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Featured Article

Multicenter Alzheimer's and Parkinson's disease immune biomarker verification study

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Abstract

Introduction: Multiple immunity biomarkers have been suggested as tracers of neuroinflammation in neurodegeneration. This study aimed to verify findings in cerebrospinal fluid (CSF) samples of Alzheimer's disease (AD) and Parkinson's disease (PD) subjects from the network of the European, Innovative Medicines Initiative–funded project AETIONOMY.

Methods: A total of 227 samples from the studies/centres AETIONOMY, ICEBERG, and IDIBAPS were used to analyse 21 selected immunity biomarkers in CSF. Results were compared to data of an independent cohort of 399 subjects previously published.

Results: Immunity markers were predominantly and reproducibly associated with pathological levels of tau isoforms, but also with amyloid levels, aging, sex, *APOE* genotype, and center-specific factors.

Discussion: Immunity biomarker levels in CSF reflect molecular and cellular pathology rather than diagnosis in neurodegenerative disorders. Assay standardization and stratification for age and other covariates could improve the power of such markers in clinical applications or intervention studies targeting immune responses in neurodegeneration.

Keywords: Alzheimer's disease; Parkinson's disease; Mild cognitive impairment; Cerebrospinal fluid; Biomarker; Inflammation; Amyloid; Tau; Aging; Multicenter

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1. Background

Cerebrospinal fluid (CSF) levels of beta amyloid (Aβ) and tau isoforms are routinely used markers of Alzheimer's disease (AD) and at present recommended for inclusion in research and clinical practice [1–3]. Concordant with these recommendations are approaches to stratify subjects by combinations of molecular markers, such as the amyloid/tau/neurodegeneration (A/T/N) scheme [4]. These suggestions originate from a currently intensively discussed concept that neurodegenerative disorders should be defined by their underlying molecular and cellular mechanisms, while noticeable symptoms occur in later stages of disease [5]. A common notion between facilitators and critics of this concept is that current biomarkers, although powerful for monitoring of specific pathological features, are still insufficient to reflect the complexity of various neurodegenerative disorders [6]. AETIONOMY is a European, Innovative Medicines Initiative–funded project dedicated to further development of mechanism-based, molecular taxonomies of AD and Parkinson's disease (PD). One candidate mechanism within this project was neuroinflammation, the reaction of central nervous system (CNS) immune cells to pathological stimuli [7,8]: Neuroinflammation probably begins during early presymptomatic stages with the sensing of miss-folded and/or aggregated proteins like Aβ or α-synuclein which represent danger-associated molecular patterns and activate microglia and astroglia. Later, immune reactivity propagates in response to neuronal death and the respective damage signalling. Tracing biomarkers specific for these processes is a prerequisite in the attempt to monitor neuroinflammation as a taxonomy feature. Though many typical proinflammatory proteins are hard to detect in the CSF of dementia subjects, research has nonetheless led to several immunity markers that can be reliably analysed and therefore constitute reasonable candidates for functional neuroinflammation panels [2,9–13]. This study aimed to verify observations for 21 selected markers in CSF samples derived from a multicenter cohort of 227 subjects (nondemented/ND, mild cognitive impairment/MCI, AD and idiopathic PD/IPD) provided by the AETIONOMY network. All data acquired in this study are accessible via the AETIONOMY knowledge base (https://data.aetionomy.scai.fraunhofer.de/).

The panel consisted to one third of signalling molecules such as cytokines/chemokines and other messengers (YKL-40, TGF-β1, IP-10, MCP-1, MIF, MIP-1β), to one third of soluble immune receptors and shedded receptor ectodomains (sIl-1RAcP, sAXL, sTyro3, sTREM2, sTNF-RI/II, sICAM-1), and to one third of complement and innate immunity factors (CRP and the complement factors C1q, C3, C3b, C4, B, H, and properdin). A brief overview of previous findings for these markers including respective literature is provided in Supplementary Table 1. This study furthermore investigated critical covariates that have to be considered for modelling of mechanisms, characterization of subject subgroups, and potentially translation to clinical diagnostic or interventional approaches.

2. Methods

2.1. Study design and approval

An overview of the study design is given in Supplementary Fig. 1. Samples and data were provided from the cohorts/studies of AETIONOMY (https://www.aetionomy.eu/en/vision.html), ICEBERG (https://icm-institute.org/en/scientific-projects/), IDIBAPS (http://www.idibaps.org/qui-som/en_index.html), and UKB (The university clinic of Bonn Department of Neurodegenerative Diseases & Geropsychiatry/Neurology, Germany, https://neurodeg.uni-bonn.de/).

Subjects were recruited following local authorities' ethical approval and by informed consent.

2.2. Sampling and preanalytical procedures

CSF samples were obtained by lumbar puncture performed by trained medical personal following standardized procedures and good clinical practice guidelines. Preanalytical sample handling procedures differed between AETIONOMY/ICEBERG (protocol 1), IDIBAPS (protocol 2), and UKB (protocol 3) by centrifugation, method of freezing, type of polypropylene storage tubes, and time point of freeze/thaw cycles within the procedure: Protocol 1 included no centrifugation of samples, preparation of aliquots before freezing, and freezing as well as storage in liquid nitrogen. Protocol 2 included centrifugation at 2000 × g for 10 minutes at 4°C, followed by preparation of aliquots and freezing and storage in a −80°C freezer. Protocol 3 included snap-freezing in liquid nitrogen immediately after LP without centrifugation, and subsequent storage in a −80°C freezer. Then, samples underwent one freeze-thaw cycle for subfractionation into smaller aliquots. Independent of origin, all samples had a total of 2 freeze-thaw cycles on analysis.

