A new blood-based biomarker of Aβ clearance – the monocyte Aβ mid-domain assay

Wettergreen M[1], Torsetnes SB[1], Christensen E [2] and Fladby T[1, 3]

1. Akershus University Hospital, Norway, 2. Pre Diagnostics AS, Norway, 3. University of Oslo, Norway

Introduction

Deficient cerebral Amyloid beta (Aβ) clearance is implicated in the pathogenesis of Alzheimer's disease (AD), and Aβ clearance systems are potential therapeutic targets in AD. Aβ phagocytosis and degradation are important components of clearance. Innate immune-linked genetic risk factors contribute to AD, and innate immune cells; microglia in the brain and monocytes/macrophages in the peripheral blood (PB), phagocytose Aβ and contribute to clearance. We propose that Aβ34 represents a marker of amyloid clearance and may be helpful for the characterization of Aβ turnover in clinical samples (Liebsch 2019).

Several proteases degrade Aβ both *in vitro* (Rogeberg et al, 2014) and *in vivo* (CTAD 2019 poster P76), with intracellular, cell-membrane and extracellular activities. Protease activity may reflect cellular activation states, and could be manipulated for therapeutic purposes. Activity of intracellular proteases give rise to Aβ mid-domain peptides (Rogeberg et al, 2015). Two frequent cleavage sites between amino acid residue 19 (Phe) and 20 (Phe), and 34 (Leu) and 35 (Met) in the Aβ 1-42 nomenclature, generate the Aβ 20-34 peptide.

An intracellular blood monocyte assay for mid domain peptides was developed as a proxy for cerebral Aβ clearance.

Goal

Our objective was to develop a blood-based immunoassay enabling Aβ mid-domain peptide measurements in relevant biological specimens. The intracellular concentration of peptides containing mid-domain fragments reflects phagocytic activity or Aβ degradation efficiency, and may be measured in microglia and in circulating peripheral blood monocytes/macrophages.

Method

We developed an immunoassay on the Quanterix Single Molecule Array (Simoa) platform using a proprietary anti-mid-domain sheep monoclonal antibody as capture antibody (Fladby US patent: 9,625,474). The detector reagent consists of an anticomplex antibody, (Bio-Rad HuCAL technology) aiding an optimal Aβ mid-domain peptide specificity and assay sensitivity (CTAD 2019 poster P76) (Figure 1).

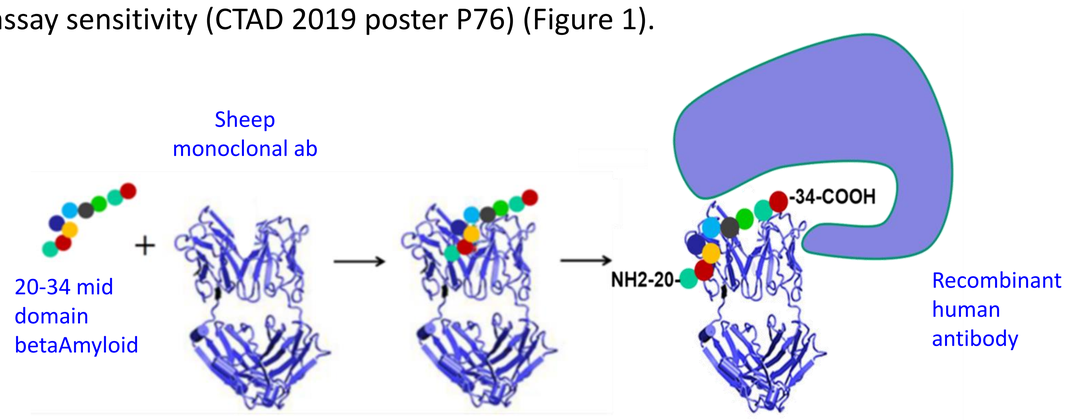

Figure 1. Sandwich assay with anti-complex antibody

Monocyte lysates from blood donors were isolated from the leukocyte fraction using a combination of density gradient centrifugation and RosetteSep antibodies from StemCell (Ca). A monocyte count was measured before cell lysis, and the monocyte concentration was adjusted to 20 000 cells/uL.

Results

The new assay with the two anti-Aβ mid-domain antibodies analyzed on the Simoa platform gave a sensitive immunoassay detecting Aβ mid-domain containing peptides with a limit of detection (LOD) of 0,78 pg/mL, and a lower limit of quantification (LLOQ) of 1,38 pg/mL* resulting in measurable levels of the peptide in monocyte lysates (Figure 2 and Table 1).

