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Plasma A β 42 and A β 40 as markers of cognitive change in follow-up — a prospective, longitudinal, population-based cohort study

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ABSTRACT

Background: Single measurements of plasma A β are not useful in the diagnostics of Alzheimer's disease (AD). However, changes in plasma A β levels during repeated testing may be helpful in the prediction and evaluation of progression of the incipient AD or mild cognitive impairment.

Objective: To examine the relation of baseline and serial plasma A β levels to cognitive change in follow-up.

Methods: 269 subjects (52 cognitively impaired and 217 controls) from a population-based cohort were clinically followed up from three to six years. Serial plasma samples were available from 70 subjects who were followed up for three years and 43 subjects followed for six years. The plasma A β levels were measured using ELISA.

Results: Subjects who declined cognitively during the follow-up had lower levels of plasma A β 42 at the baseline. Plasma A β 42 and the A β 42/A β 40 ratio decreased (-2.4 pg/ml for A β 42 in six years) in those who declined in follow-up whereas A β 42 and the A β 42/A β 40 ratio increased in the subjects who remained cognitively stable or improved in follow-up. Subjects using acetylsalicylic acid, dipyridamole, antidiabetic or anticoagulant drugs as well as subjects with coronary heart disease had higher levels of A β 40.

Conclusions: Low or decreasing plasma A β 42 during the follow-up is associated with cognitive decline. Serial measurement of plasma A β 42 may be useful in the detection of the subjects who are at risk for cognitive decline.

INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia. At present, its diagnosis is based on clinical criteria and the exclusion of other causes. However, objective biomarkers for the early diagnosis and monitoring of the disease process are clearly needed because symptomatic treatments are available and disease-modifying drugs are already in phase III trials. The presence of amyloid β deposition in senile plaques is one pathological hallmark of Alzheimer's disease (AD) together with neurofibrillary tangles. [1] The amyloid β is a peptide secreted by neurons [1] and platelets [2], derived from amyloid precursor protein APP via the activity of proteases β and γ secretase. [3] Most of the $A\beta$ deposited in the brain is constituted of 42 amino acids ($A\beta_{42}$) form. [4] $A\beta_{42}$ has also been shown to be first amyloid form to accumulate with $A\beta_{40}$ being deposited later in the process of AD pathogenesis. [5] The level of $A\beta_{42}$ in cerebrospinal fluid (CSF) is reduced in patients with mild cognitive impairment (MCI) and AD [6,7] and a combination test of CSF $A\beta_{42}$ and tau or phospho-tau has been claimed to be helpful in the early diagnosis of AD. [8]

$A\beta$ is present in plasma but it is still unknown whether it originates from peripheral sources or from the brain. In Tg2576 transgenic mouse, plasma $A\beta$ levels decline in parallel with their increasing accumulation in the brain. [9] Since $A\beta$ can be transported bidirectionally across the blood-brain-barrier, it has been hypothesized that there may be an equilibrium between CSF and plasma pools of $A\beta$. [10,11] Seeing that it is well established that CSF $A\beta_{42}$ levels decrease in conjunction with the cognitive decline, it has been postulated that plasma $A\beta_{42}$ may decrease similarly. [12] If so, plasma $A\beta$ would offer a straightforward, non-invasive and economical biomarker for AD. However, patients with known mutations

in chromosome 21 causing early-onset familial AD as well as patients with trisomy 21 have increased plasma A β 42 levels which are detectable before the onset of the symptoms of dementia. [13,14] Also the first-degree relatives of late-onset AD patients exhibit elevated A β levels measured in plasma. [15]

Previous studies have suggested that the levels of plasma A β 40 are increased before the onset of sporadic AD. [16,17] However, one recent study concluded that low plasma A β 40 level predicted AD in elderly men. [18] Other studies have found elevated A β 42 levels in patients who later develop dementia [19], particularly in MCI amnesic type. (aMCI) [20] Finally, several studies have not been able to detect any significant difference of A β levels between AD converters and cognitively stable controls. [21,22]

It seems that a single measurement of plasma A β is not useful whereas the change in plasma A β levels observed in repeated testing may be of help in the prediction and evaluation of progression of incipient AD or MCI. [23] However, only a limited number of longitudinal studies have been performed. [24-26] Our aim was to examine whether the change in plasma A β levels during follow-up would be more predictive of cognitive decline than straightforward baseline plasma A β levels in a population-based cohort of MCI and cognitively intact controls.