2.3. Biomarker analysis

The AD standard biomarkers (beta-amyloid [Aβ] 42, total-tau [t-tau], and phospho-tau [p-tau]-181) were determined at IDIBAPS using Fujirebio GmbH INNOTEST® assays and local cutoff values (Aβ42, 550 pg/ml; t-tau, 450 pg/ml; p-tau-181, 65 pg/ml; and the ratio Aβ42/p-tau-181, 7.5). For determination of immunity biomarkers, samples were processed on ice until application to the assay. Samples and calibrators were run in duplicates, and samples with a duplicate coefficient of variance >15% were repeated. To normalize for interrun variances, a pooled and aliquoted CSF sample was run as an internal control on each assay plate. Details on the assays used are found in Supplementary Table 2. YKL-40 was analyzed at IDIBAPS and all other markers at UKB/DZNE.

2.4. Statistical analysis

The statistical workflow is depicted in Supplementary Fig. 2. Data analysis and visualization were done using Prism 7 (GraphPad Software Inc., La Jolla, CA) and IBM SPSS Statistics 21 (IBM Corporation, Armonk, NY). Nonparametric statistics were preferably used for group comparisons (Kruskal-Wallis or Mann-Whitney *U* tests) and correlation matrix calculation (Spearman correlation analysis). For parametric tests such as ANCOVA and partial correlation, log-transformed values were used. Covariance analysis was done stepwise including first all covariates and in the next step only those significant were included. Distribution of sex and *APOE* genotype was tested using Pearson's chi-squared test. Significance level was defined as $\alpha = 0.05$, and the Bonferroni method was used to control for multiple testing of the 21 immunity markers and pairwise comparisons in multigroup tests. Comparison of linear regression functions was calculated using the method described by J.H. Zar [14].

3. Results

3.1. Normalization of center-specific effects

Demographic data were heterogeneous between the included cohorts/studies, and subject groups were obtained from different centres using different preanalytical protocols and recruiting from different populations (for detailed descriptive statistics, see Supplementary Tables 3-6). We therefore addressed potential influence of center-specific effects and found 10 markers affected (Supplementary Table 7): Samples obtained from IDIBAPS (centre 1) had lower values of sTREM2, IP-10, MCP-1, MIF, C1q, C4, factor B, and properdin than those from AETIONOMY/ICEBERG (centre 2). By contrast, C3 and C3b levels were higher for samples from IDIBAPS compared to those from AETIONOMY/ICEBERG (Supplementary Fig. 3). Values of affected markers were adjusted using normalization factors calculated from the median values within the ND subjects dichotomized by centre of origin. Effects observed for these normalized values were reflected by trends in the nonnormalized data dichotomized by centre.

3.2. Influence of age, sex, and APOE genotype

Correlation analysis (Fig. 1) showed positive correlations of age and immune biomarker levels for most markers. Five markers differed significantly by sex, of which the complement factors H and properdin were robustly elevated in males (details in Supplementary Table 8). A total of 9 markers differed between *APOE* ε4–positive and *APOE* ε4–negative individuals (Supplementary Table 8). All these markers were slightly higher in *APOE*-positive individuals with the exception of CRP, which was significantly lower in *APOE* ε4–positive individuals.

3.3. Clinical and pathological stratification

When stratified by clinical diagnosis, complement factors C1q, C3, and B as well as sTREM2 and IP-10 differed significantly after adjustment for covariates and multiple testing (see Supplementary Table 9 for extensive description and weaker trends). In no comparison, the IPD group differed from the ND group, but MCI and/or AD groups showed differences compared to ND and/or IPD groups. Next, we used pathological AD biomarker levels for stratification approaches: First, by use of single markers, second, by use of the Aβ42/p-tau-181 ratio, and finally, by use of the A/T scheme combining amyloid and t-tau classification (Supplementary Tables 10-14). Observed effects were highly redundant between use of t-tau or p-tau-181 as stratification variables. Most informative and congruent with single-marker-based approaches was stratification by A/T scheme (Figs. 2 and 3). Significant markers can be divided into 3 groups: First, those influenced primarily by amyloid but not tau showing decreased median in amyloid-positive groups (only complement C3). Second, those primarily influenced by tau but not amyloid with increased median levels in tau-positive groups (YKL-40, MIF, sTNF-RI, sTNF-RII, sTREM2, C1q, C4). Third, those with potential influence of both amyloid and tau, resulting in reduced median in the A+ T- group compared to A- T- and A+ T+, but equal or slightly higher median in A+ T+ compared to A-T- (YKL-40, sTyro3, sAXL, sICAM-1, C3b, factor H). This observation, however, is limited by a low number of only 9 subjects within the A- T+ group.

3.4. Sex-specific effects on pathology-based comparisons

Sex was a strong covariate of several complement factors, YKL-40 and TGF-β1 in all comparisons that were based on pathological AD marker levels: When stratified by both sex and pathological amyloid, YKL-40 and the complement factor H showed more pronounced effects in male than in female subjects (Supplementary Table 15). In similar manner, when combining stratification by sex with tau pathology, the complement factors C1q, C4, and B as well as TGF-β1 were significantly influenced by both: The elevation of these proteins in tau-positive subjects (either t-tau or p-tau-181) was more pronounced in males than in females (Supplementary Table 16).

3.5. Age-dependent pathological trajectories

Given that many of the pathology-related observations were significant, but of small effect size, and the frequency of age as covariate, we plotted immune marker levels against age dichotomized by pathological versus nonpathological amyloid or t-tau values (Figs. 4 and 5). For all markers significantly correlated to both aging and the pathology marker, we compared intercept and slope of the respective linear regression models (Supplementary Tables 17 and 18). In general, these

