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Title page

Plasma βamyloid 40 levels are positively associated with mortality risks in the elderly

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Abstract

Background: We evaluated if plasma Aβ levels were associated with mortality risks in a subsample of the French Three-City (3C) prospective cohort study.

Methods: Analyses were based on 1254 participants randomly selected from the initial 3C cohort stratified by center, sex and age in the context of a nested case-cohort study to investigate biological variables. Associations between plasma Aβ and mortality were assessed with the Cox regression model with delayed entry including various potential confounding factors and testing possible mediators.

Results: A relationship between high plasma $A\beta_{1-40}$ concentrations and risk of mortality (HR 1.15; 95% CI [1.01-1.31], p=0.03) was unveiled independently from age, educational level, vascular risk factors, diet, physical activity, cognitive impairment or frailty status. It was only modified when we included Cystatin C levels.

Conclusions: Further investigations are needed to determine precisely the pathophysiological roles of plasma $A\beta_{1-40}$ and Cystatin C and before envisioning any future clinical applications.

Background

The role of plasma β-amyloid biomarkers has been increasingly reported in Alzheimer's disease (AD) diagnosis and prognosis of cognitive decline both in vascular and neurodegenerative amyloidopathies[1-8]. From the onset of neurodegenerative disorders throughout the temporal freeze of the AD process,[9 10], it seems clear that cerebrospinal fluid (CSF) and plasma amyloid markers are highly relevant regardless of the stage of the disease: from memory complaints to loss of autonomy status and/or mortality[1]. In order to improve diagnostic criteria at an early stage, some studies have focused on determining Aβ markers profile in population-based studies in subjects free of cognitive impairment as well as the curve of plasma β-amyloid concentrations over a person's lifetime and prognostic usefulness of plasma β-amyloid biomarkers in aging populations.

This domain raises new questions about the role of amyloid biomarkers in terms of the gaining prognosis. Surprisingly, very little is known about the physiological "role" of amyloid in plasma. As described in a few studies, concentrations of amyloid peptides increase with age[11-13]. The ratio between the two forms $A\beta_{1-40}$ and $A\beta_{1-42}$ is inversely associated with blood pressure values and risk of hypertension in the elderly[14] and also with depression[15]. Vascular risk factors including cholesterol and HbA1c are associated with higher concentrations of $A\beta_{1-42}$[16]. A recent report has suggested that higher dietary intakes of ω3-Poly-Unsaturated Fatty Acids were associated with lower plasma concentrations of $A\beta_{1-42}$ in cognitively healthy elderly subjects[17].

It has been hypothesized that high plasma amyloid level was associated with mortality. To address this issue, we studied whether plasma Aβ concentrations were associated with mortality risk in a subsample of the French Three-City (3C) prospective cohort study of men and women aged 65 and over. Being in this cohort framework enabled us to test if this

association was independent from other factors previously linked to plasma Aβ concentrations and to potential mediators.

Methods

The study design, population and methods used have been described in our accompanying paper. Details of the 3C (three French cities: Bordeaux, Montpellier and Dijon) population-based prospective study were also previously published[3 18]. Analyses were based on 1254 participants randomly selected from the initial cohort and stratified by center, sex and age in the frame of a nested case-cohort study to investigate biological variables ***(Figure 1, flow chart)***. Baseline amyloid concentrations were available for 1244 of them. We also ended up excluding 73 subjects for whom data on confounding factors were missing as well as 24 prevalent dementia cases diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria.

The standardized interview included questions on sociodemographic and lifestyle characteristics including hypertension, diabetes, history of vascular pathologies (stroke, angina pectoris, myocardial infarction and cardio-vascular surgery), diet (fruit, vegetables, fish, olive oil consumption), physical activity and chronic respiratory disorders as well as an inventory of all drugs used. For each participants, MCI status at baseline was defined according to the revised algorithm (MCI-R) based on a cross-sectional evaluation as suggested by an international consensus group[19]. In a subsample of the cohort (Bordeaux and Dijon centers), physical frailty was defined according to the Fried frailty criteria[20], as indicated by the presence of at least three of the following criteria: weight loss, weakness, exhaustion, slowness and low activity level[21].

The plasma Aβ peptide assay was performed using the INNO-BIA kit (Innogenetics, Ghent, Belgium), based on a multiplex xMAP technique with a LABScan-100 system

(Luminex BV, The Netherlands). Analyses were performed in a unique centralized laboratory in Lille (SS, LB). Fibrinogen was measured using the kinetic method of Clauss (Dade Behring, Paris, France), fibrin D-dimer by ELISAs from Diagnostica Stago (Asserachrom VWF and D-Di; Asnières-sur-Seine, France). As previously described[22] we used equations to standardize all serum creatinine values from the Jaffe assay to obtain IDMS- traceable measurements. Then, we estimated eGFR in mL/min/1.73 m^2 using the CKD-EPI equation. Cystatin C factor was measured with the turbidimetric method based on measurements of immunoprecipitation at 540nm with the konelab™ analyzer (Thermo Fischer Scientific Oy, Finland).

