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 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, respectivelyat 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 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

CLAIMS

1. 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 (Aβ) 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 Aβ peptide directly grafted to at least one surface of said core, said at least one receptor for the Aβ peptide being antibodies capable of specific and conformationally independent binding to the Aβ peptide, at least one flux of a body fluid with soluble Aβ 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, respectivelyat 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 Aβ 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 Aβ peptide and of the Tau protein is indicative for the disease stage.

2. The method of claim 1, wherein the infrared transparent material of the first and second IR cell is independently selected from silicon, germanium, zinc selenide, gallium selenide and diamond, and preferably is germanium.

3. The method of claim 1, wherein said first and second infrared sensor elements comprise a germanium internal reflection element being of trapezoid or parallelogram shape, and at least one receptor for the Aβ peptide or for the Tau protein being antibodies capable of specific and conformationally independent binding to the Aβ peptide or to the Tau protein, respectively, and being directly grafted to at least one surface of said internal germanium 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 Aβ peptide or the Tau protein, respectively.

4. The method of claim 1 or 3, wherein the internal reflection element

(i) is a germanium monocrystal, preferably is a trapezoid cut germanium monocrystal; and/or

(ii) allows for or provides for more than one passages of the infrared light through the reflection element; and/or

(iii) is further suitable for the alternative or parallel analysis by another optical method including detection of fluorescence at different wavelengths; and/or

(iv) the blocking substance not cross-reacting with the Aβ peptide or the Tau protein is selected from casein, ethanolamine, L-lysine, polyethylene glycols, albumins and derivatives thereof.

5. The method of claim 1, 3 or 4, wherein the silane and thiol linkers include homogenous silane and thiol linkers, mixtures of silane linkers and mixtures of thiol linkers, and have an effective linker chain length of not more than 20 atoms or not more than 15 atoms, preferably

the silane linkers have one of the following formulas:

(i) $X_3Si-(CH_2)_n-Y-(CH_2)_{n'}-Z$,

(ii) $X_2R^1Si-(CH_2)_n-Y-(CH_2)_{n'}-Z$ or

(iii) $X(R^1)_2Si-(CH_2)_n-Y-(CH_2)_{n'}-Z$,

and the thiol linkers have the following formula:

(iv) $WS-(CH_2)_n-Y-(CH_2)_{n'}-Z$,

wherein W is R^1S- or H, X at each occurrence is independently selected from halogen and C1-6 alkoxy, n is an integer of 1 to 10, n' is an integer of 1 to 5, R^1 at each occurrence is independently selected from C_{1-6} alkyl, Y is selected from a chemical bond, -O-, -CO-, $-SO_2-$, $-NR^2-$, -S-, -SS-, $-NR^2CO-$, $-CONR^2-$, $-NR^2SO_2-$ and $-SO_2NR^2-$ (wherein R^2 is H or C_{1-6} alkyl), and Z is an amine-reactive group including $-CO_2H$, $-SO_3H$ and ester derivatives thereof.

6. The method of claim 5, wherein the infrared sensor element is obtainable by

(i) silanization and in the linkers of formulas (i) to (iii) X is independently selected from C_{1-6} alkoxy-groups, preferably from methoxy and ethoxy groups, Y is -NHCO-, Z is $-CO_2H$ or an ester derivative thereof, and n is an integer of 1 to 5 and n' is an integer of 1 to 3, preferably n is 3 and n' is 2; or

(ii) thiolation and in the linkers of formula (iv) W is H, Y is a chemical bond, Z is $-CO_2H$ or an ester derivative thereof, and n is an integer of 1 to 8 and n' is an integer of 1 to 5, preferably n is 8 and n' is 4.

7. The method of any one of claims 1 to 6, wherein

(i) the receptor binding to the Aβ peptide is an antibody, preferably is an antibody specifically binding to

the central epitope of the Aβ peptide, including antibody A8978; or

(ii) the receptor binding to the Tau protein is an antibody, preferably is an antibody specifically binding to the middle epitope of Tau and to an epitope present in all Tau variants, including antibody Tau-5.

8. The method of any one of claims 1 to 7, wherein the method provides the differentiation of Alzheimer's disease into early/prodromal, moderate, and severe disease stages, and wherein

(i) amide I maxima of the mentioned biomarkers, Aβ from CSF, Aβ from blood plasma, and Tau from CSF, are all below the discriminative threshold (1643 cm^{-1} ± 5 cm^{-1}) which are indicative for a severe disease stage,

(ii) amide I maxima of two biomarkers, one is Aβ from CSF or blood plasma and the other one is Tau from CSF, below the discriminative threshold (1643 cm^{-1} ± 5 cm^{-1}) are indicative for a moderate disease stage,

(iii) amide I maxima of one or two biomarkers (Aβ from CSF and/or blood plasma), but not Tau from CSF, below the discriminative threshold (1643 cm^{-1} ± 5 cm^{-1}) indicative for an early disease stage.

9. The method of any one of claims 1 to 7, wherein the differential diagnosis provides for an assured clinical profile of the dementia type, preferably the method comprises the detection of the secondary structure distribution of Aβ from CSF (A), Aβ from blood plasma (B), and Tau from CSF (C), most preferably the method enables the differential diagnosis of Dementia Alzheimer type (DAT) and a Disease Control (DC), wherein DAT patients being sub-classified into early, moderate, and severe DAT, and DC patients being separated into healthy controls, other diseases, and dementia due to another origin than Alzheimer's disease.

10. The method of claim 9, wherein

(i) for both biomarkers, (A) and (B) for Aβ and (C) for Tau, a discriminative threshold (1643 cm^{-1} ± 5 cm^{-1}) separates Alzheimer's disease and DC patients; and/or

(ii) the combination of (A), (B), and (C) provides a biomarker panel applicable for an assured DAT diagnosis.

11. The method of any one of claims 1 to 10, which provides information about the patient disease state based on the analysis of bodily fluids, wherein a shift of the amide I band maximum of the biomarker protein is a classifier indicative for the progression of the disease.

12. The method of claim 11, wherein a threshold classifier with a value of 1638-1648 cm^{-1} is a classifier indicative for the progression of the disease.

13. 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 in any one of claims 1 to 7.

14. 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 in any one of claims 1 to 7.

15. Use of the first and second infrared sensor element of any one of claims 1 to 7, the kit of claim 13 or the device of claim 14 for direct analysis of the secondary structure distribution of a soluble Amyloid-beta (Aβ) peptide fraction and a soluble Tau protein fraction in bodily fluids.