Vascular biomarkers of cognitive performance in a community-based elderly population: the Dublin Healthy Ageing study

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Abstract

Background: population studies suggest that cardiovascular risk factors may be associated with cognitive impairment. Epidemiological studies evaluating individual markers of vascular disease as risk factors for cognitive dysfunction have yielded inconsistent results. Homocysteine has emerged as a marker consistently associated with poorer outcomes. Existing studies have largely examined individual vascular risks in isolation and have tended to ignore patient psychological status.

Objective: to investigate the association between markers of vascular disease and cognition in a community-dwelling non-demented elderly population while adjusting for vascular and non-vascular confounds.

Design: cross-sectional community based assessment.

Participants: 466 subjects with mean age 75.45 (s.d., 6.06) years. 208 (44.6%) were male.

Results: higher levels of homocysteine were consistently associated with poorer performance in tests assessing visual memory and verbal recall. No other vascular biomarker was found to be associated with cognitive performance. Factors such as alcohol use, tea intake, life satisfaction, hypertension and smoking were positively correlated with global cognitive performance. Negative correlations existed between cognitive performance and depression, past history of stroke, intake of fruit and use of psychotropic medication.

Conclusions: homocysteine was the only vascular biomarker associated with poorer function in a number of domains on neuropsychological testing, independent of vascular and non-vascular confounds. Other psychosocial factors may need to be taken into account as potential confounds in future studies investigating cognition.

Keywords: epidemiology, vascular, cognition, elderly, homocysteine

Introduction

The ageing of populations worldwide raises concern regarding the prevalence of cognitive dysfunction in the future. Therefore, it becomes increasingly important to identify risk factors associated with cognitive impairment. Epidemiological studies have linked cardiovascular disease, vascular comorbidities and specific vascular biomarkers such as cholesterol, HbA1c, homocysteine and C-reactive protein (CRP) [1–3] with cognitive decline and dementia. These factors are highly prevalent in the elderly and are potentially modifiable. Population studies evaluating individual markers of vascular disease as risk factors for cognitive dysfunction have been inconclusive. One reason for this uncertainty may be that most existing studies examined individual vascular risks in isolation and tended to ignore patient psychological status when adjusting for potential confounds. Individual vascular risk factors or biomarkers may influence each other [4], leading to over- or underestimation of their individual effects on cognition. In addition, other biophysical and psychosocial patient factors may influence observed associations between vascular risks, biomarkers and cognition [5].

The potential for prevention of cognitive decline and dementia through modifying vascular risk factors is therefore dependent on an understanding of their effects individually and collectively. Similar examples include the observed benefit of anti-oxidants and oestrogen hormone replacement.
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therapy on vascular disease—this may have been due to disease-avoiding behaviour on the part of those taking such therapies [6, 7]. In order to better evaluate the correlations between vascular biomarkers and cognition, we carried out a population-based study adjusting for known vascular co-morbid and psychosocial factors while testing for cognitive dysfunction in a community-dwelling population.

**Methodology**

**Study population and design**

The Dublin Healthy Ageing study is a community-based study examining physical, psychiatric, cognitive and social health characteristics of older people. It is a cross-sectional examination of 466 non-demented elderly subjects, stratified for age, and randomly selected from the patient lists of four general practitioners in the catchment area of St. James’s Hospital, Dublin. All medically stable, non-demented community-dwelling individuals aged over 65 years who were able to provide consent and cooperation with neuropsychological testing were eligible for recruitment. Data were collected between 2003 and 2005. Ethical approval was obtained from both the Hospital and GP ethics committees. Subjects were contacted by post and offered participation. A total of 1349 letters to individuals were sent. Of these, 419 (31%) individuals refused participation. A total of 159 (11.8%) did not meet inclusion criteria; 175 (13%) of these, 419 (31%) individuals refused participation. A total of 159 (11.8%) did not meet inclusion criteria; 175 (13%) had passed away and 71 (5.3%) were no longer at their given addresses; 59 patients were no longer on the GP list. A research psychologist and a doctor visited individuals who agreed to participate in their own home on a single occasion. The duration of each assessment was approximately 2 h.

**Assessments**

A structured interview recorded self-reported information on demographic details, education, medical history, current medications, diet, smoking status, alcohol use, exercise, psychosocial history and family history. Lifetime alcohol intake was estimated from self-report and calculated in the number of units/week. Pack years were calculated by dividing the number of cigarettes smoked per day by 20 and multiplying this by the number of years smoked.

