Amyloid-β-Secondary Structure Distribution in Cerebrospinal Fluid and Blood Measured by an Immuno-Infrared-Sensor: A Biomarker Candidate for Alzheimer’s Disease

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Supporting Information

ABSTRACT: The misfolding of the Amyloid-beta (Aβ) peptide into β-sheet enriched conformations was proposed as an early event in Alzheimer’s Disease (AD). Here, the Aβ peptide secondary structure distribution in cerebrospinal fluid (CSF) and blood plasma of 141 patients was measured with an immuno-infrared-sensor. The sensor detected the amide I band, which reflects the overall secondary structure distribution of all Aβ peptides extracted from the body fluid. We observed a significant downshift of the amide I band frequency of Aβ peptides in Dementia Alzheimer type (DAT) patients, which indicated an overall shift to β-sheet. The secondary structure distribution of all Aβ peptides provides a better marker for DAT detection than a single Aβ misfold or the concentration of a specific oligomer. The discrimination between DAT and disease control patients according to the amide I frequency was in excellent agreement with the clinical diagnosis (accuracy 90% for CSF and 84% for blood). The amide I band maximum above or below the decisive marker frequency appears as a novel spectral biomarker candidate of AD. Additionally, a preliminary proof-of-concept study indicated an amide I band shift below the marker band already in patients with mild cognitive impairment due to AD. The presented immuno-IR-sensor method represents a promising, simple, robust, and label-free diagnostic tool for CSF and blood analysis.

Alzheimer’s disease (AD) is the most common cause of dementia and affects over 35 million individuals worldwide. Clinical and research evidence indicates that the neuropathology starts 10–20 years before AD becomes clinically overt.1–4 The amyloid cascade hypothesis postulates5 that misfolding and subsequent aggregation of Aβ peptides is responsible for neurodegeneration. However, it is an ongoing discussion, whether Aβ aggregation is the cause or epiphenomenon of AD.6 Nevertheless, aggregation of Aβ peptides into neurotoxic soluble oligomeric species and finally into insoluble fibrillar structures is associated with AD.7,8 Thus, misfolding precedes or accompanies aggregation.

Widely accepted criteria for the clinical diagnosis of Alzheimer’s disease were published by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer’s Disease and Related Disorders Association (ADRSA) in 1984.9,10 Updated and revised diagnostic guidelines were elaborated 2007–2011.11–15 The two most important differences to the original NINCDS-ADRSA criteria were (i) the definition of three phases of the disease: preclinical, symptomatic predementia (mild cognitive impairment due to AD, MCI-AD), and dementia due to AD (DAT = Dementia Alzheimer type) and (ii) the incorporation of biomarkers of Aβ accumulation and biomarkers of neurodegeneration or injury.12 Of particular clinical relevance for the development of therapies is the reliable identification of preclinical or predementia stages and the clear distinction between DAT and other forms of dementia premortem.16 Even today, a definite AD diagnosis can only be given post-mortem. However, the performance of current diagnostic tools for early stages is limited17–19 and novel diagnostic biomarker candidates20–22 remain inadequate or have not fully entered clinical routine yet.14,15,23 In this context, several biomarker studies have shown that Aβ-aggregates are rich in β-sheets,24–28 as compared to the unfolded or partially α-helical individual peptides. Additionally, the presence of individual oligomeric Aβ species in CSF and blood was described, but the concentration of these species itself may not correlate with early stages of AD.29–31 Here, we present the application of a novel immuno-
infrared-sensor (Figure 1), which monitors the secondary structure distribution of all Aβ states extracted from CSF and blood, especially its α-helix and β-sheet secondary structure distribution.

For the analysis of the secondary structure distribution of peptides and proteins, Fourier transform-infrared (FT-IR) spectroscopy is an excellent tool. Secondary structure analysis of proteins associated with neurodegenerative disorders has already been performed on purified, isolated proteins. In these studies, the infrared amide I absorbance band between 1700 and 1600 cm\(^{-1}\) was analyzed. The amide I band originates from the protein backbone C=O stretching vibration. Its frequency depends on the respective secondary structure.