SUBJECTS AND METHODS

Subjects

Study subjects were participants in the population-based study (n=806, aged 60-76 years) examining the risk factors and predictors of dementia in the elderly [27,28] (Table 1). At baseline (years 1997-1998), 52 subjects were cognitively impaired (CDR 0.5 n=51 and CDR 1 n=1). Group 1 of this study included all of the cognitively impaired subjects from the original cohort who provided a plasma sample (n=52). For each of them, we randomly selected 4 - 5 cognitively unimpaired (n=217) age- and sex-matched controls from the same cohort. These subjects were clinically re-evaluated after three (n=197) and six years (n=60).

The longitudinal marker group (group 2) included 70 subjects of the original cohort of 269 subjects who provided a 3 year follow-up plasma sample and 43 of them a 6 year follow-up sample. The group 2 included 11 cognitively impaired (at baseline), non-demented subjects (CDR 0.5) who provided at least one follow-up plasma sample and 59 cognitively intact (at baseline) age- and sex-matched controls with at least one follow-up plasma sample (Figure 1). Drop-outs occurred mainly due to refusal of the participants to continue the study. Written informed consent was obtained from all the subjects and the study was approved by the local Ethical Committee.

Table 1 The baseline demographic information of the subjects.

	Population-based cohort	Group 1 (cognitive follow-up)		Group 2 (plasma follow-up)	
n	806	269		70	
Age	68 (60 – 76)	70 (60 - 77)	p > 0.1	71 (61 - 77)	p > 0.1
Men / Women	321 / 485 47 % / 53 %	121 / 148 45 % / 55 %	p > 0.1	25 / 45 36 % / 64 %	p > 0.1
APOE ε4 -/+	414 / 207* 67 % / 33 %	169 / 99† 63 % / 37 %	p > 0.1	37 / 33 53 % / 47 %	p < 0.05
MMSE	26 (7 - 30)	27 (13 - 30)		26 (17 - 30)	
CDR = 0	731	217		59	
CDR = 0.5	70	51		11	
CDR‡ = 1	3	1		0	

The data is given as median values (range) or as number of subjects with the percentage of all subjects in the group.

*APOE data missing from 185 subjects.

†APOE data missing from one subject.

‡One subject had CDR 2. Data missing from one subject.

Clinical evaluation

The evaluation included a structured detailed interview including demographic information, medical history, medication, smoking habits and alcohol consumption, and a subjective assessment of memory disturbances and depression. The evaluation also included clinical examination as well as an assessment of cognitive impairment by applying the Clinical Dementia rating (CDR) and using a battery of neuropsychological tests. *Memory*: Visual Reproduction Test from Wechsler Memory Scale [29], Word List Recall from the CERAD Neuropsychological Assessment Battery [30], Logical Memory Test from Wechsler Memory Scale-Revised [31], NYU Paragraph Recall [32], Delayed Recall of the Constructional Praxis from CERAD [30]; *Language*: vocabulary subtest from the Wechsler Adult Intelligence Scale-Revised [33], Abbreviated (15 items) Boston Naming Test [34]; *Attention and executive function*: Trail Making Test [35] parts A and B, Verbal Fluency Test [36,37]; *Visuospatial skills*: Block Design from the WAIS-R [33], Constructional Praxis from CERAD [30]; *Global functioning*: Mini-Mental State Examination [38] (MMSE) Clock Drawing Test. [30].

Cognitive decline was defined by the CDR change from 0 to 0.5 or 0.5 to 1.

Measurement of A β 40 and A β 42

The 269 baseline samples (group 1) were measured in year 2002-2003. After completing the six year follow-up, we re-analysed the baseline samples of 70 subjects (group 2) together with their follow-up samples in year 2006-2007 (group 2).

A venous blood sample was obtained into heparine tubes and plasma was separated using the standard methods. The samples were aliquotted and stored in polypropylene

tubes at -70°C until analyses. $\text{A}\beta_{40}$ was measured by the ELISA method modified from a well established method. [16] The capture antibody was 6E10 (Sigma, Saint Louis, MO) and the detection antibody was a biotin labelled G2-10 antibody (The Genetics Company, Schlieren, Switzerland). The synthetic $\text{A}\beta_{1-40}$ peptide (Bachem, Bubendorf, Switzerland) was used as the standard. Before the analyses, 0.05% Tween 20 - 0.5% BSA was added to the samples. $\text{A}\beta_{42}$ was measured by a high sensitivity method of a commercially available ELISA (Innogenetics, Gent, Belgium) which we modified to be suitable for the measurements of more than 7 pg/ml concentrations. Before the analyses, 0.5M guanidine chloride was added to the standards and the samples. The detergents were used to avoid coagulation of samples and to release $\text{A}\beta$ peptides from plasma proteins.

