A NEW DECISION TREE COMBINING ABETA 1-42 and p-TAU LEVELS IN ALZHEIMER'S DIAGNOSIS

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ABSTRACT :

The objective of this work was to improve the clinical diagnosis of Alzheimer's disease (AD) by proposing a simple decision tree based on three major biomarkers of AD found in the cerebrospinal fluid (CSF): amyloid peptide Aβ1-42, total Tau (t-Tau) and Tau phosphorylated at Thr181 (p-Tau). Two consecutive cohorts comprising 548 patients in total were recruited by the Memory and Neurology Clinics at Lille University Hospital (France). These included 293 patients with AD, 171 patients with other dementias and 84 healthy controls. All patients underwent lumbar puncture for the assessment of CSF concentrations of Aβ1-42, t-Tau and p-Tau. International criteria for dementias were used for diagnosis by investigators blind to CSF test results. To identify the combination of biomarkers that best predicted the 3 diagnoses, we used the CHAID decision tree method with the first cohort. Our analysis yielded a two-step decision tree, with a first stratification step based on the Aβ1–42/p-Tau ratio of the CSF, and a second step based on CSF p-Tau concentrations. The second cohort was then used to determine the power (0.618), sensitivity (82%) and specificity (81%) of this tree in AD diagnosis. These were found to be at least as high as those of other known algorithms based on the three CSF biomarkers, Aβ1-42, t-Tau and p-Tau.

For the first time, diagnostic rules for AD based on CSF variables were compared in a single study. Our findings indicate that the measurement of Aβ1-42 and p-Tau levels in the CSF is sufficient to diagnose AD.

Keywords : Dementia, Tau, amyloid peptide, ratio, indice, sensitivity, specificity

Abstract: 250 words (250 max)

INTRODUCTION:

Alzheimer's disease (AD) is the most common cause of dementia in the elderly. With the aging of the population, there is an increasing need to develop objective tests for the early diagnosis of AD, since disease-modifying treatments are more likely to be effective when started early, before neurodegeneration is too advanced. To improve the accuracy of clinical diagnosis, clinical practice at present includes the assessment of biomarkers in the cerebrospinal fluid (CSF) [1]. The three biomarkers currently validated for routine clinical use are proteins that are found in the two brain lesions specific to AD patients: senile plaques (SPs) and neurofibrillary tangles (NFTs). SPs consist for the most part of the hydrophobic amyloid peptide Aβ1-42, whereas the principal components of NFTs are the Tau proteins, which occur mainly in an abnormally hyperphosphorylated state. These biochemical changes in the brain are reflected by characteristic changes in the CSF of AD patients, including elevated levels of total Tau (t-Tau) and Tau phosphorylated at Threonine 181 (p-Tau), and decreased levels of Aβ1-42. Previous studies have shown that these biomarkers can be used to distinguish between AD patients and healthy controls with good sensitivity and specificity, but cut-off levels differ between laboratories [2, 3, 4, 5, 6]. Furthermore, results from CSF studies of neuropathologically validated dementias do not differ significantly from those that are based exclusively on a clinical diagnosis of dementing illness [7, 8]. In order to increase their diagnostic power, over the last few years, several studies have proposed the use of new indices consisting of ratios of CSF biomarkers or formulae combining the levels of 2 or more of these biomarkers. However, to our knowledge, no single study has compared the diagnostic value of the three biomarkers, singly or in combination. Our objective here is to propose a clear and simple decision tree that combines these CSF biomarkers and can be used to diagnose AD in routine clinical practice.

We assessed CSF concentrations of Aβ1-42, t-Tau and p-Tau in a cohort of patients with dementia and healthy controls drawn from our Memory and Neurology Clinics. We first used an independent cohort to determine the major diagnostic indicators of AD by comparing the sensitivity, specificity and diagnostic power of the CSF biomarkers described in the literature, alone or in combination [9, 10, 11, 12]. Next, we established a simple decision tree based on 2 of these biomarkers, Aβ1-42 and p-Tau. Finally, using a second, independent, cohort, we compared our decision tree to several others used routinely in clinical practice to discriminate between AD and other dementias.

