Impaired cognitive performance in asymptomatic peripheral arterial disease: relation to C-reactive protein and D-dimer levels

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Abstract

Background and purpose: asymptomatic peripheral arterial disease (APAD), a highly prevalent condition in the general older population, is associated with an increased risk of cerebrovascular events because of co-existing clinical or subclinical cerebral atherosclerosis. The purpose of this study was to investigate whether cognitive function is impaired in stroke- and...
Impaired cognitive performance in asymptomatic peripheral arterial disease

Peripheral arterial disease (PAD), a manifestation of atherosclerosis, has been found to affect approximately 18% of the population aged 55–74 years [1]. Although intermittent claudication is considered the earliest and the most common presenting symptom of PAD [2], community-based epidemiological studies showed that most people with PAD are asymptomatic [3]. PAD patients, whether symptomatic or asymptomatic, have an increased risk of death and cardiovascular events because of co-existing clinical or subclinical atherosclerosis in the coronary and cerebral arteries [1, 4], and a decreased ankle brachial index (ABI) of <0.90 is a risk factor for ischaemic stroke in the elderly [5].

Some [6, 7, 8], but not all [9], clinical studies have found cognitive deficits on tests of attention, psychomotor speed, executive function, visuospatial ability and visual memory among patients with symptomatic moderate-to-severe PAD [7, 8] and PAD amputees [6, 7], who were free of clinical stroke and transient ischaemic attacks (TIAs). Two longitudinal cohort studies [10, 11] have also shown in a general stroke-free population that intermittent claudication was associated with poor performance on various cognitive function tests.

To our knowledge, no previous studies have investigated cognitive function in patients with asymptomatic peripheral arterial disease (APAD), which is usually a milder form of the disease. Thus, it is not known whether the finding of cognitive deficits applies to the whole spectrum of PAD patients. Correlates of poorer cognitive performances have been reported to be PAD severity [7], ischaemic heart disease [7], diastolic blood pressure [8] and plasma glucose levels [8]. However, the relationship between cognitive abilities of PAD patients and the levels of inflammatory and haemostatic variables, such as C-reactive protein (CRP), D-dimer and fibrinogen, has not yet been evaluated.

Increased levels of CRP [12, 13] and D-dimer and fibrinogen [12, 14] have been reported in PAD patients. Evidence from prospective studies shows that CRP [15] and D-dimer and fibrinogen [16] are each linked to raised risk of stroke in the general population, and are predictive of cardiovascular events, including stroke, in patients with PAD [17–19]. Furthermore, CRP [20, 21], D-dimer [22, 23] and fibrinogen [24] have been significantly associated to increased risk of cognitive decline and dementia in population-based, cross-sectional [24] and prospective [20, 21, 22] studies and in one cross-sectional case–control study [23].

Therefore, in this study, we investigated whether cognitive function is impaired in patients with APAD who had not experienced previous clinical stroke or TIA. We also examined the blood levels of CRP, D-dimer and fibrinogen as potential correlates of neuropsychological performance in these patients.

Materials and methods

Study participants

One hundred and sixty-four well-functioning, community-dwelling Caucasian patients with APAD (Leriche-Fontaine stage I) were identified from screening for PAD with the ABI among asymptomatic at-risk subjects seeking medical advice at the study facility, during the period of 1 January 2003 to 31 July 2004. Risk factors for PAD were age >55 years, smoking, diabetes, hypertension and hypercholesterolaemia. The patients with PAD were considered asymptomatic if they had no history of intermittent claudication or ischaemic rest pain and did not present with ischaemic skin lesions.

Exclusion criteria included history or clinical evidence of stroke, TIA, neurological or psychiatric disorder, alcohol and/or substance abuse, uncontrolled diabetes (fasting blood glucose >11.1 mmol/l), uncontrolled hypertension (>180/110 mmHg), concomitant treatment with psychotropic drugs, use of anti-inflammatory drugs other than low-dose aspirin, use of anticoagulants and other conditions (e.g. cancer, inflammatory processes, hepatic, renal or respiratory failure) known to interfere with cognitive function and/or inflammatory and coagulatory response. Potential
participants using statins and other drugs reported to have some anti-inflammatory effects were included in the study. One hundred and sixty-four healthy control subjects were recruited in the same period from the local community and matched to the patients with APAD with respect to age, gender and level of education.