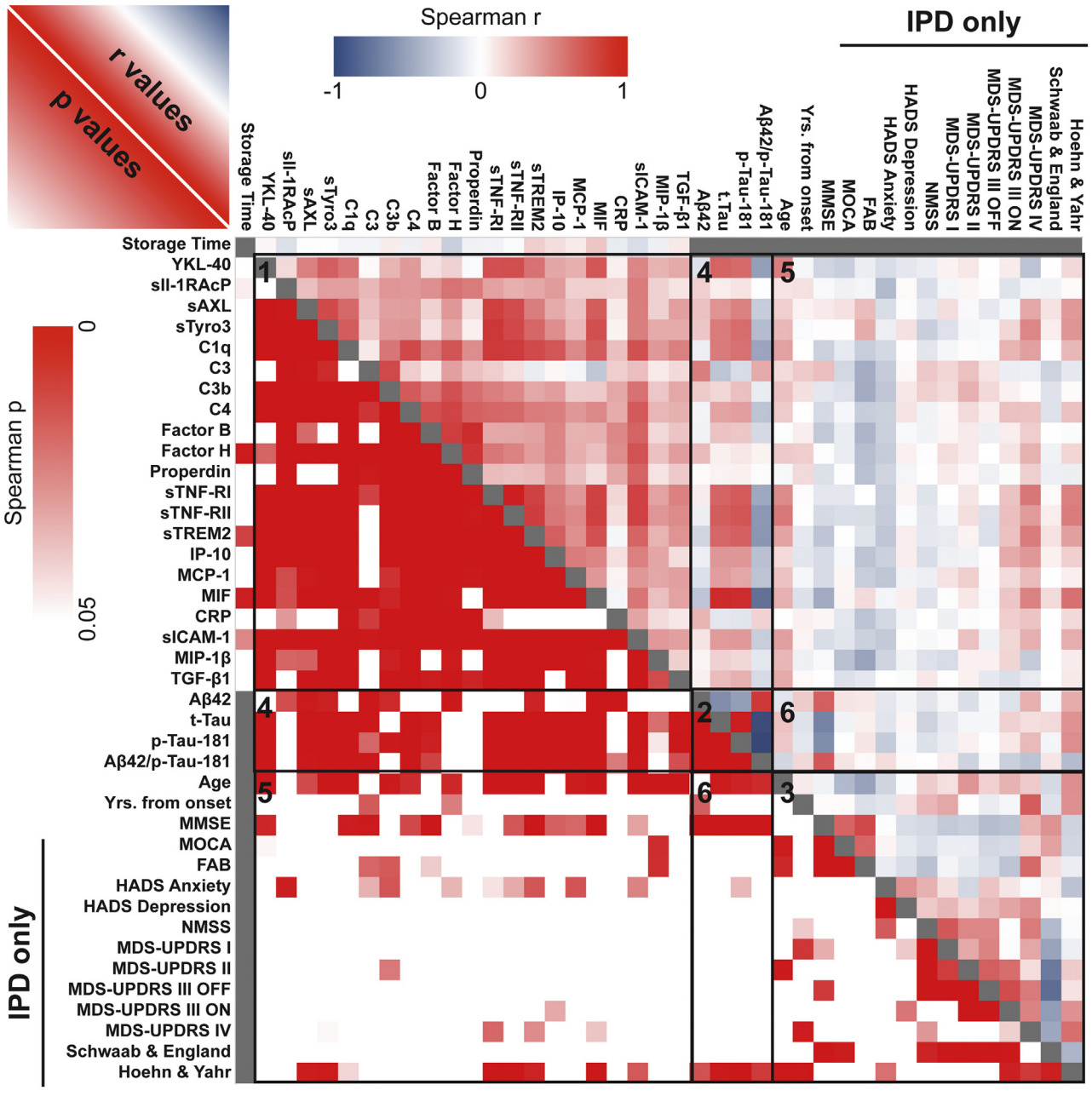

Fig. 1. Correlation matrix of immune markers, AD markers, and clinical features. Results of Spearman correlations visualized as heat map: Lower left part = *P* values; upper right part = r-values. Storage time applied only to the 21 markers investigated in this study. (1-3) Immune markers, AD markers, and clinical features correlated against each other, respectively. (4) Immune markers versus AD markers. (5) Immune markers versus clinical features. (6) AD markers versus clinical features. Within the clinical features, only age, years from onset, and MMSE were available for all groups of subjects. All other clinical features were available for IPD subjects only. Abbreviations: AD, Alzheimer's disease; FAB, Frontal Assessment Battery; HADS, Hospital Anxiety and Depression Scale; IPD, idiopathic Parkinson's disease; MDS-UPDRS, International Parkinson and Movement Disorder Society Unified Parkinson's Disease Rating Scale; MMSE, Mini–Mental State Examination; MOCA, Montreal Cognitive Assessment; NMSS, Non-Motor Symptoms Scale.

functions would follow a more or less steep age-dependent trajectory, and intercept, but not slopes of theses trajectories differed significantly. Where available, data were compared between this study and the previously published UKB data set. YKL-40 was the strongest and most reproducible correlate to amyloid and age and on lower trajectory in amyloid positive in both data sets. Age trajectories dichotomized by t-tau were more pronounced: In both data sets, levels of YKL-40, sTREM2, complement C1q, and MIF were on elevated tracks. MCP-1 and sICAM-1 had elevated intercepts only within the AETIONOMY/ICEBERG/IDIBAPS data, but not in the UKB data set. Of the markers available within the AETIONOMY/ICEBERG/IDIBAPS cohorts only, sAXL, sTyro3, complement factors C3 and C4, TGF-β1, and the soluble TNF receptors I and II had elevated trajectories. In comparison,

