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Validation of the Erlangen Score Algorithm for the Prediction of the Development of Dementia due to Alzheimer's Disease in Pre-Dementia Subjects

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Abstract.

Background: In previous studies, a dichotomous stratification of subjects into "cerebrospinal fluid (CSF) normal" and "CSF pathologic" was used to investigate the role of biomarkers in the prediction of progression to dementia in pre-dementia/mild cognitive impairment subjects. With the previously published Erlangen Score Algorithm, we suggested a division of CSF patterns into five groups, covering all possible CSF result combinations based on the presence of pathologic tau and/or amyloid-β CSF values.

Objective: This study aimed to validate the Erlangen Score diagnostic algorithm based on the results of biomarkers analyses obtained in different patients cohorts, with different pre-analytical protocols, and with different laboratory analytical platforms.

Methods: We evaluated the algorithm in two cohorts of pre-dementia subjects: the US-Alzheimer's Disease Neuroimaging Initiative and the German Dementia Competence Network.

Results: In both cohorts, the Erlangen scores were strongly associated with progression to Alzheimer's disease. Neither the scores of the progressors nor the scores of the non-progressors differed significantly between the two projects, in spite of significant differences in the cohorts, laboratory methods, and the samples treatment.

Conclusions: Our findings confirm the utility of the Erlangen Score algorithm as a useful tool in the early neurochemical diagnosis of Alzheimer's disease.

Keywords: Alzheimer's disease, biomarkers, cerebrospinal fluid, clinical neurochemistry, results interpretation, validation

INTRODUCTION

A need to establish a tool determining the risk of developing Alzheimer's disease (AD) before the onset of dementia, including the mild cognitive impairment (MCI) and predementia stage, has recently been

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expressed in the diagnostic recommendation papers [1, 2]. Such a tool would "*open a crucial window of opportunity to intervene with disease-modifying therapy*" [1]. To achieve this goal, several studies have been performed following a strategy of first dividing patients at the MCI stage into "cerebrospinal fluid (CSF) pathologic" and "CSF normal", and then following them until the conversion to AD [3–5]. The increased percentage of MCI subjects with pathologic CSF, who convert to AD, in contrast to those having normal CSF is considered a very strong argument in favor of the application of the CSF biomarkers as predictors of the MCI-AD conversion and selection of subjects with underlying AD pathology. In other words, subjects with pathologic CSF biomarkers in the pre-dementia AD stage become demented in a shorter time period compared to those with normal CSF biomarkers. A significant drawback of this strategy is that it relies on a dichotomous classification of patients into just two groups, without leaving anything in-between. Trying to extend this strategy, we suggested an interpretation algorithm that would divide the subjects into five categories (0–4 points) which, we believe, better reflects the continuum between the entirely normal and the entirely pathologic CSF [6]. Since that time this algorithm has been successfully applied for routine diagnostic testing in our center and has inspired other research groups [2, 7].

Discrepancies between the results of the CSF biomarkers obtained by different laboratories is one of the factors limiting the acceptance of the CSF biomarkers as routine diagnostic tools in AD [8, 9]; particularly in multi-center studies it is difficult to combine the results from different laboratories into one statistical analysis if the discrepancies across them approach up to 250%. The same applies if laboratory methods used to measure CSF biomarkers and/or their reference ranges need to be changed in a given laboratory. On the other hand, we believe that it is the diagnosis-oriented interpretation of the CSF pattern, and not the raw concentrations of the biomarkers, that plays the most important role in the support of AD diagnostic procedures. This is particularly relevant if a lumbar puncture is performed at the MCI or dementia stage, when the alterations of the biomarkers (mostly reflecting amyloid-β (Aβ) pathology) have already reached a plateau, and do not correlate with the progression of disease severity anymore [10]. Therefore, we believe that the application of diagnostic-oriented interpretation algorithms, for example such as the one proposed by us, would facilitate the comparison of the outcomes obtained by different laboratories (or the same laboratory but with different methods) at least in terms of diagnosis-relevant interpretation.