Biophysical measurements such as the subjects’ height (cm), waist circumference (cm) and hip circumference (cm) were measured using a standard tape measure accurate to 1 mm. Weight (kg) was measured on a calibrated scale accurate to 0.5 kg with normal indoor clothing. Subjects’ waist : hip ratio (waist/hip) and body mass index (weight/height²) were calculated according to standard equations.

Blood pressure measurements were performed with the subject sitting, with a standard aneroid sphygmomanometer accurate to 3 mmHg. A comprehensive assessment of neurological status and mood was performed by a medical doctor. Further clinical assessments were performed if deemed necessary.

Cognitive status was assessed in a variety of domains using standardised instruments. These domains included pre-morbid intellect (National Adult Reading Test (NART-R) [8]), psychomotor processing speed (Wechsler Adult Intelligence Scale (WAIS)—III—digit symbol coding [9]), verbal fluency and category fluency (FAS test and animal fluency [10]), and verbal learning, interference, delayed recall and recognition (Wechsler Memory Scale (WMS)—III—serial word lists [11]). Visual memory was assessed using the Wechsler Memory Scale—revised visual reproduction. Working memory was tested using the Wechsler Memory Scale—III—letter number sequencing [12]. Test scores were standardised using a z-transformation (subject score minus sample mean divided by sample standard deviation) to enable comparison of tests with different ranges. A composite score consisting of the average of the sum of the standardised test scores [13] was used as a global measure of cognitive performance (global composite: GC). The Mini-Mental State Examination (MMSE) [14] was performed as a second general index of cognitive functioning. Personality was measured using the Eysenck Personality Inventory (EPI) [15] which measures two important personality dimensions, extroversion—introversion and neuroticism-stability.

Blood samples were collected by venepuncture and non-fasting samples taken for homocysteine, glucose, glycosylated haemoglobin, lipid profile and CRP. All samples were measured by a commercial laboratory. Homocysteine samples were transported on ice, spun within 30 min and stored at −20°C or below until analysed. Quantitative measurement of L-homocysteine in serum was by fluorescence polarization immunoassay (Imx system; Abbott laboratories). Sensitivity of these assays was calculated to <0.50 μmol/l corresponding to the upper limit of the 95% confidence interval. Precision of these assays was determined by assaying samples on 5 days, i.e. 2 runs/day across 10 instruments. Samples with mean value 5.9, 10.8, 21.6 μmol/l yielded within-run coefficient of variation (CV)% of 2.2, 1.9 and 1.4 respectively and total CV% of 5.2, 4.1 and 3.7 respectively. Glucose levels were determined enzymatically (bioMerieux) with CV range of 0.75–0.81%. Glycosylated haemoglobin was measured using high-performance liquid chromatography (Hi-AUTO A1c Analyser system) with a CV of 5–10%. Serum cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were measured by enzymatic clearance assay (Randox Laboratories) using a Hitachi 737 or 747 with a CV range of 0.88–1.2% for HDL, and CV 0.49–1.79% for LDL. Triglyceride levels were measured by the triglycerides liquid color test (Human Gesellschaft fur Biochemica und Diagnostica mbH) with a CV range between 2.0 and 3.5%. C-reactive protein was measured by particle enhanced immunonephelometry (BN Systems) with a CV range between 2.1 and 5.7%.

**Statistical Analysis**

Multiple linear regression models were used to investigate the relationship between vascular biomarkers and neuropsychological tests, controlling for identified covariates. The relationship between neuropsychological test scores and vascular biomarkers (homocysteine, C-reactive protein,
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Results

Demographic, clinical, biochemical, psychosocial, dietary and lifestyle characteristics are summarised in Table 1. There were 466 subjects with a mean age (SD) of 75.5 (6.1), of which 55.4% were female. Neuropsychological test characteristics are summarised in Table 2. Due to fatigue or illiteracy some subjects were unable to complete all the neuropsychological tests.

Homocysteine

Raised homocysteine was consistently associated in base models with poorer function in several domains on neuropsychological testing, specifically, tests assessing visual memory, verbal recall, psychomotor processing speed and a measure of global cognition, the MMSE (Table 3). This was independent of known confounds.

In multivariable models, controlling for other covariates, homocysteine remained consistently associated with visual memory and verbal recall (Table 3). There were no consistent associations found between other vascular biomarkers investigated and cognitive tests.