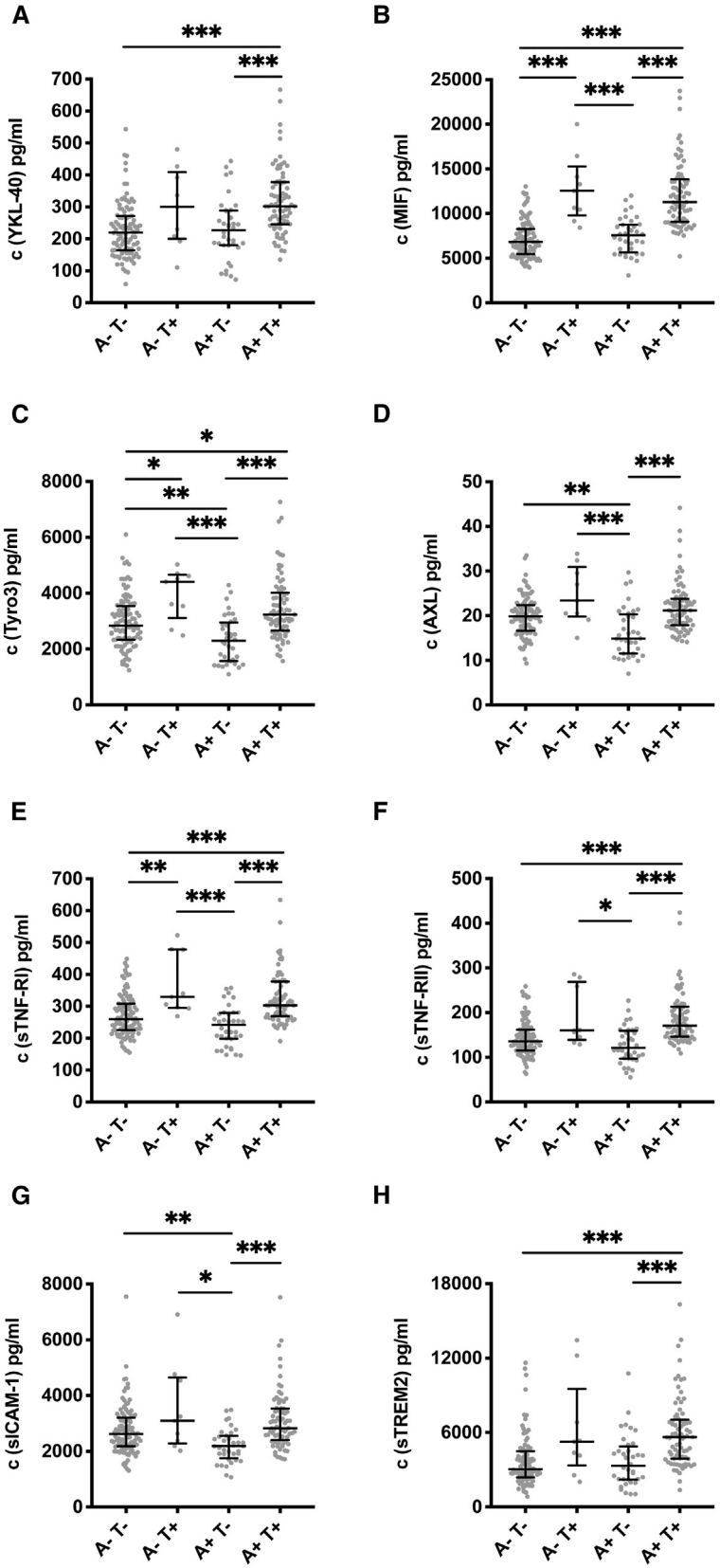

Fig. 2. Chemokines and soluble receptors stratified by A/T scheme. Biomarker values of chemokines and soluble receptors compared by A/T scheme: amyloid and t-tau nonpathological (A- T-); amyloid nonpathological, t-tau pathological (A- T+); amyloid pathological, t-tau nonpathological (A+ T-); amyloid and t-tau pathological (A+ T+). Plots show individual data points, median, and interquartile range for the 8 most significant markers. Bar graphs indicate significant Bonferroni-adjusted pairwise comparisons (* $p_{Adj.} \leq 0.05$; ** $p_{Adj.} \leq 0.01$; *** $p_{Adj.} \leq 0.001$). See Supplementary Table 14 for further details on statistics and markers not depicted in the figure. (A) YKL-40, (B) MIF, (C) Tyro3, (D) AXL, (E) sTNF-RI, (F) sTNF-RII, (G) sICAM-1, (H) sTREM2.