Correspondingly, in this paper, we present the data on the validation of our interpretation algorithm on the basis of two independent large-scale cohorts: German Dementia Competence Network (DCN) and the US Alzheimer's Disease Neuroimaging Initiative (ADNI).

MATERIALS AND METHODS

Patients; sample collection procedures

The CSF biomarkers were analyzed in two cohorts of patients: the German Dementia Competence Network (DCN, $n = 190$ subjects with MCI) [11], and the US ADNI 1 (ADNI, $n = 292$ MCI or cognitively normal (CN) subjects, the data were downloaded on 09.10.2014). The ADNI was launched in 2004 by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, the Food and Drug Administration, pharmaceutical companies, and non-profit organizations, as a multi-year public-private partnership. It is a longitudinal study, ultimately including more than 1,500 participants (aged 55 to 90) recruited from over 50 sites across the United States and Canada.

In the DCN cohort, the follow-up period of the MCI patients was 1–4 years. In the ADNI cohort, the follow-up period was one month to 8 years (average 3.24 ± 2.16 years). The basic demographic data for both cohorts are presented in Table 1.

The operating procedures for the CSF collection, storage, and shipment, significantly differed between the two studies. In the DCN, the samples were centrifuged and aliquoted at the sites where the patients recruitment took place (14 gerontopsychiatric centers in Germany), and then frozen at –80°C following

Table 1
Demographic data. Age presented as averages and standard deviations

Cohort	n (M/F)	Age (years)
ADNI Overall	292 (176/116)	75.0 (7.0)
ADNI Progressors	115 (71/44)	74.9 (6.9)
ADNI Non-Progressors	177 (105/72)	75.1 (7.1)
DCN Overall	190 (115/75)	65.5 (8.7)
DCN Progressors	45 (24/21)	68.3 (8.1)
DCN Non-Progressors	145 (91/54)	64.6 (8.7)[a]

[a]DCN Non-Progressors were significantly younger than the DCN Progressors ($p = 0.01$).

shipment on dry ice to the Laboratory in Erlangen, where the analyses took place [12]. In ADNI, baseline CSF samples were obtained in the morning after an overnight fast from 292 subjects (195 MCI and 97 CN with average (standard deviation) ages of 74.5 (7.4) and 75.6 (5.3) years, respectively) from individuals enrolled at 56 participating centers at the time the subjects entered ADNI (i.e., baseline) according to the ADNI standard operating procedures manual (http://adni.loni.usc.edu/research/protocols/biospecimens-protocols/). The CSF samples were deep frozen immediately after the lumbar puncture without centrifugation or aliquoting, and shipped to the UPENN ADNI Biomarker Laboratory in Philadelphia on dry ice, where they were thawed, aliquoted, and re-frozen.

Analysis of the biomarkers; definitions of the reference ranges and border zones

The summary of the reference ranges and the border zones are presented in the Table 2. In the DCN cohort, the analyses were performed with ELISAs: $A\beta_{1\text{-}42}$, Tau, and pTau181 from Innogenetics (currently Fujirebio Europe, Gent, Belgium), and for Aβx-42/x-40 ratio from The Genetics Co. (Zürich, Switzerland). The reference ranges were published previously [13, 14], and not modified or optimized for this study. In the ADNI study, the biomarkers were analyzed with the bead-based xMAP multiplex immunoassay, AlzBio3: $A\beta_{1\text{-}42}$, Tau, and pTau181 from Innogenetics (currently Fujirebio Europe, Gent, Belgium) on the Luminex (Austin, Texas, USA) platform as described elsewhere [5, 15]. The reference ranges used for the diagnosis-oriented interpretation of the ADNI data were taken directly from the previous study [15], and applied for this study without further modifications or optimization.