In longitudinal analysis, baseline and follow-up samples from one individual were placed on the same plate to prevent inter-assay variation. Thus, we measured baseline samples from 70 subjects twice (4 years apart). The absolute concentrations differed between these two measurements; median level for $\text{A}\beta_{40}$ 174,5 (year 2003) and 198 pg/ml (74) (year 2007), and $\text{A}\beta_{42}$ 17 (year 2003) and 49 pg/ml (110) (year 2007). However, there was a moderately good correlation between these measurements ($\text{A}\beta_{40}$ $r=0.674$, $p<0.001$ and $\text{A}\beta_{42}$ $r=0.824$, $p<0.001$). The correlation figure is presented in the supplemental data (available at www.jnnp.com).

The inter-assay variation for $\text{A}\beta_{40}$ assay was 23.8 % and for $\text{A}\beta_{42}$ assay 19.1 %. The inter-CVs were measured by using reference samples of medium concentration (~ 250 pg/ml for $\text{A}\beta_{40}$ and ~ 400 pg/ml for $\text{A}\beta_{42}$). The intra-assay variations for $\text{A}\beta_{40}$ were 0,71 % for high (~ 1200 pg/ml), 0,95 % for medium and 5,9 % for low concentrations (~ 150 pg/ml). The intra-assay CVs (median) for $\text{A}\beta_{42}$ were 1,6 % (~ 1000 pg/ml), 2,5 % and 9,8 % (~ 15 pg/ml), respectively.

APOE genotyping

The APOE allele genotyping was done by a PCR based method. (39) The subjects were subdivided into the APOE ϵ 4 negative and APOE ϵ 4 positive subjects.

Statistics

The statistical analyses were conducted by the SPSS for Windows release 14.0.1. (SPSS Inc., Chicago, IL, USA). Due to the non-normal distribution of data, Kruskal Wallis, Mann Whitney and Spearman's correlation tests were used. The categorical data was analysed by χ^2 test. The odds ratios (OR) for cognitive decline of patients in different groups were calculated by logistic regression analysis. We fitted a linear regression slope by Microsoft Excel to analyse the alteration trend of A β levels.

RESULTS

Table 1 presents the demographic information about the subjects. The baseline A β 40 and A β 42 levels of 269 individuals were generally low although some subjects exhibited extremely high A β 42 levels. The limit for the 90th percentile was 101 pg/ml but the highest measured level was 1341 pg/ml. The A β 40 levels showed a weak correlation to age ($r = 0.186$, $p = 0.002$) but this was not the case with the A β 42 levels. There were no differences in A β 40 and A β 42 levels between the sexes or between the APOE ϵ 4 carriers and non-carriers. A β 42 levels did not correlate with A β 40 concentrations.

Baseline A β levels and cognitive decline during the follow-up

No significant differences were found in plasma A β 40, A β 42 or the A β 42/A β 40 ratio between cognitively impaired ($n=52$) and cognitively intact subjects ($n=217$) at baseline. However, 197 of these subjects were clinically assessed after three years and 60 were clinically assessed after six years. The baseline A β 42 levels were significantly lower in the subjects who showed cognitive decline after three years of follow-up (cognitively stable, $n=147$: 19 pg/ml (0-1341), cognitive decline, $n= 50$: 12 pg/ml (0-276), $p = 0.001$). Baseline A β 42 levels were also lower in subjects who had declined cognitively after six years (10 pg/ml, $n=36$) compared to those who remained cognitively stable (18 pg/ml, $n=24$), $p=0.013$.

Subjects who had baseline A β 42 levels in the lowest quartile displayed an OR of 3.12 (95% CI 1.25 - 7.79, $p=0.015$) for cognitive decline after three years and 4.77 (95% CI 1.14 - 19.98, $p=0.033$) after six years in comparison to subjects who had A β 42 levels in the highest quartile. Similarly, subjects who had A β 42/A β 40 ratio in the lowest quartile had an OR of 3.26 (1.31 - 8.11, $p=0.011$) for cognitive decline after three years and 8.40 (1.83 -

83.568, $p=0.006$) after six years of follow-up when compared to the subjects in the highest quartile.

