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Head-to-head comparison of amplified plasmonic exosome Aβ42 platform and single-molecule array immunoassay in a memory clinic cohort

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Abstract

Background:

Various blood biomarkers reflecting brain amyloid-β (Aβ) load have recently been proposed with promising results. However, to date, no comparative study among blood biomarkers has been reported. Our objective is to examine the diagnostic performance and cost effectiveness of three blood biomarkers on the same cohort.

Methods:

Using the same cohort (n=68), we compared the performance of the single-molecule array (Simoa)-Aβ40 and Aβ42, Aβ42/Aβ40 and the amplified plasmonic exosome (APEX)-Aβ42 blood biomarkers using amyloid PET as the reference standard. We also determined the extent to which these blood tests can reduce the recruitment cost of clinical trials by identifying Amyloid positive (Aβ+) participants.

Results:

Compared to Simoa biomarkers, APEX-Aβ42 showed significantly higher correlations with amyloid PET retention values and excellent diagnostic performance (sensitivity=100%, specificity=93.3%, AUC=0.995). When utilized for clinical trial recruitment, our simulation showed that pre-screening with blood biomarkers followed by a confirmatory amyloid PET imaging would roughly half the cost (56.8% reduction for APEX-Aβ42 and 48.6% for Simoa-

Aβ42/Aβ40) as compared to the situation where only PET imaging is used. Moreover, with a 100% sensitivity; APEX-Aβ42 pre-screening does not increase the required number of initial participants.

Conclusions:

With its high diagnostic performance, APEX is an ideal candidate for Aβ+ subject identification, monitoring, primary care screening, and could efficiently enrich clinical trials with Aβ+ participants while halving recruitment costs.

Text:

Introduction

Alzheimer's disease (AD) is a pathophysiologically heterogeneous and complex neurodegenerative disease affecting millions of individuals worldwide[1]. Supported by neuroimaging and cerebrospinal fluid (CSF) biomarkers that capture key aspects of the pathogenesis and neuropathology, the predominant amyloid cascade hypothesis poses that dyshomeostasis of Aβ triggers a cascade of toxic events: aggregation of Aβ residues from the cleavage of the amyloid precursor protein into insoluble toxic plaques inducing tau pathology, synaptic dysfunction, neuroinflammation, neurodegeneration and ultimately cognitive decline[2-5]. The introduction of amyloid neuroimaging and CSF biomarkers led to a conceptual shift towards considering the disease as a continuum, recognizing the preclinical and prodromal phases, and reinforced the importance of a pathology and biomarker-based definition of AD in lieu of a syndromal definition[6,7]. Two conclusions can be drawn from the recent clinical trials[8,9], both supporting the use of Aβ biomarkers as a screening tool in the recruitment process. First, disease-modifying treatments are likely to be most effective in the pre-symptomatic or prodromal phase of the disease. Second, the disruption in the balance in Aβ metabolism - whether causal or

epiphenomenal - remains the earliest manifestation of the disease, which can be detected several decades before onset of clinical symptoms. The two validated AD core biomarkers for β-amyloidosis are obtained with amyloid positron emission tomography (PET and with CSF sampling for the measurement of Aβ residues[2,10,11]. However, amyloid PET is expensive and limited to specialised facilities[12,13], while CSF sampling is not widely accepted as it is perceived to be invasive and time-consuming by many practitioners, thus hampering the overall widespread application for diagnosis in primary care settings as well as for the large scale selection of individuals for clinical trials.

In this context, a widely accessible, minimally invasive and cost-effective blood-based biomarker would qualify as an ideal tool[14,15] that could be performed routinely in primary care settings for early intervention and also serve as a case selection test to increase the recruitment of Aβ+ participants in clinical trials. However, first generation techniques such as the enzyme-linked immunosorbent assays showed insufficient accuracy and sensitivity[16]. A major challenge is that blood has a more complex matrix than CSF as it includes a wide variety of molecules: proteins, peptides, lipoproteins and lipids and metabolites, among which only a fraction are brain proteins and at much lower concentrations than in CSF[1,14]. However, the field of blood biomarkers for neurodegenerative diseases is evolving quickly and much more sensitive techniques have been recently proposed[17], such as the Single molecule array (Simoa) immunoassay[14,18,19], Elecsys immunoassays[20], immunomagnetic reduction[21,22] and the Amplified Plasmonic Exosome (APEX) platform[23].

The novelty of this work was to perform in the same cohort a head-to-head comparison of the diagnostic performance of three blood biomarkers, the Simoa-Aβ42 and -Aβ42/Aβ40 tests, as well as the APEX-Aβ42 assay for the detection of significant amyloid burden, using the corresponding cortical amyloid PET results as the gold standard. Furthermore, we evaluated the extent to which

cost-effective blood tests can reduce the overall recruitment cost of anti-amyloid clinical trials by pre-selecting only participants with positive blood amyloid status for PET scans.