MATERIALS AND METHODS:

Patients:

This study was conducted at the Memory Research and Resource Center of the Neurology department of Lille University Hospital. This clinic has a local catchment area but also serves as a resource and reference center for the region, which explains the fact that not all patients examined are followed up by the clinic, and also that it recruits young and/or atypical patients. In accordance with French legislation, explicit informed consent from patients was waived since all clinical, imaging, and biological data were generated during routine clinical work-up and were extracted for the purpose of this study. For control subjects, informed consent was obtained with the approval of the local ethics committee in order to take an additional 2 ml of CSF and to conserve it for research purposes.

Regulations concerning electronic filing were followed, and patients and their relatives were informed of the possibility that individual data would be used in retrospective clinical research studies.

Between February 2004 and August 2010, 7491 new patients attended the clinic. Of these, 685 had a lumbar puncture. Those with a diagnosis of possible dementia (n=57) and those with no confirmed diagnosis (n=80) were excluded from the study.

The first cohort (training set) consisted of 233 patients recruited between February 2004 and June 2008 by the Memory and Neurology Clinics and included 91 patients presenting with probable AD, 104 patients with other dementias and 38 controls (Table 1). The second cohort (validation sample) consisted of 315 patients recruited between July 2008 and August 2010, including 202 patients presenting with probable AD, 67 patients with other probable dementias and 46 controls (Table 1). Standardized dementia assessment included medical history, informant-based history, physical and neurological examination, laboratory tests, neuropsychological testing and brain imaging. For the study, two senior physicians specialized in cognitive disorders and blind to the CSF test results assessed all participants, and their diagnoses were compared. Only patients for whom the probable diagnoses of the two physicians were in agreement were considered for the study.

A diagnosis of probable AD was made using the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association [13]. Diagnoses for non-AD degenerative dementias (referred to here as OD, other dementias), i.e. vascular dementia (VaD) [14], Lewy body dementia (LBD) [15] and frontotemporal dementia (FTD) [16], were made using the comprehensive admission criteria of international dementia associations. For Cohorts 1 and 2, respectively, the number of patients included in the OD group was as follows: VaD (n1=25, n2=25), LBD (n1=33, n2=21), FTD (n1=21, n2=17), Creutzfeldt-Jakob Disease (n1=3, n2=0), alcoholic dementia (n1=1, n2= 0) and other degenerative dementias (Corticobasal Degeneration, Progressive Supranuclear Palsy, Parkinson Dementia and semantic dementia) (n1=21, n2=4). MMSE scores were available for 82 AD and 97 OD patients.

Healthy controls consisted of individuals referred to the Neurology Clinic for psychiatric disorders according to DSM-IV criteria (n1=11, n2=19) or other neurological conditions characterized by the lack of progression of a degenerative pathology, such as headaches, dysarthria, chronic alcoholism, Parkinson's disease, multiple sclerosis or drug addiction (n1=26, n2=27). Minimental State Examination (MMSE) scores were not systematically available for controls.

CSF Samples and Measurements:

CSF was obtained from the L3/L4 or L4/L5 intervertebral space by lumbar puncture (LP), and collected in 15mL polypropylene tubes. Within 4 hours, CSF samples were centrifuged at 1000xg for 10 minutes at 4°C. aliquoted into 1.5 mL polypropylene tubes and stored at -80°C until further analysis. CSF Aβ1-42 levels were measured using the Innotest β-amyloid[1-42] sandwich immunosorbent assay (ELISA) kit (Innogenetics, Belgium) for the detection of Aβ peptides containing both the 1st and 42nd amino-acids. CSF t-Tau concentrations were determined using the Innotest hTAU-Ag sandwich ELISA kit (Innogenetics, Belgium) capable of detecting all Tau isoforms, irrespective to their phosphorylation state. Tau phosphorylated at threonine 181 (p-Tau) was measured using the Innotest Phospho-Tau[181P] sandwich ELISA kit (Innogenetics, Belgium). The interassay coefficient of variation was 16% for Aβ1-42, 8% for t-Tau and 7% for p-Tau. The LP and the collection of clinical data were carried out less than 1 month apart.

Statistical analysis:

Statistical analysis was performed using SAS software version 9.2 [SAS Institute Inc., Cary, NC USA]. The threshold for significance was set at p=0.05.

Qualitative variables were expressed as frequencies or percentages, and quantitative variables as means ± standard deviation. Comparisons between the 3 groups were performed by a chi-squared test for qualitative variables and by analysis of variance (ANOVA) for quantitative variables.