Lifestyle and psychosocial factors

Factors negatively associated with global cognition scores included increased age, lower social class and a history of stroke or depression (please see Appendix 1 in the supplementary data at Age and Ageing Online Table 1). Factors that correlated positively included education, higher life satisfaction, increased fruit intake, alcohol and tea intake and a history of hypertension. Cigarette smoking also correlated positively with cognition.

Discussion

In this elderly non-demented community population, raised homocysteine concentrations were significantly associated with poorer performance in neuropsychological tests assessing visual memory and verbal recall. This was independent of biophysical and psychosocial factors and other vascular biomarkers. There were no consistent associations found between other biochemical markers of vascular disease and cognition. Aside from the known confounds of cognitive performance such as age, educational status and social class, this study identified other potential psychosocial confounds which should be taken into consideration in studies assessing cognition.

The association found between elevated levels of homocysteine and cognitive dysfunction in this study is consistent with that observed in other population studies although this has not been universal [17–19]. Hyperhomocysteinaemia has previously been implicated in the pathogenesis of arteriosclerosis and a number of epidemiological studies have shown a relationship between vascular disease and homocysteine concentrations [20]. High homocysteine levels may contribute to cognitive decline through silent brain infarcts [21]. However, whether elevated levels of homocysteine is a causative factor in vascular disease or the consequence of tissue damage is still uncertain.

In vitro studies have shown that homocysteine potentiates β amyloid neurotoxicity [22]. Thus, homocysteine may play a
more direct role in the pathogenesis of neurodegenerative disorders [23]. While several randomised controlled trials have failed to demonstrate that reduction of homocysteine levels protects against cognitive decline [24, 25], one limitation of these studies is that the time span in which they are conducted may be insufficient to detect an effect.

Results of studies investigating the association between cognition and other vascular biomarkers such as CRP, glycosylated haemoglobin and LDL-cholesterol have been mixed [17, 26–28]. This study found no consistent association between these vascular biomarkers and cognitive performance. Potential explanations include differences in populations studied such as educational status, the degree of cardiovascular co-morbidity and the use of disease-modifying drugs such as statins. Age may also be a factor—the mean age of this sample was 75 years, and all subjects were over 65 years of age. Subjects with adverse outcomes related to these vascular biomarkers may not have survived to inclusion in this study or may have become ineligible. It is also possible that vascular biomarkers are related to cognitive performance in younger individuals only. Levels at the age of entry may not reflect levels earlier in life. Finally, it is possible that there is no association between CRP, glycosylated haemoglobin and LDL-cholesterol, and that previous positive reports could be due to residual and uncontrolled confounding.

Limitations of this study include a single timed measurement of vascular biomarkers, which lends itself to measurement error. This, if non-differential, could have contributed to an underestimation of the effect of homocysteine on cognitive performance and the lack of association found with the other vascular biomarkers. Homocysteine samples in this study were obtained from non-fasting subjects. However, studies have found no significant difference between levels of pre- and post-prandial homocysteine when measured in the same subject [29]. Our sample was an urban Caucasian community population and our findings may not be applicable to rural or other ethnic populations. Although the response rate is comparable to other studies, this does not out-rule the possibility of response bias reducing any association between the vascular biomarkers and cognitive performance. Due to the cross-sectional nature of this study, we were unable to account for survival bias or establish cause and effect.

Finally, our findings support evidence from other studies suggesting that lifestyle and psychosocial factors may be important in determining cognitive performance [5, 30]. Factors such as alcohol use, tea intake, depression, life satisfaction, hypertension, smoking, past history of stroke, intake of fruit and use of psychotropic medication were all found to be associated with global cognitive performance. These factors may need to be taken into account as potential confounds in future studies investigating cognition.

### Key points
- In a cross-sectional study of 466 community-dwelling, non-demented older people, higher serum homocysteine was associated with poorer cognitive function.
- Other biomarkers such as c-reactive protein, glycosylated haemoglobin and low-density lipoprotein were not associated with cognitive function.
- A number of psychosocial factors including alcohol use, tea intake, depression, life satisfaction, smoking and intake of fruit were all found to be associated with cognitive performance.