All-cause mortality was obtained from civil registration data with a systematic request for all subjects not seen in follow-up visits. Date of point was defined as date of death or date at last follow-up or phone contact for the 8-year follow-up. Information on cause of death was obtained medical records (based on the International Classification of Diseases, version 10, ICD-10) [23]. Mortality from cardiovascular disease (CVD) (ICD-10: I) and cancer (ICD-10: C), the two leading causes of death in this population were considered[24].

Statistical analysis

Aβ variables were evaluated as continuous characteristics. Comparisons between subjects who died and those who stayed alive were done with a Chi2 on the set of variables. We examined correlations between Aβ variables and age or biological dosage using Spearman correlation coefficients.

Associations between Aβ levels and risk of mortality were determined by the Proportional Hazards Regression Model of Cox with delayed entry, where age (in years) was used for the time axis and left truncation for age at study entry. The use of time-on-study as a time scale is

not recommended when covariates of interest are strongly associated with age; which is clearly the case for amyloid dosage[25].

We validated the linearity of all quantitative variables which were included as continuous variables in the models. Assumption of proportionality (for amyloid concentrations) was tested and met in the presence of other covariates in the full model. Multivariate-adjusted models used to probe associations between Aβ variables (for 1 Standard Deviation change) and mortality, were tested for various potential confounders. Selected confounding factors associated with mortality ($p<0.15$) were age, gender, educational level (years of schooling), occupation, body mass index (BMI), history of chronic disorders, depression, limitations of instrumental activities of daily living (IADL), olive oil consumption, physical frailty, Cystatin C, fibrinogen and plasma $A\beta_{1-40}$. Although they were not significant we also added the study center, alcohol consumption, smoking habits, hypertension, fish intake, fruits and vegetables intake and physical activity to the models as they were associated with mortality for the whole sample. ApoE was not related to mortality ($p=0.39$) and thus was not included in the models. The model named BASIC was adjusted for age (timescale), gender and study center. The CLASSIC CONFOUNDERS model also included educational level, occupation, BMI, smoking status, alcohol, history of chronic disorders, hypertension, depression, IADL, fruits and vegetables, fish, olive oil intake and physical activity.

Secondly, we tested the possible mediation effect of cognitive status and physical frailty in these elderly subjects based on two indicators: cognitive status (defined by MCI status at baseline, model MCI) and physical frailty defined only on a subsample (FRAILTY Model).

Furthermore, we examined whether associations between plasma amyloid marker and mortality observed in the CLASSIC CONFOUNDERS model were mediated by biological determinants. First, we studied renal functions through eGFR (under or over 60mg/ml) (Model KIDNEY FUNCTIONS). The role of systemic inflammation measured by fibrinogen

was assessed (Model FIBRINOGEN). Finally, Cystatin C factor, related to renal functions and CSF amyloid metabolism was considered as a potential mediating factor (Model CYSTATIN C).

Analyses were performed using SAS software (version 9.2, SAS Institute Inc., Cary, NC).

Results

Baseline Characteristics (Table 1). During the 8675 person-years of observation in a total of 1147 subjects, 215 deaths (mean follow-up 5.7 years SD=2.6) were registered while 932 participants remained alive (mean follow-up 8.0 years SD=1.4). The subjects who died were predominantly men ($p<0.0001$), older ($p<0.0001$) and current or former smoker ($p=0.01$). They more often had a history of chronic disorders ($p<0.0001$), hypertension ($p=0.01$) and IADL impairment ($p<0.0001$). They were less often fish consumers ($p=0.05$), olive oil consumers ($p=0.01$) and practiced less physical activity ($p=0.01$). They had higher level of fibrinogen (3.49 g/L vs. 3.31, $p=0.01$) and Cystatin C (0.99 unit vs. 0.87, $p<0.0001$) and lower e-GFR (71.5 mL/mn/1.73 m^2 vs. 76.7, $p<0.0001$). Plasma $A\beta_{1-40}$ concentrations increased with age ($r=0.19$, $p<0.0001$) and were correlated with biological factors linked to renal functions such as Cystatin C ($r=0.30$, $p<0.0001$) and e-GFR ($r=-0.31$, $p<0.0001$) but not with fibrinogen ($r=0.03$, $p=0.27$). Similar associations were shown for $A\beta_{1-42}$ concentrations, respectively: Age ($r=0.08$, $p=0.005$), Cystatin C ($r=0.19$, $p<0.0001$), e-GFR ($r=-0.23$, $p<0.0001$) and fibrinogen ($r=0.05$, $p=0.07$). Cystatin C is highly correlated to e-GFR ($r=-0.59$, $p<0.0001$). All correlations are presented in Table 2.