Methods

Study population

Of the 84 participants of the previous study[23], we selected 68 subjects with residual plasma samples. They were recruited from the memory clinic at the National University Hospital, Singapore from April 2016, to September 2018. All participants were administered a detailed questionnaire to collect demographic information, including age, gender and years of formal education. They subsequently underwent comprehensive physical, clinical and neuropsychological assessments as well as amyloid PET and magnetic resonance (MR) imaging. The allele frequencies of APOE ε4 were also determined. Following a standard clinical assessment protocol, each subject was classified into one of the following four diagnostic categories without use of AD specific biomarkers: no cognitive impairment (NCI), cognitive impairment no dementia (CIND), vascular dementia (VaD), or AD, at a weekly consensus meeting. NCI was defined no objective cognitive impairment on formal neuropsychological tests or functional loss. CIND was based on clinical judgment and was diagnosed in patients who were impaired in at least one cognitive domain on a formal neuropsychological test battery, but did not meet the Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition (DSM-IV) criteria for dementia. VaD was defined using the National Institute of Neurological Disorders and Stroke and Association Internationale pour la Recherche et l' Enseignement en Neurosciences (NINDS-AIREN) criteria. AD was diagnosed using the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA). Ethics approval was obtained from the National-Healthcare Group Domain-Specific Review Board of

Singapore. The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained in the preferred language of the participants or their legal representatives by bilingual study coordinators prior to their recruitment into the study.

Blood sampling and collection

Non-fasting blood was collected into tubes containing ethylenediaminetetraacetic acid as anticoagulant and centrifuged at 2000rcf for 10 minutes at 4°C, within 4 hours post-sampling. Plasma was extracted, mixed well, and aliquoted in 0.2 ml aliquots that were stored in polypropylene tubes at -80°C until use[24].

Blood exosome-bound Aβ42 measurement (APEX)

We used the values of APEX measurements from a previous study conducted with the department of Biomedical Engineering, National University of Singapore[23]. The APEX platform used for measuring exosome-bound Aβ42 in peripheral blood samples has recently been described[23]. Briefly, through (1) in situ enzymatic amplification that forms insoluble optical deposits on the sensor and subsequent enhancement of the surface plasmon resonance signal, and (2) double-layered plasmonic nanostructures, the APEX sensor is a highly sensitive analytical platform, profiling at the nanoscale. Using this platform, we enriched for Aβ42 directly from the native plasma via the Aβ42 capture antibody (Abcam ab34376) and measured the relative amount of CD63 associated with the captured Aβ42, via the CD63 detection antibody (BD Biosciences 556019). To minimize non-specific binding, 5% bovine serum albumin was used as a blocking agent for the APEX sensor. We have also included a set of sample-matched negative controls, where we incubated the same sample over a control sensor functionalized with immunoglobulin G (IgG) isotype control antibody. All measurements were made relative to this IgG control.

Simoa immunoassay

By using the residual plasma samples, Aβ40 and Aβ42 were measured simultaneously using the

commercially available Simoa Human Neurology 3-Plex A assay kit and the fully-automated HD-1 analyzer (Quanterix, Lexington, MA, USA)[19], at the Sahlgrenska Academy at University of Gothenburg in Sweden. All procedures were performed according to the manufacturer's protocol. The Simoa-Aβ40 and Aβ42 assays both utilize the same capture antibody targeting the N-terminus of Aβ and different detection antibodies specific to Aβ40 or Aβ42[25]. Briefly, samples were incubated with capture antibody coated paramagnetic beads and biotinylated detection antibodies. After washing, a conjugate of streptavidin-β-galactosidase (SβG) was supplied where SβG bound to the biotin. Following another wash, the capture beads were resuspended in a resorufin β-D-galactopyranoside substrate solution and transferred to the Simoa array disc for detection. The concentration of the target protein in each sample was interpolated from a standard curve. All samples were diluted 8-fold and 4-fold for Aβ40 and Aβ42 respectively. All sample coefficient of variations was < 15 %. Two QC levels were run in duplicates in the beginning and the end of each run. All samples showed values above our in-house quantified lower limit of quantification (LLOQ; Aβ40: 0.674pg/mL, Aβ42: 0.142 pg/mL).

All biomarker measurements were performed on one occasion using one batch of reagents by laboratory technicians who were blinded to PET data on amyloid status.

PiB-PET/MR acquisition and quantification

PET-MR imaging was performed on a mMR synchronous PET/MR scanner (Siemens Healthcare GmbH)[26] at the Clinical Imaging Research Centre of the National University of Singapore. All subjects underwent a 30-min brain PET scan, 40 min after intravenous infusion of 370 (+/-15%) MBq of ^{11}C-Pittsburgh Compound B (PiB). MR data were acquired using a 12-channel head receive coil for acquisition correction and T1-weighted Magnetization Prepared RApid Gradient Echo (MPRAGE) image (1 mm isotropic resolution, TI/TE/TR = 900/3.05/1950ms) and processed using FreeSurfer (ver. 6.0.0) to produce parcellated volumes of the cortex. Standardized Uptake

Value ratio (SUVr) were generated using the cerebellar gray matter as the reference region. This reference region was chosen as it is relatively devoid of senile amyloid plaques and showed low ^{11}C-PiB binding[27]. Regional amyloid SUVr for 11 amyloid regions of interest (Rostral anterior cingulate, Medial orbitofrontal, Posterior cingulate, Frontal, Caudal anterior cingulate, Precuneus, Insula, Isthmus cingulate, Parietal, Temporal, Occipital) were measured individually and also averaged to derive the subject's global SUVr. PET images were independently visually interpreted by three raters［T.T, Y.N, A.R］ blinded to the clinical diagnosis of each subject and following the criteria described previously[28-30]. Two conflicting cases were discussed to obtain final consensus.

