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# Anti-heparan sulphate antibodies and homocysteine in dementia: markers of vascular pathology?

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**Abstract**

Increasing evidence supports a pathogenic role of heparan sulphate (HS) in the development of dementia. Since HS proteoglycans are present in the endothelial cells and perivascular basement membrane, we wanted to assess blood titres of HS antibodies (Abs) in patients with vascular dementia (VD) and in patients with Alzheimer's disease (AD) with cerebrovascular disease (CVD) [mixed dementia (MixD)]. Moreover, plasma levels of homocysteine, an independent risk factor for the development of dementia as well as for CVD, were also determined. High HS Abs titres were present in one patient with VD and in two patients with mixed dementia, as well as in two neurological control patients (stroke and epilepsy). Increased homocysteine levels were found in 62.5% of patients with mixed dementia, in 22.2% of the VD subjects, in 54.2% of patients with CVD, and in 41.2% of patients with other neurological diseases. The present findings suggest that neither elevated HS Abs titres nor increased homocysteinemia may represent a useful biochemical marker for the diagnosis of VD.


*Keywords:* Heparan sulphate; Homocysteine; Vascular dementia

## 1. Introduction

Vascular dementia (VD), the second most common cause of cognitive impairment, accounting for up to 20–24% of all cases of dementia, includes several clinical entities [1]. Numerous sets of diagnostic criteria, with different sensitivity and specificity, have been proposed for VD, but the diagnosis still remains difficult. No reliable biochemical tests are so far available for VD. In fact, whereas CSF tau and β-42 peptide levels are sensitive markers of AD, they yield the lowest specificity in VD [2]. Recognition is also growing of a close relationship between VD and Alzheimer's disease (AD). Vascular risk factors are common to VD and AD [3,4], and vascular as well as degenerative brain alterations have been detected in pathological studies of AD populations [5]. Moreover, AD with cerebrovascular disease (CVD)—mixed dementia (MixD)—is now recognized as a common form in the elderly [6], although precise diagnostic criteria are still lacking.

Heparan sulphate (HS) is a glycosaminoglycan, a hexosamine-containing complex polysaccharide, with *o*-glycosidyl linkages to proteins to form proteoglycans. HS proteoglycans are widely distributed in the nervous system, both in the extracellular matrix and as membrane-associated molecules [7]. HS-proteoglycans are found in all types of amyloid deposits and are crucial for amyloidogenesis in AD. The sulphate moiety of HS proteoglycans plays a critical role in amyloid deposition, enhancing β-amyloid protein fibril formation, maintaining fibril stability, and affecting the proteolytic sensitivity of the fibrillar form of amyloid-β peptide [8]. HS is also the most abundant proteoglycan in endothelial cells, where it plays important roles in anticoagulation, maintenance of vessels barrier, processes of cell adhesion, and control of angiogenesis [9]. In previous

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studies, using an innovative enzyme-linked immunosorbent assay (ELISA) [10], we tested sera from more than 300 neurological patients for the presence of antibodies (Abs) to HS [11,12]. We did not find increased titres of anti-HS Abs in AD patients. Interestingly, however, high titres were present in subjects with vascular damage due to vasculitis/vasculopathy.

Since HS proteoglycans are present in the endothelial cells and perivascular basement membrane, we undertook a prospective study on patients with cognitive impairment and CVD alterations, testing for the presence of Abs to HS. Moreover, since increased plasma homocysteine level, an independent risk factor for the development of dementia [13] is also known to cause endothelial dysfunction [14], plasma homocysteine levels were also determined.

