Chalmers University of Technology

MPBME Biomedical engineering
Master thesis

Evaluating longitudinal aspects of cerebrospinal fluid biomarkers for cognitive disease

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Acknowledgement

I would like to express my gratitude to my examiner Fredrik Westerlund for accepting my masterthesis proposal and agreeing to be my examiner and giving me the possibility to conducting this study.

I would also like to express an extra and special thank you to my supervisor Petronella Kettunen, who has contributed with her expertise and time greatly, to guide and help me during this study. Without your help and guidance, the study would not have been so enjoyable and educational.

Abstract

The most common neurodegenerative disease that millions are affect by today are Alzheimer's disease (AD) and Parkinson's. Diseases and disease progression can be diagnosed and evaluated using cerebrospinal biomarkers. The aim of this project is to evaluate these biomarkers (Aβ 1-42, T-tau, P-tau, albumin ratio, soluble APPα/β) to certain variables and how they affect cognition. Patient data was obtained from the participants of in the Gothenburg Mild Cognitve Impairment Study, and contained baseline data and date from a check up 2 years after baseline. The group included 862 patients and controls. Statistical analysis was performed to calculate normality plots, distribution between groups, medians, interquartile ranges Bonferroni correction and linear regression analysis. Results showed that there were significant differences in baseline for the variables between the groups. For year 2, there were significant differences between all the group for the variables expect for sAPPα. The delta values showed significant differences between the groups in biomarkers P-tau and sAPPβ and all the cognition test. Linear regression analysis showed that all biomarkers went toward the state that is considered sick with increasing age, but biomarkers sAPPα/β were considered stable. Results showed that some biomarkers are better for diagnosing certain diseases and the new biomarkers could potentially introduce a more accurate way of predicting cognitive disease.

Contents

Monday 20th June, 2022

1 Background

1.1 Neurodegenerative diseases

Millions of people are affected by neurodegeneritve diseases around the world, with the most common ones being Alzheimer's disease (AD) and Parkinson's disease [1]. It is estimated that around 55 million people in the world suffer from dementia [2], which is an umbrella term for diseases affecting memory, cognition and behaviour [2].

1.2 Aim

The aim is to evaluate biomarkers in cerebrospinal fluid for cognitive disease to investigate how the biomarkers change depending on certain variables and how cognition is affected by these variables as well.

1.3 Subjective Cognitive Impairment and Mild Cognitive Impairment

Subjective cognitive impairment (SCI) is a condition where the person suffering is self-reporting experiences of worsening of thinking abilities or more frequent memory loss [3]. The decline however, can not be verified by standard cognition tests, which makes the condition hard to diagnose [4]. The symptoms usually consist of increased problem with memory, losing line of thought, having problems with planning and decision and depression [4]. SCI is considered a prestage for dementia but the links to disease are not fully understandable.

Mild cognitive impairment (MCI) is more severe than SCI, with a stronger decline in mental abilities, which are noticeable by friends and family [5], but does not hinder the person affected from performing everyday activities. MCI can be verified with cognition tests and can therefore be diagnosed [5]. MCI patients have similar symptoms as SCI patients but have problems with attention, language and visual depth perception [5]. MCI can develop into dementia, like AD, but for some people it can revert to normal condition or stabilize and not get worse [6].

1.4 Alzheimer's disease

One of the most common cognitive disorders in elderly is AD. Problems with nonmemory cognition are usually the first signs of AD, which includes word-finding, vision and spatial issues and impaired reasoning or judgement, as the cerebral cortex is damaged [7] [8] and also memory, as the entorhinal cortex and hippocampus are affected [8]. As the disease progresses, the disease can develop into different stages, consisting of mild, moderate and severe AD. Mild AD bring greater memory loss, troubles completing daily tasks and personality and behaviour changes [7]. When the disease has progressed and developed into moderate AD, the damage is usually found in brain regions that control language, reasoning, conscious thoughts and sensory processing. Memory loss will be even greater, and leads to trouble with recognizing family and friends [7]. With severe AD, the brains is heavily damaged and shrunken, which makes people with severe AD unable to communicate and will be dependant on others for care [7].

AD is characterized by the accumulation of extracellular plaques on the brain that are consisting of amyloid-β (Aβ 1-42) [9], where amyloid plaques seem to gather between the neurons and disrupt cell functions [8]. Another hallmark is intracellular tangles, also called neurofibrillary tangles, made of hyperphosphorylated tau (P-tau), a protein that accumulates in abnormal amounts inside the neurons which damaged the synaptic connection between neurons [8].

1.5 Subcortical small vessel disease

Subcortical small vessel disease (SSVD), is the most common form of vascular cognitive disease. The diseases affect small arteries, arterioles, venules and capillaries deep in the brain [10], which cause lacunar infarcts [11]. Characteristic for SSVD is ischemic damage in the basal ganglia and leads to atrophy in that brain region, and also white matter hyperintensities (WHM), which is the attenuation of white matter and results in deterioration in conductivity in the neural pathways [12]. SSVD is responsible for 25% of the ischemic strokes around the world [10]. Magnetic resonance imaging is used to properly identify SSVD, and the radiological features, which include global atrophy in basal ganglia, WHM, microbleeds, which are small brain hemorrhages caused from the damaged vessels, and lacunes, which are cavities filled with cerebrospinal fluid [11]. Compared to AD, SSVD does not signify with memory loss, but rather by problems with cognitive speed and attention.

1.6 Mixed dementia (Alzheimer's disease/Subcortical small vessel disease

When patients have both AD and SSVD, is called mixed dementia, and is regarded as the third most common cause of dementia [13]. In this disease the amyloid plaques and the neurofibrillary tangles are present in the brain along with the vascular problem in the brain. Studies have tried to differentiate inchoate mixed dementia with inchoate AD and inchoate SSVD [13]. It was found that patients with mixed dementia usually have an AD-like biomarker profile and a SSVD-like cognitive profile [13].

1.7 Biomarkers

Biological markers are measurable biological indicators that make it possible to observe and examine different process in the body. For example, biomarkers can be used to follow disease progress, or differentiate between diseases. For the biomarkers that will be mention next, they were analyzed in cerebral spinal fluid (CSF). CSF is found in the ventricles and subarachnoid spaces in the cranium and spine [14], and its thought to correspond to chemical changes inside the brain.

1.7.1 Amyloid-β 1-42

Amyloid-β (Aβ 1-42) are peptides that are derived from the breakdown of amyloid precursor protein (APP) with the help of β- and γ-secretase [15]. As mentioned before, the abnormal accumulation of Aβ 1-42 is the main component of the plaque on the brain causing AD. The APP that it derives from, is a type 1 transmembrane protein that is important for neuronal development, signaling and intracellular transport [15]. Lower levels of Aβ 1-42 indicate disease.

1.7.2 Phosphorylated tau

Phosphorylated tau (P-tau) is usually described as a cytsolic protein that is involved in microtubules and help regulate the axonal transport, but studies have shown that it is also involved in DNA stabilization and synaptic function [16]. As previously mentioned, P-tau is a key factor in neurofibrillary tangles which are on the causes for AD.

1.7.3 Total tau

Total tau (T-tau) can be seen as a general marker for neurodegeneration while P-tau is suggested to be seen as a more specific marker for AD [17]. Patients that have AD, usually have higher amount of T-tau and P-tau compared to healthy people, which means that higher levels could indicate disease. [17].

1.7.4 Soluble APPα/β

When the amyloid precursor proteins are cleaved in the "non-amyloidogenic" way, you get the metabolites soluble APPα/β (sAPPα/β) [18]. sAPPα is formed when APP is cleaved by the α-secretase [18] and sAPPβ when APP is cleaved by β-secretase [19]. The properties of the sAPPα/β in the central nervous system are not fully understood, but they have been linked to synaptic plasticity [19].

1.7.5 Albumin ratio

The CSF albumin/serum, or usually called albumin ratio, is a biomarker that is used to evaluate the integrity of the blood-brain barrier (BBB) [20]. The biomarker compares the amount of albumin in serum to the amount in CSF. The BBB is responsible for regulating and maintaining the ideal environment for the brain [21]. A higher ratio could indicate damage in the blood-brain barrier, which is seen as an indication of SSVD.

1.8 Cognitive tests

A way to evaluate if a persons brain is functioning as it should, is by letting the person take cognitive tests under the supervision of a neuropsychologist, which are short and quick tests that can identify cognition problems, which may be caused by dementia [22]. Cognitive tests used in this rapport will be listed and explained below.

1.8.1 Mini-Mental State Examination

A widely used test for cognitive function is the Mini-Mental State Examination (MMSE), which test the orientation, attention, memory, language and visual-spatial skills [23]. The test is quick and requires no additional equipment and provides a way to see deterioration over time [24]. Test scores range from 0-30, where scores of 24-30 mean no cognitive impairment, 18-23 corresponds to mild cognitive impairment and 0-17 is severe cognitive impairment[24]. The disadvantages with MMSE is that it is biased against people with lower education, biased at people with vision problems, examination of visuospatial cognition is limited and it has a poor sensitivity for detection of early/mild dementia [24].

1.8.2 Rey Auditory Verbal Learning Test

For evaluation of verbal memory, the Rey Auditory Verbal Learning Test (RAVLT) is used. It tests the nature and the degree of memory dysfunction and can track changes in memory over time.

RAVLT works by letting the patient listen to a list of 15 nouns, and to repeat as many words as possible afterwards. After five repetitions of that list, the patient is to listen to another list and try to recall as many words as possible. Directly after, the patient is asked to recall the words from the first list, and then again after 20 minutes [25]. Then the examiner reads out words from another list, and the patient is to indicate if the word was from the first list [25].