Each biomarker was dichotomized according to cut-off values in the literature. Only control subjects and patients with pure AD were considered for the evaluation of the sensitivity and specificity of each variable. Values for sensitivity and specificity near or greater than 0.8 were considered to be good.

Our second step was to develop rules for the prediction of AD using these biomarkers. A decision tree was constructed for the training set by means of the CHAID (Chi-squared Automatic Interaction Detection) method, using SIPINA software, version 3.7. The CHAID method is a non-linear stepwise discriminant analysis that selects the most predictive variable at each step according to the chi-squared test. The dependent variables were the three diagnostic groups, and the independent variables the three previously dichotomized biomarkers.

The diagnostic power of this decision tree was evaluated using the mean kappa coefficient, sensitivity and specificity, with the AD group on the one hand and a non-AD group consisting of OD and control subjects pooled on the other. A kappa coefficient greater than 0.8 indicated excellent diagnostic power, while a kappa coefficient between 0.6 and 0.8 indicated good agreement. The sensitivity corresponded to the probability that an AD patient would be correctly classified using the tree. The specificity corresponded to the probability that a patient without AD would be classified as a control or OD patient using the tree.

The third step consisted of an external validation study of the new decision tree, using the kappa coefficient, sensitivity and specificity on a validation sample. Two commonly used decision trees were applied to the same validation sample, as described above. For each decision tree, the kappa coefficient, sensitivity and specificity were evaluated. The sensitivity and specificity of the new decision tree were compared with the two commonly used decision trees using a McNemar test. Kappa coefficients were also compared by normal approximation.

RESULTS :

The demographic data of the two cohorts are summarized in Table 1. It is worth noting that the age of the cognitively healthy group was significantly lower than that of the two groups with dementias in the first cohort. The mean and median CSF concentrations of the three validated biomarkers, Aβ1-42, t-Tau and p-Tau, in each group are shown in Table 1. These were comparable to levels described in the literature, validating our cohorts for subsequent CSF analyses.

Diagnostic power of CSF biomarkers and indices in discriminating AD patients from controls in Cohort 1:

Healthy controls in Cohort 1 were younger than OD or AD patients ($p<0.0001$). Since the 3 groups were not matched for age, we did not compute new cut-off values but used those previously published in the literature: 500 pg/mL for Aβ1-42 [5, 12]; 450 pg/mL for t-Tau (mean age of the patients 70 years; [17]); 53 pg/mL for p-Tau; 0.8 for the Innotest Amyloid Tau Index (IATI) [9, 18]; 0.6 for t-Tau/Aβ1-42; 9 for Aβ1-42/p-Tau [10, 11] and 1 for the

Discrimination Formula (DF) described in [12]. The sensitivity and specificity of each biomarker were computed using controls and patients with pure AD (Table 2). They were higher for all ratios or formulae using a combination of at least 2 CSF biomarkers than for single CSF biomarkers.

Establishment of a simple decision tree based on the Ab42/p-Tau ratio and p-Tau cut-offs for optimal discrimination between AD patients and controls/ non-AD dementia patients (Cohort 1):

In clinical practice, the clinician has to deal with patients with various types of dementia that do not necessarily occur in isolation. We therefore analyzed a heterogeneous group of demented patients (Cohort 1; Table 1) to determine the best stratification of AD patients vs. controls and patients with other dementias, based on previously validated CSF biomarkers/indices. This analysis yielded a two-step decision tree, which we will hereafter call the Lille decision tree (Figure 1), in which the first step consisted of stratification based on the CSF Aβ1−42/p-Tau ratio, and the second step on CSF p-Tau levels. This simple decision tree allowed us to define three groups based on their biochemical signature. The first was characterized by an altered Aβ1−42/p-Tau ratio and p-Tau values, and corresponded to probable AD, consisting mostly of AD patients (74%) and to a much lesser extent of OD patients (25%). The second was characterized by an altered Aβ1−42/p-Tau ratio but normal p-Tau values, and corresponded to the possibility of AD, consisting mostly of OD patients (60%) and to a lesser extent of AD patients (30%). The third signature was characterized by normal Aβ1-42/p-Tau and p-Tau values, and corresponded to the exclusion of AD, consisting mainly of OD patients (62%) and controls (30%). Ten AD patients (9%) were identified as belonging to this group, suggesting that they were undetectable by this method (false negative). The sensitivity and specificity of this tree for the diagnosis of AD were, respectively, 86% and 81%. (Figure 1).