## 2. Patients and methods

### 2.1. Patients

Sera from a total of 75 patients (34 males, 41 females; mean age: 64.5±18.8 years, range 15–95 years) were tested. Of these, 17 patients were cognitively impaired: eight were diagnosed as VD (NINDS-AIREN diagnostic criteria [15]) and nine as MixD (possible AD with CVD [16]). As control groups with neurological diseases but without dementia, we tested sera from 24 patients (mean age 67.2±12.8 years) with CVD (12 ischemic or hemorrhagic stroke, 9 transitory ischemic attacks, and 3 chronic cerebral vasculopathy) and from 34 patients (mean age 53.8±19.5 years) with other neurological diseases (headache, neuropathy, and epilepsy). The healthy control group consisted of 40 cognitively intact healthy volunteers.

Fasting blood was collected from each participant by standard venipuncture into evacuated tubes with and without EDTA. Plasma and serum were isolated and stored at −20 °C until analyzed.

### 2.2. Enzyme-linked immunosorbent assay (ELISA) for the detection of anti-HS Abs

Anti-HS Abs were measured by ELISA using biotinylated antigens and avidin-coated microwells, as previously described [10]. Briefly, HS from porcine intestinal mucosa (Sigma, St. Louis, MO) was biotinylated using biotin hydrazide and EDC as cross-linker and applied (1 μg/well) to avidin-precoated microwells. After saturation with 1% BSA in PBS, increasing dilutions of patients' sera were added and incubated overnight at 4 °C followed by peroxidase-conjugated goat antihuman IgM or IgG (Jackson Immunoresearch Laboratories, West Grove, PA) for 2 h. Wells coated with avidin-BSA only served as control for each dilution. After washing, 100 μl of developing solution containing 0.05 M $Na_2HPO4$, 0.024 M sodium citrate pH 5, 0.08% *o*-phenylenediamine, and 0.08% $H_2O_2$ were added. Reaction products were measured spectrophotometrically at 450 nm in a Biotek EIA reader. The titer for each specimen was taken as the highest dilution at which the optical density (OD) reading was 0.1 units greater than in the corresponding avidin-BSA-coated wells. As positive control for the assay, mouse monoclonal anti-HS Abs were used (Sigma).

### 2.3. Homocysteine, folate, and vitamin $B_{12}$ dosage

Plasma total homocysteine levels were measured using an IMx analyzer (Abbott). Since homocysteine levels can be influenced by $B_{12}$ and folate levels, $B_{12}$ and folate levels were also measured by ACS:180 automated chemiluminescence analyzer (Bayer). Plasma homocysteine levels were considered elevated when >15 μmol/l, which is considered the border between normal and mildly elevated levels [17].

### 2.4. Statistical analysis

The nonparametric Mann–Whitney *U* and the Pearson's correlation tests were used to evaluate statistical differences between groups. The significance level was set at $p<0.05$.

## 3. Results

### 3.1. Anti-HS Abs

Of the 40 normal subjects, 10 had IgM anti-HS Abs titres of 12,800, one of 25,600, and two of 51,200; the remaining patients had titres ≤6400. IgG anti-HS antibody were ≤6400 in the majority of normal subjects, whereas titres of 12,800 and 25,600 were present in four subjects. We therefore considered significant values higher than 51,200 for IgM and 25,600 for IgG.

High Abs titres were present in one patient with VD and in two patients with MixD. The patient with VD was a 91-year-old woman with IgM Abs titres greater than 80,000. The patients with MixD were men aged 73 and 80 years, respectively, with an IgG anti-HS Abs titre of 51,200.

Of the neurological control groups, only two patients (a 71-year-old female with ischemic stroke and a 15-year-old boy with epilepsy) presented high Abs titres (IgG 25,600).

### 3.2. Homocysteine, folate, and $B_{12}$ vitamin levels

We did not find any significant difference in mean homocysteine level between the different subgroups of patients. Interesting observations, however, could be drawn from the analysis of the subgroups of patients when adopting the cut-off value for hyperhomocysteinemia of 15 μmol/l [17]. As shown in Fig. 1, among the cognitively impaired patients, 62.5% of the MixD patients and 22.2% of the VD subjects had homocysteine levels above 15 μmol/l.