In our study, an immuno-IR-sensor was applied to complex body fluids. Briefly, an antibody is attached to the surface of the internal reflection element of a flow-through attenuated total reflection (ATR) setup to extract Aβ peptides out of the body fluid. Thereby, the secondary structure distribution of the surface attached Aβ peptides from CSF and blood plasma was measured without further purification or concentration. A sufficient signal/noise ratio of the amide I band of Aβ extracted from CSF or blood plasma was achieved.

The immuno-IR-sensor indicated that the overall Aβ secondary structure distribution was shifted mostly toward the misfolded β-sheet in DAT patients. Thus, the amide I band frequency above or below a decisive spectral marker frequency was used to discriminate between DAT and disease control (DC) patients. By analyzing 99 CSF samples, we observed a sensitivity of 94% and a specificity of 88%, whereas an analysis of 86 blood samples resulted in a sensitivity of 75% and a specificity of 88%. The CSF results were validated with an additional set of patient samples. An accuracy of 90%, sensitivity of 88%, and specificity of 93% were determined.

As compared to conventional ELISA tests, the sensor has the advantage to monitor not the concentration of a single Aβ folding state or of selected oligomers, but the overall distribution of the Aβ fraction in CSF and blood. Thereby, the secondary structure distribution of all soluble Aβ forms is detected in the amide I band as an averaged signal. We propose this average distribution as an easily accessible, novel biomarker candidate for DAT and potentially for MCI-AD.

**EXPERIMENTAL SECTION**

The prospective study fulfills the international standards for studies of diagnostic test accuracy in dementia.

**Patient Cohorts and Clinical Phenotyping.** The patient cohort was divided into three subgroups, probable dementia due to Alzheimer’s disease (Dementia Alzheimer type, DAT), mild cognitive impairment due to AD (MCI-AD), and disease controls (DC). The DC group comprised patients with dementia of other origin and nondemented patients with heterogeneous neurological or psychiatric diseases but without memory complaints. Patients with probable dementia due to Alzheimer’s Disease (DAT, n = 33), MCI-AD (n = 11), and disease controls (DC, n = 66) were admitted to the gerontopsychiatric unit of the department of psychiatry and psychotherapy, LVR Clinics, University of Duisburg-Essen, for dementia diagnostics from 07/2009 to 12/2013. CSF and blood samples were collected and stored at one clinical center adhering to one standard operation procedure to minimize storage and sampling handling artifacts. The patient cohort consisted of 46 male (10 DAT, 33 DC and 3 MCI-AD, mean age 67 ± 11 years) and 64 female (23 DAT, 33 DC, and 8 MCI-AD, mean age 70 ± 12) patients, as summarized in Table S-1A. Clinical diagnosis of DAT was made according to the criteria of the National Institute for Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA). The majority of patients with DAT presented with early AD (18/33) and 10 patients were seen within a moderate clinical disease stage. Diagnosis of MCI-AD was performed according to the 2011 recommendations from the National Institute on Aging. Memory complaints in MCI patients had to be associated with a performance in the mini mental state examination test (MMSE) and a minimum of 25 MMSE test score, preserved activities of daily living, and a CSF biomarker pattern (elevated phospho-Tau/total-Tau and decreased Aβ peptide ratio 1-42/1-40) indicating the AD predementia stage. The clinical diagnosis of DC was performed according to the International Classification of Diseases (ICD-10). The DC cohort included patients without dementia and patients with schizophrenia, bipolar disorders, depression, and dementia not due to AD. The diagnosis of DC patients was cross validated by their CSF dementia biomarker and neuroimaging panel, that is patients with a biomarker signature indicating DAT (decreased Aβ peptide ratio 1-42/1-40 and/or neuroimaging) were excluded. Standardized clinical assessment was conducted by gerontopsychiatrists and neuropsychologists within the setting of an University Memory Clinic, and the clinical diagnosis was independently validated by an expert gerontopsychiatrist (JW). Gerontopsychiatrists and neuropsychologists had access to all available clinical, neuroimaging, psychometric, and conventional CSF dementia biomarker data but were blinded for the FT-IR-analysis results. Patients were investigated by psychometric testing (mini mental state examination, MMSE, and/or extended neuropsychological
evaluation) and CSF-guided neurochemical dementia diagnostics (CSF-NDD). For a subset of patients, in addition, neuroimaging (cranial computed tomography or 3 T magnetic resonance imaging) data were available. For some patients the complete set of diagnostic measures could not be performed (for details see Table S-2). The standard operating procedures for sampling of lumbar CSF and EDTA-blood plasma (sample taking, preanalytical sample handling, biomaterial banking, shipment) followed the guidelines of the German Competence Net Dementias.\textsuperscript{40} CSF-concentrations of \(\text{A}^{\beta}_{1-42}, \text{A}^{\beta}_{1-40}\), total-Tau (tau), and phospho-Tau181 (ptau) were measured in duplicate by commercially available ELISA in an accredited expert laboratory for CSF-NDD (P. Lewczuk, University of Erlangen-Nürnberg, Germany).\textsuperscript{41,42} Since not for every single patient the CSF \(\text{A}^{\beta}\) peptide concentration ratio \(\text{A}^{\beta}_{1-42}/\text{A}^{\beta}_{1-40}\) as determined by the Erlangen laboratory was available (compare Table S-2), all CSF samples were additionally assessed with the MSD V-Plex \(\text{A}^{\beta}\) peptide panel multiplex kit employing monoclonal antibody 6E10 for detection (Meso-Scale Discovery, Rockville MD). The \(\text{A}^{\beta}\) peptides \(\text{A}^{\beta}_{38, 40, 42}\) were determined according to the manufacturer’s instructions after 16-fold dilution of the CSF samples with “Diluent 35” (MSD). The \(\text{A}^{\beta}_{42/40}\) ratios calculated from the multiplex- assay data were in good agreement with the CSF-NDD ELISA data (Spearman \(r = 0.81, p < 0.001, 80\) pairs). Table S-2 summarizes the clinical, psychometric (MMSE) and CSF biomarker phenotyping data of the patient collective, including cutoff values, mean, standard deviation, and MIN-MAX values for MMSE and the CSF dementia biomarkers.