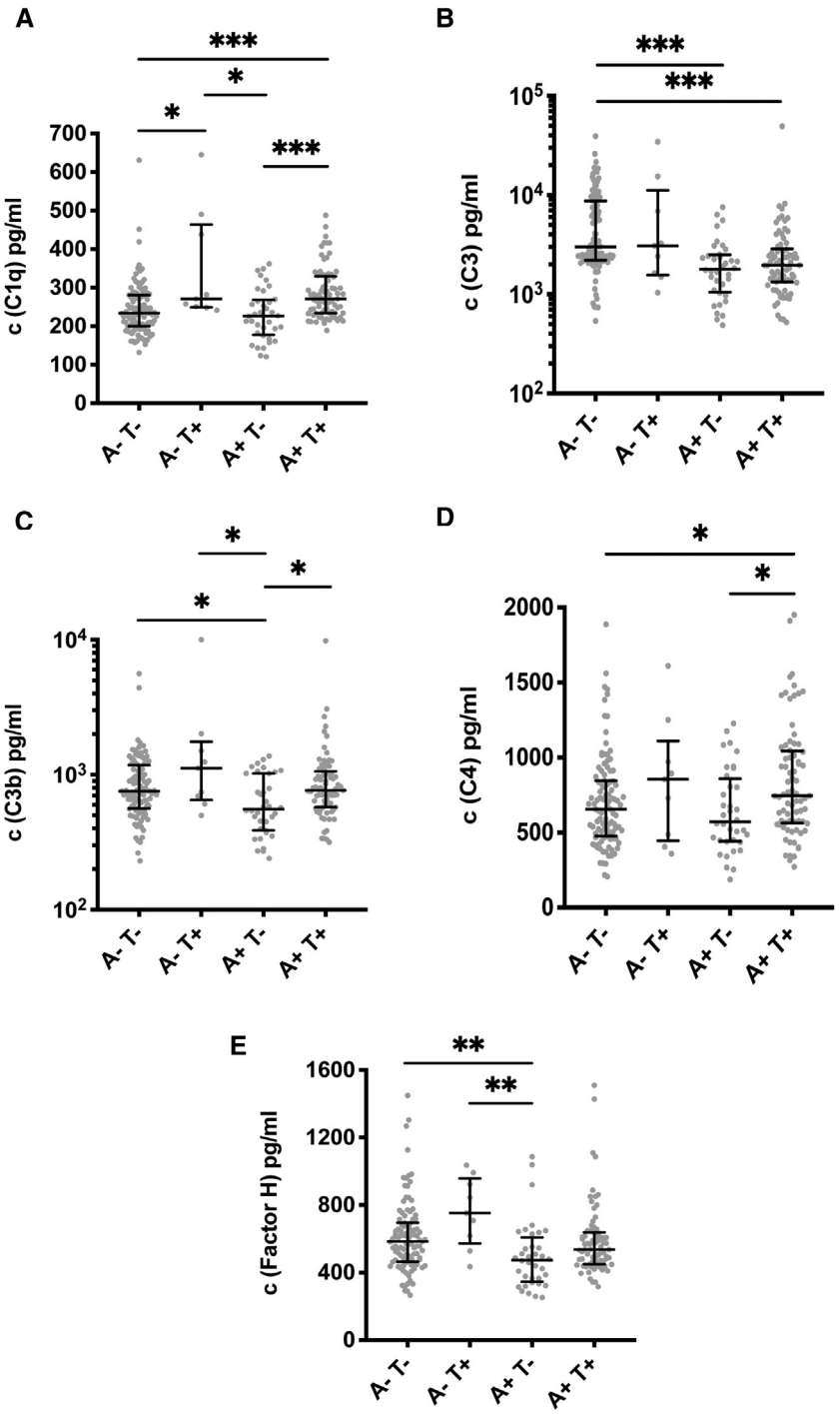

Fig. 3. Complement factors stratified by A/T scheme. Complement factor level values compared by A/T scheme: amyloid and t-tau nonpathological (A- T-); amyloid nonpathological, t-tau pathological (A- T+); amyloid pathological, t-tau nonpathological (A+ T-); amyloid and t-tau pathological (A+ T+). Plots show individual data points, median, and interquartile range. Bar graphs indicate significant Bonferroni-adjusted pairwise comparisons (* $p_{Adj.} \leq 0.05$; ** $p_{Adj.} \leq 0.01$; *** $p_{Adj.} \leq 0.001$). See Supplementary Table 14 for further details on statistics and markers not depicted in the figure. (A) C1q, (B) C3, (C) C3b, (D) C4, (E) Factor H.

effects were most significant for YKL-40, sTREM2, sTNF-receptors I and II, and MIF. As visualized by the 95% confidence intervals within the plots, this analysis was limited by increasing degrees of uncertainty for the youngest and oldest areas on the age axis.

Not all markers that correlated to both pathology and aging showed such additive effects when stratifying trajectories: For MIP-1β and complement C3b, there was no difference in age-dependent linear models when stratified by pathology biomarker levels.

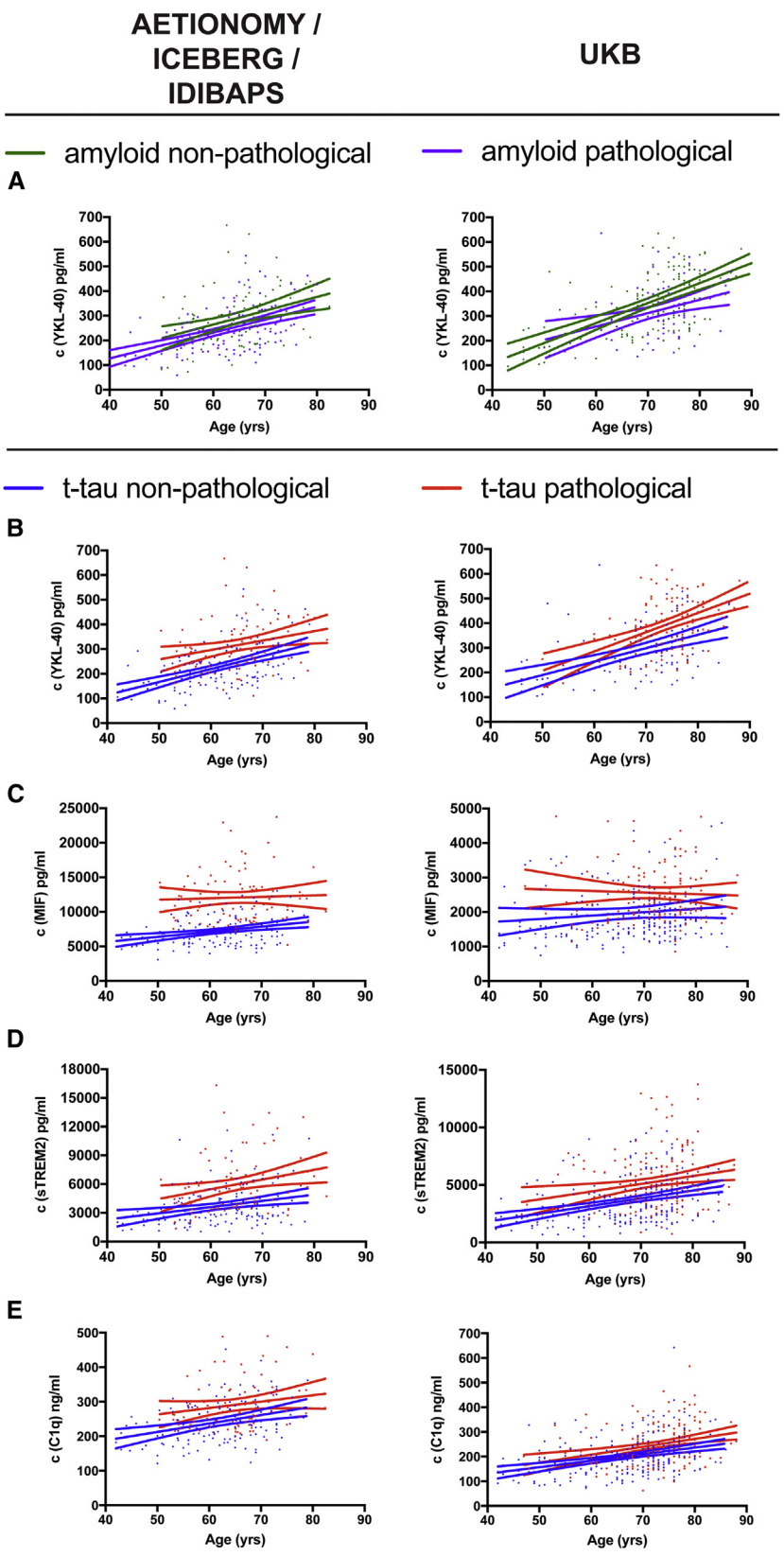

Fig. 4. Pathological aging signatures comparable between previous and current subject cohorts. Immune biomarker values were dichotomized based on pathological/nonpathological amyloid or t-tau and plotted against subject age (see Supplementary Tables 17 and 18). Results are compared between the current AETIONOMY/ICEBERG/IDIBAPS and the previously analysed UKB cohort. The figure depicts results for 4 markers in which these trajectories differed, and which were available in both data sets: (A) YKL-40 dichotomized by amyloid. (B–E) YKL-40, MIF, sTREM2, and C1q dichotomized by t-tau.