Plasma Aβ concentrations associated with mortality.

Baseline $A\beta_{1-40}$ level and $A\beta_{1-40}/_{1-42}$ ratio were significantly higher in the plasma samples of deceased subjects than in survivors' samples (mean $A\beta_{1-40}$ = 247.7 pg/mL vs. 229.7 pg/mL,

p<0.0001 and mean $A\beta_{1-40}/_{1-42}$ = 6.7 vs. 6.3, p=0.03). The Kaplan-Meier survival curves (Figure 2) graphically show the crude difference in survival between subjects according to their Aβ1-40 level (above or under the tertile 3 cut-off). A gradient of mortality risk was evident for an increase of 1 SD of $A\beta_{1-40}$ plasma level and $A\beta_{1-40}/_{1-42}$ ratio at baseline (Table 3) taking into account factors known to be involved in mortality (Model BASIC and CLASSIC CONFOUNDERS). No difference was observed for $A\beta_{1-42}$ regardless of the survival curve or models.

Association of plasma Aβ concentrations with cause-specific mortality.

Regarding cause-specific mortality, baseline Aβ1-40 level was associated with mortality from cancer (Model CLASSIC CONFOUNDERS n=76, HR=1.26, 95% CI: 1.02-1.57, p=0.04) and to a lesser extent with mortality from CVD (n=44, HR=1.30, 95%CI: 0.97; 1.73, p=0.08). No difference was observed for Aβ1-42 or Aβ1-40/1-42 ratio regardless of the specific cause.

Is the association between plasma $A\beta_{1-40}$ concentrations and mortality explained by cognitive status and/or physical frailty? (Table 4)

Adjusting for MCI, our primary results were not modified for plasma $A\beta_{1-40}$ (HR =1.15; 95% CI [1.02-1.31], p=0.03) or for $A\beta_{1-40}/_{1-42}$ ratio (HR 1.08; CI 95% [1.02-1.15], p=0.01). Adjusting for physical frailty did not substantially alter the observed correlations between mortality and plasma $A\beta_{1-40}$ (HR = 1.21; 95% CI [1.04-1.40], p=0.02) or the $A\beta_{1-40}/_{1-42}$ ratio (HR 1.09; CI 95% [1.02-1.17], p=0.02).

Are correlations between plasma Aβ concentrations and mortality explained by biological systemic abnormalities? (Table 4)

The association between risk of mortality and plasma Aβ concentrations was not modified by the macroscopic inflammatory process measured by fibrinogen (Model FIBRINOGEN), or kidney functions analyzed by e-GFR (Model KIDNEY FUNCTION). Associations between plasma $A\beta_{1-40}$ and mortality were partially explained by the Cystatin C level ($A\beta_{1-40}$: HR 1.10; CI 95% [0.96-1.25], p=0.17; Model CYSTATIN C). Additional adjustments on fibrinogen or e-GFR did not change the CYSTATIN C model results (*data not shown*).

Discussion

To the best of our knowledge, this is the first report highlighting that plasma concentrations of $A\beta_{1-40}$ and $A\beta_{1-40}/_{1-42}$ ratio are associated with an increased risk of mortality in the elderly. Our study also brings forward arguments to try and answer the central question raised by our analyses: How can we explain such a relationship?

One of the first explanations might be that elderly subjects presented intra-cerebral lesions of AD at a prodromal stage of the disease, since we know that AD-related pathophysiological changes can occur many years before the onset of clinical dementia syndrome. First, we excluded all prevalent dementia cases from the analysis sample (n =24) and our complementary analyses did not validate this point.

When the Alzheimer's process is taken into account by including in all models baseline MCI status, the relationships between plasma amyloid $A\beta_{1-40}$ and $A\beta_{1-40}/_{1-42}$ ratio and risk of mortality were not modified. Additionally, the pathological link between plasma $A\beta_{1-40}$ concentrations and mortality was not observed for the $A\beta_{1-42}$ isoform, which is specifically altered in the Alzheimer’s process. In the complementary analysis we also excluded the 72 incident dementia cases (Alzheimer’s disease and other dementia), confirming the absence of an association between $A\beta_{1-42}$ and mortality which was previously evidenced in the Northern Manathan cohort in a three-year follow-up[1].