The study was approved by the ethical board of the University of Duisburg-Essen (ID 12 S160 BO) and the research use of the samples and data was in accord with the terms of the informed consents.

From continued patient recruitment within the study, CSF samples of 31 similarly well-characterized patients (14 DC, 17 DAT) were available. Age and gender matched well with the main cohort (Table S-1B). These samples were used as additional validation set.

\section*{Workflow.}

For the FT-IR-spectroscopic analysis of \(\text{A}^{\beta}\) peptides present in body fluids, our published protocol\textsuperscript{32} had to be slightly optimized.

Briefly, a Vertex 70 V FT-IR-spectrometer (Bruker, Ettlingen, Germany) with external MIR-source, equipped with a 24 h IN2-cooled MCT-detector and a vertical variable angle ATR-setup (Specac, Orpington, U.K.), with a trapezoid Ge-crystal (52 mm \(\times 20 \text{ mm} \times 2 \text{ mm}\), Korth Kristalle, Altenholz, Germany) as an internal reflection element was used (see Figure S-1 for a schematic drawing).

The total volume of the flow-cell including all connection tubes was reduced to 450 \(\mu\text{L}\). Thus, the volume dependent concentration sensitivity for \(\text{A}^{\beta}\) was optimized. For each analysis, one sensor element per sample was functionalyzed with silane-monolayers\textsuperscript{43} and antibodies were covalently attached. The surface was blocked with casein to generate an inert surface against other CSF and blood components. Within our optimized method, the monoclonal antibody A8978 (Sigma-Aldrich, aa13-28) was used instead of 1E8, which had been applied in the original protocol\textsuperscript{32} for \(\text{A}^{\beta}\) capturing. For the analysis, 50 \(\mu\text{L}\) of CSF or 150 \(\mu\text{L}\) of EDTA-blood plasma were thawed immediately before analysis and added to the circulating buffer, respectively. The flow-rate was set to 1 \(\text{mL}/\text{min}\). All procedural steps needed 4 h in total.