Table 2
The biomarkers reference ranges and their border zones (in brackets) used in the two cohorts; biomarkers concentrations in pg/mL

Biomarker	US-ADNI	DCN
$A\beta_{1\text{-}42}$	192 (173–192)	600 (540–600)
$A\beta_{42/40}$ ratio	Not done	0.11 (0.1–0.11)
Tau	93 (93–102)	300 (300–330)
pTau181	23 (23–25)	60 (60–66)

Interpretation algorithm

The interpretation algorithm is described in detail elsewhere [6], and summarized in Table 3. Briefly, depending on the pattern of the biomarker alterations, the CSF results of a given patient are scored between 0 and 4 points. A CSF result with all biomarkers entirely normal is scored 0 points; a pattern with only marginal alterations in one biomarkers group (either Aβ or Tau, but not both) results in the score of 1; a CSF result with the alterations in either Aβ metabolism (decreased $A\beta_{42}$ concentration and/or decreased $A\beta_{42/40}$ ratio) or Tau metabolism (increased concentrations of Tau and/or pTau) but not both is scored 2 points; a result with clear alterations in one biomarkers' group (either Aβ or Tau) accompanied by marginal alterations in the other group is scored 3 points; clear alterations in both Aβ and Tau/pTau result in 4 points. In practical terms, this algorithm was implemented as a Microsoft Office Excel macro, written in Visual Basic. The script of the macro is presented in Supplementary Table 1.

Statistical analyses

The percentage of patients with a given score who progressed to AD is presented as the ratio of patients who developed AD within the follow up period to the total number of the patients with this score. The scores of the progressors and the non-progressors were compared using an ANOVA test followed by the *post-hoc* Scheffe test. Cox proportional-hazards model was used to estimate the effects of different baseline scores

Table 3
Summary of the interpretation algorithm. The original algorithm was simplified, because in the two cohorts considered in this study there were no patients with extremely high Tau concentrations, suggesting rapidly progressing neurodegeneration, which would require more complex interpretation

Pattern of the NDD biomarkers	Score	Interpretation
All CSF AD biomarkers normal	0	No evidence for organic CNS disease
Only slightly altered results of either Aβ OR Tau/pTau, but not both	1	AD improbable
Clearly pathologic results of either Aβ OR Tau/pTau, but not both	2	AD possible
Slight alterations of both, Aβ AND Tau/pTau	2	AD possible
Clearly pathologic results of either Aβ OR Tau/pTau accompanied by slight alterations of the biomarker(s) of the other group[a]	3	AD possible
Aβ AND Tau/pTau clearly pathologic	4	AD probable

[a]For example, a clearly decreased $A\beta_{42/40}$ ratio and/or $A\beta_{1\text{-}42}$ concentration and a slightly increased (border zone) Tau and/or pTau181.

(covariate) on the relative risk of conversion to AD, whereas for this analysis only subjects with the follow-up period between 1 month and 8 years were included. Curves for different groups in the Cox model must have hazard functions that are proportional over time (proportional hazards assumption). To test this assumption, we analyzed the correlation between the Schoenfeld residuals and survival time, which was not met by group 3. Therefore we calculated a hinge function, to estimate independent hazards function for the two segments of group 3. The differences between the trajectories of the groups with different baseline scores were analyzed with Wald test; Kaplan-Meier estimates illustrate progression to AD depending on the baseline score. The analyses were performed with MedCalc 13.1 and Statistica 12.0. For all analyses, a two-tailed p-value <0.05 was considered significant.

RESULTS

Correlation between the conversion percentage and the Erlangen Score

The barplot of the percent of the DCN subjects who have progressed to AD during the follow up time and their initial score is presented in Fig. 1. The dark grey bars represent the scores calculated on the basis of four biomarkers ($A\beta_{1\text{-}42}$, $A\beta_{42/40}$ ratio, Tau, and pTau181), and the light grey bars correspond to the score calculated with three biomarkers only ($A\beta_{1\text{-}42}$, Tau, and pTau181), i.e., after the omission of $A\beta_{42/40}$ ratio. The barplot of the percent of the ADNI subjects who have progressed to AD during the follow up time and their initial score is presented in Fig. 2.

