Rapid Communication

Cerebrospinal Fluid Amyloid $\beta_{42}$/Phosphorylated Tau Ratio Discriminates Between Alzheimer’s Disease and Vascular Dementia

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Background. The differentiation of Alzheimer’s disease (AD) from vascular dementia (VaD) is hampered by clinical diagnostic criteria with disappointing sensitivity and specificity. The objective of this study was to investigate whether cerebrospinal fluid (CSF) levels of total tau protein (t-tau), amyloid $\beta_{42}$ protein (A$\beta_{42}$), and tau phosphorylated at threonine 181 (p-tau$\_181$) are useful biomarkers to distinguish AD patients from VaD patients.

Methods. We measured CSF levels of p-tau$\_181$, A$\beta_{42}$, and t-tau in 86 patients with a clinical diagnosis of AD or VaD and in 30 control participants.

Results. Optimal differentiation between AD and VaD was achieved by using the ratio of the CSF levels of A$\beta_{42}$ and p-tau$\_181$ (Q A$\beta_{42}$/p-tau) with sensitivity, specificity, positive and negative predictive values all $>85\%$.

Conclusions. Our results support further efforts to prospectively validate the use of Q A$\beta_{42}$/p-tau as a biomarker to discriminate between AD and VaD.

Differentiation of Alzheimer’s disease (AD) from other dementia disorders, such as vascular dementia (VaD), is becoming increasingly important. An accurate and early diagnosis is essential for appropriate support and treatment of dementia patients, as symptomatic drugs are specifically available for AD patients and neuroprotective drugs based on altered amyloid $\beta$ metabolism are being developed.

The clinical diagnostic criteria currently used for AD and VaD (1,2) have disappointing sensitivity and specificity (3,4), often leading to the unequivocal diagnosis “mixed dementia,” indicating clinical features of AD, but with multiple vascular lesions at brain imaging and/or cardiovascular risk factors. Although AD and VaD are clearly different diseases (e.g., as exemplified by genetics), both seem to share vascular risk factors such as atherosclerosis and smoking (5). Finally, AD may present with vascular comorbidity, which complicates the diagnostic work-up of AD patients. So, how to disentangle AD from VaD?

Cerebrospinal fluid (CSF) analysis of amyloid $\beta_{42}$ protein (A$\beta_{42}$) and total tau protein (t-tau), have been advocated as diagnostic biomarkers. T-tau levels are elevated and A$\beta_{42}$ levels decreased in CSF of AD patients compared to normal control (NC) participants (6,7). The combination of CSF t-tau and A$\beta_{42}$ yields a highly accurate differentiation between AD and NC (sensitivity 50%–94%, specificity 83%–100% (6). However, CSF-based differentiation of AD from VaD remains a challenge; specificity was only 48% versus VaD (8). Therefore, additional biomarkers are clearly needed. Quantification of hyperphosphorylated tau (p-tau) in CSF may be such a biomarker. CSF p-tau$\_181$ concentrations improve the discrimination of AD from dementia with Lewy bodies (DLB) (9), but its validity in discriminating AD from VaD has not extensively been studied. In this retrospective case–control study we analyzed CSF levels of t-tau, A$\beta_{42}$, and p-tau$\_181$ in NC participants and patients with clinical AD and VaD, to achieve an optimal differentiation between AD and VaD.

Methods

Patients

Patients with mild to moderate AD ($n = 61$) or VaD ($n = 25$) were selected from a large database containing 260 patients with cognitive impairment or dementia of various origins (e.g., degenerative, vascular, hereditary, inflammatory, metabolic) who visited our outpatient clinic between 1992 and 2004. Only patients with a diagnosis of probable AD or VaD, according to accepted criteria (1,2), were included. The standard diagnostic examination protocol included a complete geriatric assessment, neurological examination, neuropsychological testing, laboratory testing, imaging of the brain, and a lumbar puncture. As NC participants, we included 30 persons older than 50 years who visited our outpatient clinic for various reasons but turned out not to suffer from a neurological disorder. Their CSF had normal leukocyte and erythrocyte counts, normal
total protein, glucose and lactate concentrations, and no oligoclonal immunoglobulin G (IgG) bands.