Statistical methods: Performance comparison between APEX and Simoa

Relationships between global SUVr and the three plasma biomarkers (Simoa-Aβ42, Simoa-Aβ42/Aβ40 and APEX-Aβ42) were assessed with Pearson correlation analysis. The same analysis was repeated to assess the association between each regional SUVr value and the plasma biomarkers in order to investigate their association with brain regions known to show early amyloid accumulation. Receiver Operating Characteristic (ROC) analysis was conducted to evaluate the performance of each blood biomarker in predicting the Aβ+/Aβ- status as defined by the amyloid PET visual assessment. Optimal cut-off points as well the corresponding sensitivity and specificity were determined each time using the Youden's index[31]. In addition, we estimated the predictive values of the measured cut-off points with a repeated cross-validation whereby the cut-off points were determined using 58 subjects randomly selected and the sensitivity and specificity computed from the remaining 10 unused subjects. The process was repeated 50 times for each biomarker allowing us to derive mean and std. deviation. The area under the curves (AUC) obtained with the three biomarkers were compared with each other using the DeLong test[32]. We also analyzed subgroups according to APOE ε4 status. Data analysis was performed using the

JMP (ver. 12.0.1. SAS Institute Japan, Tokyo, Japan) and MedCalc Statistical Software version (ver. 19.0.5. MedCalc Software bvba, Ostend, Belgium).

Statistical methods: Cost saving for clinical trial

We estimated the cost saving that could be obtained with each blood test if used as a case selection tool to increase the proportion of Aβ+ participants in clinical trials. For this, we devised a hypothetical clinical trial in which 100 Aβ+ participants in the pre-clinical phase of AD were to be recruited. Two scenarios were studied. In the first scenario, amyloid PET imaging is used alone to select 100 positive subjects. In the second scenario, subjects are first selected based either on their Simoa or APEX-Aβ status and their positivity confirmed with amyloid PET imaging. In this hypothetical scenario, we set the cost of a PET scan to USD$4,000[33] and of each blood test to USD$200. In addition, a prevalence of 33.8% of amyloid positivity amongst the cognitively healthy elderly was assumed.

Results

Subject characteristics

The 68 participants (mean age, 74.5 years; 35 females) recruited for this study were diagnosed into the following diagnostic categories: NCI (n=14), CIND (n=30), VaD (n=9), AD (n=15). The allele frequencies of APOE ε4 gene was 38.0% and 23 subjects (33.8％) were amyloid PET positive status based on the visual assessments.

Comparison between blood biomarkers and amyloid PET retention

Demographic, cognitive, imaging and blood biomarker data results are presented in Table 1. Note that 23 amyloid PET scans out of the 68 (33.8%) were visually rated positive. Results of correlations of the global SUVr and the three plasma biomarkers (APEX-Aβ42, Simoa-Aβ42 and Simoa-Aβ42/Aβ40) are presented in scatter plots with regression in figure 1. Results showed a

strong positive correlation between the global SUVr and the plasma APEX-Aβ42 (figure 1.A, R^2=0.901, p=<0.0001) and weaker but still significant negative correlation between the global SUVr and the Simoa-Aβ42 (figure 1.B, R^2=0.117, p=0.0043) and Simoa-Aβ42/Aβ40 ratio (figure 1.D, R^2=0.123, p=0.0034). As already reported[34,35], no correlation was found between SUVr and Simoa-Aβ40 alone (figure 1.C). This later measurement is usually used as a surrogate of the total amount of Aβ secreted to plasma for inter-individual differences normalisation[6,10] as in the composite Aβ42/Aβ40 biomarker. This higher association between APEX-Aβ42 and SUVr is confirmed at the regional level as reported in Table 2, where APEX showed systematically higher correlation coefficient and lower p values. In addition, APEX-Aβ42 showed stronger correlations in regions known to be early Aβ accumulation sites (precuneus, medial orbitofrontal, and posterior cingulate cortex). Correlation analysis also showed significant association between APEX-Aβ42 and Simoa-Aβ42 and -Aβ42/Aβ40 ratio (p=0.0018, p=0.0014, respectively. Figure2).

The ROC analysis shown in Figure 3 revealed AUC of 0.995 (95% CI = 0.938–1.000) for APEX-Aβ42, 0.816 (95% CI = 0.704–0.900) for Simoa-Aβ42/Aβ40 ratio and 0.776 (95% CI = 0.658–0.868) for Simoa-Aβ42 alone. The Youden cut-off point for APEX-Aβ42 was 1.27 (nm) with a sensitivity of 100% and specificity of 93.3% revealing a high diagnostic performance (supplementary Figure 1), while the cut-off points for Simoa-Aβ42/Aβ40 ratio and Simoa-Aβ42 were 37.6 (sensitivity 69.6%, specificity 88.9%) and 9.94 (pg/ml) (sensitivity 73.9%, specificity 77.8%). DeLong's test indicated that APEX-Aβ42 was statistically significant superior to the Simoa results (APEX-Aβ42 vs Simoa-Aβ42/Aβ40 ratio, p=0.002, APEX-Aβ42 vs Simoa-Aβ42 alone, p=0.0008). The results of the APOE ε4 status-defined subgroup analyses also showed the superiority of APEX-Aβ42 (See supplementary figure2). The ROC analysis repeated using CDR global score 0.5 and 1.0 individuals only with prodromal or mild AD, a common group eligible for AD clinical trials, showed similar patterns in Figure 3B (APEX-Aβ42 vs Simoa-Aβ42/Aβ40 ratio,