Relationship between changing plasma A β levels and cognitive decline

The follow-up plasma samples were available from 70 subjects after three years and 43 subjects after six years of follow-up. The median levels of A β 42 did not change or decreased in subjects with cognitive decline ($n=27$ after three years and $n=14$ after six years) whereas they increased in those who remained cognitively stable (Table 2). No statistically significant changes were found in A β 40 levels. The A β 42/A β 40 ratio decreased significantly in the subjects who experienced a cognitive decline.

A trend analysis was undertaken by calculating a slope for each subject and this was used to assess the change of A β 42 between cognitively stable and cognitively declining subjects. During the follow-up of 3 to 6 years, the cognition of 28 out of the total 70 subjects had declined and there was a decreasing trend of the level of A β 42 in 24 subjects during the follow-up. The A β 42 level remained the same in the declining subjects and increased in the cognitively stable subjects (0.0 (4.9) pg/ml/year and 2.1 (21) pg/ml/year; $p=0.009$ for slope difference). The corresponding changes of A β 42/A β 40 ratio were also significant (0.0 (0.029) per year and 0.0036 (0.055) per year; $p=0.02$ for slope difference).

Table 2. Cognition and changes of A β between baseline and follow-ups in subjects.

Baseline	Cognitively healthy		Cognitively impaired	
N =	59		11	
A β 40	195 (88)		201 (60)	
A β 42	50 (121)		46 (92)	
A β 42/A β 40	0.236 (0.464)		0.291 (0.487)	
	Cognitively stable or improved		Cognitive decline	
Follow-up	3 years	6 years	3 years	6 years
N =	43 [†]	29 [‡]	27	14
change of A β 40	18 (55)	33 (45)	21 (24)	33 (75)
change of A β 42	3.7 (29)	12 (54)	0.0 (14)*	-2.4(50)*
change of A β 42/A β 40	0.0093 (0.169)	0.0166 (0.451)	-0.0027 (0.084)*	-0.0486 (0.251)*

The data is given as medians (interquartile range).

The values of p reflect the significance against the cognitively stable subgroup.

*p < 0.05 against "stable or improved"

[†]A β data of one subject is from a plasma sample of 4 years of follow-up.

[‡]Cognition data of two subjects is from the previous year

Plasma A β and general health

Table 3 shows the relationship between medication as well as certain diseases on the plasma A β levels. The A β 40 levels were not associated with the use of lipid lowering drugs or NSAIDs at baseline. Hormone replacement therapy was not related to A β values in women. The A β 40 levels were higher in those subjects using ASA (n = 62, p = 0.004) or dipyridamole (n = 12, p = 0.017). The A β 40 values were lowest in subjects using neither of the drugs, intermediate in subjects using either ASA or dipyridamole and highest in the subjects taking both drugs. The A β 40 levels were also higher in subjects using insulin alone (n = 5, p = 0.009) or insulin in combination with oral antidiabetic drugs (n = 13, p = 0.003). The A β 42 levels were not associated with use of any of the drugs. CHD was associated with a high plasma A β 40 level (p=0.035). There was no statistically significant difference in medication use between the cognitively stable and the cognitive decliners.

Table 3. Relation of A β 40 level to medication at baseline.

	Group 1	A β 40		pg/ml	pg/ml
	n			Users	Non-users
Sex, female	148	-			
Smoking	21	-			
CHD	61	↑	p=0.035	188 (105 - 360)	176 (0 - 780)
Diabetes	22	-			
Anticoagulants	16	↑	p=0.038	198 (144 - 360)	176 (0 - 780)
NSAIDs	22	-			
ASA	62	↑	p=0.004	194 (0 - 278)	175 (0 - 780)
Dipyridamole	12	↑	p=0.016	208 (156- 278)	176 (0 - 780)
Antidiabetics*	13	↑	p=0.003	219 (161 - 426)	179 (0 - 780)
Lipid lowering agents	24	-			

*insulin and oral antidiabetics

DISCUSSION

Our results indicated that low or decreasing plasma A β 42 levels and A β 42/A β 40 ratio were related with cognitive decline during the follow-up. Moreover, possible age-related increase in plasma A β 42 levels in serial measurements was associated with stable cognitive performance.

