A new blood assay measuring Aβ clearance - the Aβ mid-domain immunoassay

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Background

Decreased Aβ clearance may precede Alzheimer's disease (AD) amyloid plaques and clinical AD symptoms. 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 – phagocytize and degrade Aβ. Based on this, we hypothesize that intra- and extracellular Aβ fragments, specifically mid-domain Aβ, reflect phagocytic activity or Aβ degradation efficiency.

Our objective is to develop an assay measuring Aβ mid-domain peptides in relevant biological specimens with a particular focus on blood-derived samples.

Methods and analyses

Firstly, a panel of sheep monoclonal antibodies (mAbs by Bioventix plc, UK) was raised against the Aβ20-34 peptide. Secondly, an anticomplex antibody (HuCal, BioRad, CA, USA) was screened for, needing to be selective for only Aβ20-34 bound to the most sensitive sheep mAb (Fig A). Throughout this process, both immunoprecipitation and reversed phase liquid chromatography mass spectrometry (IP LCMS) and ELISA were used to map the affinity and selectivity of antibodies, guiding to the best-performing antibodies pair (sheep mAb 2A9 and HuCal H3). Thirdly and finally, an immunoassay on the Quanterix Single Molecule Array (Simoa, Quanterix, MA, USA) platform was developed, resulting in our final product.

Results

IP LCMS experiments showed that mAb 2A9 bound to a mid-domain peptide is needed for complex formation with HuCal H3 (data not shown). As a part of the selectivity mapping, a panel of Aβ variants in buffer was used as samples demonstrating preferential binding of AβX-34>>other Aβ peptides (Fig B).

The two antibodies applied on the Simoa platform gives a sensitive immunoassay; detecting Aβ mid-domain peptides with a LOD<0.5 pg/mL and LLOQ<1.54 pg/mL (Fig C). This assay detects endogenous levels not only in monocyte lysates (Fig C), but also in other blood-derived samples and csf. Various samples from healthy subjects, study participants in AD intervention studies, and other clinical samples are currently being analyzed.

Fig A. Our proprietary anti-complex antibody pair

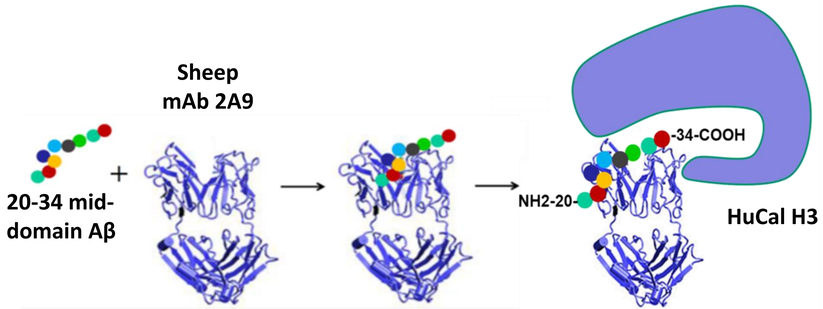

Fig B. Selectivity exploration of the antibody pair

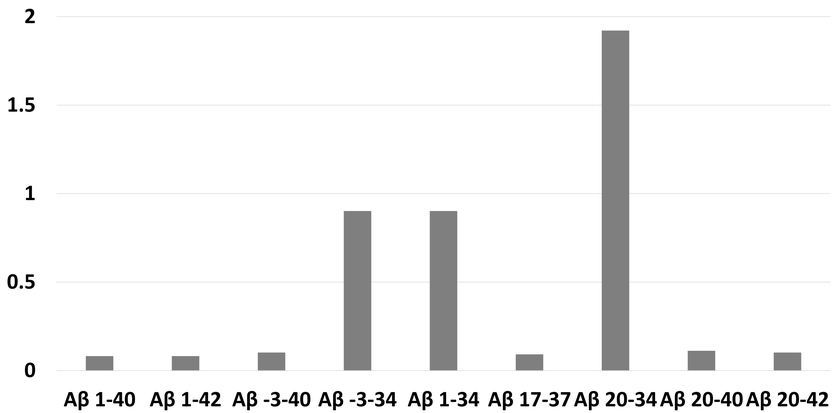

Fig C. Calibration curve and monocyte samples

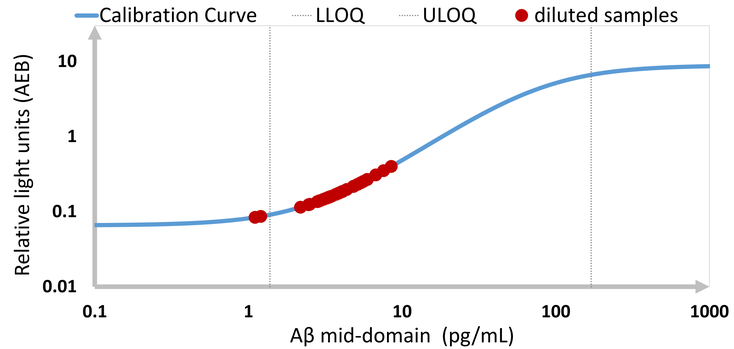

Fig A. Anti-complex antibody pair. The recombinant human antibody HuCal H3 only binds if both the proprietary antibody mAb 2A9 and an AβX-34 peptide is present.

Fig B. Sandwich immunoassay response using capture mAb 2A9 and detector antibody HuCal H3. The samples are solutions with 1 ng/mL of each of the Aβ peptides annotated by the x-axis, while the y-axis gives signal intensity responses.

Fig C. Calibration curve for monocyte samples. Sample from healthy donors (n=33) were analyzed by the use of mAb 2A9 as capture antibody and HuCal H3 as detection antibody on the Simoa platform. The samples, from 18 females and 15 males, were age matched and spanned the range of 20-70 years.

Conclusion

We present an antibody pair that forms a selective anti-complex sandwich for mid-domain AβX-34 peptides. Cleavage at the 34-bond may be relevant for intracellular Aβ detoxification, and its measurement may be useful to assess clearance. The herein presented blood immunoassay may thus serve as a tool for peripheral blood measurement in both disease progression- and intervention studies.

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