CSF Analysis

Lumbar punctures were performed after written informed consent was obtained from the patients and from the patients’ legal representatives. CSF from all participants was collected in polypropylene tubes, within 30 minutes transported to the adjacent laboratory at room temperature, centrifuged after routine investigations, and immediately aliquoted and stored at −80°C until analysis. Levels of t-tau, Aβ42, and p-tau181 in CSF were measured using enzyme-linked immunosorbent assays (all obtained from Innogenetics NV, Gent, Belgium). In five AD and five VaD patients and 10 NC participants, the amount of CSF was insufficient to measure the p-tau181 concentration.

Statistical Analysis

Statistical procedures were performed using GraphPad Prism (San Diego, CA) software. All data were normally distributed; therefore, one-way analysis of variance (ANOVA) with Bonferroni’s post hoc correction was used for multiple comparisons. Cutoff values, sensitivity, and specificity for biomarkers in different groups were calculated using receiver operating characteristic (ROC) curves. Cutoff values with the most optimal combination of sensitivity and specificity to discriminate between these two groups for each biomarker were calculated. Subsequently, positive and negative predictive values (PPV and NPV) were calculated. Correlation analysis was performed by using Pearson’s method.

RESULTS

Gender distribution was similar in the NC (47% male, 53% female) and combined dementia patient groups (45% male, 55% female). The mean age of NC participants was significantly lower than that of patients with AD and VaD ($p < .001$). There was no significant age difference between patients with AD and VaD (Table 1).

Mean CSF levels of Aβ42 were significantly decreased in AD patients compared to VaD patients and NC participants ($p < .001$; Tables 1 and 2) as well as in patients with VaD compared to NC participants ($p < .001$). Mean CSF levels of t-tau were significantly increased in AD patients and combined dementia patient groups ($p < .001$).

Table 1. Age and Levels of Cerebrospinal Fluid Markers in Patients and Control Participants

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of Patients</th>
<th>Age, y (Mean ± SD)</th>
<th>Aβ42 (pg/ml) (Mean ± SD)</th>
<th>t-tau (pg/ml) (Mean ± SD)</th>
<th>p-tau181 (pg/ml) (Mean ± SD)</th>
<th>Q Aβ42/t-tau (Mean ± SD)</th>
<th>Q Aβ42/p-tau (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td>25/36</td>
<td>68 ± 8.8*</td>
<td>419 ± 1281</td>
<td>613 ± 3261</td>
<td>103 ± 441</td>
<td>0.9 ± 0.51</td>
<td>4.9 ± 2.71</td>
</tr>
<tr>
<td>VaD</td>
<td>14/11</td>
<td>72 ± 8.4*</td>
<td>655 ± 2201</td>
<td>303 ± 3073</td>
<td>47 ± 143</td>
<td>3.3 ± 1.91</td>
<td>15.9 ± 6.53</td>
</tr>
<tr>
<td>NC</td>
<td>14/16</td>
<td>61 ± 8.3</td>
<td>869 ± 207</td>
<td>184 ± 89</td>
<td>53 ± 16</td>
<td>5.6 ± 2.4</td>
<td>16.5 ± 4.2</td>
</tr>
</tbody>
</table>

Notes: $^* p < .001$, compared to NC.