### Table 2. Neuropsychological test scores

<table>
<thead>
<tr>
<th>Neuropsychological tests: mean, (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mini-mental state examination</td>
</tr>
<tr>
<td>Pre-morbid IQ (NART)</td>
</tr>
<tr>
<td>Letter fluency (FAS)</td>
</tr>
<tr>
<td>Category fluency (Animals)</td>
</tr>
<tr>
<td>Psychomotor processing speed (WAIS-III digit symbol coding)</td>
</tr>
<tr>
<td>Working memory (WMS-III letter number sequencing)</td>
</tr>
<tr>
<td>Visual memory (immediate and delayed) (WMS-R visual reproduction)</td>
</tr>
<tr>
<td>Verbal memory (WMS-III serial words)</td>
</tr>
<tr>
<td>Learn slope</td>
</tr>
<tr>
<td>Short delay recall</td>
</tr>
<tr>
<td>Long delay recall</td>
</tr>
<tr>
<td>Recognition</td>
</tr>
<tr>
<td>Total recall list 1–4</td>
</tr>
<tr>
<td>List B recall</td>
</tr>
<tr>
<td>Proactive interference</td>
</tr>
<tr>
<td>Retroactive interference</td>
</tr>
</tbody>
</table>

Note: a National Adult Reading Test. b Wechsler Adult Intelligence Scale. c Wechsler Memory Scale—III. d Wechsler Memory Scale—Revised Visual Reproduction.
Table 3. Associations between cognitive score and blood tests-adjusted for age, gender, education and social class (model 1) adjusted for age, gender, education, social class alcohol, tea intake, depression, life satisfaction, hypertension, use of psychotropic medications, smoking, stroke, fruit intake (model 2)

<table>
<thead>
<tr>
<th></th>
<th>Homocysteine</th>
<th>C-reactive protein</th>
<th>Glycosylated haemoglobin</th>
<th>Low-density lipoprotein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model 1</td>
<td>Model 2</td>
<td>Model 1</td>
<td>Model 2</td>
</tr>
<tr>
<td></td>
<td>Standard beta</td>
<td>Standard beta</td>
<td>Standard beta</td>
<td>Standard beta</td>
</tr>
<tr>
<td></td>
<td>$P$</td>
<td>$P$</td>
<td>$P$</td>
<td>$P$</td>
</tr>
<tr>
<td>FAS test</td>
<td>0.010</td>
<td>0.836</td>
<td>0.028</td>
<td>0.620</td>
</tr>
<tr>
<td>Animals</td>
<td>-0.31</td>
<td>0.504</td>
<td>0.026</td>
<td>0.620</td>
</tr>
<tr>
<td>Visual reproduction1</td>
<td>-0.127</td>
<td>0.008</td>
<td>-0.317</td>
<td>0.038</td>
</tr>
<tr>
<td>Visual reproduction2</td>
<td>-0.127</td>
<td>0.008</td>
<td>-0.139</td>
<td>0.012</td>
</tr>
<tr>
<td>Letter number sequencing</td>
<td>-0.078</td>
<td>0.144</td>
<td>-0.089</td>
<td>0.148</td>
</tr>
<tr>
<td>Digit symbol score</td>
<td>-0.134</td>
<td>0.006</td>
<td>-0.084</td>
<td>0.121</td>
</tr>
<tr>
<td>Verbal immediate free recall</td>
<td>-0.086</td>
<td>0.080</td>
<td>-0.114</td>
<td>0.033</td>
</tr>
<tr>
<td>Verbal Short delay recall</td>
<td>-0.057</td>
<td>0.243</td>
<td>-0.091</td>
<td>0.093</td>
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<tr>
<td>Verbal recognition</td>
<td>-0.076</td>
<td>0.129</td>
<td>-0.088</td>
<td>0.119</td>
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<tr>
<td>Verbal list B recall</td>
<td>-0.125</td>
<td>0.011</td>
<td>-0.127</td>
<td>0.022</td>
</tr>
<tr>
<td>Composite global score</td>
<td>-0.105</td>
<td>0.053</td>
<td>-0.082</td>
<td>0.178</td>
</tr>
<tr>
<td>MMSE</td>
<td>-0.107</td>
<td>0.025</td>
<td>-0.086</td>
<td>0.117</td>
</tr>
</tbody>
</table>

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Conflict of interest
None

Ethics approval
Ethics approval was obtained from both the Irish College of General Practitioners Research Ethics Committee and the St. James’s Hospital and Federated Dublin Voluntary Hospitals Joint Research Ethics Committee.

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References

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