\section*{RESULTS AND DISCUSSION}

\section*{Analysis of \(\text{A}^{\beta}\) Peptide Secondary Structure in Body Fluids.}

For the present analysis, our sensor was functionalized with antibody A8978 recognizing a core epitope of \(\text{A}^{\beta}\) peptides (aa \(\text{A}^{\beta}_{13-28}\) to extract all \(\text{A}^{\beta}\) variants at the same time (Figure 1A,B). All preparative steps were highly reproducible and controlled by corresponding infrared spectra. Neither albumin (HSA), nor IgG2, nor \(\alpha\)-synuclein bound unspecifically to the sensor surface (Figure S-2). In addition, \(\text{A}^{\beta}\) binding was confirmed with fluorescence-labeled antibody 1E8 (Figure S-3).

No induction of specific secondary structures was observed during \(\text{A}^{\beta}\) binding to the antibody (Figure S-4 and Nabers, Ollesch et al.\textsuperscript{32}). Hence, neither A8978 nor 1E8 were selective for a specific secondary structure (Figure S-5 and Nabers, Ollesch et al.\textsuperscript{32}).

\(\alpha\)-Helical and random coil monomeric synthetic \(\text{A}^{\beta}_{1-42}\) or \(\beta\)-sheet rich fibrillar synthetic \(\text{A}^{\beta}_{1-42}\) peptide bands showed the characteristic amide I bands at 1652 \text{ cm}^{-1} \text{ for } \alpha\text{-helix and 1627 cm}^{-1} \text{ for the } \beta\text{-sheet, respectively (Figure 1C,D). Purified monomeric, oligomeric, and prefibrillar preparations of synthetic \(\text{A}^{\beta}_{1-42}\) and \(\text{A}^{\beta}_{1-40}\) could be easily distinguished by their characteristic amide I band (Figure S-5A–D). In contrast, the overall amide I band of \(\text{A}^{\beta}\) extracted out of CSF or blood plasma reflects the overall distribution of secondary structures of all \(\text{A}^{\beta}\) peptide variants present in the sample. For instance, scaled linear in silico combinations of different secondary structure distributions illustrated an amide I band shift to lower wavenumbers by increasing the \(\beta\)-sheet content (Figure S-5E). This effect became more evident by adding synthetic fibrillar \(\text{A}^{\beta}_{1}\) (1 ng/mL) to the sensor-surface immobilized \(\text{A}^{\beta}\) fraction extracted from CSF of a DC patient. As a result, fibrillar \(\text{A}^{\beta}\) attached to the antibody layer and contributed to the overall amide I band. Consequently, the amide I band of \(\text{A}^{\beta}\) shifted from 1648 to 1641 \text{ cm}^{-1} \text{ (Figure S-6). The combination of immunological specificity of the ATR surface with an ATR flow-through system provides the amide I absorbance band of the extracted \(\text{A}^{\beta}\) fraction with a sufficient signal/noise ratio under physiological and non-denaturing sample conditions.\textsuperscript{32} In summary, the applied sensor monitors the overall secondary structure distribution of the total \(\text{A}^{\beta}\) peptide fraction extracted from CSF and blood.
Spectral Discrimination of DAT and DC Patients Based on CSF. In Figure 2A, the amide I frequencies of DC and DAT patients are shown. DAT patients exhibited a significantly lower average amide I band maximum position as compared to the DC group \( \left( p < 0.0001 \right) \). The average amide I maximum was 1645 ± 3 cm\(^{-1}\), with a median value of 1646 cm\(^{-1}\) for the DC group. The 25–75% percentiles were between 1644 and 1647 cm\(^{-1}\). The average amide I maximum of DAT patients was downshifted to 1640 ± 3 cm\(^{-1}\) with a median of 1641 cm\(^{-1}\). The 25–75% percentiles were between 1639 and 1642 cm\(^{-1}\) (Figure 2A). The amide I positions of the CSF samples did not show a Gaussian normal distribution. Therefore, we used the nonparametric Kruskal-Wallis analysis of variance to indicate a significant difference of the distributions to a confidence level of 0.05. The signal downshift indicates an accumulation of \( \beta \)-sheet structures of aggregated \( A\beta \) conformations in CSF of DAT patients. The amide I band above or below a marker band can be used for the discrimination between DC and DAT. The classifying marker frequency was determined by 30 iterative ROC-curve analyses of 30 randomly selected eight vs eight DC/DAT training-data sets out of the total patient collective (details see methods). Each repetition on the training-data sets yielded a best discriminating marker frequency value for maximal accuracy. This frequency was consistently determined at 1643 cm\(^{-1}\). Thus, a robust and simple marker band for DC/DAT discrimination was determined. ROC curve analysis of the total patient collective indicated an area under the curve (AUC) of 0.90 (Figure 2B) with a maximum accuracy consistent at 1643 cm\(^{-1}\). Assigning every patient with an amide I band maximum frequency <1643 cm\(^{-1}\) as DAT patient, an accuracy of 90\%, a sensitivity of 94\%, and a specificity of 88\% was achieved for CSF samples, with regard to the clinical diagnosis (Table 2A). Thus, an accurate discrimination between the two diagnostic groups was achieved. Only 2 out of 33 clinical DAT patients were falsely assigned negative, and only 8 out of 66 control patients were falsely assigned positive.