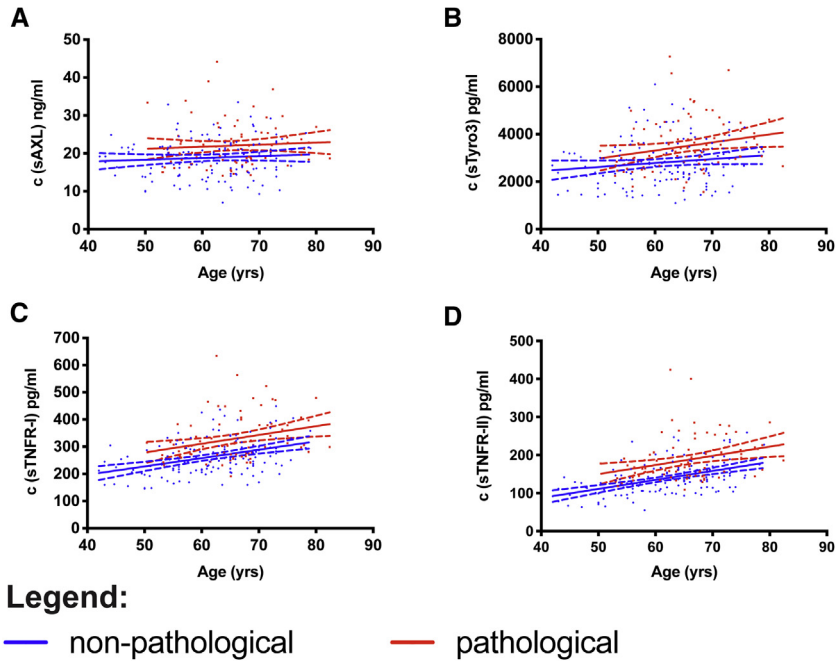

Fig. 5. Pathological aging signatures within the AETIONOMY/ICEBERG/IDIBAPS cohort. Immune biomarker values were dichotomized based on pathological/nonpathological t-tau and plotted against subject age (see Supplementary Tables 17 and 18). Results are shown for the 4 most significant markers available in the current AETIONOMY/ICEBERG/IDIBAPS cohort only. The figure depicts results for (A) sAXL, (B) sTYro3, (C) sTNF-RI, (D) sTNF-RII.

3.6. Immune markers associated with PD staging independent of aging

The strongest correlations of immune markers (in particular, sAXL, sTYro3, sTNF-RI, sTNF-RII, sTREM2, and MIF) to clinical features were those with H&Y staging within the IPD group (Fig. 6). Further important correlates of the H&Y stage were age and tau isoforms, which were also co-correlated to all the 6 relevant immune markers. When adjusting the 6 correlations for aging by partial correlation analysis, the correlations were robust though strength of correlation weakened (Supplementary Table 18). Compared by the linear functions of these correlations, t-tau showed the strongest increase throughout the H&Y stages, with successively weaker effects of the respective immune markers.

4. Discussion

4.1. Key findings

This study provided three main findings: First, a high reproducibility of effects observed for the selected markers (see Section 4.2). Second, the predominant association of immunity markers with pathological levels of amyloid or—to a greater extent—neuronal degeneration (as measured by t-tau and p-tau-181) independent of diagnosis. These results are supportive of the use of mechanism-based disease taxonomies in addition to clinical features. Third, that ageing is a major covariate of immunity markers (see Section 4.3) and therefore constitutes a potentially powerful component of models to improve applicability of these markers in medical practice or studies.

4.2. Reproducibility of findings

For the purpose of conciseness, an overview of previous findings for comparison, including the respective literature, is given in Supplementary Table 1.

YKL-40: Within this study, YKL-40 was not influenced by centre, storage time, and *APOE* genotype and only partly by sex, while age was a significant covariate in all comparisons. It was replicated to be associated with clinical diagnosis, pathological biomarkers, and in IPD with H&Y staging. Among these, the relation to tau isoforms was the strongest. The association to MCI or AD diagnosis was less strong in this study, and elevation in amyloid positive individuals was probably driven by the amyloid- plus tau-positive subpopulation. When plotted against age, YKL-40 was on lower trajectory in amyloid positive in both the AETIONOMY/ICEBERG/IDIBAPS as well as the UKB data set, though elevated in amyloid-positive subjects when not adjusted for age. Furthermore, YKL-40 levels were on a (more pronounced) higher trajectory in tau positive. This could be in line with previous reports of potential bidirectional regulation of YKL-40 throughout disease, though more research will be necessary for validation [15].