1.8.3 TMT A and TMT B

The Trail Making Test (TMT) is also on of the methods for evaluating cognitive ability. The TMT consist of two parts, where the aim is to complete the tests as quickly and accurately as possible, so a longer time means a poor result [26]. For the first test, TMT A, the person is given a paper with 25 circles, all with a number from 1-25. The person is to draw a line between the circles in ascending order, from 1-25 as fast as they can [26]. This evaluates the patient's thinking speed.

For the second test, TMT B, the person is given a paper with 24 circles, with half containing number from 1-12, and the other half containing letters from A-L. The person is then asked to draw a line in ascending order of both numbers and letters, e.g 1-A-2-B-3-C etc. [26]. This test gives a picture of the patients executive function.

2 Material and methods

For this project, various methods have been used for obtaining the biomarker data and cognition data, and evaluating them using software. These will be explained in the sections below.

2.1 Control-patients cohort

The patient data was obtained from the participants in the Gothenburg Mild Cognitive Impairment (MCI) study [27]. The patient group include 136 controls, 221 patients diagnosed with SCI, 318 patients diagnosed with MCI, 98 patients diagnosed with AD, 30 patients diagnosed with SSVD and 59 patients diagnosed with mixed AD/SSVD. The data consisted of baseline values and values recorded at the follow up visit 2 years after baseline. Patients that were excluded were diagnosed with different diseases than cognitive diseases or did not have enough data to be evaluated.

2.2 Microsoft Excel

Microsoft Excel was used to clean the data and tables with all the patient data for easier handling, as well as calculating the differences in baseline values to year 2 values in each patient. Those with missing values on either of time points, were excluded as the results were invalid. The values for TMT A and B shown in seconds. A new worksheet was created, and data was selected and transferred from an original worksheet containing all patient data, including data not studied in this project. Using the original worksheet would have been unnecessary complicated. The structured by the patient first (ID-number, age, gender and diagnosis), biomarker variables secondly, and thirdly the cognitive variables. For the later columns, data from year 2 was transferred in to the new worksheet, excluding patient data. And further more, were the calculated delta values between the cells.

2.3 Statistical Package for the Social Sciences (SPSS)

Statistical Package for the Social Sciences (SPSS) was used to calculate all statistical related values, which includes normality plots, distribution between patient groups, medians, interquartile ranges, Bonferroni correction and linear regression analysis. Firstly, the Microsoft Excel worksheet was imported to SPSS. The second step was to generate normality plots using Shapiro Wilk test to find out if the selected variable was normally distributed across the patient groups. When the p-value was below 0.05, the distribution was not regarded as normally distributed. As majority of variables were not normally distributed, the median for all variables was used to facilitate calculations. The medians also included the calculated interquartile ranges for the variables.

Then non-parametric test was used, such as Kruskal–Wallis, for the variables in all the groups. Pairwise comparison between the groups were done using Mann-Whitney U test. The baseline and year 2 values were compared using 2-Related samples Wilcoxon signed-rank test. Bonferroni correction of significance scores was used for the pairwise comparisons,

and referred to as p-values throughout the text. Significant differences (p-values ¡ 0.05) were indicated in graphs by the use of letters. Groups sharing the same letter did not show statistically significant differences. All results were transferred to different Microsoft Excel worksheets. The worksheet included demographic tables for baseline and year 2, tables for the pairwise comparison between the groups and differences.

3 Results

This section will present the most interesting results, in tables and figures, while the rest can be seen in the appendix A. Significant values are marked with bold text.

3.1 Baseline

First the demographics as well as biomarker levels and cognitive test scores of the cohort was investigated for the controls (CTRL), patients with SCI, MCI, AD, mixed AD/SSVD (MIX) and SSVD at baseline. In Table 1, it can be seen that there were significant differences between the variables in the different patient groups

Table 1: Demographics of the controls and patient groups at baseline

	Diagnosis						P-value between groups
Variables	**Control**	**SCI**	**MCI**	**AD**	**Mixed AD/SSVD**	**SSVD**	
Number of participants	n = 136	n = 221	n = 318	n = 98	n = 59	n = 30	
Age, median (IQR)	65 (61-69)	61 (57-67)	66 (59-72)	67 (61-72)	71 (67-75)	70 (65-75)	**< 0.001**
Number of males/females (%)	48/79 (35/58)	90/131 (41/59)	137/181 (43/57)	33/65 (34%/66%)	23/36 (39%/61%)	20/10 (67%/33%)	**< 0.001**
Years of education, median (IQR)	12 (10-14)	14 (11-16)	12 (9-14)	10 (8-14)	10 (8-14)	12 (9-13)	**< 0.001**
Aβ 1-42, median (IQR)	676 (500-915)	661 (530-804)	568 (401-750)	360 (281-453)	390 (320-500)	580 (473-700)	**< 0.001**
T-tau, median (IQR)	292 (200-397)	274 (200-386)	352 (240-550)	610 (413-861)	685 (480-850)	323 (221-425)	**< 0.001**
P-tau, median (IQR)	48.5 (34-60.3)	47 (36-59)	55 (43-75.8)	82.5 (59.5-114.5)	84 (61.3-116.8)	52.5 (35.8-62.8)	**< 0.001**
sAPPα, median (IQR)	306 (221-369)	289 (229-395)	297 (233-403)	292 (221-351)	286 (214-396)	232 (170-305)	**0.025**
sAPPβ, median (IQR)	526 (400-676)	570 (439-715)	552 (397-801)	553 (385-719)	478 (375-644)	371 (300-480)	**< 0.001**
Albumin ratio, median (IQR)	6 (4.8-7.2)	5.5 (4.5-7.5)	6 (4.7-7.8)	5.3 (4.3-7.6)	6.5 (5.1-8.7)	7.8 (5.7-9.3)	**0.002**
MMSE total, median (IQR)	29.5 (29-30)	29 (29-30)	28 (27-29)	25 (23-27)	25 (23-26.5)	25 (24-27)	**< 0.001**
RAVLT recognition, median (IQR)	15 (15-15)	15 (15-15)	15 (13-15)	12 (12-14)	13 (9-14)	14 (12-15)	**< 0.001**
TMT-A, median (IQR)	33 (27-40)	35 (30-42)	43 (33-55)	59 (45.5-80)	54 (43-75.5)	73 (44.5-86.8)	**< 0.001**
TMT-B, median (IQR)	77.5 (66.3-92.8)	75.5 (63-94.8)	107 (81.5-141.5)	155 (106-214)	180 (129.3-255.3)	202 (132-256)	**< 0.001**

SCI: Subjective cognitive impairment, MCI: Mild cognitive impairment, AD: Alzheimer's disease, MIX: Mixed Alzheimer's disease and subcortical small-vessel disease, SSVD: subcortical small-vessel disease, IQR: Interquartile range, Aβ: amyloid-beta, T-tau: total tau, P-tau: phospho-tau, sAPPα: Soluble amyloid precursor protein alpha, sAPPβ: Soluble amyloid precursor protein beta, MMSE: Mini-Mental State Examination, RAVLT: Rey Auditory Verbal Learning Test, TMT-A: Trail making test part A, TMT-B: Trail making test part B

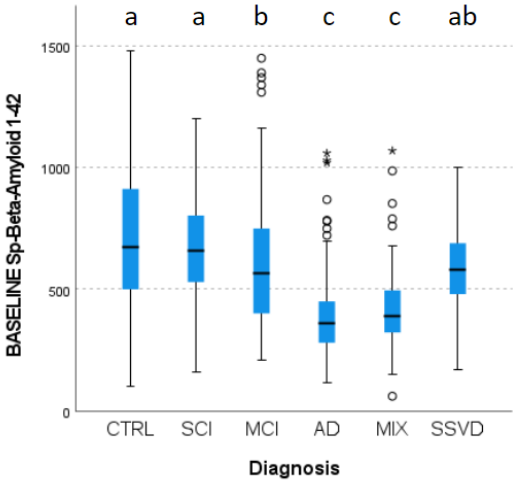
Further comparisons between all the patient groups were done for each variable. As seen in Appendix A.1 (A.1), there was a significant difference between the ages of the cohort groups. The SCI group had the youngest participants, while the MIX group had the oldest participants.

When the number of years of education (A.2) were compared for the patient groups, the participants in the SCI group had significantly longer than all the other groups, while the AD group has the shortest education (although not significantly different from the other disease groups).

In Figure 3(A), it was observed that the CTRL group has the highest amount of CSF Aβ 1-42, which is to be expected as they are classified as healthy. The AD group has the lowest amount of Aβ 1-42, which fits well with the notion that this biomarker is reduced in AD. A declining trend in Aβ 1-42 levels could be seen in patients ranging from CTRL to SCI to MCI and to AD, presumably showing the disease progression of amyloid pathology. SSVD levels of Aβ 1-42 were significantly different from AD and MIX patients. Aβ 1-42 levels below 600 are said to be strong indications for AD.

Next, the CSF levels of T-tau and P-tau were explored (Figure 1(B) and (C)). The AD and MIX groups showed the highest amount of T-tau compared to the other groups, whilst the CTRL and SCI groups has the lowest, as can be seen in Figure 1(B). A similar trend was seen for P-tau (Figure 1(C)), where the AD and MIX groups have the significantly highest amount of CSF P-tau, and the CTRL and SSVD groups has the lowest levels of P-tau. For T-tau, it is said that a value over 350 is a strong indication of AD, but for P-tau it is unsure, but generally a higher value is negative.