Kaplan-Meier estimates

Results of the Cox proportional hazards analyses are summarized in Table 4. Since there were no statistical differences between the subjects with 0 and 1 points ($p = 0.94$), the two groups were combined into a single group *a posteriori* ("0 or 1"). The Kaplan-Meier estimates for the ADNI subjects stratified based on baseline Erlangen scores are presented in Fig. 3. In the ADNI cohort, the median survival time for the progression to AD dementia was 7.2, 7.3, 5.2, 3.9, and 3.7 years in the groups with 0, 1, 2, 3, and 4 points, respectively.

Table 4
Statistical analysis of the estimates of the rate of progression to AD in the US-ADNI cohort presented in Fig. 3. Considered are only the subjects with the follow-up period between 1 month and 8 years

Score (covariate)	Wald	p[a]	Hazard Ratios (95% CI)[a]
1	0.006	0.94	1.38 (0.67–2.87)
2	9.36	0.0022	3.34 (1.90–5.87)
3	18.44	<0.0001	6.90 (2.64–18.06)
4	41.70	<0.0001	7.68 (5.00–11.81)

[a]Compared to the group with the Erlangen score "0".

Scores comparison between the progressors and the non-progressors in the two cohorts

The results of the scores of the progressors and the non-progressors of the two cohorts are presented

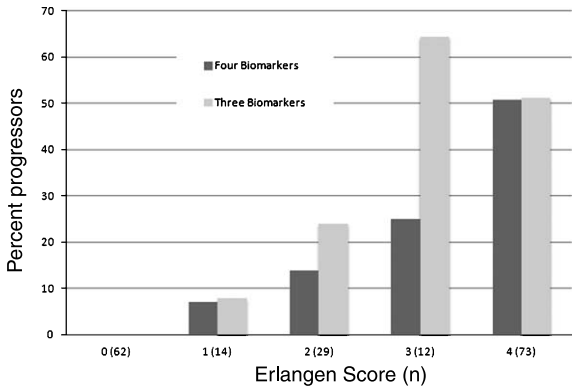

Fig. 1. Percentage of the DCN subjects in the MCI stage progressing to AD in the follow-up time (1–4 years). Light-grey bars indicate the results when only three biomarkers ($A\beta_{1\text{-}42}$, Tau, and pTau181) were considered; dark-grey bars indicate the results when four biomarkers ($A\beta_{1\text{-}42}$, $A\beta_{42/40}$ ratio, Tau, and pTau181) were considered. In the brackets, the total number of patients with a given score is presented in the four-biomarkers model.

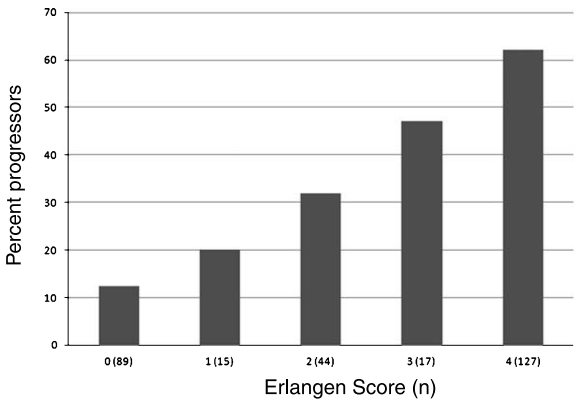

Fig. 2. Percent of the ADNI subjects in the preclinical and MCI stages progressing to AD in the follow up time (average, 3.24 ± 2.16 years). In the brackets, the number of patients with a given score is presented.