We found no differences in plasma A β levels between cognitively intact and impaired subjects in the cross-sectional analysis at baseline. Previous cross-sectional studies comparing plasma A β levels in patients with sporadic AD and controls have reported contradictory results. Elevations of plasma A β 40 [16] or A β 42 [19] have been described whereas other studies have found no differences between AD and controls. [21] One recent study found increased levels of plasma A β 42 in women with amnesic MCI compared to healthy controls or affected men. [40] However, low A β 42 levels at baseline were associated with a cognitive decline occurring during the follow-up. In line with our results, a recent study detected an association between a low A β 42/A β 40 ratio and cognitive decline. [12] Respectively, a prospective three-city study of 257 dementia patients found an association of high A β 42/A β 40 ratio with a lower risk of dementia in follow-up. [41] Another population-based case-cohort study claimed that individuals with a combination of low A β 42 and high A β 40 measured from plasma at baseline had an over 10-fold risk of dementia, but found no association between the A β 42 or A β 40 levels alone with cognitive decline. [17] Other studies have reported different results. The VITA study found no association between baseline A β levels and cognitive decline during the follow-up. Other studies have found elevated concentrations of A β 42 at the baseline in subjects

who developed AD during the follow-up [19,42] although plasma A β levels were not associated with AD in the fully adjusted multivariate model. [42]

Differences in study cohorts, for example timing with respect to cognitive decline, assessment of cognitive functioning (different tests) and presence of confounding factors such as medication and other diseases, are all factors that can influence the results. The outcome in some studies has been conversion to dementia [17,19] whereas the outcome in our study as well as in some other studies [12] was cognitive decline. The difference in the selected outcome and the timing of the sample collection may partially explain differences in the results.

Single measurement of plasma A β may not be a suitable marker for AD due to many confounding factors. The timing of the A β measurement in terms of the natural history of AD may be critical. Experimental studies on transgenic animals suggest that plasma A β levels decrease at the time when accumulation of A β begins in the brains. [9] It is possible that the increased plasma A β concentration is related to the development of AD as suggested by the findings of elevated plasma A β levels in AD gene mutation carriers [43] and in the first degree relatives of the patients with late onset AD. [15] However, since amyloid pathology in the brain begins years before the appearance of the first symptoms, the possible increase of plasma A β may not be detected in the symptomatic individuals. This hypothesis can only be addressed in longitudinal studies. In line with our results, previous studies have suggested that decreasing A β levels are associated with cognitive decline [19,24] and A β 42 levels were lower in patients diagnosed having AD than in those with MCI. [44] Many studies have shown that plasma A β levels increase with age, as was found in the cognitively intact subjects in our study. [19,25,42] In one study, the age-

related increase was smallest in those subjects who converted to AD from MCI [25]. It is possible that age-related changes of plasma levels of A β 40 and A β 42 differ in subjects with AD. In this respect, the A β 42/A β 40 ratio may be a better predictor for AD than the single markers.

Differences in study cohorts make the comparison between different studies difficult. There are many confounding factors that may influence plasma A β levels. Renal dysfunction may increase plasma since plasma A β is excreted through the kidneys [45]. Many studies have suggested an association between vascular disease and plasma A β levels. [46-48] The levels of plasma homocysteine, a possible marker for vascular disease, correlate positively with plasma A β 40 and A β 42 levels. [49] Previous studies have also suggested that certain drugs may influence plasma A β . [50] We found elevated levels of A β 40 in the subjects who were using ASA and dipyridamole, i.e. drugs that directly influence platelet function and activation, and subjects who used anticoagulation and antidiabetic drugs, whereas there was no association between the use of NSAIDs or lipid lowering agents and plasma A β levels. The association between ASA and plasma A β 40 was seen also in subjects without cardiovascular diseases. In line with previous studies [50,51], no relationship was found between A β levels and lipid lowering agents has been found in previous studies.

Differences in methodology and experimental conditions may also influence results. Erythrocytes and plasma proteins, for example albumin and lipoproteins, bind A β and denaturing conditions liberate A β into the free pool of plasma. [52] Also, the different antibodies used in the immunological assays may detect different fractions of A β . Previous studies suggested that the absolute levels of A β vary across different ELISA batches [53]. We also noticed a difference between absolute levels in 70 samples that were measured

twice four years apart. Due to these methodological difficulties, the diagnostic value of a single measurement is limited.