The profile of the CSF/serum albumin ratio differed from the other biomarkers. When comparing the albumin ratio between the groups as a sign of blood-brain-barrier damage, it could be observed that the SSVD group has the highest level albumin ratio, significantly different from levels of SCI and AD patients. An increasing trend in albumin ratio could be seen in the AD - MIX - SSVD spectrum, where MIX groups also had high ratios, as seen in Figure 1(D).



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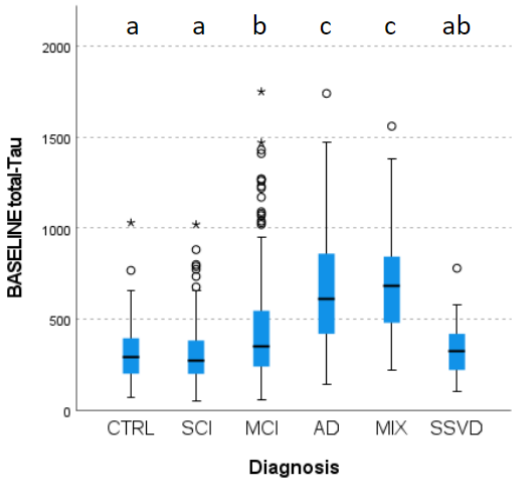

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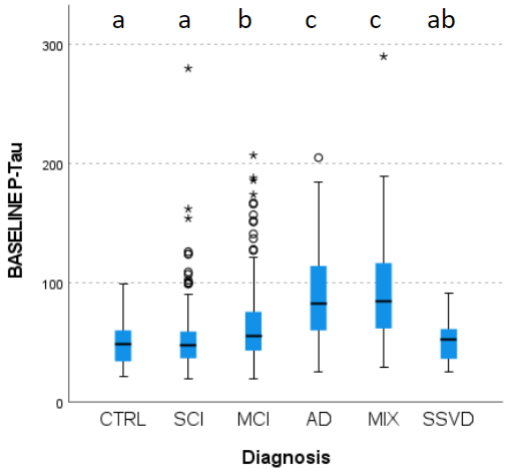

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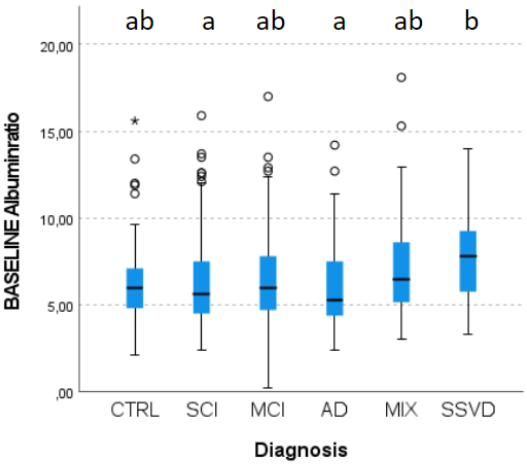
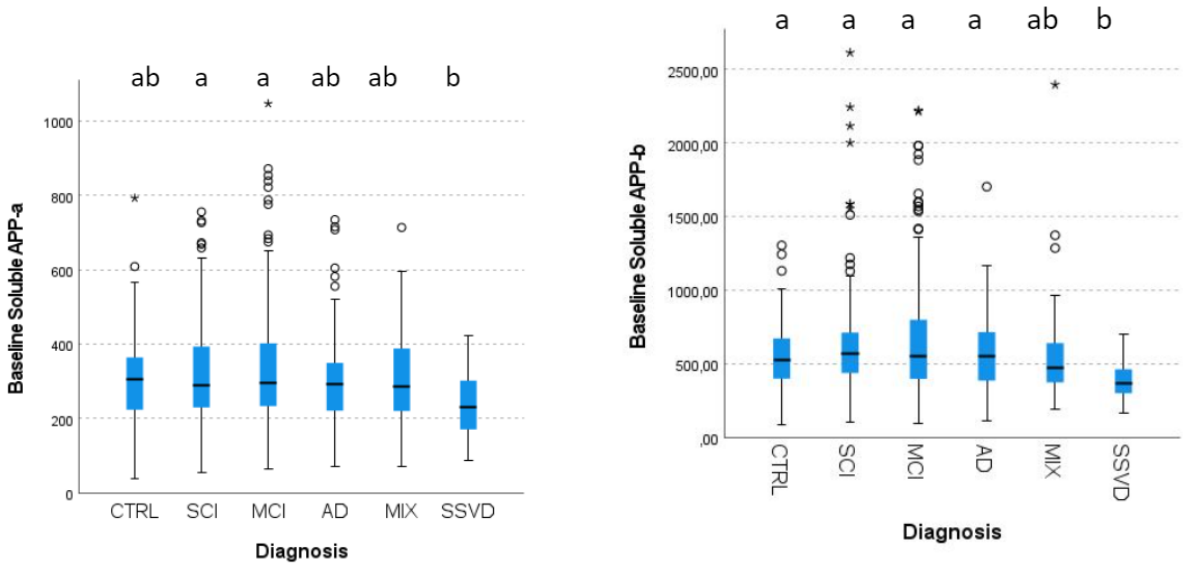


(D)

Figure 1: Graphs showing the values of the biomarker variables between the groups, **(A)** Aβ 1-42, **(B)** T-tau, **(C)** P-tau and **(D)** Albumin ratio.

The last CSF biomarkers to explore were sAPPα and sAPPβ. In Figure 2(A), it can be seen that the SSVD group had the lowest amount of sAPPα, while the CTRL group had the highest median of sAPPα, although the SCI and MCI groups were significantly different from the SSVD group. Similarly, when comparing the diagnosis groups in Figure 2(B), it can be observed that SSVD had the lowest level of sAPPβ, significantly different from CTRL and all other patient groups apart from the MIX group.



(A) **(B)**

Figure 2: Graphs showing the values of the biomarker variables between the groups, **(A)** sAPPα and **(B)** sAPPβ.

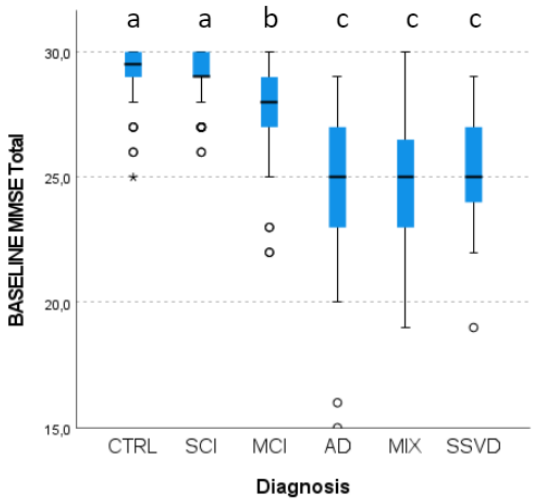
Next, we explored how cognition varied in the different participants of the study. We had selected a general cognitive screening test (MMSE; Figure 3(A)) as well as a verbal memory test (RAVLT; Figure 3(C)), and the tests for speed (TMT-A, time given in seconds, Figure 3(C)) and executive function (TMT-B, time given in seconds; Figure 3(D))

In Figure 3(A), AD and MIX patients had the lowest scores of MMSE, with SSVD slightly behind, which is to be expected as these groups classify as disease groups with confirmed cognitive dementia. It could also be observed that the MCI group was in a decline, significantly different from all the other groups, indicating that this group is in the process of developing cognitive disease.

In Figure 3(B), it could be observed that CTRL and SCI participants had nearly perfect scores, whilst AD and MIX have the lowest. Again, the MCI group showed a position between CTRL/SCI and AD/MIX, but resembling to the memory performance of SSVD patients. A declining trend in RAVLT scores could be observed in SSVD to AD groups, confirming the observations that memory impairments are most prominent in AD, exist in MIX but appear later in the disease process of SSVD.

Finally, the speed and executive function at baseline was explored. In figure (C), an increasing trend in TMT-A processing time could be seen in the spectra of disease groups, from CTRL to SSVD, with SSVD having the longest time to finish the TMT-A test. The CTRL group has the shortest times, confirming the intact cognitive speed of these participants.

The image of the TMT-B test times (Figure 3(D)) was similar. It was observed that CTRL and SCI groups had significantly shorter TMT-B times than the other groups, while there is an increasing trend in TMT-B time in the AD to SSVD groups, where the SSVD group has the longest TMT-B times.