MIF: Of the other cytokines or growth factors investigated, MIF showed the strongest effects, in particular in its

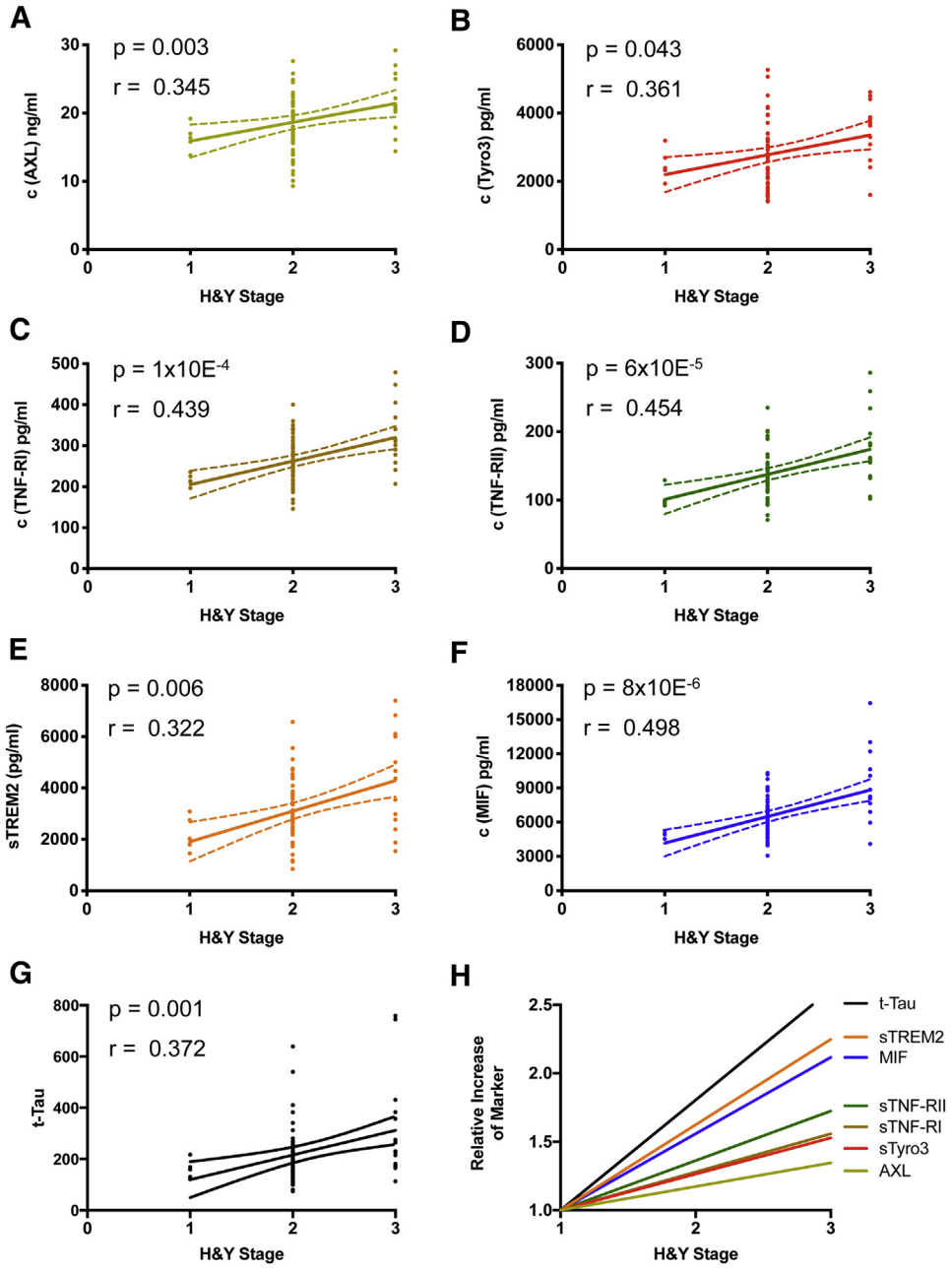

Fig. 6. Correlations of immune markers and t-tau with Hoehn & Yahr stage in IPD. Correlations in figure were robust against aging as covariate of the Hoehn & Yahr stage (see Supplementary Table 19). Individual values with linear correlation function and 95% confidence intervals. Results are shown for the IPD group only: (A) sAXL, (B) sTYro3, (C) sTNF-RI, (D) sTNF-RII, (E) sTREM2, (F) MIF, (G) t-tau. To compare effect strength of the correlations, relative functions of these markers were plotted together against H&Y stage (H). Abbreviation: IPD, idiopathic Parkinson's disease.

reproducible association with tau and ageing, but also with H&Y stage similar to YKL-40. MIF is known to be released upon brain injury, and a similar reaction might be triggered in neurodegeneration. The resulting proinflammatory signalling might be aggravating the disease and even increase key pathological hallmarks such as tau hyperphosphorylation [16]. Nonetheless, MIF is a multifunctional protein that has cytokine and enzyme properties, is involved in wound healing processes, and can act as a neurotrophic factor [17,18]. An advantageous aspect of MIF as biomarker is that it is well detectable in CSF, in contrast to many of its downstream signal mediators.

Soluble receptors: Like MIF and YKL-40, soluble TAM and TNF receptors as well as sTREM2 were reproducibly associated with tau and ageing. For soluble TAM receptors, there might be additional influence of amyloid similar to that observed for YKL-40. These findings are well in line with previous observations. Noteworthy, the ectodomains of these

receptors are released upon cleavage by sheddases like ADAM10 and ADAM17, and conditions of shedding as well as the function of the soluble ectodomains are incompletely understood [19–22]. The shedded receptors can have antagonistic function or act as signal mediators. Hence, closer understanding of the age- and neurodegeneration-reactive increase of soluble receptor levels will be required to understand the potential for intervention in this process.

For the soluble coreceptor sIL-1RAcP, we observed weaker effects associated with diagnosis or amyloid load but did not confirm previous results of higher levels in IPD previously reported [9].

Complement factors: In this study, C1q was associated with pathological tau and ageing less dependent of amyloid pathology, while C3, C3b, and factor H were related to pathological amyloid levels rather than tau pathology. Hence, biomarker levels of these markers might depend on different mechanisms in different stages of disease. Critical covariates were age and sex (confirmative with many studies), but in contrast to a study by Bonham et al. [23] not the *APOE* status. As described in previous studies, observed effect sizes were small and accompanied by high interindividual variance. Furthermore, differences between antibodies or differing proteomics detection techniques might lead to detection of different isoforms, increasing heterogeneity. Although it is still not entirely clear how exactly complement mechanisms in the degenerating CNS translate into CSF biomarker levels, further investigation and standardization—in particular for C3 and C1q—could help to monitor complement system activation in AD and PD.