p=0.019, APEX-Aβ42 vs Simoa-Aβ42 alone, p=0.016). The cut-off points obtained for the Simoa-Aβ42/Aβ40 ratio was slightly higher (37.9) in this subset of subjects than in the whole cohort. However, the cut-off points for Simoa-Aβ42 and APEX-Aβ42 were the same. Moreover, in the repeated cross-validation analysis, we found that thresholds computed for the APEX-Aβ42 was highly predictive with a sensitivity of 98.7% (± 9.4) and a specificity of 92.8% (± 9.3) which compare well with sensitivity of 100% and specificity of 93.3% when computed using the whole cohort.

Cost saving

Estimating an amyloid positivity prevalence of 33.8% in the studied population, a total of 100/0.338 = 296 participants (100 positive, 196 negative) on average should be recruited for amyloid PET imaging to identify 100 Aβ+ subjects (Figure 4). This PET-only screening scenario would lead to a total recruitment cost of 296*USD$4,000 = USD$1,184,000. With a sensitivity of 100% and a specificity of 93.3%, the same number of subjects (296) has to undergo the APEX-Aβ42 pre-screening blood test, leading to the selection of 100 Aβ+ subjects subsequently confirmed with amyloid PET imaging and to 196*(1 - 0.933) = 13 Aβ negative subjects (false positive) determined by PET. The total recruitment cost for this 2-step screening scenario with the APEX-Aβ42 test would be 296*USD$200 + 113*USD$4,000 = USD$511,200, corresponding to a 56.8% cost reduction. The sensitivity and specificity obtained with the Simoa-Aβ42/Aβ40 are 69.6% and 88.9% respectively, thus increasing the number of subjects required for blood tests to 296/0.696 = 425 (144 positive, 281 negative), amongst which 100 true positive would pass the blood screening and be confirmed with PET imaging along with 31 false positives that would be rejected by PET-imaging. Pre-screening with Simoa-Aβ42/Aβ40 would lead to a total recruitment cost of USD$609,000, corresponding to a 48.6% reduction. Following the same logic, pre-screening with the Simoa-Aβ42 biomarker would require 400 subjects to be blood tested, 159

amyloid PET scans, amongst which 59 would be rejected, leading to a total cost reduction of 39.5%.

Discussion

Amyloid biomarkers can play a central role to identify prodromal or presymptomatic subjects that are at risk for progression to AD for inclusion in clinical trials or for early intervention. Compared to the two current validated amyloid biomarkers: amyloid PET imaging and CSF tests, a blood biomarker would be a less invasive and more affordable screening or monitoring tool that could easily be deployed in clinical settings, without further introduction and training for health-care professionals[1]. We performed for the first time and on the same cohort a head-to-head comparison of the diagnostic performance of three blood biomarkers, the Simoa-Aβ42 and -Aβ42/Aβ40 as well as the APEX-Aβ42 and using the corresponding cortical amyloid PET retention as the gold standard.

Firstly, we showed that the performance of the Simoa biomarkers in predicting the Aβ+/Aβ- amyloid PET status of our cohort is generally slightly above published values. Verberk et al. reported, an AUC, sensitivity and specificity of 0.68, 70% and 78%, respectively, for the Simoa-Aβ42/Aβ40, while an AUC of 0.66 was calculated for the Aβ42 alone[14]. Similarly, AUC, sensitivity and specificity of 0.794, 78.1% and 74.9% as well as of 0.681, 52.3% and 79.7% were reported for Simoa-Aβ40/Aβ42 and Simoa-Aβ42 respectively by Vergallo et al.[18]. Note that in this latter report, Aβ40/Aβ42 ratio replaced the Aβ42/Aβ40 ratio for improved distribution normality[21]. However, we observed exactly the same prediction performance using both ratios on our cohort.

Compared with Simoa biomarkers, APEX-Aβ42 showed higher correlation with amyloid PET retention values measured globally but also in regions known to be initial deposition sites, possibly indicating its capacity to detect abnormal amyloid changes at an early stage of the

disease[36]. ROC analysis was conducted to assess the performance of APEX-Aβ42 in predicting Aβ status. The results showed very high diagnostic performance with an AUC of 0.995, a sensitivity of 100% and a specificity of 93.3%, outperforming Simoa-Aβ biomarkers.