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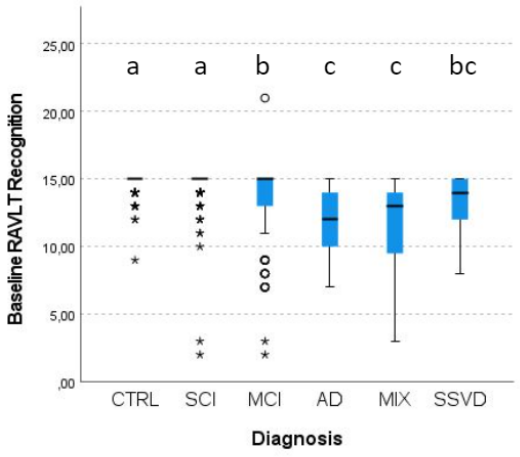

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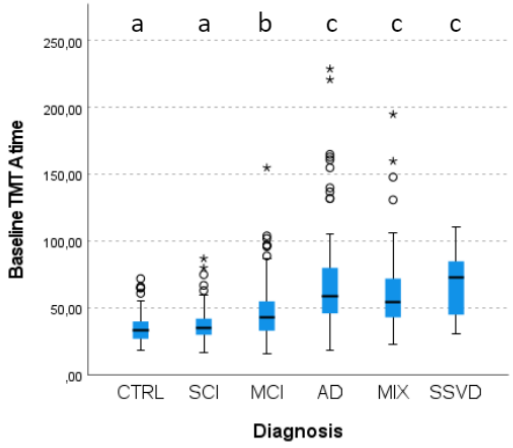

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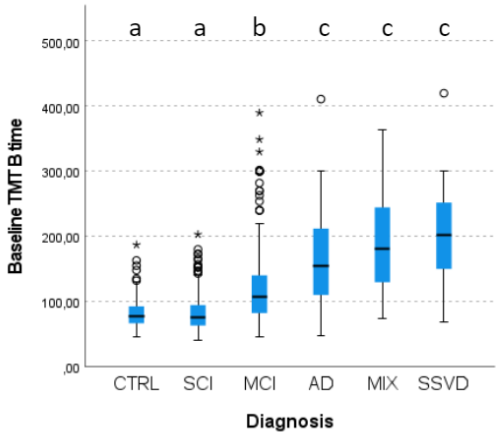


(D)

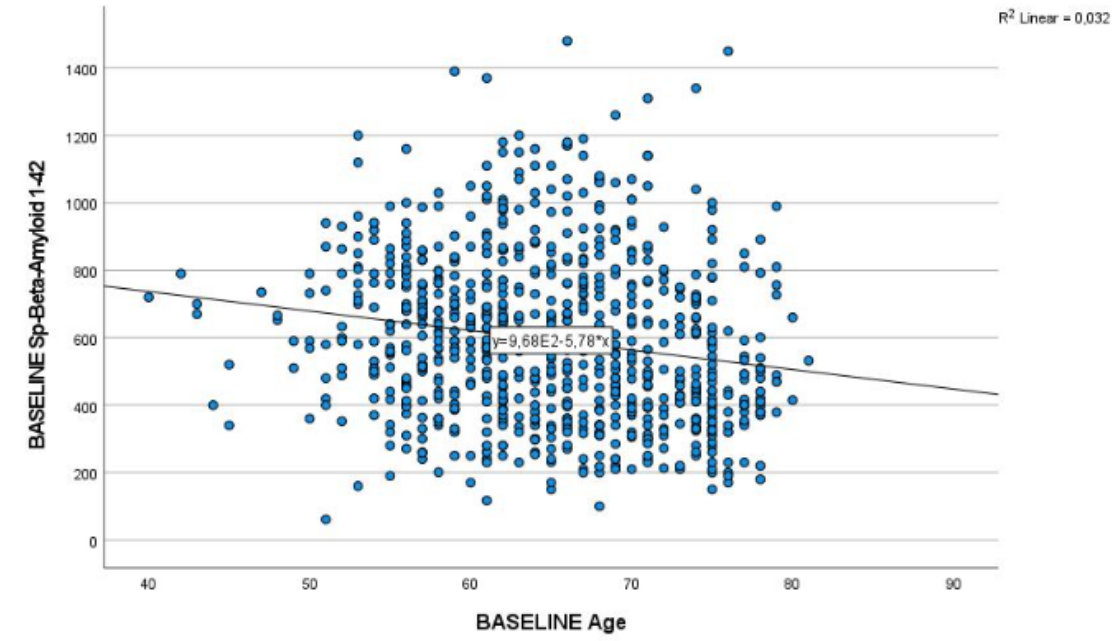
Figure 3: Graphs showing the value of the cognitive variables between the groups, **(A)** MMSE scores, **(B)** RAVLT scores, **(C)** TMT-A completion times and **(D)** TMT-B completion times.

3.2 Linear regression analysis

To better understand how the biomarkers and cognitive scores correlated with patient variables such as patient age, length of education and with each other, linear regression analysis of baseline values were performed on the whole cohort.

First, biomarker values were explored in relation to age. In Figure 4(A), a linear regression analysis showed that Aβ 1-42 decreases as age increases. This indicates that the pathological processes of AD patients corresponding to low Aβ 1-42 values could be enhanced with age.

Figure 4(B) shows an increase in T-tau as age is increasing in the total cohort. P-tau is presenting the same trend, with an increase in amount of P-tau as age increases, as seen in A.3. It is known that tau-proteins are signs of brain damage and this could be increased and diseases enhanced as age progresses.



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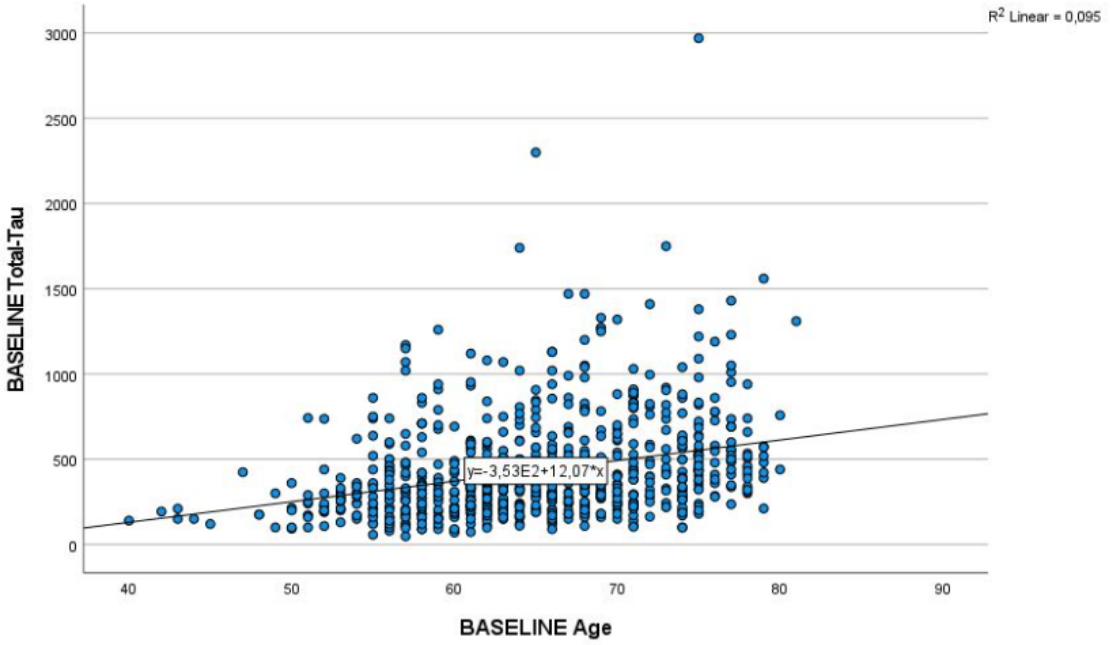


(B)

Figure 4: Linear regression analysis graphs showing how with changing Age, the variables **(A)** Aβ 1-42 and **(B)** T-tau are affected.

In A.4, the CSF/serum albumin ratio was also slightly increasing as the participant's ages were increasing.

The sAPPα seemed to have a slight increase as age increases, as seen in A.5. Even sAPPβ was having an increasing trend as age increased in the cohort, which is seen in A.6.

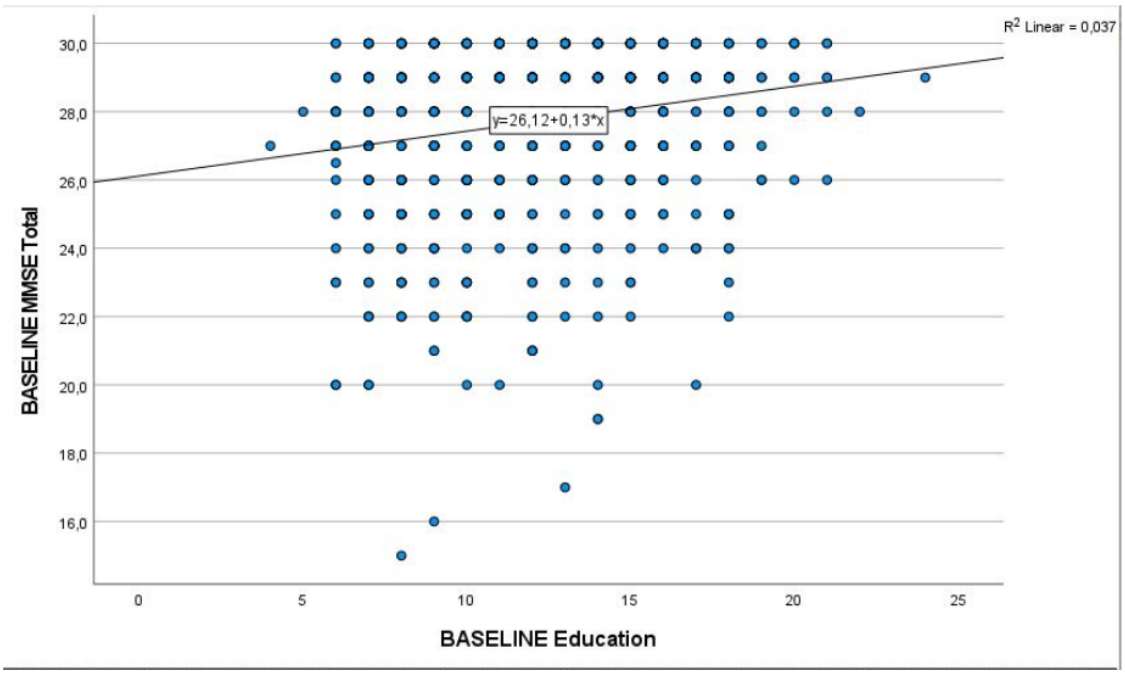
Next, the cognitive scores were explored in relation to age. In A.7, the MMSE total score was shown to be decreasing as the participant group age was increasing, although the variability was large. This indicates that global cognitive scores were reduced with age.

