur in the course of several autoimmune disorders, due to vitamin B₁₂ or folate deficiency, or secondary to therapy with some drugs [13]. Nowadays, hyperhomocysteinaemia is considered an independent risk factor for atherosclerosis, and might play a role in anterior ischaemic optic neuropathy, silent brain infarction and stroke, thoracic aortic arteriosclerosis, abdominal aortic aneurysm and acute coronary syndromes [14–20]. All of these manifestations might occur with a variable frequency during the course of GCA. Whatever the cause of hyperhomocysteinaemia, it can be prevented by a safe and inexpensive approach using vitamin supplements of folic acid, vitamin B₁₂ and/or vitamin B₆ [21, 22]. Therefore, hyperhomocysteinaemia is a treatable risk factor for ischaemic complications. Furthermore, patients with PMR and GCA, in addition to advanced age, are treated for prolonged periods of time with corticosteroids, a well-known factor that can accelerate atherosclerosis [23].

The purposes of the present study were: (i) to analyse the levels of homocysteine in patients with PMR and GCA, (ii) to determine the influence of corticosteroid therapy on the homocysteine levels and (iii) to analyse whether the levels of homocysteine may predict the development of ischaemic complications in patients with GCA.

**Methods and patients**

**Patients**

Fifty-six patients with active PMR/GCA before steroid treatment were enrolled in the present study: 39 had isolated PMR and 17 GCA. In 30 of these patients (18 PMR and 12 GCA) a second analysis was done when they were in clinical remission with steroid treatment. Twenty-three healthy age-matched volunteers were used as controls. PMR was diagnosed according to the criteria proposed by Chuang et al. [24]. In patients with PMR, the possibility of GCA was excluded either by a normal temporal artery biopsy, or by the absence of manifestations of GCA and cure with low-dose prednisone after a long-term follow-up. All the patients with GCA had a positive temporal artery biopsy, consisting of interruption of the internal elastic laminae with infiltration of mononuclear cells into the arterial wall with or without giant cells and fulfilled the American College of Rheumatology 1990 classification criteria for the disease [2, 25–27]. Patients with GCA were considered to have major ischaemic manifestations if they had jaw claudication (defined when the patient had pain on
chewing that improved after stopping mastication), visual involvement (defined as diplopia, transient visual loss and/or permanent visual loss), cerebrovascular accident (when the patient had stroke and/or transient ischaemic attacks) or extracranial large vessel involvement [2, 25, 28]. Corticosteroid treatment for PMR patients was started with prednisone at 10 mg daily (5 mg twice daily). Treatment for GCA was begun with prednisone at 40–60 mg daily (15–20 mg three times a day). After initial control of the disease, the dose of cortico-
steroids was reduced according to clinical disease activity (10 mg daily (5 mg twice daily). Treatment for GCA was begun
with prednisone at 40–60 mg daily (15–20 mg three times a
day). After initial control of the disease, the dose of cortico-
stereoids was reduced according to clinical disease activity [25, 29–31]. Remission was defined on a clinical basis as the absence
of clinical symptoms and signs of the disease associated with
normal laboratory values.

**Laboratory assessment**

All the patients had blood samples taken between 8.00 am and 9.30 am, before the morning dose of their treatment. For PMR and GCA patients studied prior to onset of steroid treatment, follow-up samples were taken after a minimum of 3 months of treatment and when they were in clinical remission. Total white cells, lymphocytes, haemoglobin and platelet counts were measured by routine techniques. The erythrocyte sedimentation rate (ESR) was measured in the routine haematology laboratory by the Westergren method (readings taken at 1 h) and the C-reactive protein (CRP) was measured by nephelo-
meter. Fasting glucose, albumin, urea, creatinine, thyroid function, total cholesterol, triglycerides, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol were also measured in the routine biochemistry laboratory. Anti-
cardiolipin antibodies were measured by an in-house enzyme-
linked immunosorbent assay (ELISA) in the immunology
laboratory. The total plasma homocysteine concentration was measured using a commercial fluorescent polarization immuno-
assay (IMX; Abbott Laboratories, Abbott Park, IL, USA). The assay was subjected to regular quality control and had an
interassay variability of 4%. At our hospital, the upper normal
limit of homocysteine was 15 µmol/l.