Another explanation for this association between plasma β-amyloid level and mortality risk in elderly subjects might be frailty. Frailty has been associated with an increased risk of death or adverse clinical outcomes independently of cognitive impairment[21 26-29]. Even though the potential association between frailty and plasma β-amyloid has not yet been observed in our sample (*data not shown*), some elements pointed to this possibility. Recently, β-2-microglobulin has been described as a marker of frailty in older adult[30]. Interestingly, β-2-microglobulin is known to be a component of amyloid fibrils in peripheral amyloidosis[31 32] and $A\beta_{1-40}$ fragment is more involved as a potential marker in amyloidosis and peripheral or cerebral angiopathy disorders. In our population, the frailty confounding factor did not alter the relationship between mortality and plasma $A\beta_{1-40}$ levels. However, we are aware of the limits of this frailty concept and the frailty composite score used in this study. So, the hypothesis that frailty is in fact the missing link between plasma Aβ level and mortality risk remains unanswered.

A mediator metabolic process either by an increased production of β-amyloid peptide via pro-inflammatory mechanisms or by a defect of the amyloid peptides clearance secondary to kidney function alteration could be another hypothesis. The notion of pro-inflammatory process caused by potential atherosclerotic lesions seems to quite relevant on many levels: (1) atherosclerosis plaques commonly seen in the elderly[33]; (2) the major mechanism of atherosclerosis seems to be a chronic inflammation of the vessels[34]; (3) chronic inflammation/atherosclerosis induces activated platelets[34]; (4) part of plasma β-amyloid peptides is produced by platelets in a peripheral pool; (5) both aorta and platelets contains predominantly $A\beta_{1-40}$ peptides whereas $A\beta_{1-42}$ plasma reservoir constitutes a minor contribution to atherosclerotic plaques[34]; (6) the $A\beta_{1-40}$ fragment seems to play a pathophysiological role in disrupting endothelial vascular functions as suggested by results showing that plasma $A\beta_{1-40}$ concentrations are independently associated with the diffuse

small-vessel disease subtype[35]. However, the relationship we observed is in fact independent from the inflammation process assessed through fibrinogen as biomarkers; this was also true considering CRP levels (*data not shown*).

Interestingly, we unveiled that the positive relationship between higher plasma $A\beta_{1-40}$ levels and increased mortality risk could be at least partially explained by the Cystatin C marker. Cystatin C belongs to the super family of the Cystatin type 2 and acts as an inhibitor of the Cystein protease (ie Cathepsins). Cystatin C is known to modulate the inflammatory response[36] and can be considered as a systemic inflammatory marker. However, further adjustments on fibrinogen did not modify the model CYSTATIN results. Secondly, we could have imagined that since Cystatin C was exploring kidney functions[37] it might be implied in the renal clearance of amyloid-β peptide. However, this link seems to be independent from kidney function alterations, as validated by the e-GFR. There is also evidence of Cystatin C role in Aβ metabolism and development of clinical AD[38]. Cystatin has been shown to be involved in Aβ metabolism through different mechanisms, regulating soluble Aβ concentrations as transporter of soluble Aβ isoforms[39] and regulating the Aβ fibrillization process in the brain. Cystatin C interacts with both $A\beta_{1-40}$ and $A\beta_{1-42}$, via a specific and high-affinity binding[40]. The role of Cystatin on amyloid fibril formation was originally suggested by its co-localization with Aβ in senile plaques and in amyloid fibril deposits in vascular walls of AD brains[41]. Cystatin C does not dissolve preformed amyloid-β fibrils or oligomers[40] [42] but its association with Aβ inhibits *in vivo* amyloid-β oligomerization and fibril formation[39] [43]. Unlike a complete deletion of Cystatin C, either reduced or enhanced levels of Cystatin C expression inhibit the aggregation of amyloid-β[39] [43]. These puzzling effects on aggregation may be explained by the different mechanisms underlying Cystatin C regulation of soluble and insoluble Aβ. Finally, the effect on amyloid aggregation depends on the balance between Aβ species, Cystatin C levels and Cathepsin B (CatB). Based on all these arguments, how can

we explain Aβ concentrations variations in the light of Cystatin C levels? An increase in plasmatic $A\beta_{1-40}$ but not $A\beta_{1-42}$ is associated with increased mortality with a correlation with Cystatin C. Increased Cystatin C inhibits degradation of amyloid peptides by CatB and can explain the increase in soluble brain $A\beta_{1-40}$ and $A\beta_{1-42}$, passing in extracellular fluids like CSF or plasma. The 40/42 ratio decreases at the limit of significance. As Cystatin C inhibits Aβ oligomerization (concerning mainly $A\beta_{1-42}$ species) the amount of soluble $A\beta_{1-42}$ should increase relatively to $A\beta_{1-40}$ explaining a higher $A\beta_{1-42}/_{1-40}$ ratio.