Additionally, the identified marker band criterion, amide I frequency <1643 cm\(^{-1}\) denotes DAT, was validated using an independent data set of 31 patients not included into the main analysis. Thus, 14 DC and 17 DAT patients were discriminated with an accuracy of 90\%, sensitivity of 88\%, and specificity of 93\%. In the next step we compared the amide I band frequency with other clinically established markers as the CSF concentrations of \( A\beta \)1-42 (Figure S-7A), ptau \( \left( r = -0.57, p = 1.3 \times 10 ^{-10}, \text{Figure S-7C} \right) \), and ttau \( \left( r = -0.54, p = 1.2 \times \right) \).
10−9, Figure S-7D) and with the Aβ42/40 ratios as determined with two assays (r = 0.58, p = 2.1 × 10−6 (ELISA) and r = 0.58, p = 2.0 × 10−11 (MSD), Figure S-7B and Table 1).

Table 1. Summary of the Spearman Rank Correlation (rS) and the Significance Value (p Value for a Niveau of α = 0.05) between the Amide I Maximum Positions and Neurochemical Biomarkers of CSF Samples

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<td>Aβ1-42/Aβ1-40 (ELISA, Erlangen lab)</td>
<td>0.5771</td>
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<td>Aβ1-42/Aβ1-40 ratio (m essoscale)</td>
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<td></td>
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The Spearman correlations indicate that a higher CSF concentration of ptau, ttau (both are considered biomarkers of neuronal destruction), or the decreased Aβ42/40 ratio is accompanied by the amide I band downshift (Table 1, Figure S-7). The amide I maximum frequency of Aβ did not correlate with the age of participants or the Aβ1-40 CSF-concentration (Table 1).

Spectral Discrimination of DAT and DC Patients Based on EDTA-Blood Plasma. Next, we applied the novel infrared-sensor to the analysis of EDTA-blood plasma, which is more easily accessible than CSF. Within the study, samples of 28 DAT and 58 DC patients were available. The DC group showed an average amide I maximum of 1647 ± 4 cm−1 (median 1649 cm−1), whereas the average maximum of the DAT group was significantly downshifted to 1642 ± 4 cm−1 (median 1641 cm−1, p < 0.0001, Figure 2C). Again, the amide I positions of the plasma samples did not show a Gaussian normal distribution, but the nonparametric Kruskal–Wallis analysis of variance indicated a significant difference of the distributions to confidence level 0.05. The identical marker frequency classifier as used before (<1643 cm−1) was determined by a randomized 30-times repeated 8 + 8 versus 76 patient cross validation, performed as described. It resulted in a discrimination of the diagnostic groups with an accuracy of 85%, a sensitivity of 75% and a specificity of 88%. Thus, only 7 false positive of 58 DC patients and 7 false negative of 28 participants with DAT were falsely classified. ROC analysis yielded an AUC of 0.83 (Figure 2D). In comparison to the analysis of Aβ from CSF, the accuracy and sensitivity were lower in blood.

Discrimination of DC and MCI-AD Patients. In the next step we investigated the capability of the method to identify AD patients in the MCI-stage (MCI-AD). However, because of a comparatively small number of patients with MCI-AD (n = 11) our data have to be regarded as a preliminary proof-of-concept study. We analyzed CSF and plasma of 11 MCI-AD patients. As explained above, the MCI-AD patients presented elevated tau and decreased Aβ1-42 levels in CSF, indicating incipient Alzheimer’s disease.