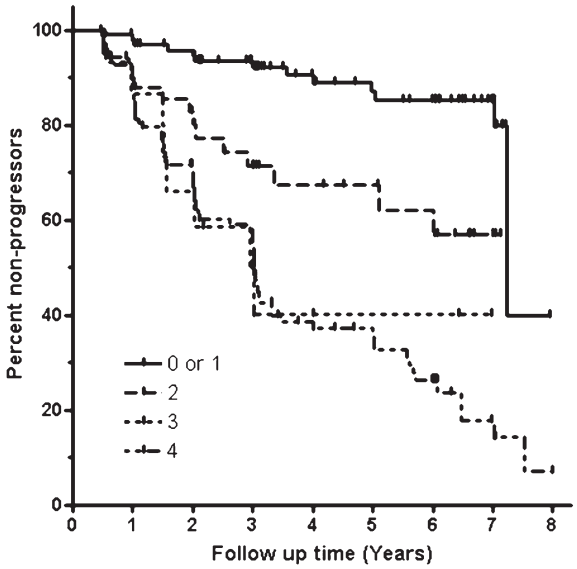

Fig. 3. Kaplan-Meier estimates of the rate of progression to AD in the US-ADNI cohort based on the baseline scores. Considered are only the subjects with the follow-up period between 1 month and 8 years. The results of the proportional hazards are presented in the Table 4.

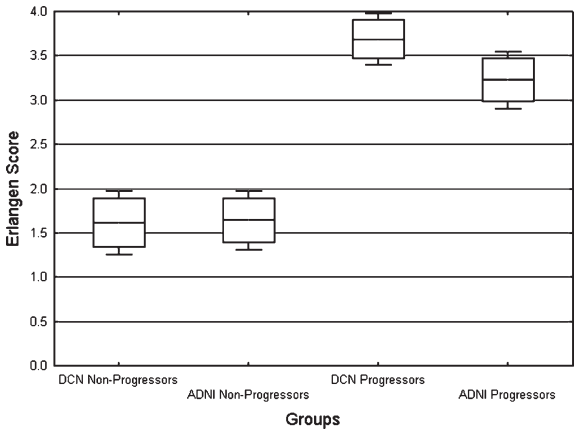

Fig. 4. The scores of the progressors and the non-progressors of the two cohorts. Presented are the medians (horizontal bars), 5th and 95th percentiles (boxes) and 1st and 99th percentiles (whiskers).

in Fig. 4. Neither between the progressors of the two cohorts nor between the non-progressors of the two cohorts were statistically significant differences observed. DCN progressors had significantly higher score than DCN non-progressors (3.7 versus 1.6, $p < 0.001$). Similarly, ADNI progressors had significantly higher scores than ADNI non-progressors (3.2 versus 1.7, $p < 0.001$).

DISCUSSION

In contrast to the previously published studies, including those from our group [3, 4, 14–16], the Erlangen Score algorithm [6] enables the categorization of the CSF results into five discrete groups, reflecting different degrees and constellations of pathological findings, instead of dichotomous division into only "normal" and "pathologic" categories. This leads to at least three features, which have not been demonstrated using other interpretational approaches:

(a) It enables more precise estimation of the risk to develop dementia in a given person in a pre-dementia AD stage, at least within 4-5 years after the lumbar puncture. As an example, a MCI subject with the score of 2 points or less is unlikely to develop AD dementia within 4 years, and his progression risk is lower than that of a person with 3 points. Note that both cases would be classified as "CSF pathologic" by all other approaches;

(b) If treated as a "biomarker", the Erlangen Score characterizes with high consistency across laboratories, methods, and preanalytical sample handling procedures, because it can be applied using the specific cut-offs developed for the different analytes in each laboratory. For example, a person (or group of persons) with the score of 3 points obtained in one laboratory will likely have the same score when tested in another laboratory or with different analytical methods, irrespective of the fact that the raw concentrations of the single biomarkers can significantly differ across laboratories. Indeed, our algorithm was first described and validated based on the findings obtained with ELISA and in an entirely different cohort of subjects [6]. In this study we compared the interpretation of the data obtained in two different cohorts that: (i) employed different preanalytical sample handling protocols and (ii) utilized two different immunoassay methods and (iii) with entirely different reference ranges. It is perhaps also worth stressing, that these reference ranges were established *a priori* and were not optimized for this study. This finding reconfirms our original hypothesis that this algorithm can be easily adopted by laboratories irrespective of their analytical platform and the reference ranges.

