To the Editor:

We thank Dr. Le Bastard and colleagues for their interest in our study, and their additional investigations based on our findings. Similar to our study (1), they calculated the ratio of cerebrospinal fluid (CSF) levels of amyloid $\beta_{42}$ and tau phosphorylated at threonine 181 ($Q\ A\beta_{42}/p\tau181$) in patients with Alzheimer’s disease (AD), and vascular dementia (VaD). These diagnoses were established after post mortem examination, which is an important addition to our study in which we used a clinical diagnosis. In contrast to our findings, they found unexpectedly low specificity levels (38% to 52%) when applying our proposed $Q\ A\beta_{42}/p\tau181$ cutoff levels to differentiate between AD and VaD. This discrepancy thus needs further attention and clarification.

Critical review of the data provided by Le Bastard and colleagues revealed several striking findings. Mean CSF $A\beta_{42}$ levels were lower in both AD and VaD patients compared to our study (Table 1), and compared to other studies using the same methods (2–5). Furthermore, the minimum CSF $A\beta_{42}$ concentration they reported was 67 pg/ml in the AD group, and 46 pg/ml in the VaD group, which is surprisingly low since the analytical range of the enzyme-linked immunosorbent assay (ELISA) was reported to be...
produced, although previous investigations have shown that information about the material of which the test tubes were lower) than recommended. Second, the type of test tubes stored their CSF samples at a higher temperature (–20°C or lower) than recommended. Second, the type of test tubes used to collect and store CSF: The authors provided no information about the material of which the test tubes were produced, although previous investigations have shown that this affects results, particularly those of Aβ42. When CSF is collected in glass or polystyrene tubes, the measured concentration of Aβ42 decreases. Therefore, nonadsorbing plastic (polypropylene) tubes should be used (7).

Another important finding was the high mean CSF p-tau181 level in VaD patients compared to our study (Table 1). Moreover, two VaD patients and one AD patient had extremely high p-tau181 levels, even exceeding the upper limit of the ELISA (500 pg/ml). To our knowledge, CSF p-tau181 levels equal to, or higher than, 500 pg/ml have never been reported; the highest reported CSF Aβ42 level was 342 pg/ml in a patient with AD (8). This is consistent with our own experience, since the highest CSF p-tau181 level measured in our laboratory was 370 pg/ml (AD patient), of a total of 324 p-tau181 analyses in patients with cognitive impairment or dementia. When the out-of-range p-tau181 concentrations were considered as outliers and omitted from the analysis, the CSF p-tau181 level in the VaD group fell to 48 ± 31 pg/ml, which is comparable with our study. Clearly, these two VaD patients, as well as the AD patient with exceptionally high CSF p-tau181 levels are remarkable, and we suggest alternative diagnostic considerations.

As a result of the above-mentioned discrepancies, the outcome of the calculations of Q Aβ42/p-tau181 sensitivity, and specificity were also different, and our proposed cutoff levels could not be applied. Therefore, although we acknowledge the importance of autopsy confirmation of clinical studies, we encourage that investigators take great care in the preanalytical and analytical conditions required for optimal CSF analysis of Aβ42, and p-tau181. Furthermore, the diagnosis of patients with out-of-range levels of CSF biomarkers should be carefully reevaluated.

125–2000 pg/ml. There are a few possible explanations for the low CSF Aβ42 levels. First, the method of storage of CSF samples: It is recommended to store CSF samples at –80°C, and it is known that in CSF samples stored at 4°C, Aβ42 decreases by approximately 20% during the first 2 days compared with the baseline value (–80°C) (6). The authors stored their CSF samples at a higher temperature (–20°C or lower) than recommended. Second, the type of test tubes used to collect and store CSF: The authors provided no information about the material of which the test tubes were produced, although previous investigations have shown that this affects results, particularly those of Aβ42. When CSF is collected in glass or polystyrene tubes, the measured concentration of Aβ42 decreases. Therefore, nonadsorbing plastic (polypropylene) tubes should be used (7).

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