The median Aβ amide I band recorded from MCI-AD CSF samples exhibited a downshift below the same 1643 cm−1 marker band as shown for DAT patients (Figure 3). A total of 8 out of 11 MCI-AD patients were correctly classified by our sensor. Three of the MCI-AD patients exhibited an amide I frequency >1643 cm−1 and thus appear to be “false negative”. MCI-AD patients yielded an average amide I maximum at 1642 ± 3 cm−1 (below the marker band), with the median identical to the DAT group at 1641 cm−1 (Figure 3A).

Box-plots indicate that the MCI-AD and DC group (same as Figure 2A,C) statistically significantly differ in the amide I band distributions (p < 0.005). The marker frequency criterion of the amide I maximum below 1643 cm−1, an accuracy of 86%, sensitivity of 73%, and a specificity of 88% was reached for the discrimination of MCI-AD patients from the DC based on CSF (Table 2B). ROC analysis yielded an AUC of 0.79 (Figure 3B).

We further analyzed EDTA-blood plasma of MCI-AD patients. Again, the Aβ amide I bands showed downshifts below 1643 cm−1 for 5 out of 10 patients. Box-plots comparing the amide I maxima of MCI-AD and DC patients indicate a

Figure 3. Distributions of the amide I maximum frequencies as recorded of CSF and plasma indicate a separable spectral downshift associated with MCI-AD progression (A). A marker band at 1643 cm−1 separates DC with 86% accuracy for CSF and 83% for plasma from (dashed line) MCI-AD. 25/50/75% quantiles are displayed in box-plots as horizontal lines, the average band position (square), ± standard deviation (whiskers), and observed minimum/maximum values (x) are displayed. A ROC curve was obtained by variation of the marker frequency (B). An AUC of 0.79 was achieved for CSF (yellow) and 0.71 for plasma (orange).
A hand, no correlation was found with the less amyloidogenic pathological misfolding of Aβ. This supports the idea that the amide I marker band in DAT patients was inversely sensitive amide I band frequency. The observed downshift of indicated by a spectral downshift of the secondary structure conformations in CSF and blood of DAT patients. This was available 130 CSF DAT and DC samples with an accuracy of downshifted and might be predictive for AD before clinical

β-amyloid peptides in CSF and in plasma of MCI-AD patients were enriched Aβ an increased level of misfolded states, especially of peptides in CSF and blood samples of AD patients may show an increased level of misfolded states, especially of β-sheet enriched Aβ conformations. Employing a novel immuno-IR-sensor, we con

In summary, the average amide I band positions of Aβ peptides in CSF and in plasma of MCI-AD patients were downshifted and might be predictive for AD before clinical symptoms appear.

CONCLUSIONS

The accumulation of amyloid in the brain of AD patients is a characteristic feature of Alzheimer’s disease and starts years before cognitive decline manifests.1 4 This suggests that Aβ peptides in CSF and blood samples of AD patients may show an increased level of misfolded states, especially of β-sheet enriched Aβ conformations in CSF and blood of DAT patients. This was indicated by a spectral downshift of the secondary structure sensitive amide I band frequency. The observed downshift of the amide I marker band in DAT patients was inversely correlated with the decrease of Aβ1-42 in CSF. On the other hand, no correlation was found with the less amyloidogenic Aβ1-40. Moreover, the amide I band correlated positively with the tau protein concentration in CSF, which is considered a biomarker of neuronal damage. This supports the idea that pathological misfolding of Aβ is associated with neurotoxicity. However, the analysis of CSF samples with the novel immuno-IR-sensor revealed an accuracy of 90%, whereas the analysis of blood plasma yielded an accuracy of 84% for the identification of DAT patients (Table 2). Thus, the results obtained with the novel infrared-sensor using CSF and blood samples were in excellent agreement with the CSF-NDD supported clinical AD diagnosis.