(c) The algorithm enables a comprehensive interpretation and a semi-quantitative presentation of

all possible patterns of the results of the CSF AD biomarkers, including such situations when Aβ and Tau pathologies are "inconsistent" with the described AD biomarker model [17]. This is, in our opinion, a substantial advantage over such approaches that leave out results of Aβ and Tau not fitting each other (for example, a decreased $A\beta_{42}$ and normal Tau) as "conflicting" or "non-interpretable" [2]. Taken together the available literature, and the results of this study, we think that such patterns of CSF results are interpretable; they may, for example, occur in the early stages of the disease, when pathologic Aβ results precede pathologic Tau results in CSF [18]. Correspondingly, all possible patterns of CSF results can be interpreted as neurochemically "normal", "improbable AD", "possible AD", and "probable AD".

For this study, since there were no patients with extremely high Tau concentrations, which would suggest rapidly progressing neurodegeneration (for example, prion cases), there was no need to take such a special situation into consideration.

Comparison of the scores of the DCN cohort obtained with three and with four biomarkers clearly shows that the results based on four-biomarkers correlate better with the percent of the subjects progressing to AD dementia than the scores calculated with three biomarkers only (compare the light gray versus dark gray bars in Fig. 1). Omitting the $A\beta_{42/40}$ ratio results in an illogic impression that the proportion of the subjects progressing to AD dementia is larger in the group with a lower score. This might be cautiously considered as another argument favoring four-biomarker approach, which also is in agreement with other studies confirming better diagnostic performance of the $A\beta_{42/40}$ ratio compared to the $A\beta_{42}$ concentration alone [19–23]. We hypothesize that the concentration of $A\beta_{1\text{-}42}$ depends not only on the brain burden of amyloid pathology, but also on the total amount of the Aβ peptides in the patient's CSF. Previously we found that the concentrations of $A\beta_{40}$, the most abundant isoform in the human CSF, follow almost an ideal Gaussian distribution [19]. As a consequence, there are subjects with either very low or very high concentrations of Aβ isoforms in the CSF. A better diagnostic performance of the $A\beta_{42/40}$ ratio compared to the $A\beta_{1\text{-}42}$ concentration might be explained by the assumption that the subjects with either extraordinary low or high concentrations of Aβ peptides in the CSF characterize with the respectively low or high $A\beta_{1\text{-}42}$, and consequently, a normalization of the $A\beta_{1\text{-}42}$ concentration by the application of the $A\beta_{42/40}$ ratio improves the interpretation of the biomarkers.

In the ADNI, but not in the DCN cohort, 12.4% of subjects with a normal CSF (scored 0) developed dementia during the follow up time. If the clinical diagnoses were entirely accurate, this might stay in contrast with the assumption that the alterations in the CSF biomarkers, particularly in Aβ, are pathologic already decades before the onset of the clinical symptoms [10], however, clinical diagnosis of AD has been clearly shown to be often inaccurate, and according to the literature the inaccuracy rate is an average of about 13% (up to 32%) based on autopsy data [24–26]. Alternatively, one of the possible explanations could be that only $A\beta_{1\text{-}42}$ CSF concentrations were measured in this cohort, $A\beta_{42/40}$ concentration ratios were not available. This means that a given percent of the subjects classified here as having "normal" CSF (0 points) has actually Aβ pathology that could have been revealed if $A\beta_{42/40}$ ratios were available. Such subjects would be correspondingly re-classified as having 1 or 2 points, although another study that compared xMAP AlzBio3 $A\beta_{1\text{-}42}$ to another vendor's $A\beta_{42/40}$ ratio showed no improvement by the latter on the former [27]. On the other hand, the percent of the CSF-negative subjects having converted to AD in this study roughly corresponds to the ratio of MCI subjects without evidence of Aβ pathology on positron emission tomography who convert to AD within 1–3 years (7%, reviewed in [28]).