Although our results concern a single measurement of plasma β-amyloid concentrations their relevance is reinforced by the large number of potential confounding factors documented at baseline including markers of renal function and inflammation. We controlled for many factors known to be associated with mortality or plasma Aβ concentrations, such as gender[44], age[1 11 44-46], educational level[47], cardiovascular factors[4 44], hypertension[14], atherosclerosis risk factors[16], physical activity and diet[48], depression[15], body mass index[49], tobacco and alcohol consumption[50], diabetes and limitations in at least one of the IADL or impaired kidney functions[44 51]. Furthermore there was no interaction between amyloid levels and gender.

To conclude, plasma $A\beta_{1-40}$ is associated with mortality in our elderly population. Further investigations are needed to determine more precisely plasma $A\beta_{1-40}$ and Cystatin C pathophysiological roles. In our era of preventive care management, the possibility of monitoring changes in plasma biomarkers after specific health/preventive interventions opens new opportunities in this field.

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Details of contributors: **Individual contributions to the manuscript**

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S. Schraen: data acquisition; analysis and interpretation of the data; revising the manuscript.

L.A Gutierrez: acquisition of data; analysis and interpretation of the data; statistical analysis; drafting/revising the manuscript.

C. Pays: data acquisition; revising the manuscript.

O. Rouaud: drafting/revising the manuscript.

L. Buée: drafting/revising the manuscript.

J. Touchon: study supervision; drafting/revising the manuscript.

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JC. Lambert: data acquisition; study supervision; drafting/revising the manuscript.

C. Berr: data acquisition; analysis and interpretation of the data; statistical analysis; study concept; study supervision; drafting/revising the manuscript.

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All patients gave their written informed **consent** to participate in this research study.

The study protocol was approved by the **Ethical Committee** of the Institutional Review Board at Kremlin-Bicêtre University Medical Center.

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TABLES LEGENDS Gabelle et al., manuscript

Table 1: Characteristics of the 3C sub-cohort population according to the 8-year vital status.

		Alive n=932	**Deceased n=215**	**p**
		%	**%**	
Sex	Men	37.12	52.56	<.0001
Educational level : Years of schooling	≤ 5 years	24.03	22.33	0.45
	6-9 years	36.37	37.67	
	10-12 years	20.82	24.65	
	>12	18.78	15.35	
Occupational status	Manual workers	30.47	30.70	0.52
	Clerks	50.00	53.02	
	White collars	19.53	16.28	
BMI [a]	Underweight	4.40	7.91	0.10
	Normal	62.77	59.53	
	Overweight	32.83	32.56	
Smoking	Former and current	38.20	47.44	0.01
Alcohol	≥ 0gr/day	80.58	80.93	0.91
History of chronic disorders [b]	Yes	40.45	59.53	<0.0001
Hypertension [c]	Yes	55.26	64.65	0.01
APOE 4	At least one	20.60	18.14	0.42
Depression [d]	Yes	26.82	31.63	0.16
IADL [e]	>1	5.79	13.95	<0.0001
Fruit and vegetables [f]	Yes	32.83	29.77	0.39

Fish [g]	Yes	90.13	85.58	0.05
Olive oil [h]	No	21.46	30.70	0.01
	Moderate	40.45	37.67	
	High	38.09	31.63	
Physical activity [i]	No	58.58	69.30	0.01
	Yes	32.40	22.79	
	Unknown	9.01	7.91	
Mild Cognitive Impairment	Yes	42.93	45.75	0.46
Physical frailty *	Yes	4.55	12.75	0.0002
		m (SD)	m (SD)	
Age (years)		73.00 (4.85)	77.23 (5.18)	<0.0001
Fibrinogen (g/L)**		3.31 (0.67)	3.49 (0.72)	0.01
e-GFR (mL/mn/1.73m2)		76.70 (12.28)	71.54 (14.82)	<0.0001
Cystatin C (unit)		0.87 (0.18)	0.99 (0.28)	<0.0001
$A\beta_{1-40}$ (pg/mL)		229.7 (51.0)	247.7 (52.8)	<0.0001
$A\beta_{1-42}$ (pg/mL)		38.12 (10.19)	39.43 (12.15)	0.17
$A\beta_{1-40}/_{1-42}$		6.31 (1.86)	6.70 (2.16)	0.02

* n=787 with 149 deceased

**n=1118 with 208 deceased

[a] Body mass index (BMI, underweight <20 kg/m², normal, overweight ≥27 kg/m²);

[b] History of chronic disorders at inclusion (cardiovascular diseases, diabetes, chronic bronchitis, dyspnea, thyroid disease and cancer);

[c] Hypertension (SBP>160 mmHg or DBP >95mmHg, or treatment)

[d] Presence of depressive symptoms assessed with the Center for Epidemiological Studies-Depression Scale (CES-D)[49] including a cut-off of >16 or current treatment for depression;

[e] Limitations in at least one of the instrumental activities of daily living (IADL);

[f] Fruits or vegetables intake at least once a day;

[g] Fish/seafood intake at least once a week;

[h] Olive oil: “no use”, “moderate use”: using olive oil for cooking or dressing alone or “high” use: using both for cooking and dressing;

[i] Physical activity defined as regular when doing sport, active leisure or household physical activity regularly.