Table 2. Discriminative Value of Cerebrospinal Fluid Markers Between Groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of Patients</th>
<th>Biomarkers and Cutoff Values</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD vs VaD</td>
<td>61 vs 25</td>
<td>Aβ42 cutoff = 520 pg/ml*</td>
<td>82</td>
<td>76</td>
<td>89</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>61 vs 25</td>
<td>t-tau cutoff = 321 pg/ml*</td>
<td>80</td>
<td>76</td>
<td>89</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>56 vs 20</td>
<td>p-tau181 cutoff = 68.5 pg/ml*</td>
<td>75</td>
<td>95</td>
<td>98</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>61 vs 25</td>
<td>Q Aβ42/t-tau cutoff = 1.2*</td>
<td>82</td>
<td>92</td>
<td>96</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>56 vs 20</td>
<td>Q Aβ42/p-tau cutoff = 10.95*</td>
<td>95</td>
<td>90</td>
<td>96</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>56 vs 20</td>
<td>Q Aβ42/p-tau cutoff = 12.7*</td>
<td>100</td>
<td>85</td>
<td>95</td>
<td>100</td>
</tr>
<tr>
<td>AD vs NC</td>
<td>61 vs 30</td>
<td>Aβ42 cutoff = 603 pg/ml*</td>
<td>93</td>
<td>93</td>
<td>97</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>61 vs 30</td>
<td>t-tau cutoff = 352 pg/ml*</td>
<td>79</td>
<td>97</td>
<td>98</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>56 vs 20</td>
<td>p-tau181 cutoff = 68 pg/ml*</td>
<td>75</td>
<td>85</td>
<td>93</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>61 vs 30</td>
<td>Q Aβ42/t-tau cutoff = 1.895*</td>
<td>95</td>
<td>97</td>
<td>98</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>56 vs 20</td>
<td>Q Aβ42/p-tau cutoff = 8.7*</td>
<td>89</td>
<td>95</td>
<td>98</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>56 vs 20</td>
<td>Q Aβ42/p-tau cutoff = 13.2*</td>
<td>100</td>
<td>85</td>
<td>95</td>
<td>100</td>
</tr>
<tr>
<td>VaD vs NC</td>
<td>25 vs 30</td>
<td>Aβ42 cutoff = 814 pg/ml*</td>
<td>80</td>
<td>67</td>
<td>67</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>25 vs 30</td>
<td>t-tau cutoff = 174.5 pg/ml</td>
<td>76</td>
<td>57</td>
<td>59</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>20 vs 30</td>
<td>p-tau181 cutoff = 45.5 pg/ml</td>
<td>55</td>
<td>70</td>
<td>65</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>25 vs 30</td>
<td>Q Aβ42/t-tau cutoff = 3.5*</td>
<td>68</td>
<td>87</td>
<td>81</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>20 vs 20</td>
<td>Q Aβ42/p-tau cutoff = 14.15</td>
<td>55</td>
<td>80</td>
<td>73</td>
<td>64</td>
</tr>
</tbody>
</table>

Notes: Sensitivity and specificity to differentiate between Alzheimer’s disease (AD), vascular dementia (VaD), and normal control participants (NC) are listed, using different cerebrospinal fluid markers and cutoff values. In addition, positive predictive values (PPV) and negative predictive values (NPV) are shown.

Aβ42 = amyloid β42 protein; t-tau = total tau; p-tau181 = tau phosphorylated at threonine 181; Q Aβ42/t-tau = ratio of Aβ42 and t-tau; Q Aβ42/p-tau = ratio of Aβ42 and p-tau181.

*p < .001.
compared to the other groups \((p < .001)\). Only small, nonsignificant differences were found between the mean CSF t-tau levels of patients with VaD and those of NC participants. Mean \(p\)-tau\(_{181}\) levels were significantly increased in CSF of patients with AD compared to patients with VaD \((p < .001)\). There were no differences in mean CSF \(p\)-tau\(_{181}\) levels between patients with VaD and NC participants. A positive correlation was observed between levels of \(p\)-tau\(_{181}\) and t-tau in NC participants \((r = 0.88, p < .0001)\) and in AD patients \((r = 0.84, p < .0001)\), but not in VaD patients \((r = 0.31, p = .2)\).

The \(\beta\_42/p\)-tau\(_{181}\) ratio \((Q \, \beta\_42/p\)-tau\) was significantly lower \((p < .001)\) in patients with AD compared to NC participants and patients with VaD. Furthermore, the \(\beta\_42/t\)-tau ratio \((Q \, \beta\_42/t\)-tau\) was significantly different in all three studied groups (Tables 1 and 2).

High sensitivity and specificity for the discrimination between AD and VaD were obtained at two optimal cutoff levels of \(Q \, \beta\_42/p\)-tau (Table 2). At a cutoff level of 12.7, sensitivity was 100% and specificity 85% with a PPV of 95% and an NPV of 100%. At a slightly lower cut-off level (10.95), sensitivity and specificity were 95% and 90%, respectively (PPV 96% and NPV 86%). Sensitivity and specificity of any of the other biomarkers were less than those of \(Q \, \beta\_42/p\)-tau\(_{181}\).