These results remained unchanged when a lower cut-off value for t-Tau (350 pg/mL) was used for analysis, independent of age, as described in several studies [12].

Validation of the decision tree using an independent cohort (Cohort 2):

To avoid a circularity bias, we tested the Lille decision tree on a validation sample (Cohort 2). This second cohort included significantly more AD patients than the Cohort 1 (Table 1). The subgroup with altered Aβ1−42/p-Tau ratios and p-Tau values again corresponded mainly to AD patients (89%) and a few OD patients (9%). The subgroup characterized by an altered Aβ1−42/p-Tau ratio but normal p-Tau values consisted of an equal number of AD and OD patients (37.5% each). Finally, the subgroup with normal Aβ1-42/p-Tau ratios and p-Tau values consisted mainly of equal numbers of controls (34%) and OD patients (34%). However, there were many more misidentified AD patients (25%) in this group than in Cohort 1. These results were in good agreement with the clinical diagnosis (κ=0.648). Finally, compared to Cohort 1, the specificity of the tree did not change for Cohort 2 (81%) and its sensitivity was slightly reduced (82%).

Comparison of the Lille decision tree with two commonly used decision trees (Cohort 2) :

Two other decision trees are commonly used for AD diagnosis. In Tree A, a combination of 2 or more altered biomarkers has been shown to predict the development of AD with a sensitivity of 82% and a specificity of 97% [19]. In Tree B, widely used in France, the consensus for interpreting CSF parameters is based on a combination of CSF p-Tau levels and IATI (a function of t-Tau and Aβ1-42 levels): the biochemical profile is in favor of probable

AD when both parameters are abnormal and excludes AD when both are normal. For intermediate results, the biochemical profile is not conclusive. We used the validation cohort (Cohort 2) and previously determined cut-off values to analyze the capacity of these two decision trees to discriminate between AD patients and controls or OD patients (Figure 2A and 2B). There was no significant difference between these trees and the Lille tree in terms of diagnostic power (Figure 2C), as revealed by the kappa coefficient. When considering the sensitivity and specificity of the trees, Tree A was not significantly different from the Lille tree. However, Tree B, in spite of its similar diagnostic power, showed a significantly greater sensitivity (85%; p=0.025) but reduced specificity (77%), a difference that was almost significant (p=0.058).

DISCUSSION:

In the present study, we investigated the diagnostic performance of various indices based on the levels of three validated CSF biomarkers of AD: t-Tau, p-Tau and Aβ1-42. We first analyzed single CSF biomarker levels in the first cohort to compare them with those described by large multicenter studies [12][5]. While our cohort size was similar to that of the first of these studies [12], t-Tau and p-Tau levels were lower in our control group (244 vs. 280 pg/mL and 37 vs. 51 pg/mL respectively), a finding that could be explained by the considerably lower mean age of our control group (49 years) compared to that of the multicenter study (67 years). In AD patients, in spite of a similar mean age (71 years), t-Tau and p-Tau levels were higher in our study (710 vs. 559 pg/mL and 101 vs. 82 pg/mL, respectively), with a correspondingly worse memory performance (mean MMSE score: 18 vs. 22). Aβ1-42 levels were not significantly different between Cohort 1 and the multicenter study, either with respect to the control group (628 vs. 675 pg/mL) or with respect to the AD group (407 vs. 370 pg/mL), when considering an intra-assay variability of 16%.

We next tested the diagnostic performance (sensitivity and specificity) of these CSF markers and indices derived from them, since it has been suggested that the use of a combination of 2 or 3 CSF biomarkers improves AD diagnosis. We demonstrated that indices consisting of ratios or formulae combining at least 2 CSF biomarkers performed better than single CSF biomarkers. Using Cohort 1, we therefore established a new decision tree for AD diagnosis with a sensitivity of 86% and specificity of 81%. Moreover, to avoid a circularity bias, we analyzed the performance of this Lille tree with a second independent cohort, the validation cohort or Cohort 2. The diagnostic power and specificity of our decision tree were stable with Cohort 2, with only a slight reduction in sensitivity (82%).