**LOD and LLOQ evaluated by the Simoa 4PL Assay development tool.*

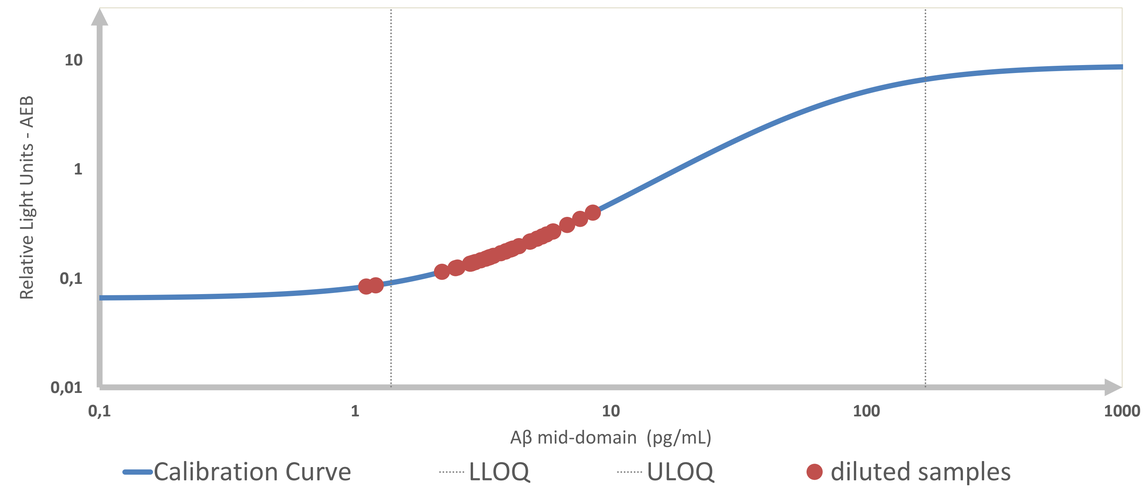

Figure 2. Calibration curve with monocyte samples.

Samples	Mean conc pg/mL	Mean CV %
Monocyte lysates (n=33, 18 female / 15 male) Age between 20-70 years	8,5 Range 4,4– 17,0	7,9 Range 0,5 – 20,8

**Of 33 samples, 1 sample below LOD, 1 sample below LLOQ*

Table 1. Results monocyte lysates.

Sample	Dilution Factor	Spike w. Aβ 20-34	Fitted conc, pg/mL	Corrected fitted conc, pg/mL	Recovery, %
Monocyte lysate pool	1:2	n.a.	8,0	16,0	100%
Monocyte lysate pool	1:4	n.a.	4,3	17,2	108%
Monocyte lysate pool	1:2	3 pg/mL	10,1	n.a.	92%
Monocyte lysate pool	1:2	6 pg/mL	12,7	n.a.	91%
Monocyte lysate pool	1:2	12 pg/mL	15,5	n.a.	78%

Table 2. Dilution factor and spike and recovery in monocyte lysates.

Sample	Untreated	Prot G bead depletion	Prot G -2A9 bead depletion	Unspecific LB509 ab depletion	2A9 depletion
Monocyte lysate pool	6,42 pg/mL	7,61 pg/mL	5,17 pg/mL	6,67 pg/mL	0 pg/mL
Percent of untreated	100%	118%	81%	100%	0%
Lysate pool + 12 pg/mL Ab 20-34	13,78 pg/mL	13,22 pg/mL	6,27 pg/mL	11,11 pg/mL	0 pg/mL
Percent of untreated	100%	96%	46%	100%	0%

Table 3. Depletion studies.

Conclusion

Aβ clearance is thought to be a key mechanism in Alzheimer disease progression, and decreased Aβ clearance precedes the clinical AD symptoms by a decade or more. Here, we present, for the first time, an immunoassay able to detect intracellular Aβ clearance.

The assay quantitated Aβ mid-domain peptides in monocytes from healthy blood donors and demonstrated good recovery in spiking and dilution studies (Table 2). In addition, depletion studies with excess antibodies removed the signals (Table 3). Thus, demonstrating acceptable assay specificity in biological samples.

This highly sensitive Simoa-assay detects Aβ mid-domain containing peptides in monocyte lysates, and may serve as a tool for peripheral blood measurement of Aβ clearance in both disease progression studies and intervention studies.

References

Liebsch, F., Kulic, L., Teunissen, C. et al. Aβ34 is a BACE1-derived degradation intermediate associated with amyloid clearance and Alzheimer's disease progression. Nat Commun 10, 2240 (2019) doi:10.1038/s41467-019-10152-w

Fladby T, Johnsen L, Blennow K. Diagnostic method for Alzheimer's disease. US patent: 9,625,474

Rogeberg M, Furlund CB, Moe MK, Fladby T. Identification of peptide products from enzymatic degradation of amyloid beta. Biochimie. 2014 Oct;105:216-20.

Rogeberg M, Wettergreen M, Nilsson LN, Fladby T. Identification of amyloid beta mid-domain fragments in human cerebrospinal fluid. Biochimie. 2015 Jun;113:86-92.

Torsetnes SB, Wettergreen M, Christensen E, Fladby T. Assessing Aβ clearance aided by mass spectrometry. CTAD 2019 Poster P76

UiO : University of Oslo

Pre Diagnostics

Akershus University Hospital