**Statistical analysis**

Analyses were performed with the STATISTICA software package for Macintosh. We used the 2-tailed Student t-test for the continuous variables with a normal distribution and non-
parametric tests (median and Mann–Whitney U-test) for those
without a normal distribution. The χ²-test or the Fisher exact
test was used to compare the dichotomous variables. Correla-
tions were analysed by the Spearman test. Groups were
compared with respect to baseline total plasma homocysteine levels by using the Wilcoxon rank-sum test. Statistical
significance was defined as P < 0.05.

**Results**

**Demographic features and vascular risk factors in patients with PMR/GCA**

As shown in Table 1, the mean age of the different study
groups was similar, with a predominance of females, especially in the GCA group. We did not find any significant
difference regarding previous traditional vascular risk factors for atherosclerosis between patients and controls except for a previous history of high blood pressure in both patient groups. Hypertension was more
frequent in GCA (53%) and PMR (36%) patients than

Table 1: Main demographic features and cardiovascular risk factors of PMR/GCA patients and controls according to the medical history

<table>
<thead>
<tr>
<th></th>
<th>PMR</th>
<th>GCA</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>39</td>
<td>17</td>
<td>23</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>72.1 ± 7.8</td>
<td>73.3 ± 5.6</td>
<td>73.2 ± 6.6</td>
</tr>
<tr>
<td>Sex (% female)</td>
<td>51</td>
<td>76</td>
<td>60</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>28</td>
<td>12</td>
<td>17</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>36a</td>
<td>53b</td>
<td>13a,b</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>10</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>Hypercholesterolaemia (%)</td>
<td>26</td>
<td>6</td>
<td>26</td>
</tr>
<tr>
<td>Ischaemic cardiopathy (%)</td>
<td>13</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Cerebral vascular accident (%)</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Peripheral vascular disease (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Previous thyroid disease (%)</td>
<td>8</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

4PMR vs age-matched controls, P < 0.05.
5GCA vs age-matched controls, P < 0.05.
6PMR vs GCA, P < 0.05.

in controls (P < 0.05). However, there were no signifi-
cant differences in the levels of homocysteine in the study
groups according to the presence or absence of hyper-
tension. Major complications of atherosclerosis were
more frequent in patients with PMR and GCA, but the
differences were not significant (Table 1).

As expected, patients with PMR and GCA showed a
significant increase in those parameters that reflect an
acute-phase response (haemoglobin, platelets, ESR and
CRP) compared with age-matched controls. Patients
with PMR were also characterized by lower levels of
acute-phase reactants compared with GCA patients
(Table 2). Within the GCA group, we found a negative
expression of homocysteine in the study
levels of glucose, renal function and total
Homocysteine levels in PMR and GCA

Table 2: Main laboratory data of PMR-GCA patients and controls

<table>
<thead>
<tr>
<th></th>
<th>PMR</th>
<th>GCA</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>12.8 ± 1.5ac</td>
<td>11.7 ± 1.4bc</td>
<td>13.4 ± 1.7ab</td>
</tr>
<tr>
<td>Platelets (× 10^12 cells/mm³)</td>
<td>269 ± 99ac</td>
<td>341 ± 101bc</td>
<td>188 ± 37b</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>46 ± 33ac</td>
<td>78 ± 34bc</td>
<td>8 ± 5b</td>
</tr>
<tr>
<td>CRP (mg/ml)</td>
<td>3.5 ± 3.2ac</td>
<td>7.1 ± 5.3bc</td>
<td>0.3 ± 0.6b</td>
</tr>
<tr>
<td>Albumin (g/ml)</td>
<td>3.9 ± 0.5</td>
<td>3.6 ± 0.5</td>
<td>3.9 ± 0.2</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>102 ± 24</td>
<td>112 ± 22</td>
<td>103 ± 24</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1 ± 0.2</td>
<td>1 ± 0.2</td>
<td>1 ± 0.1</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>45.7 ± 13.9</td>
<td>43.5 ± 13.3</td>
<td>40.1 ± 9.6</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>194 ± 54</td>
<td>191 ± 36</td>
<td>208 ± 35</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>40 ± 12a</td>
<td>39 ± 14b</td>
<td>57 ± 14b</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>137 ± 63</td>
<td>126 ± 42</td>
<td>136 ± 33</td>
</tr>
<tr>
<td>LDL/HDL ratio</td>
<td>3.4 ± 1.1</td>
<td>3.9 ± 1.2</td>
<td>2.7 ± 1.3</td>
</tr>
<tr>
<td>Abnormal TSH values (positive/perform)</td>
<td>1/31</td>
<td>0/14</td>
<td>ND</td>
</tr>
<tr>
<td>Anticardiolipin antibodies (positive/perform)</td>
<td>6/28</td>
<td>7/13</td>
<td>ND</td>
</tr>
</tbody>
</table>