Table 2: Correlations between biological parameters and age: Spearman coefficient (p value)

	Aβ1-40 (pg/mL)	Aβ1-42 (pg/mL)	Cystatin C (unit)	Fibrinogen (g/L)	e-GFR (mL/mn/1.73m2)
Age	0,19 (<,0001)	0,08 (0,0048)	0,34 (<,0001)	0,11 (0,0001)	-0,32 (<,0001)
Aβ1-40 (pg/mL)	1	0,48 (<,0001)	0,3 (<,0001)	0,03 (0,27)	-0,31 (<,0001)
Aβ1-42 (pg/mL)		1	0,19 (<,0001)	0,05 (0,07)	-0,23 (<,0001)
Cystatin C (unit)			1	0,15 (<,0001)	-0,59 (<,0001)
Fibrinogen (g/L)				1	-0,09 (0,004)

All analyses were performed on 1147 subjects except for fibrinogen performed on 1118 subjects.

Table 3 (former table 2): Risk of mortality associated with plasma amyloid peptides. (N= 1147 with 215 deceased)

Adjusted hazard ratios (HRs) and 95% confidence intervals (CI) associated with 1 SD increase.

	BASIC				**CLASSIC CONFOUNDERS**			
	HR	95% CI		p	HR	95% CI		p
$A\beta_{1-40}$	1.16	1.02	1.32	0.02	1.15	1.01	1.31	0.03
$A\beta_{1-42}$	1.02	0.90	1.16	0.73	1.00	0.88	1.13	0.97
Ratio $A\beta_{1-40/1-42}$	1.08	1.01	1.15	0.02	1.08	1.02	1.15	0.012

BASIC= Age, gender, study center

CLASSICAL CONFOUNDERS = BASIC + educational level, occupation, BMI, smoking status, alcohol, history of chronic disorders, hypertension, depression, IADL, fruits and vegetables, fish, olive oil intake and physical activity.

Table 4 (former table 3): Risk of mortality associated with plasma amyloid peptides: impact of possible mediators.

Adjusted hazard ratios (HRs) and 95% confidence intervals (CI) associated with 1 SD increase.

	MCI				**FRAILTY**			
	HR	95% CI		p	HR	95% CI		p
$A\beta_{1-40}$	1.15	1.01	1.31	0.03	1.21	1.04	1.40	0.02
$A\beta_{1-42}$	1.00	0.88	1.13	0.95	1.03	0.89	1.19	0.70
Ratio $A\beta_{1-40}/_{1-42}$	1.08	1.02	1.15	0.01	1.09	1.02	1.17	0.02
	CYSTATIN C				**FIBRINOGEN**			
	HR	95% CI		p	HR	95% CI		P
$A\beta_{1-40}$	1.10	0.96	1.25	0.17	1.17	1.02	1.33	0.02
$A\beta_{1-42}$	0.97	0.86	1.10	0.62	1.00	0.88	1.13	0.94
Ratio $A\beta_{1-40}/_{1-42}$	1.08	1.01	1.15	0.02	1.09	1.03	1.16	0.007
	KIDNEY FUNCTION							
	HR	95% CI		P				
$A\beta_{1-40}$	1.14	1.00	1.31	0.05				
$A\beta_{1-42}$	0.98	0.87	1.11	0.77				
Ratio $A\beta_{1-40}/_{1-42}$	1.08	1.02	1.15	0.01				

All analyses were performed for the whole sample (n=1147 with 215 deceased) except for the two models: FRAILTY (n=787 with 149 deceased) and FIBRINOGEN (n=1118 with 208 deceased).

CLASSICAL CONFOUNDERS = BASIC + educational level, occupation, BMI, smoking status, alcohol, history of chronic disorders, hypertension, depression, IADL, fruits and vegetables, fish, olive oil intake and physical activity.

MCI= CLASSICAL CONFOUNDERS + MCI or prevalent dementia status.

FRAILTY= CLASSICAL CONFOUNDERS + frailty status.

CYSTATIN= CLASSICAL CONFOUNDERS + Cystatin C.

FIBRINOGEN= CLASSICAL CONFOUNDERS + Fibrinogen.

KYDNEY FUNCTION= CLASSICAL CONFOUNDERS + e-GFR.

FIGURES LEGEND Gabelle et al., manuscript

Figure 1: Flow Chart of the present study.