Moreover, the RAVLT scores were showing a decreasing trend as age was increasing, which is seen in A.8. Also here, a large spread was seen at each age.

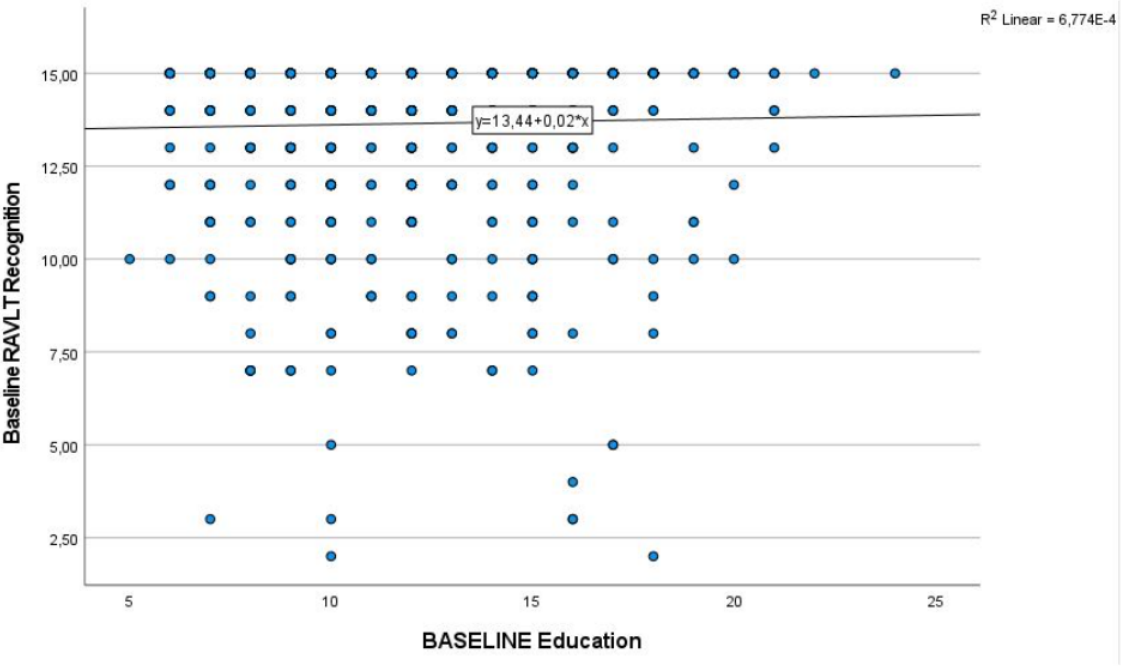
As age was increasing, the cohort was taking longer time to perform the TMT-A test, as shown in A.9. Similarly, an increase in age led to longer finishing times when performing the TMT-B test, as seen in A10. These data indicate that cognition is indeed dependent on the patient's age, although there was a large variability.

Next, we explored how the cognitive test scores were correlated to ages of education in the cohort. Figure 5(A) shows the linear regression analysis for MMSE and education, and it could be noted that education has some effect on the MMSE scores, as an higher education seemed to lead to higher MMSE scores. However, a large spread of MMSE scores could be seen for each education time.

In Figure 5(B), it could be seen that longer education leads to slightly higher RAVLT scores in the cohort, although the correlation was not convincing. This indicates that verbal memory is not sensitive to the time you spend in school.



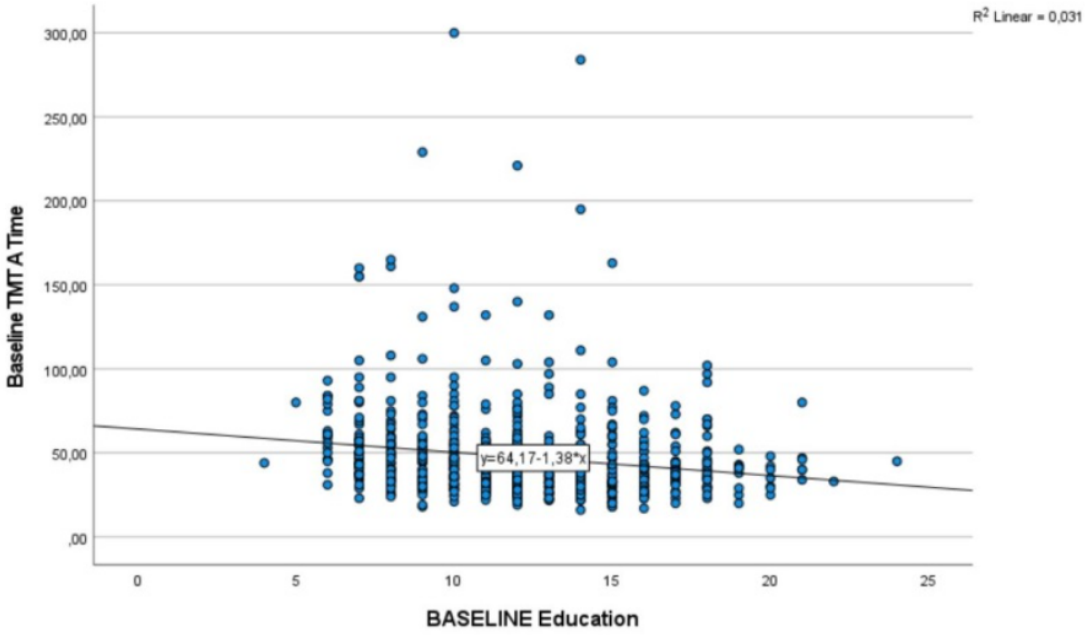
(A)



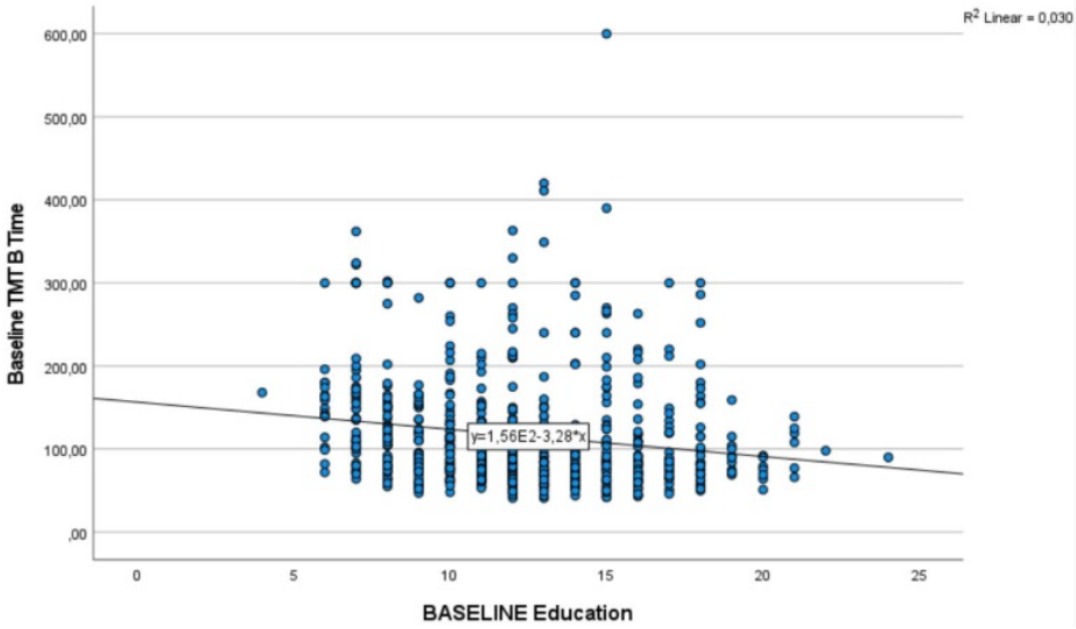
(B)

Figure 5: Linear regression analysis graphs showing how with increasing Education, the cognitive scores for **(A)** MMSE and **(B)** RAVLT are affected.

In Figure 6(A), patients and controls that had a longer education, seemed to complete the TMT-A test at a faster rate than those with shorter education. This was similar to data in Figure 6(B), where patients with longer education, completed the TMT-B test faster than those with shorter education. These correlations indicate that education either helps the brain to develop speed and executive function, or that long education builds a resilience that prevents against the vascular damages often giving rise to impairment of speed and executive function


R² Linear = 0,031
Baseline TMT A Time
300,00
250,00
200,00
150,00
100,00
50,00
,00
y=64,17-1,38*x
0
5
10
15
20
25
BASELINE Education

(A)


R² Linear = 0,030
Baseline TMT B Time
600,00
500,00
400,00
300,00
200,00
100,00
,00
y=1,56E2-3,28*x
0
5
10
15
20
25
BASELINE Education