The significance of the standardization of the conditions in storing and handling the samples is reported in a work by Vanderstichele et al. (2000). To better utilize these analyses, the assays used should be commercially available, well standardized and thoroughly validated.

We conclude that plasma A β is not a diagnostic marker for AD but the decreasing levels of A β 42 in serial measurements may associate with cognitive decline and indicate the development of AD.

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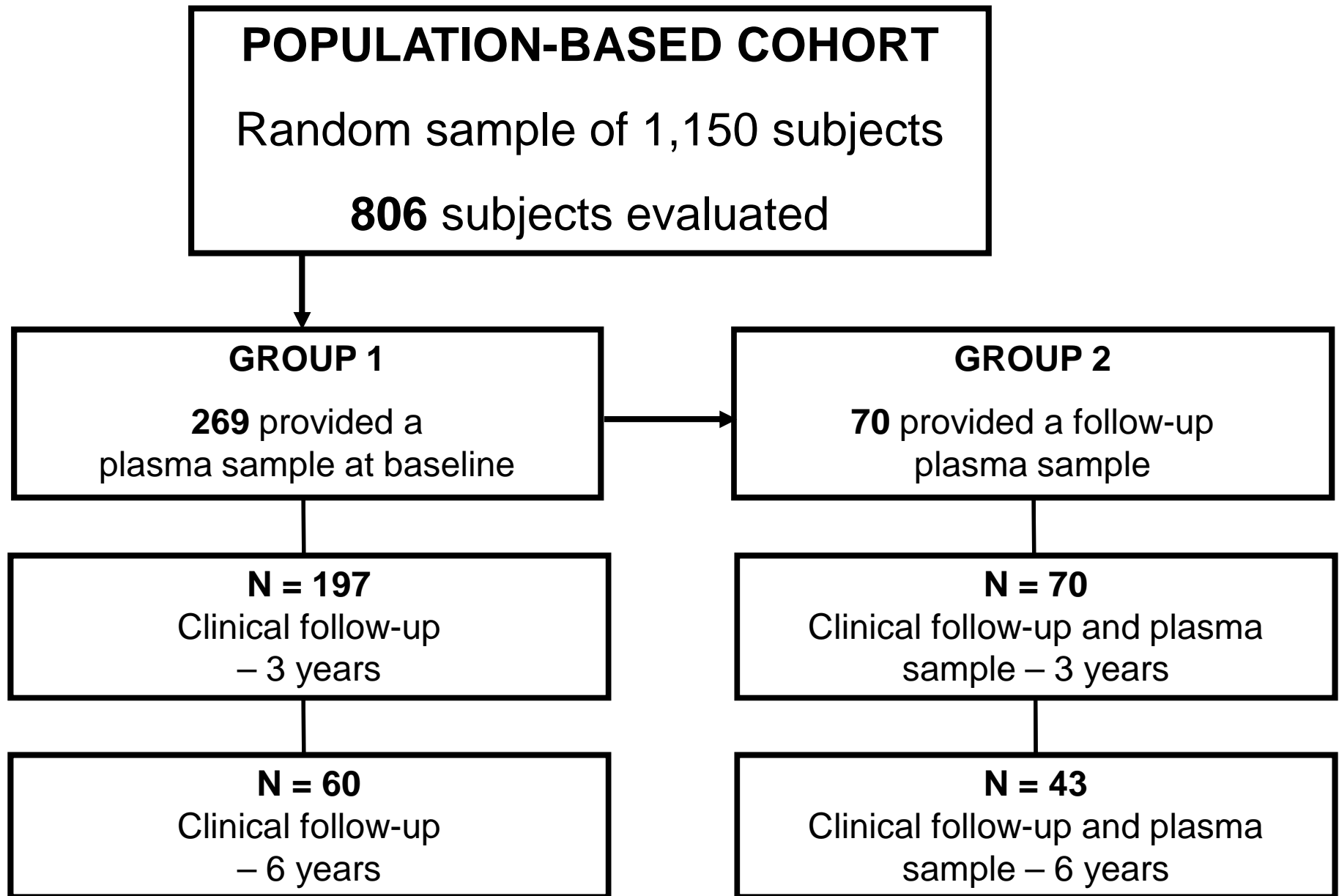


Fig. 1. Formation of the study population.