Diabetes, hypertension and hypercholesterolaemia were defined by current diagnostic criteria [25–27]. These disorders were also considered to be present if the patients were receiving treatments for them. Smoking behaviour was assessed by self-report.

All participants underwent blood testing for CRP, D-dimer and fibrinogen, ABI measurement and, on a separate day, neuropsychological examination. All subjects had detailed history and clinical assessment, haematologic and biochemical screens and 12-lead ECG evaluation. History included medication details, and drug dose, type and duration of use were recorded. Our institutional ethics committee approved the study, and informed consent was obtained from all study participants.

**Ankle brachial index**

The diagnosis of PAD was made based on an ABI of <0.90 on either leg, which is 95% sensitive and 99% specific for angiographically significant PAD [28]. The ABI was performed using a sphygmomanometric cuff and a hand-held 8-MHz Doppler probe (Hadeco Bidop Es-100V3, Hayashi Denki, Japan). The right and left ABI values were calculated by dividing the higher of the dorsalis pedis and posterior tibial systolic pressures in each leg by the higher of the two arm pressures, and the worse of the two values was used to define the ABI for each patient [29].

**Neuropsychological testing**

All study participants completed a battery of six neuropsychological tests assessing cognitive domains of attention and verbal working memory (Digit Span Forward and Backward) [30], perceptuomotor speed, attention and mental flexibility (Trail Making Test, Parts A and B) [31], and visuoconstructive skills and visual memory [Rey–Osterrieth Complex Figure (ROCF) Copy and ROCF Delayed Recall] [32, 33]. The 30-item Mini-Mental State Examination (MMSE) [34], a measure of global cognitive functioning, and the 15-item Geriatric Depression Scale (GDS), a depression screening device [35], were also administered to all participants.

**Biochemical measurements**

Blood samples were drawn after a 12- to 14-h overnight fast. After separation, plasma and serum aliquots were stored at −80°C until analysis. Serum CRP was determined using a highly sensitive particle-enhanced immunonephelometric assay (N High Sensitivity CRP, Dade Behring, Marburg, Germany) on the Behring BN II analyser. The detection limit of this assay system is 0.175 mg/l; intra- and inter-assay coefficients of variation were less than 5 and 6%, respectively.

D-dimer was measured by a latex-enhanced turbidimetric assay (D-dimer PLUS, Dade Behring, Marburg, Germany) using the automated BCS analyser (Dade Behring). Intra- and inter-assay coefficients of variation were less than 4%. Fibrinogen was measured according to the thrombin-time method of Clauss using reagents from Dade Behring. Plasma glucose and serum total cholesterol were measured enzymatically using kits from Roche Diagnostics (Milan, Italy).

**Statistical analysis**

Sample size estimation and power analysis were performed using the StatMate Package by GraphPad Software, San Diego, CA, USA. Continuous normally distributed data are reported as mean ± standard deviation (SD), continuous non-normally distributed data as median (25th–75th percentile) and categorical data as percentages. Continuous variables were compared between groups using unpaired t-test or Mann–Whitney U test, as appropriate, and categorical variables were compared using Fisher’s exact test. Univariate relationships between CRP, D-dimer, fibrinogen and the cognitive performances shown to be impaired among patients with APAD were tested by Spearman’s correlation coefficient. Other variables previously shown or thought to correlate with cognitive performances within patients with APAD were also analysed. They included diabetes, hypertension, hypercholesterolaemia, coronary artery disease, smoking, ABI, systolic blood pressure, diastolic blood pressure, fasting glucose and total cholesterol. Multivariate linear regression analyses were used to assess which variables were significantly and independently related to impaired cognitive measures (dependent variables) within patients with APAD. Variables found to be significantly (P<0.05) associated with impaired cognitive measures in the above univariate analyses (independent variables) were selected for inclusion into the multivariate linear regression models. Age, gender, level of education and depression score were also included in these models. In all analyses, a two-tailed P-value <0.05 was considered significant.