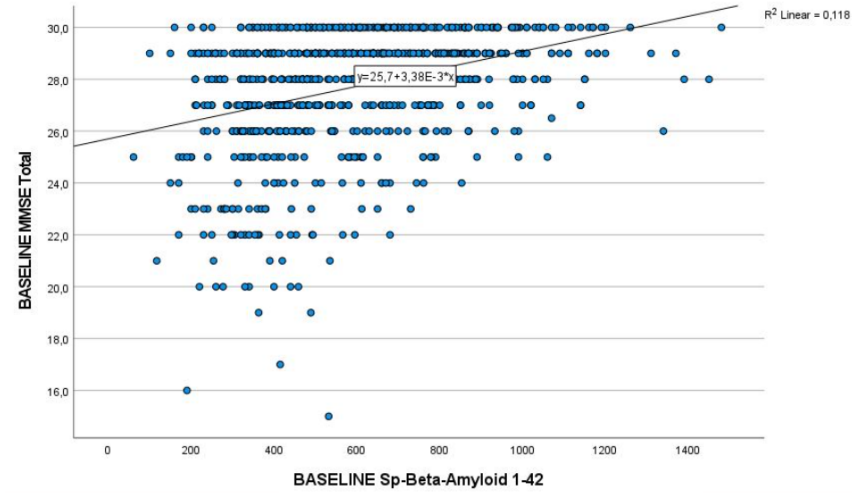
(B)

Figure 6

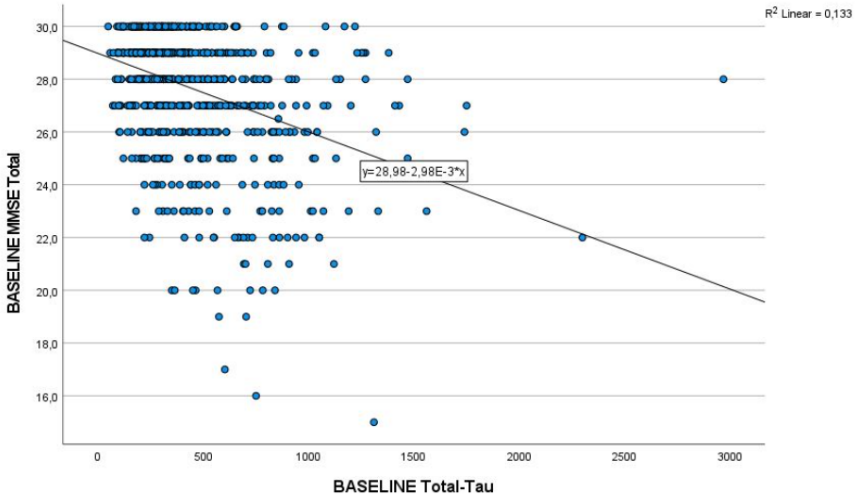
Since the cognitive scores seemed to be correlated to age and education, we were interested in further exploring how the CSF biomarkers correlated to the cognitive scores. In this way, we could get information regarding disease processes and how they affected the cognitive capacity.

With a higher amount of Aβ 1-42, the higher the MMSE scores, which indicated a correlation between them, as seen in Figure 7(A). This is not surprising as higher levels of A 1-42 indicates that the patient is not suffering from AD, a disease that affects MMSE.

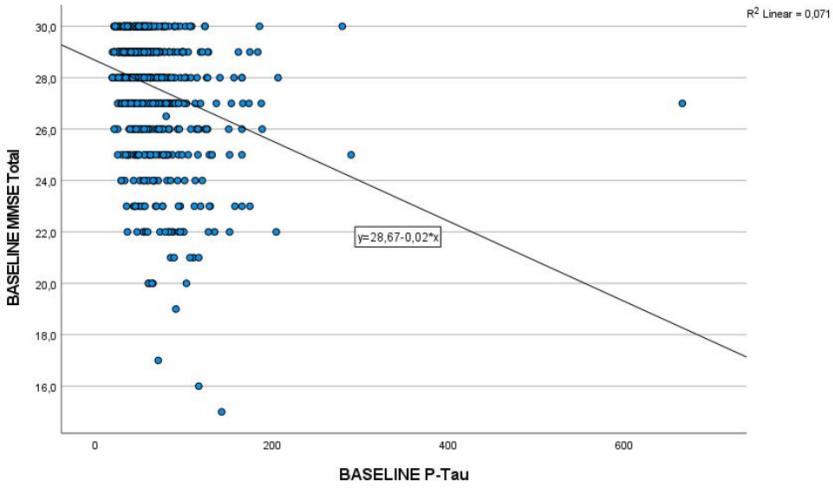
In Figure 7(B), an increase in amount of CSF T-tau protein pointed towards a decrease in MMSE score. Moreover, it was noted that an increase in P-tau was correlated with a decrease in MMSE total score among the participants, as seen in Figure 7(C).



(A)



(B)



(C)

Figure 7: Linear regression graphs showing how MMSE scores are affected by biomarker variables **(A)** Aβ 1-42, **(B)** T-tau and **(C)** P-tau.

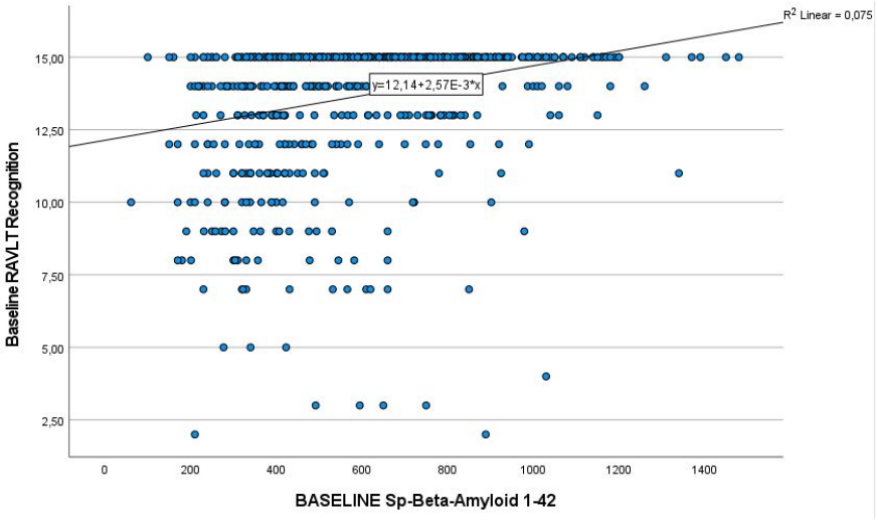
As the albumin ratio, a sign of vascular damage, increased in the group, the MMSE score decreases, as shown in A.11. This resonates well with the global cognitive impairment seen in vascular cognitive disease.

When it comes to the "newer" APP metabolites, an increase in sAPPα seemed to lead to a slight increase in the MMSE score, as observed in A.12.

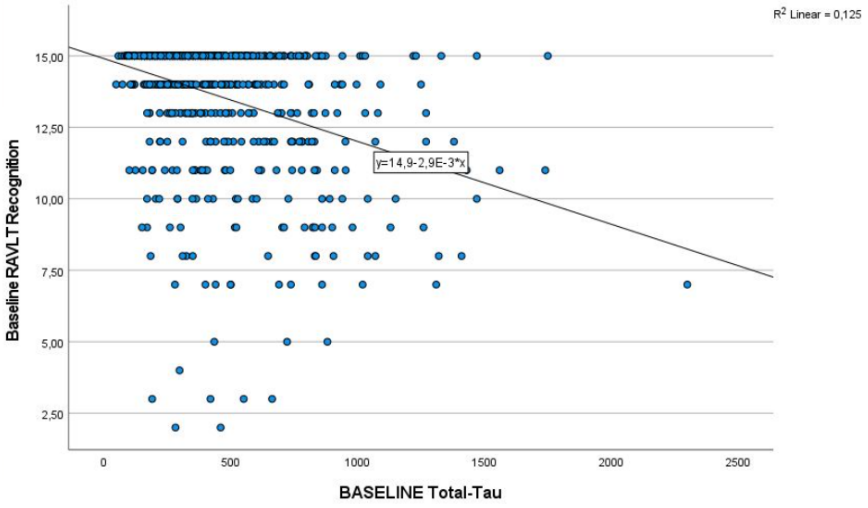
Also in A.13 did the linear regression analysis show that MMSE scores increased slightly as the amount of sAPPβ increased. Considering that the sAPPα/β biomarkers were reduced in patients with SSVD, could this data indicate that high sAPPα/β levels correspond to a cognitively healthy brain.

Next, a deeper analysis of memory performance in relation to the biomarkers was done. Figure 8(A) shows that higher amounts of Aβ 1-42 in patient correlated to higher RAVLT scores, and low amounts correlated to low scores, indicating that AD pathology affects verbal memory.

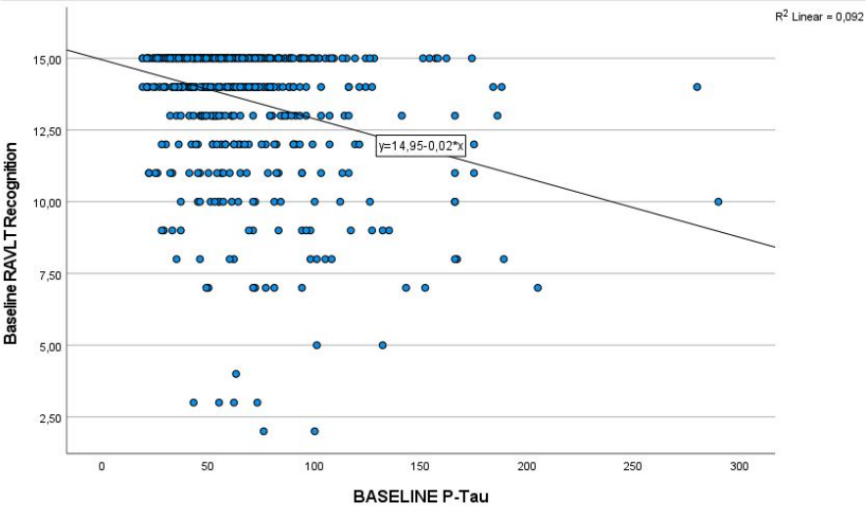
Similarly, as T-tau levels, that indicate general brain damage, increased in the patient group, the RAVLT scores decreased, as seen in Figure 34. This could also be seen with P-tau, a more specific AD biomarker, that as the amount of P-tau increased, the RAVLT score decreased (Figure 35).



