including the secondary structure change of Tau out of CSF for Alzheimer's disease detection provides more than an additive effect on the diagnostic accuracy. Analyzing the secondary structure distribution of Aß (e.g. in CSF and/or blood plasma), and Tau (e.g. in CSF) enables the sub-classification of Alzheimer's disease in mild to severe disease stages, and the differentiation between AD and other dementia types.

Summary

In one aspect, there is provided a method for the differential diagnosis and sub-classification of Alzheimer's disease into different disease stages by direct analysis of the secondary structure distribution of a soluble Amyloid-beta (Aß) peptide fraction and a soluble Tau protein fraction in bodily fluids, comprising the steps

(a) conducting, in a first IR cell comprising a first infrared sensor element having an internal reflection element with a core of an infrared transparent material and at least one receptor for the Aß peptide directly grafted to at least one surface of said core, said at least one receptor for the Aß peptide being antibodies capable of specific and conformationally independent binding to the Aß peptide, and being directly grafted to at least one surface of said internal reflection element by silanization with short silane linkers or by thiolation with short thiol linkers, reacting freely accessible amine groups of said at least one receptor with amine-reactive groups on the short silane/thiol linkers, and blocking remaining amine-reactive groups on the short silane/thiol linkers with a blocking substance not cross-reacting with the Aß peptide, at least one flux of a body fluid with soluble Aß peptide; submitting an IR beam through said first IR cell; and obtaining an infrared spectrum therefrom;

(b) conducting, in a second IR cell comprising a second infrared sensor element having an internal reflection element with a core of an infrared transparent material and at least one receptor for the Tau protein directly grafted to at least one surface of said core, said at least one receptor for the Tau protein being antibodies capable of specific and conformationally independent binding to the Tau protein, respectively, and being directly grafted to at least one surface of said internal reflection element by silanization with short silane linkers or by thiolation with short thiol linkers, reacting freely accessible amine groups of said at least one receptor with amine-reactive groups on the short silane/thiol linkers, and blocking remaining amine-reactive groups on the short silane/thiol linkers with a blocking substance not cross-reacting with the Tau protein, at least one flux of a body fluid with soluble Tau protein; submitting an IR beam through said second IR cell; and obtaining an infrared spectrum therefrom; and

(c) analyzing the obtained infrared spectra to determine the secondary structure distribution of the soluble Aß peptide and of the soluble Tau protein in the bodily fluids for the differential diagnosis, wherein a downshift of the amide I band of the Aß peptide and/or of the Tau protein is indicative for the disease stage.

In another aspect, there is provided a kit for the differential diagnosis and sub classification of Alzheimer's disease into different disease stages comprising a first and second infrared sensor element as defined herein.

In another aspect, there is provided a device for the differential diagnosis and sub classification of Alzheimer's disease into different disease stages, said device comprising a first and second infrared sensor element as defined herein.
In another aspect, there is provided use of the first and second infrared sensor element defined herein, the kit defined herein or the device defined herein for direct analysis of the secondary structure distribution of a soluble Amyloid-beta (Aß) peptide fraction and a soluble Tau protein fraction in bodily fluids.

Short Description of the Figures

Figure 1: Scheme of the combined immuno-infrared assay and principle of the analysis. (A) The total fraction of Aß (1) and Tau (2) present in CSF and/or plasma were separately extracted using an antibody functionalized immuno-infrared sensor. The detected Aß and Tau secondary structure distribution is indicated by the infrared amide I maximum position.

Figure 2: Distribution of the amide I maximum position as displayed in box-plots for DC and DAT discrimination based on the analysis of Aß in CSF and plasma, and Tau in CSF. Both diagnostic groups showed a high significant difference in the amide I maximum of Aß out of CSF ($p=2.5*10^{-11}$, Kruskal-Wallis ANOVA, confidence level $a=0.05$) and EDTA-plasma ($p=3.4*10^{-9}$), and a moderate significance for Tau out of CSF ($p=1.6*10^{-3}$). In Box-plots 25/50/75% quantiles are displayed as horizontal lines, the average band position as square, ± standard deviation as whiskers, and observed minimum/maximum values as cross.

Figure 3: 3D-scatter plot of the amide I maximum position as determined for Tau in CSF, Aß in CSF and Aß in EDTA-plasma for 61 DC (grey) and 39 DAT (black) samples. Data points within the transparent black box indicate subjects which are identified as DAT in all three assays.

Figure 4: ROC-curve analysis for DC (n=61) and DAT (n=39) differentiation based on the determination of the Aß secondary structure distribution in CSF (A), Aß in EDTA-plasma (B), and Tau protein in CSF (C). In this order an AUC of 0.90, 0.85, and 0.67 was achieved. Thus, a diagnostic accuracy of 92 % (Aß, CSF), 85 % (Aß, plasma), and 68 % (Tau, CSF) was calculated (D). Based on these data, the Tau secondary structure distribution alone seems more to be a general biomarker for dementia than for the specific DAT detection.

Figure 5: Diagram of the procedure for the differential diagnosis and disease stage classification of DAT and other types of dementia by the combined immuno-infrared assay. The assay is based on the determination of the Aß peptide and Tau protein