The results obtained from CSF samples were validated using a limited, but independent sample set, indicating a prediction accuracy of 90%. More importantly, the deciding marker frequency appears to robustly distinguish the DAT and DC classes. The marker frequency 1643 cm⁻¹ distinguished the available 130 CSF DAT and DC samples with an accuracy of 90%, a sensitivity of 92%, a specificity of 89%, and an ROC of 0.89 (Figure S-8).

Still, these findings have to be validated in a larger, independent clinical study. Interestingly, a similar spectral downshift was observed in CSF and blood of a small number of MCI-AD patients. The identical discriminative marker frequency, which was applied for clinically overt AD, separated the MCI-AD and DC group, however, with a reduced accuracy of approximately 83%. Therefore, the Aβ amide I band frequency appears to provide a highly promising novel biomarker candidate and our assay may identify AD at a predementia stage. The immuno-IR-sensor comprises several advantages: The label-free analysis does neither rely on the misfolding of a single, specific Aβ peptide species nor on the quantification of a specific soluble oligomeric form. In addition, our assay provides an intrinsic spectroscopic control of each preparative step. Thereby, preparative errors can be easily identified and subsequently avoided.

For optimum reproducibility of the results, we did not reuse sensors after partial removal of the surface functionalization. Naturally, a reusable sensor element would increase the analytical throughput. Methods for regeneration remain to be researched.

Regarding the patient collective, our study is limited by a challenge bias (STARDdem criteria38) since only patients with predominantly early or prodromal AD have been investigated, and only patients with probable DAT were included (possible DAT excluded). Moreover, only patients were included whose diagnoses were cross-validated by CSF dementia biomarkers. Finally, patients attending an expert university memory clinic may not be representative for the general AD population.

Summing up, the application of an immuno-IR-sensor to measure the Aβ secondary structure distribution in CSF and blood plasma was demonstrated. Our novel findings indicate Aβ misfolding into β-sheet enriched conformations in body fluids of DAT and even MCI-AD patients. Using the sensor, excellent accuracies of 84–90% were obtained and validated. Thus, the secondary structure distribution of Aβ in CSF and blood provides a promising novel biomarker candidate to support the clinical diagnosis. Notably, these results were achieved with a single test and a single readout parameter. In general, the patients were phenotyped clinically and by well-established CSF dementia biomarkers (total-Tau, phospho-Tau, and the Aβ peptide ratio 1-42/1-40), which were measured by a single expert laboratory (P. Lewczuk, Erlangen). Like F18-Amyloid-PET, CSF guided neurochemical dementia diagnostics can be regarded as a state-of-the-art cross validation of the clinical AD diagnosis.

Follow-up studies are planned to (i) verify the findings in an independent cohort, to (ii) further evaluate the diagnostic potential with emphasis on preclinical stages, to (iii) research whether a combination of the amide I maximum position with one or more of the established biomarkers may further increase the accuracy of AD diagnosis, and to (iv) evaluate the potential of the technology to investigate the secondary structure distribution of other proteins known to be associated with neurodegenerative disorders like α-synuclein for Parkinson disease. Thus, the immuno-sensor may also support the biomarker guided diagnosis of other protein misfolding diseases, such as Parkinson disease, Parkinson dementia, dementia with Lewy bodies, subphenotypes of Frontotemporal

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Lobar Degeneration, Chorea Huntington, and prion diseases. Furthermore, this sensor could potentially also be used to monitor the therapeutic efficacy of drug candidates which support the refolding of Aβ back to the less neurotoxic α-helical form.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.6b04286.

Schematic drawing of the immuno-IR-setup; reproducibility of the immuno-IR-sensor and evidence of Aβ extraction from CSF and blood by fluorescence spectroscopy; antibody A8978 did not induce a secondary structure change; Aβ monomers, oligomers and fibrils display different amide I maxima; increased β-sheet isomers shift the amide I band of Aβ in disease control patients; correlation of AD biomarkers with the Aβ amide I maximum position; validation of the marker band criterion for DAT identification on CSF; table of summary of sample specifications; and phenotyping data of the sample collective and comparison of CSF and blood-plasma IR-analysis (PDF)

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**Notes**

*For questions regarding the clinical issues in the Supporting Information, please contact J.W. at jens.wiltfang@med.uni-goettingen.de.*

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## REFERENCES