**Results**

Table 1 shows the sociodemographic and clinical characteristics of patients with APAD and control subjects. Neuropsychological performances in the two groups are shown in Table 2. Patients with APAD scored significantly worse (P<0.0001) than control subjects on five cognitive tests: Digit Span Backward, Trail Making A, Trail Making B, ROCF Copy and ROCF Delayed Recall. In univariate analysis for patients with APAD, CRP was significantly negatively correlated with Digit Span Backward scores (r = −0.194; P = 0.0124), ROCF Copy scores (r = −0.176; P = 0.0239) and ROCF Delayed Recall scores (r = −0.174; P = 0.0256) and positively correlated with Trail Making A scores (r = 0.235; P = 0.0024) and Trail Making B scores (r = 0.181; P = 0.0200); D-dimer was significantly negatively correlated with Digit Span Backward scores (r = −0.301; P<0.0001) and positively correlated with Trail Making A scores (r = 0.365; P<0.0001) and Trail Making B scores (r = 0.2800; P = 0.0003); systolic blood pressure was significantly negatively correlated with ROCF Copy scores (r = −0.191; P = 0.0142) and ROCF Delayed Recall scores (r = −0.282;
Table 1. Characteristics of the study population

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>APAD (n = 164)</th>
<th>Controls (n = 164)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>70.0 ± 3.4</td>
<td>70.3 ± 3.7</td>
</tr>
<tr>
<td>Men, n (%)</td>
<td>122 (74)</td>
<td>120 (73)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.0 ± 3.7</td>
<td>25.1 ± 3.8</td>
</tr>
<tr>
<td>Education, years</td>
<td>7.8 ± 3.5</td>
<td>7.9 ± 3.6</td>
</tr>
<tr>
<td>GDS-15 score</td>
<td>4.5 ± 2.1</td>
<td>4.3 ± 2.2</td>
</tr>
<tr>
<td>MMSE score [median (25th–75th)]</td>
<td>28 (27–29)</td>
<td>28 (27–29)</td>
</tr>
<tr>
<td>Current smoking, n (%)</td>
<td>74 (45)</td>
<td>–</td>
</tr>
<tr>
<td>Prior smoking, n (%)</td>
<td>58 (35)</td>
<td>50 (30)</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg*</td>
<td>135.0 ± 18.2</td>
<td>124.2 ± 8.6</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg*</td>
<td>85.4 ± 9.7</td>
<td>75.7 ± 6.8</td>
</tr>
<tr>
<td>Fasting glucose, mmol/l*</td>
<td>6.5 ± 1.3</td>
<td>5.1 ± 0.5</td>
</tr>
<tr>
<td>Total cholesterol, mmol/l*</td>
<td>5.3 ± 0.7</td>
<td>4.7 ± 0.3</td>
</tr>
<tr>
<td>CRP, mg/l [median (25th–75th)]*</td>
<td>3 (2–4)</td>
<td>2 (1–2)</td>
</tr>
<tr>
<td>D-dimer, ng/ml*</td>
<td>194.8 ± 25.4</td>
<td>168.2 ± 29.0</td>
</tr>
<tr>
<td>Fibrinogen, g/l*</td>
<td>0.29 ± 0.04</td>
<td>0.24 ± 0.04</td>
</tr>
<tr>
<td>ABI*</td>
<td>0.76 ± 0.05</td>
<td>1.1 ± 0.09</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>58 (35)</td>
<td>–</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>82 (50)</td>
<td>–</td>
</tr>
<tr>
<td>Hypercholesterolaemia, n (%)</td>
<td>56 (34)</td>
<td>–</td>
</tr>
<tr>
<td>Coronary artery disease, n (%)</td>
<td>30 (18)</td>
<td>–</td>
</tr>
</tbody>
</table>

Values are mean ± SD unless otherwise indicated.