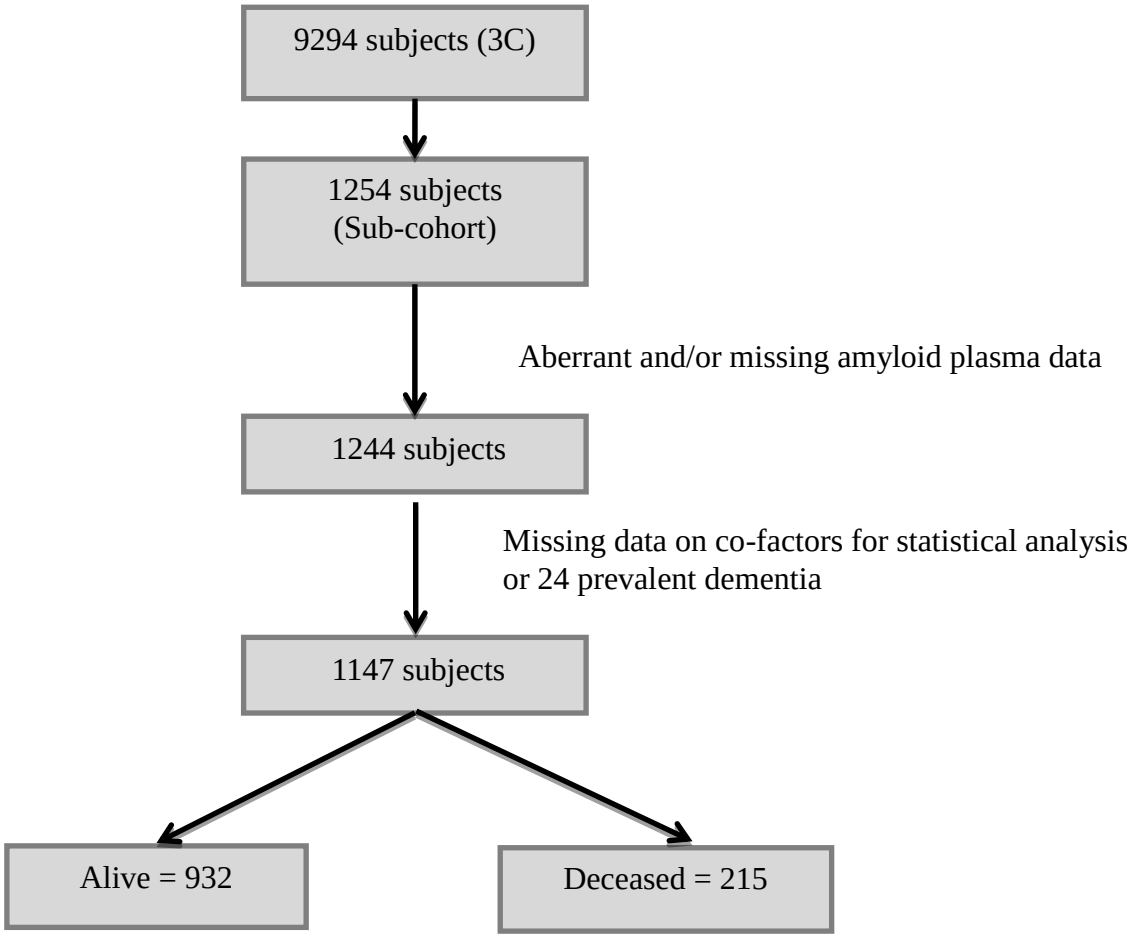
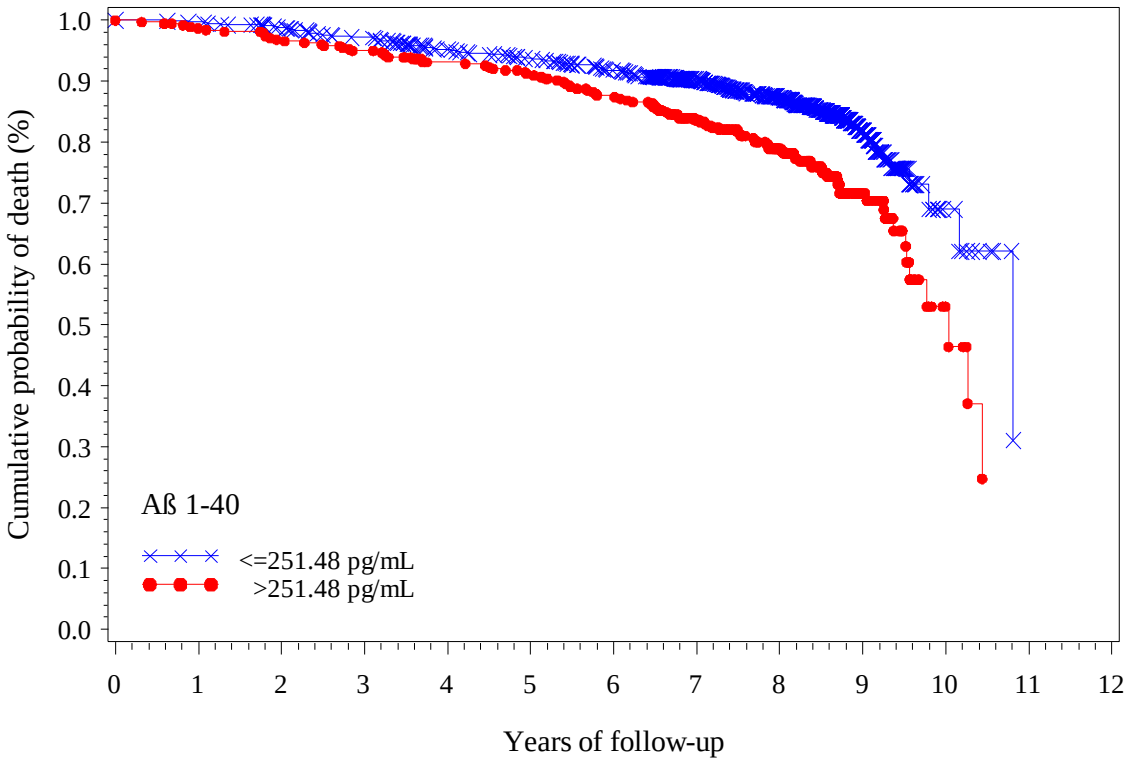