The Alzheimer's Precision Medicine Initiative Working Group recently estimated that an ideal biomarker for primary care screening should have a Positive Predictive Value (PPV) above 50% and a Negative Predictive Value (NPV) above 95%[1]. On the other hand, to select Aβ+ subjects for clinical trials, a high PPV and an acceptable NPV are more desirable. According to our results, the APEX biomarker largely meets both these criteria for utilization as a biomarker with a PPV and a NPV of 88.5% and 100% respectively. With a PPV of 76.2% and a NPV 85.1%, the Simoa-Aβ42/Aβ40 technique is acceptable for Aβ+ subject selection for clinical trials but is not an ideal candidate for primary care screening. When utilized for clinical trial recruitment, our simulation showed that pre-screening with blood biomarkers followed by a confirmatory amyloid PET imaging would roughly halve the cost (56.8% reduction for APEX-Aβ42 and 48.6% for Simoa-Aβ42/Aβ40) as compared to the situation where only PET imaging is used. However, with a 100% sensitivity; APEX-Aβ42 pre-screening does not increase the required number of initial participants, while Simoa-Aβ42/Aβ40 lead to an increase of 43.6%, possibly lengthening the recruitment period and increasing the management cost and burden that were not considered in this simulation. Of note, APEX-Aβ42 showed an AUC around 100%, which seems to be a promising gold standard test for amyloid status. If used as a replacement of amyloid PET imaging in subject selection, a cost saving as high as 95% could be expected. Further studies are needed to validate APEX- Aβ42 platform.

The first limitation of our study lies in the small sample size (n=68) used for measuring and comparing the performance of the three biomarkers. Further, validation of the results in an additional independent clinical cohort will also be important. However, we performed a direct

comparison using measurements from the same set of subjects thus eliminating important sources of variability that are inherent to comparative studies performed using different cohorts. In addition, the repeated cross-validation analyses supported the validity of our results. The second limitation is that the current data included more severely demented patients which might not be included in current clinical trials for disease modifying interventions. However, this choice did not affect our results which were obtained using the whole cohort and the ROC analysis with subjects having prodoromal dementia (CDR 0.5 and 1.0 only), who might be eligible for clinical trials, lead to similar results. The third limitation is that we assumed a 33.8% amyloid positivity rates for the cost saving estimation because our cut-off points were determined on the all subjects. If we used a 45% positivity rates (derived from CDR 0.5 and 1.0 only of our data [n=40]), prescreening with APEX-Aβ42 would lead to a total recruitment cost of USD$419,600, corresponding to a 47.3% reduction.

In conclusion, this comparative study conducted on the same cohort for increased confidence demonstrated the potential of using Simoa-Aβ42/Aβ40 and APEX blood tests as pre-screening tools for enriching clinical trials with Aβ+ subjects in the pre-clinical phase of AD and for reducing the related cost. Further validation and comparative studies will be required to fully characterize and validate the APEX blood biomarker, however, our results show its significant superiority over Simoa-Aβ blood biomarkers. In the event of such a blood biomarker is recognized by research guidelines for its diagnostic utility and included in the core AD biomarker list, subject identification based on blood test alone would reduce the recruitment cost to a fraction of the current cost when PET imaging only is utilized as a subject selection tool. Finally, with its exceptional diagnostic performance, APEX is also the ideal candidate for primary care screening.

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Disclosure of conflicts of interest

HZ has served at scientific advisory boards for Wave, Samumed, CogRx and Roche Diagnostics, has given open lectures in symposia sponsored by Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg. The other authors report no conflicts of interests specifically related to this manuscript.

Data availability

All data generated for this work and which supports the findings are available upon reasonable request.

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Figure legends

Figure 1

Correlations of the Investigated Plasma Biomarkers with Amyloid PET

Scatter plots with regression line of plasma and SUVr amyloid PET biomarkers. Reported R^2 and p values are from Pearson's correlation analysis. Simoa-Aβ42/Aβ40 ratio was multiplied by 1,000. The clinical diagnoses are pointed with different colors (black: Alzheimer's disease (AD), blue: Vascular dementia (VaD), orange: cognitive impairment no dementia (CIND), green: no cognitive impairment (NCI), respectively).

Abbreviations: SUVr, Standardized Uptake Value ratio; Aβ, amyloid Beta; PET, Positron Emission Tomography; APEX, Amplified Plasmonic Exosome; Simoa, Single molecule array.

Figure 2

Correlation among plasma biomarkers

Scatterplots with regression line of APEX-Aβ42 versus Simoa-Aβ42 (A) and Simoa-Aβ42/ Aβ42 ratio (B). R2 and p values are from Pearson's correlation analysis. Simoa-Aβ42/Aβ40 ratio was multiplied by 1,000. APEX-Aβ42 were negatively correlated with Simoa-Aβ42 and Aβ42/Aβ40 ratio (Aβ42: R2=0.116, p=0.0018; Aβ42/Aβ40 ratios: R2=0.145, p=0.0014). Simoa-Aβ42/Aβ40 ratio was multiplied by 1,000. The clinical diagnoses are pointed with different colors [black: Alzheimer's disease (AD), blue: Vascular dementia (VaD), orange: cognitive impairment no dementia (CIND), green: no cognitive impairment (NCI), respectively].

Abbreviations: Aβ, amyloid Beta; APEX, Amplified Plasmonic Exosome; Simoa, Single molecule array.

Abbreviations: Aβ, amyloid Beta; PET, positron emission tomography; APEX, Amplified Plasmonic Exosome; Simoa, Single molecule array.