ABI, ankle brachial index; APAD, asymptomatic peripheral arterial disease; BMI, body mass index; CRP, C-reactive protein; GDS-15, 15-item Geriatric Depression Scale; MMSE, Mini-Mental State Examination.

*P = 0.0001.

Table 2. Neuropsychological performance in patients with APAD and control subjects

<table>
<thead>
<tr>
<th>Test</th>
<th>APAD (n = 164)</th>
<th>Controls (n = 164)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digit Span Forward</td>
<td>6 (6–7)</td>
<td>6 (6–7)</td>
<td>0.2718</td>
</tr>
<tr>
<td>Digit Span Backward</td>
<td>4 (3–4)</td>
<td>4 (4–5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Trail Making A</td>
<td>50 (38–70)</td>
<td>35 (29–44)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Trail Making B</td>
<td>165 (110–198)</td>
<td>90 (78–112)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ROCF Copy</td>
<td>22 (21–25)</td>
<td>29 (28–30)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ROCF Delayed Recall</td>
<td>9 (8–10)</td>
<td>16 (14–18)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Values are median (25th–75th).

APAD, asymptomatic peripheral arterial disease; ROCF, Rey–Osterrieth Complex Figure.

P = 0.0002); fasting glucose was significantly negatively correlated with ROCF Copy scores (r = −0.182; P = 0.0196) and ROCF Delayed Recall scores (r = −0.196; P = 0.0117).

When these variables were entered into multiple linear regression analyses, the following variables were found to be significant independent predictors of poorer performances on the five cognitive tests: for Digit Span Backward, CRP and D-dimer; for Trail Making A, CRP and D-dimer; for Trail Making B, CRP and D-dimer; for ROCF Copy, CRP, fasting glucose and systolic blood pressure; for ROCF Delayed Recall, fasting glucose and systolic blood pressure (Table 3).

Discussion

These findings extend previous studies by demonstrating that clinically stroke- and TIA-free patients with APAD showed cognitive impairment in five neuropsychological tests assessing cognitive domains of verbal working memory, attention, perceptuomotor speed, mental flexibility, visuoconstructive skills and visual memory. They also show that CRP, a marker of inflammation, and D-dimer, an index of ongoing thrombus formation and lysis, were significant negative predictors of performance on four and three cognitive tests, respectively, independently of conventional risk factors. CRP and D-dimer predicted poorer performance of cognitive tests of verbal working memory, attention, perceptuomotor speed and mental flexibility. CRP also was a significant, independent predictor of impaired visuoconstructive performance.

Among conventional risk factors, fasting glucose and systolic blood pressure were found to be independent negative predictors of cognitive performance on two tests of visuoconstructive skills and visual memory.

Our findings do reinforce the notion that PAD is a marker for cognitive impairment in clinically stroke-free patients, but the exact underlying mechanisms are unknown. PAD has been reported to be related to a spectrum of asymptomatic, vascular-related brain injury, including silent stroke, atrophy and white matter hyperintensities [36–38]. These morphologic brain changes are, in turn, associated with cognitive impairment [38–40]. Although only one study has assessed cognitive functioning in relation to neuroimaging and PAD [38], these findings suggest that vascular brain damage is the most likely mediator linking PAD to cognitive impairment. Recently, however, atherosclerosis and its risk factors have been associated with an increased risk of Alzheimer’s disease (AD) [41]. Thus, it is possible that AD pathology contributes to the cognitive deficits in PAD patients.
Brain imaging was not performed in the current investigation, and therefore no information is available on the brain pathology underlying the cognitive dysfunction observed in patients with APAD. However, neuropsychological tests revealed that these patients had impaired executive function, and early decline in executive control is often seen in vascular cognitive impairment, but not in early AD [42]. Further studies are required to investigate cognitive functioning in relation to neuroimaging in patients with APAD.