In our approach to the interpretation of the CSF AD biomarkers, for the first time we suggested introduction of the "border zones" results. This concept is in accordance with the statement published in the recent diagnostic recommendations: "*biomarker standardization must also include gaining a broad consensus on how to obtain results that are interpretable as clearly normal, clearly abnormal, and perhaps intermediate*" [29]. A good example is a situation when only one biomarker is marginally abnormal, with the remaining entirely normal. For example, an $A\beta_{1\text{-}42}$ concentration slightly lower than the cut-off cannot be mathematically interpreted as "normal", but it should neither be interpreted entirely pathologic as in such a case when it would be decreased for example by half. A rigid interpretation of a slightly decreased $A\beta_{1\text{-}42}$ concentration, without consideration of a "border zone" concept would be "neurochemically possible AD", with all the consequences for the patient and his family. "Border zone" or "Intermediate results" concept would lead to scoring such a pattern with 1 point, leading to the interpretation "neurochemically improbable AD"

(whereas "improbable" does not mean "excluded"). Furthermore, all laboratory methods characterize with some degree of imprecision, and the number defining "border zones" in this paper (10%) is derived from the average inter-assay variation of the methods applied for the AD diagnostics; we strongly encourage all laboratories to modify it according to their needs or experiences. Finally, in our opinion, as long as a disease modifying therapy is lacking, it is better, from the ethical point of view, to under-diagnose some AD patients having marginally pathologic CSF results than to over-diagnose healthy subjects having the results normal but close to the reference ranges. This is the reason why the "border zones" of the neurochemical biomarkers of AD spread in the direction of the pathologic results (for example, in case of $A\beta_{1\text{-}42}$, the reference range minus 10%) and are not symmetrical around the reference ranges. We believe that as soon as disease modifying therapies can be offered, exactly opposite strategy will apply of the "border zones" spreading into the direction of normality. This will classify some subjects as "perhaps ill", who otherwise would be classified as "normal", and enable offering therapy to a larger population, also to patients with minimal, but non-neglectable, risk of having the disease.

Our study has at least two limitations. First, due to an increased drop-out of patients with the increasing follow-up time, it is currently difficult to make any conclusions on the correlation of the Erlangen Score and the progression hazard beyond 5-6 years. This is clearly visible on Fig. 3, where after 7-8 years the ratio of the progressors with normal CSF (scored 0 or 1 points) is paradoxically higher than the ratio of progressors with pathologic CSF (scored 2 or 3 points). Second, the current version of the Erlangen Score Algorithm treats all CSF AD biomarkers equally. Intuitively we think that this might be not optimal approach, since, for example, alterations in Aβ are considered earlier than these of Tau/pTau, and alterations of pTau are probably more specific for AD that these of the total Tau. The detailed discussion of all possible variants is definitely beyond the scope of this validation paper, particularly since we are currently working on the modification of the algorithm that would consider different weighting of the biomarkers.

Summarizing, our current evaluation reconfirms the utility of the diagnostic interpretation algorithm, described by our group in 2009 and successfully applied for the routine diagnostic since then, on the ground of the results obtained with different analytical platforms, different reference ranges (not optimized for this study), different procedures of sample handling, and in entirely different large-scale cohorts of subjects.

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Authors' disclosures available online (http://j-alz.com/manuscript-disclosures/15-0342r1).

SUPPLEMENTARY MATERIAL

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