We also demonstrated that our simple decision tree, based on the Aβ1-42/p-Tau ratio and p-Tau levels, possessed the same diagnostic power as other strategies described in the literature for discriminating between AD patients and controls or patients with other dementias. The Lille decision tree was similar in sensitivity and specificity to the previously described Tree A [19], whereas Tree B was more sensitive (85%) but less specific than ours, with a specificity of less than 80%, insufficient for accurate diagnosis. This suggests that CSF t-Tau levels are not useful for the diagnosis of AD as they result in no improvement of diagnostic power, sensitivity or specificity when compared to p-Tau and Aβ1-42 levels alone. These results are in accordance with the study of Tapiola et al. [20], which shows that the best correlation of AD-related pathologic changes in the brain is seen with the Aβ1-42/p-Tau ratio. Finally, the specificity of our tree for AD (81%) was really good, given the 45% of OD patients in our first

cohort. Considering the high incidence of comorbidity, we could not exclude the possibility that some of these patients also suffered from Alzheimer's disease.

This study also raises certain questions. When comparing our results and those in the literature, we observe considerable variability in outcome measures, especially in specificity and sensitivity. What is the underlying reason for these differences? Does the percentage of OD patients affect these measures? In Cohorts 1 and 2, the percentage of OD patients was significantly different, being 45% and 21%, respectively. In previous studies, they were, respectively, 20% [9], 56% [10], 25% [11] and 8% [12]. Cohort 1 is therefore comparable to the cohort of Welge et al. [10] whereas Cohort 2 is comparable to the cohorts used by Hulstaert et al. [9] and Ibach et al. [11]. As would be expected by an effect of the percentage of OD patients, the sensitivity and specificity of the Lille discrimination tree for Cohort 1 (86% and 81% respectively) were similar to those of the Welge study (85% and 85% respectively). However, in Cohort 2, these values (82% and 81% respectively) were significantly different from those of the Hulstaert study (85% and 58% respectively) but comparable to the results of Ibach et al. (79% and 76% respectively) [11]. Thus, the increased percentage of OD patients in Cohort 1 relative to Cohort 2 only slightly affected the sensitivity of the Lille decision tree. The accuracy of AD diagnosis decreases with age (for a stable sensitivity level of 85%, the specificity of AD diagnosis is significantly lower in younger populations [21]). The specificity values yielded by our study are thus probably overestimations, since our controls are younger than our AD patients. However, despite a significantly higher mean age of controls in Cohort 2 (although still lower than in the the two patient groups), the specificity was not significantly different whereas sensitivity decreased slightly. Finally, the severity of the disease in the study population could account for differences between studies. In our study, the mean MMSE scores of the patient groups in both cohorts were comparable, reflecting similar disease severity in the two groups. These scores were slightly lower than those seen in previous studies for AD patients (18 vs. 18.7-22) and comparable for OD patients (20 vs. 19.8-21). Some variations could also arise from preanalytical or analytical sources [22, 5, 23], and it has not so far been possible to eliminate them. It should be possible to reduce this variability by improving the standardization of biomarker-based tests in the future.

CONCLUSION:

Our results suggest that Aβ1-42 and p-Tau are the principal biomarkers for AD diagnosis, and that CSF concentrations of t-Tau do not improve this diagnosis. However, CSF t-Tau appears to be of clinical interest for the prognosis of dementia, higher t-Tau levels being associated with more rapid progression from mild cognitive impairment to AD [21] or with a more severe cognitive profile [22]. It can therefore be assumed that CSF t-Tau levels are instructive in evaluating the progression of neurodegeneration. It would be of interest to validate the Lille decision tree in a multicenter study in the future, and to further improve it by the addition of new biomarkers for the differential diagnosis of AD and other dementias.

Conflicts of interest:

Dr. A. Duhamel, Dr. J. Saleron, Dr. V. Deramecourt, Dr. M.-A. Mackowiak, V. Deken, Dr. N. Sergeant, Dr. L. Buée and Dr. B. Sablonnière have no conflicts of interest to declare.

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Dr. Pasquier serves/has served on the scientific advisory boards of Bayer Schering Pharma, Eisai Inc. and Janssen-Cilag.

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Author contributions: SSM, SB and AD conceived and designed the experiments; SB, AMM, VD and FP carried out patient inclusion/diagnosis; SSM performed experiments; SSM, AD, JS and VD analyzed data; LB, BS and AD contributed reagents/materials/analytical tools and provided scientific advice; SSM and JS wrote the paper.

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