4PMR vs age-matched controls, P < 0.05.
5GCA vs age-matched controls, P < 0.05.
6PMR vs GCA, P < 0.05.
significant correlation between the total cholesterol levels and homocysteine in the PMR group \((r = 0.41; P = 0.01)\), and between triglycerides and homocysteine in GCA patients \((r = 0.88; P = 0.02)\). Although anticardiolipin antibodies were more frequent in GCA patients \((54\%)\) than in PMR \((21\%)\), we did not find any correlation with the development of ischaemic manifestations in patients with GCA and the presence of these antibodies. Furthermore, we did not find any correlation between the levels of homocysteine and the titres of anticardiolipin antibodies.

**Plasma homocysteine concentrations were higher in patients with PMR/GCA than in age-matched controls**

As shown in Fig. 1, homocysteine concentrations were significantly higher in PMR \((P = 0.03)\) and GCA patients \((P = 0.04)\) than in the age-matched controls. Patients with GCA had slightly higher levels of plasma homocysteine than those with isolated PMR \((13.6 \pm 4.3 \text{ vs } 12.7 \pm 3.1 \mu\text{mol/l}, P = 0.6)\), although the differences between the groups did not reach statistical significance.

**Corticosteroid therapy increased plasma homocysteine levels in patients with GCA**

In 30 patients \((12 \text{ GCA and } 18 \text{ PMR})\) we were able to determine the plasma levels of homocysteine before and after therapy with corticosteroids. As shown in Fig. 2, the post-treatment levels of homocysteine were increased in GCA \((13.4 \pm 3.3 \text{ vs } 17.8 \pm 5 \mu\text{mol/l}, P = 0.003)\) rather than PMR patients \((12.5 \pm 2.7 \text{ vs } 13.6 \pm 3.5 \mu\text{mol/l}, P = 0.07)\).

**Treatment with supplements of folic acid and/or vitamin B\(_{12}\) significantly reduced the homocysteine concentrations**

In 13 patients with homocysteine levels above the normal upper limit of our laboratory \((15 \mu\text{mol/l})\), therapy with folic acid and/or vitamin B\(_{12}\) was started. At the time of starting vitamin supplements all the patients were on steroid therapy. There were three patients with PMR and 12 with GCA with a concentration of homocysteine of \(19.2 \pm 3.1 \mu\text{mol/l}\). After 3 months of treatment with vitamin supplements, the homocysteine concentration significantly decreased to \(13.6 \pm 3.2 \mu\text{mol/l} (P = 0.001)\). As shown in Fig. 3A, the decrease in homocysteine concentration was less marked in the PMR patients \((20.7 \pm 2 \text{ vs } 16 \pm 5.9 \mu\text{mol/l}, P = 0.1)\), with only one patient reaching normal homocysteine values. On the other hand, patients with GCA responded much better to therapy \((18.8 \pm 3.3 \text{ to } 12.9 \pm 1.8 \mu\text{mol/l}, P = 0.005)\), with eight out of the 10 patients showing a decline of homocysteine levels to normal limits (Fig. 3B).
The levels of homocysteine did not correlate with the development of ischaemic complications in patients with GCA

Ten out of the 17 patients with GCA had ischaemic manifestations of the disease. As shown in Fig. 4, the levels of homocysteine were higher in GCA patients with ischaemia than in those without ischaemic manifestations although the difference did not reach statistical significance (15.4 ± 4.9 vs 11.6 ± 1.9 μmol/L, P = 0.1). We did not find any significant correlation between the number of ischaemic manifestations of the disease and the levels of homocysteine (r = 0.1, P = 0.6). Furthermore, no significant correlation between the presence of ischaemia and the previous history of vascular risk factors or anticardiolipin antibodies was found (data not shown).

Discussion

To the best of our knowledge, the role of homocysteine as a contributing factor for the pathogenesis or as a predictive factor of thrombosis in patients with GCA and PMR has not been investigated previously.