CRP was lower in *APOE* ε4–positive individuals and robustly associated with amyloid load, but not diagnosis or tau pathology, as described in our previous study [9].

4.3. Influence of biological confounders

Age was the most striking covariate throughout this study. It was positively correlated to levels of most immune proteins and—naturally—with severity of disease in AD and PD as measured by AD biomarkers or H&Y stage. Although a critical confounder, associations found for immune markers and pathological features were robust against ageing. Combined stratification for ageing and pathology markers showed modified aging trajectories and could significantly improve the power of immune markers, while unadjusted age influence otherwise can obscure effects caused by pathological processes.

Sex was a significant confounder predominantly of complement factors, but also TGF-β1 and YKL-40 in some tests. These were elevated in male compared to female donors, which mixed with effects caused by pathology in similar manner as for ageing. Findings for complement factors were also more pronounced in males than in females. In mouse models and human serum, complement activity is lower in females compared to males [24,25]. Intriguingly, AD is more prevalent in females, while PD is more prevalent in males [26]. Therefore, in particular for the complement system, but also for other markers, sex should be considered as important as age as potential confounder.

Positivity for one or two *APOE* ε4 alleles was associated with slightly higher levels of many cytokines or soluble receptors (in contrast to complement factors). When comparing pathological groups, however, *APOE* genotype was less frequently significant compared to age or sex, though consistently observed as highly influential covariate of CRP. The latter finding is of good congruence with our previous findings [9].

4.4. Limitations

When analyzing data from this multicentre collection of samples, we found differences between levels of sTREM2, chemokines, and 6 complement factors that were apparently derived from center-specific factors. To address this issue, we assumed that biomarker levels of nondemented subjects matched by age, genotype, storage time, and sex should be within the same median range, and to calculate normalization factors on this basis. Although this strategy led to reasonable results, it does not allow for conclusions on the origin of observed variances. Potentially, differences in preanalytical protocols between the centres could be causative for the observed discrepancies, as the impact of preanalytical factors on biomarker levels is acknowledged well in research and assay manufacturer's instructions for standard AD markers in CSF and for immune markers in blood samples (though barely investigated for immune markers in CSF) [27,28]. This would indicate that standardization and characterization of preanalytical confounders is of importance not only for the routinely used amyloid or tau isoforms but also for several immune markers frequently studied in CSF. Yet, this work did not include systematic assessments of preanalytical factors, and observed differences could also be caused by population-based factors that differed between the centres but were not tracked in this study.

Aside of these centre-specific effects, this study had other limitations. While sample size enabled groupwise comparisons or combinations of up to two variables (e.g., tau and aging), further subdivision of the overall cohort led to low numbers of samples that would not allow for reliable evaluation. Yet, the results of this study suggest that the discriminative power of immune biomarkers in neurodegeneration could be enhanced when combining multiple covariates such as age and sex and stratifying subjects by combinatorial biomarker approaches, such as in the A/T scheme. This concept could only be verified in drastically larger cohorts of subjects with sufficiently large numbers of CSF samples. Another limitation to the interpretation of our results is that

most cytokines and soluble receptors investigated in this work are also found in peripheral blood in higher concentrations than in CSF. Disruption of the blood-brain barrier during aging and in CNS neurodegenerative/neuroinflammatory diseases might lead to infiltration of peripheral immune cells and diffusion of proteins from the blood into the CSF [29,30]. Within this study, there were no measures for blood-brain barrier integrity. Hence, the data do not allow for a calculation of the influence of cells or proteins of peripheral origin on observed CSF biomarker measures.

Mini–Mental State Examination was the only neurocognitive measure included in this study and showed several significant, but weak correlations to immunity markers, thereby limiting comparability to studies on interaction with cognitive performance.

4.5. Outlook

With YKL-40 and sTREM2, astroglial or microglial biomarkers emerged, which were found to be associated with AD and its pathological hallmarks in multiple studies [2,9,10,31–34]. Several further immunity markers investigated in this work also showed reproducible effects, though more data are required for validation. However, discriminatory power of these markers is still limited when comparing subjects based on diagnosis only [9]. In addition, findings still vary in details such as time course of changes and strength of interaction with amyloid or tau, respectively. Findings within this study underlined the importance of standardization of procedures and stratification for pathological subgroups and covariates. Most striking were differences in age-dependent trajectories of immune markers between tau-positive or -negative individuals that were independent of clinical diagnosis of MCI, AD, or PD. These proteins might present a pattern of response to neurodegeneration present in different disorders, proofing the concept to characterize subjects not only symptomatic, but also by molecular/cellular taxonomies of disease. Furthermore, they could serve as readout markers in interventional studies targeting neuroinflammation, but only when important confounders such as age or sex are considered. In conclusion, future study designs should enable such multivariate stratification of cohorts to increase the discriminatory power of immunity markers as this could be decisive for the applicability of these biomarkers in the clinical context.

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Supplementary Data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jalz.2019.07.018.

RESEARCH IN CONTEXT

1. Systematic review: This study was designed based on previous articles and PubMed literature search. It was further embedded in the European, Innovative Medicines Initiative project AETIONOMY that facilitated interdisciplinary exchange between clinicians and researchers.
2. Interpretation: In a multicenter cohort, the study verified many previous findings for immunity-associated biomarkers in cerebrospinal fluid. However, it also showed that immune marker levels were associated with severity of neurodegeneration (reflected by tau levels) rather than clinical diagnosis of Alzheimer's disease or Parkinson's disease. Age and sex of patients, but also center-specific factors, had strong influence on the immunity markers.
3. Future directions: (A) To investigate and standardize preanalytical factors not only for amyloid and tau but also for immunity biomarkers in cerebrospinal fluid. (B) To characterize immune biomarker levels in dependence of pathology markers plus aging, sex, and genetic factors in large cohorts. (C) To characterize patients not only by symptoms but also by molecular markers representative of multifactorial brain pathologies.

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