Optimal separation of the AD and NC groups using \(QA\_42/4\)-tau was achieved at a cutoff level of 1.895, with a sensitivity of 95% and specificity of 97%. In addition, very high PPV (95%) and NPV (100%) were reached with \(QA\_42/p\)-tau at a cutoff level of 13.2. Discrimination between VaD and NC participants using a \(QA\_42/4\)-tau cutoff value of 3.5 resulted in a combination of 68% sensitivity and 87% specificity.

**DISCUSSION**

In the present study, we found decreased CSF \(\beta\_42\) levels and increased CSF t-tau levels in patients with AD compared with those with VaD and normal participants, consistent with the literature \((6–10)\). Also, the levels of \(\beta\_42\) and tau in patients with VaD are in line with another study \((8)\). Our main finding, however, is that the ratio of \(\beta\_42\) to p-tau\(_{181}\) \((Q \, \beta\_42/p\)-tau\) distinguishes between AD and VaD patients with high discriminatory power. Mean p-tau\(_{181}\) levels are doubled in the AD group, but normal in VaD, consistent with the literature \((6–10)\). Also, the levels of \(\beta\_42/p\)-tau181 \((Q \, \beta\_42/p\)-tau\) distinguishes between AD and VaD patients with high discriminatory power. Mean p-tau\(_{181}\) levels are doubled in the AD group, but normal in VaD, similar to other observations \((11,12)\). Identical results were found using tau protein phosphorylated at serine 199 \((p\)-tau\(_{191}\)) and threonine 231 \((p\)-tau\(_{231}\)) \((9,11,13)\).

Q \(\beta\_42/p\)-tau was significantly decreased in the AD group compared to the VaD group. Previously, it has been reported that in AD patients a low \(\beta\_42/p\)-tau\(_{181}\) ratio was observed compared to healthy NC participants, patients with non-AD dementias, and patients with other neurological disorders \((14)\). In this study we demonstrated that Q \(\beta\_42/p\)-tau has excellent diagnostic value in the differentiation of AD from VaD. According to a consensus report \((15)\) a useful biomarker should be reliable, reproducible, and have both a sensitivity and a PPV greater than 80% for detecting AD and a specificity greater than 80% for distinguishing other dementias. Q \(\beta\_42/p\)-tau fulfills these requirements, as sensitivity, specificity, PPV, and NPV are all well above 85%. For the discrimination between AD and NC groups, Q \(\beta\_42/p\)-tau may also be a useful biomarker, because at a cut-off level of 1.895 high sensitivity, specificity, PPV, and NPV (each 91% or higher) were obtained. As all patients’ diagnoses were based on clinical criteria and not neuropathologically confirmed, improvement of sensitivity and specificity awaits prospective, neuropathology-supported studies.

A potential confounder of this study is the age difference between patients and NC participants. However, t-tau is the only CSF biomarker known to be positively correlated with age \((r = 0.60, p < .001)\) \((16)\). Thus, differences in mean CSF t-tau levels between the NC and AD groups might be smaller than has been described before. Because the results of our study do not suggest an important role for the analysis of t-tau in the discrimination of AD from VaD, and because there was no significant age difference between the AD and VaD groups, this finding does not affect our main results.

In the VaD group, one patient had exceptional high tau levels \((1603 \, \text{pg/ml})\) with normal p-tau\(_{181}\) levels \((56 \, \text{pg/ml})\). This patient underwent the lumbar puncture only 5 weeks after he suffered a stroke—a known cause of a transient (3–5 months) increase in CSF t-tau, but not of p-tau \((17)\).

In many previous studies the focus of the application of CSF biomarkers was the differentiation between AD and NC. Only a few studies addressed the truly relevant discrimination between AD and other dementia disorders, particularly VaD. As recent studies suggested that vascular risk factors, including atherosclerosis, diabetes, and smoking, might significantly contribute to the pathogenesis of AD \((5,18)\), the conventional distinction between AD and VaD has become controversial. Our study suggests that (i) there are biological differences between what we call AD and VaD; and (ii) Q \(\beta\_42/p\)-tau in CSF may detect such differences in relevant clinical situations. The true contribution of \(\beta\_42/p\)-tau CSF assessments to the clinical management of patients with late-onset dementia disorders remains to be established, preferably through a prospective randomized masked validation study with appropriate control populations.

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