(A)



(B)



(C)

Figure 8: Linear regression graphs showing how RAVLT scores are affected by biomarker variables **(A)** Aβ 1-42, **(B)** T-tau and **(C)** P-tau.

As the albumin ratio was increasing, the RAVLT scores seemed to be decreasing, which can be observed in Figure 9. This means that also vascular damage detectable by this measure, can affect memory function.

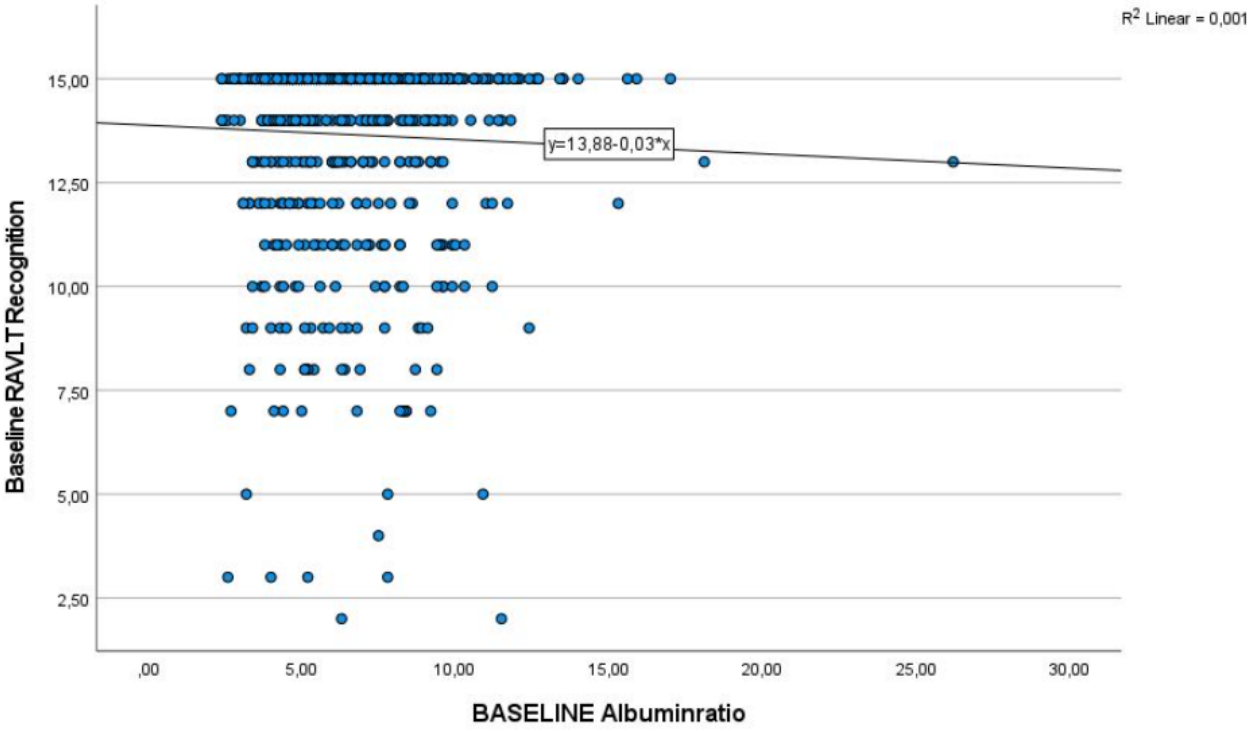


Figure 9: Linear regression graph showing how RAVLT scores are affected by the biomarker variable albumin ratio.

In A.14, the regression analysis showed a slight increase in RAVLT scores as sAPPα/ was increasing. Similarly, the regression analysis showed a slight increase in RAVLT scores as sAPPβ was increasing, depicted in A.15.

Next, correlations of processing speed with CSF biomarkers were explored.
With an increasing amount of Aβ 1-42 in the population, the time for completing the TMT-A test was decreasing, as seen in A.16.

A.17 shows that as T-tau increased so did the time it took to complete the TMT-A test. The regression analysis also showed that as P-tau was increasing, so did the time for completing the TMT-A test in the cohort, as seen in A.18. This indicates that the classical AD biomarkers were correlated to reduced processing speed.

Next, A.19 shows that increased albumin ratio led to increasing times for the completion of the TMT-A tests. This means that also vascular damage reduced cognitive speed.

In A.20, as sAPPα increased, the time for completing the TMT-A test slightly decreased, and a low amount of sAPPα was leading to slightly longer completion times.

The regression analysis also shows that TMT-A test completion times are slightly decreasing as the sAPPβ decreases, as seen in A.21. However, these correlations did not appear so strong.

Linear regression analysis of executive function, was performed with CSF biomarkers. With increasing amount of Aβ 1-42, the participants completed the TMT-B test faster than those with lower amount of Aβ 1-42, as seen in A.22.
A.23 shows that the increase in T-tau led to an increase in the time taken to complete the TMT-B test. A.24 displays that when P-tau was increasing, so was the completion time for TMT-B test in the cohort.

In Figure 10, it can be observed that increasing albumin ratios were correlated to slightly longer TMT-B test completion times. Slightly unexpected results, as effect should be stronger.

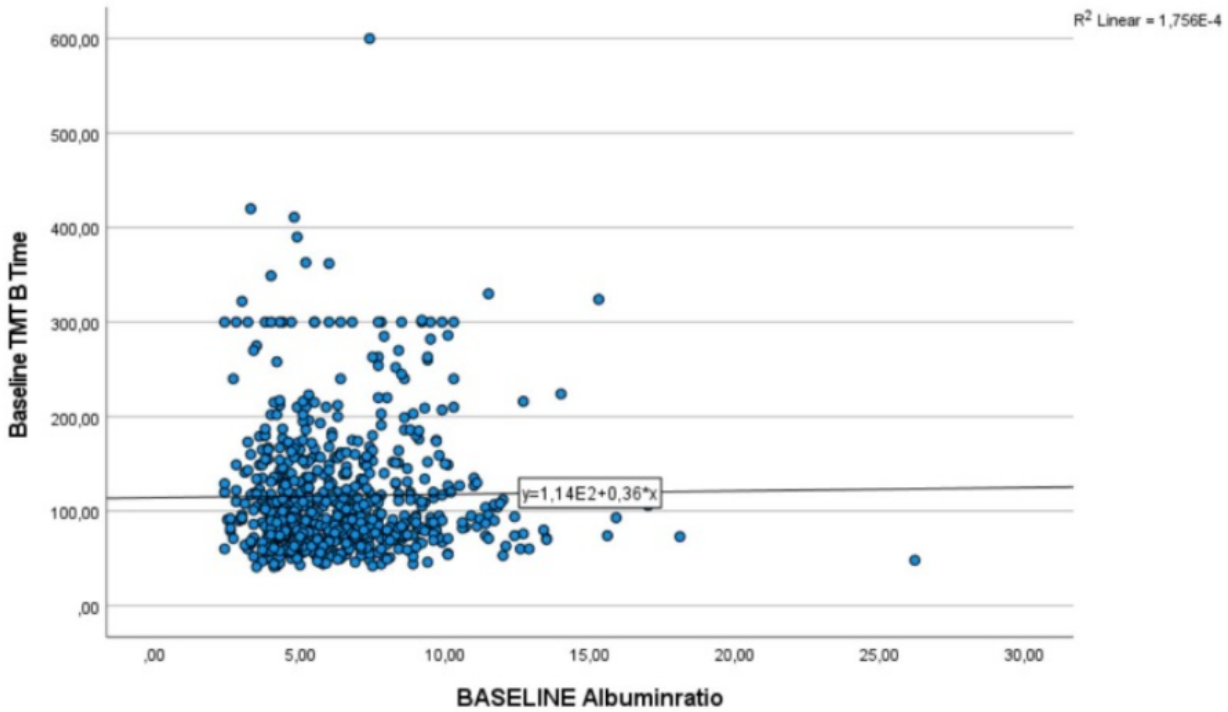


Figure 10: Linear regression graph showing how TMT-B completion times are affected by the biomarker variable albumin ratio.

The increasing sAPPα levels seemed to be correlated to a slight decrease in the TMT-B test completion time, as seen in A.25.

Here as well, the increase in sAPPβ seems so lead to slightly faster TMT-B test completion times, as seen in A.26.

3.3 Year 2

Since the Gothenburg MCI study is a longitudinal study, it was possible to compare the same patient variables over time, including biomarkers and cognitive scores. First, the participant data was explored at the first follow-up time, at 2 years after the baseline visit (Table 2). In Table 2, all variables showed significant differences between the participant groups except for sAPPα. This indicates that there were no longer any differences between controls and patient groups regarding this CSF biomarker.