There are several plausible mechanisms potentially mediating relationships between CRP, D-dimer and poorer cognitive functioning in patients with APAD. Higher CRP levels may merely reflect the inflammatory component of underlying subclinical atherosclerotic cerebrovascular disease (CVD) [43]. Alternatively, CRP may causally contribute to the progression of atherothrombotic lesions and related brain damage and cognitive deficits by activating complement [44] and by enhancing the production of inflammatory cytokines interleukin-1 (IL-1)α, IL-1β and tumour necrosis factor-α (TNF-α) [45]. CRP also affects coagulation by inducing monocytes to express tissue factor, a potent procoagulant [46].

Higher D-dimer levels may reflect activation of the coagulation–fibrinolysis pathway, and may be linked to cognitive impairment through thrombus formation within cerebral vessels [47]. On the other hand, D-dimer may stimulate inflammatory processes and acute-phase responses by inducing the production of inflammatory mediators, including cytokines IL-1β and interleukin-6 (IL-6) [48].

Our observation that fasting glucose and systolic blood pressure were independent negative predictors of performance on two cognitive tests among patients with APAD is in line with previous findings [8] and in accordance with the adverse effects of diabetes [49] and hypertension [50] on cognitive function.

The results of the present study have several potential implications. The finding that APAD is associated with cognitive impairment, a predictor for both declining functional abilities [51] and future dementia [52, 53], suggests the need for screening for APAD among at-risk subjects. In this study, based on epidemiological data on PAD [1], age >55 years was considered as a risk factor for PAD. However, screening for PAD among asymptomatic people over 55 years of age without other major risk factors could be limited to subjects older than 70 years [54]. In addition to decreasing systemic cardiovascular risk, early detection and treatment of patients with APAD might potentially prevent or slow functional decline and dementia. Interventions to prevent cognitive decline among PAD patients should target CVD and the related cognitive consequences. Because CVD also increases the risk for AD, preventing vascular brain damage should also reduce the risk for AD [42]. Effective measures for prevention of CVD include blood pressure control, smoking cessation, use of anticoagulant therapy in atrial fibrillation, antiplatelet treatment, statin therapy, and treatment of extracranial carotid artery stenosis [55]. Data specific to PAD patients are also available showing the efficacy of antiplatelet drugs, statins and angiotensin-converting enzyme inhibitors in reducing cardiovascular events, including stroke, in these patients [2]. Evidence that antihypertensive therapy [56, 57], lipid lowering with statins [58] and antithrombotic medication [59] may protect against cognitive impairment provides further support for the implementation of preventive cardiovascular interventions in PAD patients.

Our observation of an association between CRP, D-dimer and cognitive impairment in patients with APAD allows speculation that inflammation and hypercoagulability may contribute to cognitive decline, thus raising the possibility that cognitive function might benefit from therapies modulating the inflammatory and coagulatory response, including those with aspirin and non-aspirin non-steroidal anti-inflammatory drugs, statins and warfarin [59, 60]. The findings also suggest the potential use of these biological markers in risk stratification for cognitive decline in patients with APAD.

**Conclusion**

This study provides novel information on the link between PAD and cognitive function by showing that (1) cognitive deficits are present even in asymptomatic patients in the absence of clinical stroke or TIA and (2) CRP and D-dimer are associated independently and inversely with performance on three tests of verbal working memory, attention, perceptuo-motor speed and mental flexibility. CRP is also associated independently and inversely with poorer visuococonstructive performance. These data highlight the need for screening for APAD among at-risk subjects. They also support the hypothesis that inflammation and hypercoagulability are implicated in the pathophysiology of cognitive impairment in APAD.

**Key points**

- Patients with APAD show cognitive impairment.
- CRP and D-dimer levels are independent negative predictors of cognitive performance in patients with APAD.
- These findings highlight the need for screening for APAD among at-risk subjects in order to identify patients to be treated for prevention of functional decline and dementia.
- This study supports the hypothesis that inflammation and hypercoagulability are implicated in the pathophysiology of cognitive dysfunction associated with APAD.

**Acknowledgements**

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**Conflicts of interest**

The authors are not aware of any conflict of interest.

**References**

Only the most important references are listed here and are represented by bold type throughout the text. The full list of references is available as supplementary data on the journal website (www.ageing.oxfordjournals.org).
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