Figure 3

Receiver Operating Characteristic (ROC) Analysis of Plasma Biomarkers

ROCs of APEX-Aβ42, Simoa-Aβ42 and Aβ42/Aβ40 ratio in predicting Aβ+/Aβ- PET status in our all subjects (n=68) (A) and in CDR global score 0.5 and 1.0 only (n=40) (B). Amyloid status was based on visual assessment results. APEX-Aβ42 led to the highest AUC of 0.995 (using all subjects) whereas the Simoa-Aβ42/Aβ40 ratio and Aβ42 showed AUC of 0.816 and of 0.776, respectively. APEX-Aβ42 was found statistically significant superior to the Simoa results

(DeLong's test). In the sensitivity analysis (B), The AUC of APEX-Aβ42 was 0.997, Simoa-Aβ42/Aβ40 ratio was 0.828, Simoa-Aβ42 was 0.803.

Abbreviations: CDR, Clinical dementia rating; Aβ, amyloid Beta; ROC, Receiver Operating Characteristic; APEX, Amplified Plasmonic Exosome; Simoa, Single molecule array; VaD, vascular dementia. CDR

Figure 4

Potential Cost Savings with Plasma Amyloid Pre-screening in clinical trials

Analysis of the potential cost savings plasma amyloid pre-screening would generate in the hypothetical case of a clinical trial requiring the recruitment of 100 amyloid PET positive subjects. Without plasma pre-screening (PET only screening), 296 PET scans would have to be performed to identify 100 amyloid PET positive subjects leading to an estimated total cost of $1,184,000. The plasma pre-screening with APEX-Aβ42, Simoa-Aβ42/Aβ40 and Simoa-Aβ42 would reduce the recruitment cost by 56.8%, 48.6% and 39.5% respectively. The number of initial subjects required with the Simoa-Aβ42/Aβ40 and Simoa-Aβ42 would increase from 296 to 425 and 400 respectively, while the required number of initial subjects remained unchanged with Exosome Aβ42. To compare the cost-savings, we assumed the following costs: USD$4000 per 1 amyloid PET scan, USD$200 per Simoa immunoassay and USD$200 per APEX.

Abbreviations: Aβ, amyloid Beta; PET, positron emission tomography; APEX, Amplified Plasmonic Exosome; Simoa, Single molecule array; P, Positive; N, Negative; TP, True Positive; FP, False Positive.

Table

Table.1

Demographic Features, Clinical Characteristics and Biomarker Results

	Overall (n=68)	NCI (n=14)	CIND (n=30)	VaD (n=9)	AD (n=15)
Age, mean (SD), years	74.5 (8.1)	73.9 (7.4)	73.5 (7.1)	76.1 (12.4)	76.1 (7.8)
Females, n (%)	35 (52)	6 (43)	12 (40)	4 (44)	13 (87)
Education, mean (SD), years	6.9 (4.5)	7.1 (4.8)	8.4 (4.4)	5.8 (4.4)	4.5 (3.8)
APOE ε4 carrier, n (%)	26 (38)	4 (29)	11 (37)	2 (22)	9 (60)
Cognitive tests					
CDR global, mean (SD)	0.6 (0.6)	0.1 (0.2)	0.4 (0.2)	1.3 (0.5)	1.3 (0.5)
CDR sum of boxes, mean (SD)	3.0 (3.9)	0.1 (0.4)	0.7 (0.7)	7.0 (2.8)	8.0 (3.2)
MMSE, mean (SD)	21.9 (6.1)	27.5 (2.0)	24.4 (3.4)	17. (4.0)	14.6 (4.7)
MOCA, mean (SD)	18.4 (7.2)	25.7 (2.6)	20.7 (4.9)	12.0 (5.4)	10.9 (4.7)
PiB-PET					
Global SUVr, mean (SD)	1.48 (0.44)	1.21 (0.13)	1.43 (0.41)	1.15 (0.12)	2.03 (0.32)
Amyloid positive (visual assessment), n (%)	23 (33.8)	0 (0)	9 (30.0)	0 (0)	14 (93.3)
Blood biomarkers					
APEX-Aβ42, mean (SD), nm	1.02 (0.79)	0.51 (0.37)	0.96 (0.78)	0.50 (0.19)	1.94 (0.50)
Simoa-Aβ40, mean (SD),	282.8	255.8	270.4	428.7	245.1

pg/ml	(123.7)	(104.9)	(68.1)	(223.3)	(92.8)
Simoa-Aβ42, mean (SD), pg/ml	11.2 (3.6)	12.1 (3.8)	10.8 (2.7)	16.8 (10.8)	8.9 (3.6)
Simoa-Aβ42/40 ratio, mean (SD)	41.8 (11.6)	51.6 (16.6)	40.7 (9.5)	38.9 (8.7)	36.4 (5.3)

Abbreviations: NCI, no cognitive impairment; CIND, cognitive impairment no dementia; VaD, vascular dementia; AD, Alzheimer's disease; APOE, apolipoprotein E; CDR, clinical dementia rating; MMSE, Mini Mental Status Examination; MoCA, Montreal Cognitive Assessment; SUVr, Standardized Uptake Value ratio; APEX, Amplified Plasmonic Exosome; Simoa, Single molecule array; SD, Standard deviation.