First of all, we found a significant elevation of homocysteine levels in patients with active GCA and PMR compared with an age-matched population. These data add new support to the concept that patients with GCA may share a common pathway with atherosclerosis [7–9]. The same might be true for at least a subgroup of patients with PMR, where subclinical vascular inflammation seems to play a role in the pathogenesis of the disease. Although our study was not designed for this purpose, we also found a higher frequency of hypertension and lower HDL-cholesterol levels in patients with active disease compared with the control population. However, we found no significant correlation between the concentration of homocysteine and the levels of HDL-cholesterol or the presence of hypertension, suggesting that in our patient population the increase in homocysteine is not clearly related to other well-known vascular risk factors. Nevertheless, the hypothesis that patients with GCA (and probably PMR) may share a common pathway with atherosclerosis should be confirmed in larger studies.

The increase in homocysteine levels was also not related to the inflammatory process per se. In fact, we found a negative correlation between acute-phase reactants and homocysteine levels within the GCA group. Also, remission of the disease after steroid therapy was not accompanied by a parallel decrease in homocysteine concentrations despite the normalization of the acute-phase response.
The plasma homocysteine concentrations are determined by genetic and nutritional factors. In this regard, genetic mutations of enzymes, such as methylene tetrahydrofolate reductase and cystathionine-β-synthase, may cause hyperhomocysteinemia [32]. Patients with liver disease and renal failure also have an elevated homocysteine level [33]. Furthermore, deficiencies of vitamin B₆, B₁₂ and/or folic acid are probably the most frequent causes of hyperhomocysteinemia [34]. Although we did not check the concentrations of vitamin B₁₂ and folic acid routinely in those individuals with an increase in the corpuscular volume of red cells or with levels of homocysteine above the normal value, we subsequently assessed the levels of vitamin B₁₂ and folic acid. All of them, except one GCA patient with a very high homocysteine concentration, had normal levels of these two vitamins. This patient had pernicious anaemia and was excluded from the study.

Of interest, treatment with corticosteroids induced an increase in homocysteine levels in both groups of patients. This increase was only significant in patients with GCA, and might just reflect the higher doses of steroids used to treat these patients. Corticosteroids could also increase the risk of atherosclerosis via deleterious effects on lipids, glucose metabolism and blood pressure. In this regard, our results might add a new atherogenic mechanism to steroids. A growing body of evidence supports the role of inflammation in the initiation and progression of atherosclerosis [35]. Several studies have demonstrated a clear relationship between increased CRP levels (determined by highly sensitive immunoassay) and risk of future complications of atherosclerosis [36, 37]. Furthermore, systemic inflammation may also be associated with hypercoagulability, owing to several factors such as thrombocytosis and elevation of fibrinogen and von Willebrand factor [38, 39]. PMR and GCA constitute two very good examples of diseases characterized by a strong acute-phase response which might contribute to the progression of atherosclerosis in the elderly population that is the target of these processes.

In contrast to other risk factors for atherosclerosis or thrombosis, hyperhomocysteinaemia is correctable with vitamin B₁₂ and/or folic acid supplementation. All our patients with levels of homocysteine above the upper normal limit of our laboratory were started on folic acid, and only a minority of them (those who failed to respond to folic acid) also received vitamin B₁₂ supplements. With this approach, we obtained a significant decrease in basal homocysteine levels in 12 out of the 13 treated patients.

The exact mechanism by which hyperhomocysteinaemia causes vascular disease is unknown, although there are several hypotheses to explain its deleterious effect on the vessels [40]. First, hyperhomocysteinaemia has deleterious effects on endothelial cells, causing cell damage, smooth muscle cell proliferation and increased oxidative stress [41]. Second, hyperhomocysteinaemia induces an interference with coagulation mechanisms, inducing a procoagulant state [42, 43]. Disease severity in GCA patients is usually the consequence of ischaemic complications of the disease that mainly affect the cranial arteries, as such as visual or cerebrovascular manifestations. These manifestations are usually present at the beginning of the disease and, in general, are prevented with corticosteroid therapy [25]. As shown here, the levels of homocysteine were slightly higher in GCA patients with ischaemia than in those without ischaemic manifestations although the difference did not reach statistical significance, probably due to the small size of the sample. Larger studies are necessary to clarify whether homocysteine levels are related to the development of ischaemic complications in patients with GCA.

In summary, the elevated concentrations of homocysteine found in patients with active disease support the hypothesis of a common pathway between GCA (and to a lesser extend PMR) and atherosclerosis. Treatment with supplements of folic acid and/or vitamin B₁₂ significantly reduced the homocysteine concentrations. Corticosteroid therapy induced a significant increase in homocysteine levels, especially in GCA patients, and suggests a new atherogenic mechanism of corticosteroids.

Acknowledgements

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References