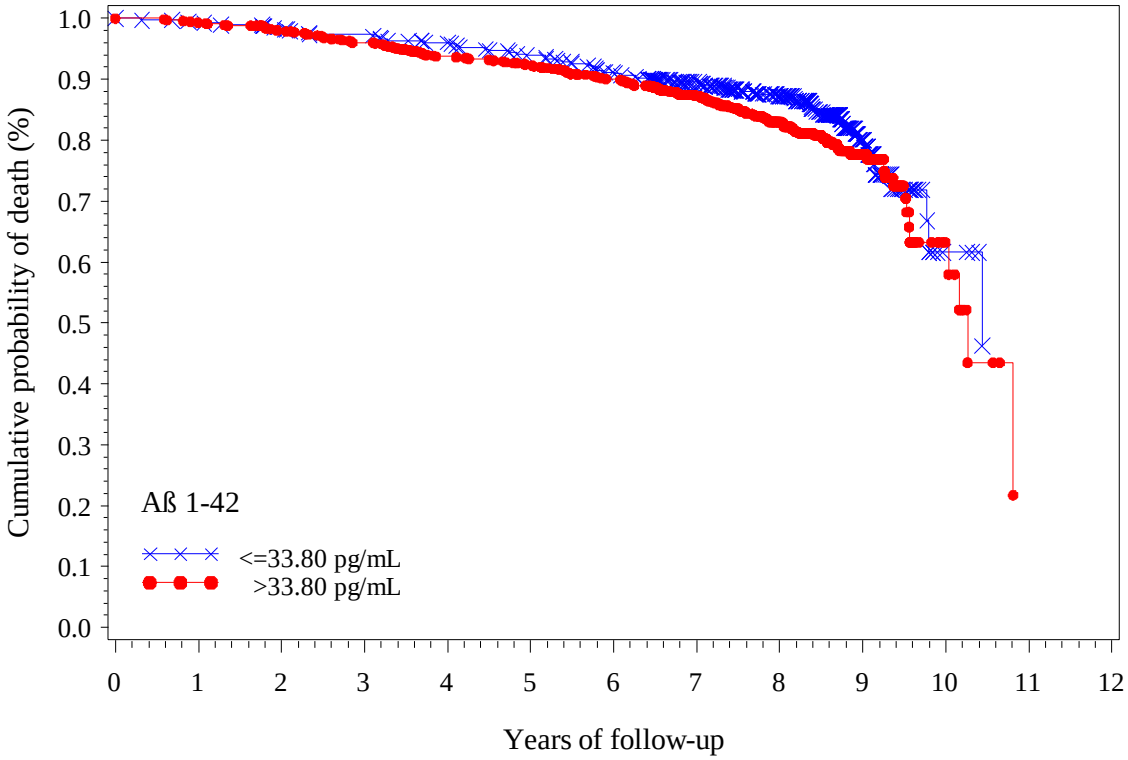
Figure 2: Kaplan-Meyer survival curves for death in 1147 of the 3C study subjects (215 died during follow up) according to plasma β amyloid levels (Figure 2A for Aβ $_{1-40}$, Figure 2B for Aβ $_{1-42}$).

Figure 2 A



(Tertile 1 + Tertile 2) versus Tertile 3

Figure 2 B



Tertile 1 versus (Tertile 2 + Tertile 3)