Note. APEX-Aβ42/Aβ40 ratio was multiplied by 1,000.

Table.2

Correlation between Regional Amyloid SUVr and Plasma Amyloid Biomarkers

	APEX-Aβ42			**Simoa-Aβ42/40**			**Simoa-Aβ42**		
	R2	**p value**	**ranking**	**R2**	**p value**	**ranking**	**R2**	**p value**	**ranking**
Rostral anterior cingulate cortex, R	0.893	<0.0001***	1	0.141	0.0016*	1	0.121	0.0037*	4
Rostral anterior cingulate cortex, L	0.882	<0.0001***		0.123	0.0034*		0.137	0.0019*	
Medial orbitofrontal, R	0.891	<0.0001***	2	0.138	0.0018*	2	0.12	0.0038*	5
Medial	0.869	<0.0001*		0.117	0.0042*		0.134	0.0022	

orbitofrontal, L		**						
Frontal cortex, R	0.885	<0.0001***	3	0.116	0.0044*	8	0.13	0.0005**	3
Frontal cortex, L	0.853	<0.0001***		0.102	0.0079*		0.142	0.0015*	
Posterior cingulate cortex, R	0.857	<0.0001***	4	0.124	0.0032*	6	0.144	0.0014*	1
Posterior cingulate cortex, L	0.866	<0.0001***		0.098	0.0093*		0.155	0.0009**	
Precuneus, R	0.859	<0.0001***	5	0.119	0.004*	4	0.1	0.0085*	8
Precuneus, L	0.861	<0.0001***		0.107	0.0064*		0.107	0.0064*	
Caudal anterior cingulate cortex, R	0.869	<0.0001***	6	0.142	0.0015*	3	0.13	0.0025*	2
Caudal anterior cingulate cortex, L	0.839	<0.0001***		0.104	0.0072*		0.168	0.0005**	
Parietal cortex, R	0.856	<0.0001***	7	0.104	0.0074*	10	0.104	0.0074*	7
Parietal cortex, L	0.819	<0.0001***		0.091	0.0124*		0.116	0.0045*	
Temporal cortex, R	0.846	<0.0001***	8	0.12	0.0039*	5	0.098	0.0094*	9
Temporal cortex, L	0.82	<0.0001***		0.104	0.0073*		0.103	0.0077*	
Insula, R	0.846	<0.0001***	9	0.112	0.0052*	9	0.119	0.0039*	6
Insula, L	0.817	<0.0001***		0.091	0.0124*		0.134	0.0021*	
Isthmus cingulate cortex, R	0.811	<0.0001***	10	0.115	0.0048*	7	0.093	0.0113*	10
Isthmus cingulate cortex, L	0.808	<0.0001***		0.105	0.0071*		0.097	0.0098*	

Occipital cortex, R	0.649	<0.0001***	11	0.089	0.0134*	11	0.042	0.0945	11
Occipital cortex, L	0.683	<0.0001***		0.075	0.0236*		0.063	0.0386*	

Abbreviations: Aβ, amyloid Beta; R, right; L, left; APEX, Amplified Plasmonic Exosome; Simoa, Single molecule array.

NOTE. The Pearson's correlation coefficients R^2 and p value were used to measure the statistical relationship between regional SUVr and plasma biomarkers. P value significant level < 0.05*, < 0.001**, <0.0001***, two tailed. The ranking of the regions is based on their mean correlation coefficients (left, right) obtained with APEX-Aβ42 (descending order).

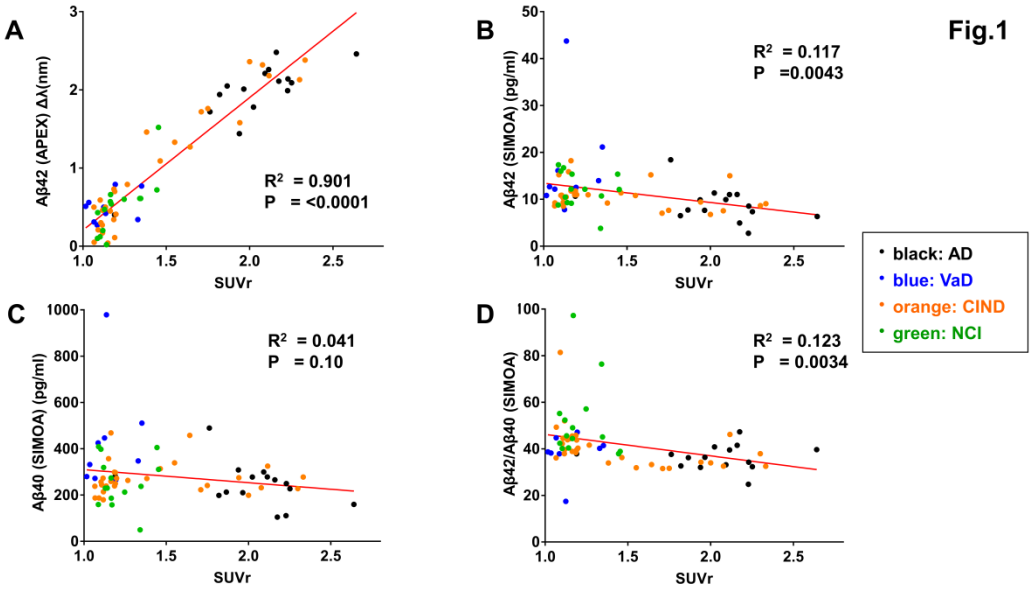

Fig.1

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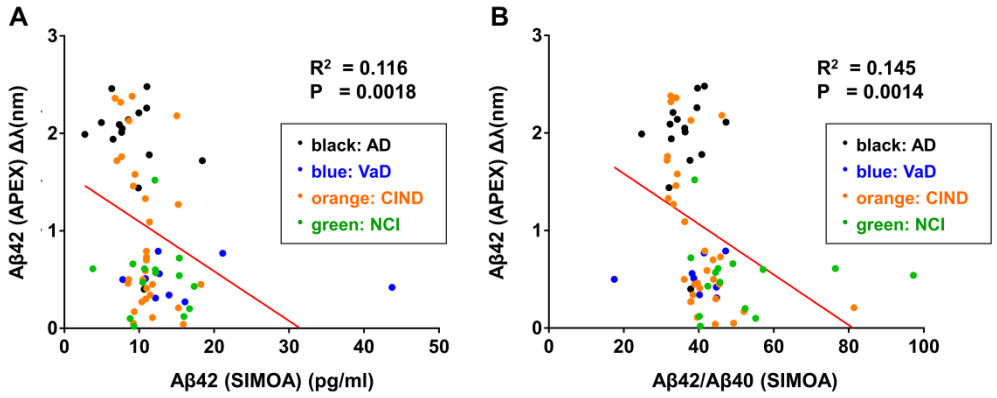
Fig.2



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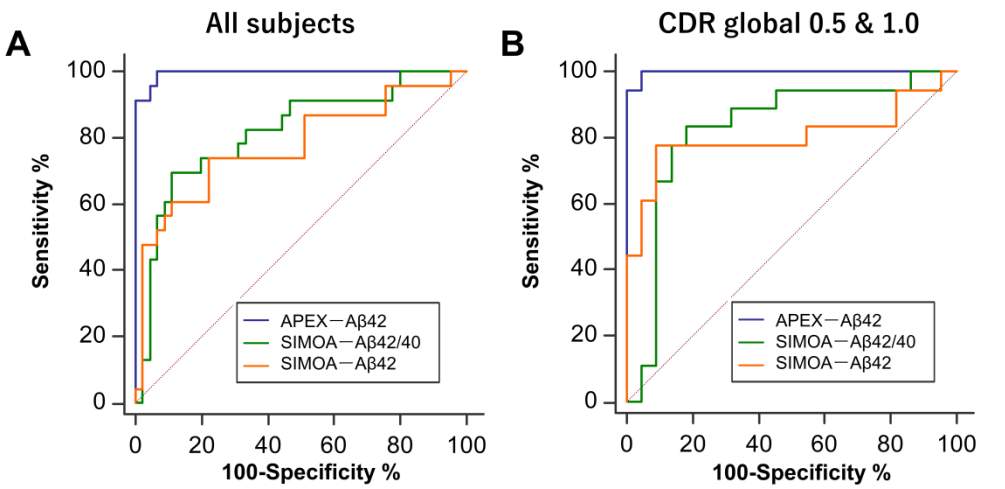

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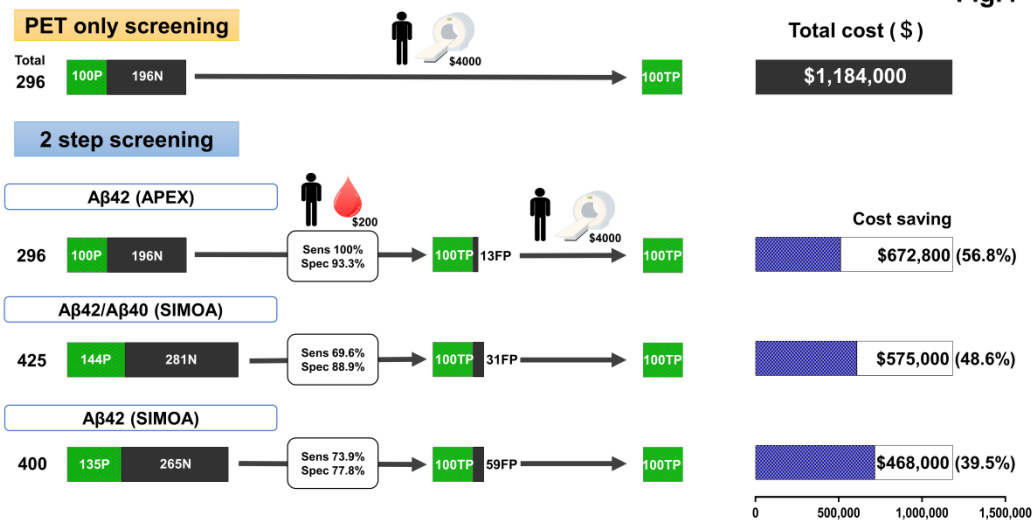

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