Table 2: Demographics of the controls and patient groups at follow-up at year 2

	Diagnosis						P-value between groups
Variables	**Control**	**SCI**	**MCI**	**AD**	**Mixed AD/SSVD**	**SSVD**	
Aβ 1-42, median (IQR)	720 (565-832)	640 (480-757)	540 (380-740)	375 (276-509)	380 (296-480)	572 (398-779)	**< 0.001**
T-tau, median (IQR)	280 (202-415)	280 (190-430)	398 (251-590)	630 (371-915)	653 (443-970)	305 (234-418)	**< 0.001**
P-tau, median (IQR)	49.5 (34-71)	52 (35.5-70	59 (45-84)	72 (50-109)	73 (60-127)	47 (33.8-60)	**< 0.001**
sAPPα, median (IQR)	255 (181-380)	284 (201-355)	290 (220-398)	282 (176-352)	266 (223-315)	239 (169-318)	0.331
sAPPβ, median (IQR)	505 (350-748)	558 (404-696)	570 (400-800)	463 (358-725)	504 (354-763)	406 (232-512)	**0.017**
Albumin ratio, median (IRQ)	6.4 (5.3-7.3)	5.7 (4.4-7.8)	5.9 (4.8-7.6)	5.2 (4.3-7.1)	6.5 (5.1-8.7)	6.8 (5.3-9.7)	**0.047**
MMSE total, median (IQR)	30 (29-30)	29 (29-30)	28 (26-29)	21 (17-25)	23 (18-25.5)	25.5 (21.3-28.8)	**< 0.001**
RAVLT recognition, median (IQR)	15 (15-15)	15 (14-15)	15 (12-15)	10.5 (8-14)	10 (8-12.8)	14 (11.3-15)	**< 0.001**
TMT-A, median (IQR)	32 (27.3-39.8)	34.5 (27-43)	44 (33-60)	62 (42-77.5)	50 (38-84)	64 (38.5-140)	**< 0.001**
TMT-B, median (IQR)	73 (60-90)	72 (61-95)	109 (80-148)	141 (102-179)	164 (110-223)	175 (116-300)	**< 0.001**

SCI: Subjective cognitive impairment, MCI: Mild cognitive impairment, AD: Alzheimer's disease, MIX: Mixed Alzheimer's disease and subcortical small-vessel disease, SSVD: subcortical small-vessel disease, IQR: Interquartile range, Aβ: amyloid-beta, T-tau: total tau, P-tau: phospho-tau, sAPPα: Soluble amyloid precursor protein alpha, sAPPβ: Soluble amyloid precursor protein beta, MMSE: Mini-Mental State Examination, RAVLT: Rey Auditory Verbal Learning Test, TMT-A: Trail making test part A, TMT-B: Trail making test part B

3.4 Pairwise comparisons

To better understand the differences between baseline and year 2, we compared median values for each variable (Table 3). Here, multiple significant differences were found between the baseline and year 2 variables, which present a higher interest in further researching these variables for potential markers to be used in diagnosing the disease. Overall, generally no variables were changed in the CTRL, SCI and SSVD groups, indicating that these patients were stable in their neurochemical processes and cognition. Interestingly, the "new" APP biomarkers sAPPα/β were reduced in SCI, MCI and AD, indicating that these were part of the disease progression towards AD. Several of the cognitive scores were also altered in MCI, AD and MIX patients, indicating that these patient groups were still undergoing pathological changes towards more severe disease.

Table 3: Pairwise comparisons between baseline and year 2

Variables	Diagnosis																	
	Control			SCI			MCI			AD			MIX			SSVD		
	Baseline	Year 2	p-value	Baseline	Year 2	p-value	Baseline	Year 2	p-value	Baseline	Year 2	p-value	Baseline	Year 2	p-value	Baseline	Year 2	p-value
Aβ 1-42, median	676	720	0.819	661	640	0.631	568	540	0.094	360	375	0.289	390	380	0.153	580	572	0.959
T-tau, median	292	280	0.977	274	280	0.96	352	398	0.188	610	630	0.512	685	653	0.166	323	395	0.756
P-tau, median	48.5	49.5	0.111	47	52	0.366	55	59	**0.005**	82.5	72	0.102	84	73	0.695	52.5	47	0.326
sAPPα, median	306	255	0.082	289	284	**0.013**	297	290	**0.013**	292	282	**0.006**	286	266	0.064	232	239	0.421
sAPPβ, median	526	505	0.476	570	558	0.278	552	570	**0.038**	553	463	**0.032**	478	504	0.277	371	406	0.196
Albumin ratio, median	6	6.4	0.471	5.5	5.7	0.09	6	5.9	0.544	5.3	5.2	0.528	6.5	6.5	**0.038**	7.8	6.8	0.312
MMSE total, median	29.5	30	0.28	29	29	0.2	28	28	**< 0.001**	25	21	**< 0.001**	25	23	**< 0.001**	25	25	0.292
RAVLT recognition, median	15	15	0.415	15	15	0.679	15	15	**< 0.001**	12	10.5	**0.009**	13	10	**0.016**	14	14	0.194
TMT-A, median	33	32	0.747	35	34.5	0.081	43	44	0.056	59	62	**0.007**	54	50	0.1	73	64	0.083
TMT-B, median	77.5	73	0.097	75.5	72	0.243	107	109	**0.006**	155	141	0.186	180	164	**0.0258**	202	175	0.176

SCI: Subjective cognitive impairment, MCI: Mild cognitive impairment, AD: Alzheimer's disease, MIX: Mixed Alzheimer's disease and subcortical small-vessel disease, SSVD: subcortical small-vessel disease, IQR: Interquartile range, Aβ: amyloid-beta, T-tau: total tau, P-tau: phospho-tau, sAPPα: Soluble amyloid precursor protein alpha, sAPPβ: Soluble amyloid precursor protein beta, MMSE: Mini-Mental State Examination, RAVLT: Rey Auditory Verbal Learning Test, TMT-A: Trail making test part A, TMT-B: Trail making test part B

3.5 Delta values

To be able to explore the direction of changes better, we calculated the differences between baseline and year 2 as delta values in percent of the investigated patient variables (Table 4). When analyzing this data, it was seen that both biomarkers (P-tau and sAPPβ) as well as all cognitive scores differed significantly between cohort subgroups. What was surprising was that not all trends pointed towards a more severe phenotype in all patient groups, and all changes did not point in the same direction.

Table 4: Changes of the variables (delta values) in percentages between baseline and year 2

Variables, % change between baseline and year 2	Diagnosis						P-value between groups
	Control	SCI	MCI	AD	MIX	SSVD	
Aβ 1-42, median (IQR)	0 (-25.4-26.4)	-5 (-17.3-20.8)	-4.6 (-22.1-20.1)	0 (-13.9-37.5)	-12.5 (-22.1-11.9)	1.9 (-16-14.5)	0.273
T-tau, median (IQR)	0 (-11.3-11.8)	0 (-20.1-20.6)	1.8 (-12.8-20)	1.1 (-13.4-21)	9.4 (-9.7-28.8)	6.5 (-18.1-22.6)	0.79
P-tau, median (IQR)	-3.9 (-14.9-6.2)	0.9 (-9.5-17.7)	2.5 (-7.1-14.3)	-4.6 (-18.1-8.9)	-1.7 (-9.4-18.5)	-3 (-10-5.2)	**0.035**
sAPPα, median (IQR)	10.1 (-3.2-37.1)	-4.5 (-14.2-4.1)	-2.7 (-13.2-7.8)	-11.4 (-20.5-7.5)	-6.9 (-16.4-2.6)	-1.2 (-11.7-5.2)	**0.031**
sAPPβ, median (IQR)	-0.3 (-30.2-10.5)	-1.8 (-12.1-7.6)	-3.3 (-17.4-12.4)	-7.1 (-19.1-8.3)	-9.7 (-21.7-10.2)	-11.1 (-19.2-9.8	0.866
Albumin ratio, median (IQR)	0.2 (-7-14.3)	3.3 (-7.6-13.9)	2.3 (-10.3-15.2)	2.8 (-11.2-11.6)	8.2 (-3-20.1)	6 (-7.2-15.6)	0.709
MMSE total, median (IQR)	0 (0-1.5)	0 (-3-3)	0 (-7-3)	-12 (27-0)	-9 (-26.5-1.5)	0 (-18.8-6.3)	**< 0.001**
RAVLT recognition, median (IQR)	0 (0-0)	0 (0-0)	0 (-13-0)	-14 (-25.5-0)	-20 (-30-(-3.5))	0 (0-17)	**< 0.001**
TMT-A, median (IQR)	-3 (-16.3-20.8)	-7 (-22.5-12.5)	3 (-13-26)	18.5 (-8.8-64.8)	8 (-6.5-47)	11 (-7-34)	**< 0.001**
TMT-B, median (IQR)	-3 (-23.5-15)	-4 (-14-14.5)	8 (-14-28.3)	7 (-12-57)	16.5 (-15.8-46.5)	13.5 (-12.8-52)	**0.006**

SCI: Subjective cognitive impairment, MCI: Mild cognitive impairment, AD: Alzheimer's disease, MIX: Mixed Alzheimer's disease and subcortical small-vessel disease, SSVD: subcortical small-vessel disease, IQR: Interquartile range, Aβ: amyloid-beta, T-tau: total tau, P-tau: phospho-tau, sAPPα: Soluble amyloid precursor protein alpha, sAPPβ: Soluble amyloid precursor protein beta, MMSE: Mini-Mental State Examination, RAVLT: Rey Auditory Verbal Learning Test, TMT-A: Trail making test part A, TMT-B: Trail making test part B

Next we plotted the delta values for all the variables, as divided into controls and patient groups. Although not statistically correct, we performed post-hoc analysis of the sAPPα even if the overall general comparisons between groups did not show significant differences (Figure 11), but these comparisons can be overlooked.

For changes in biomarkers Aβ 1-42, T-tau, P-tau, albumin ratio, sAPP (A.27-A.31 respectively) there were no differences found at the pairwise comparisons, and the majority of these variables did not change substantially over time.

For change of sAPPα, the CTRL group increased its levels significantly more than the AD group, where sAPPα levels had reduced over time.

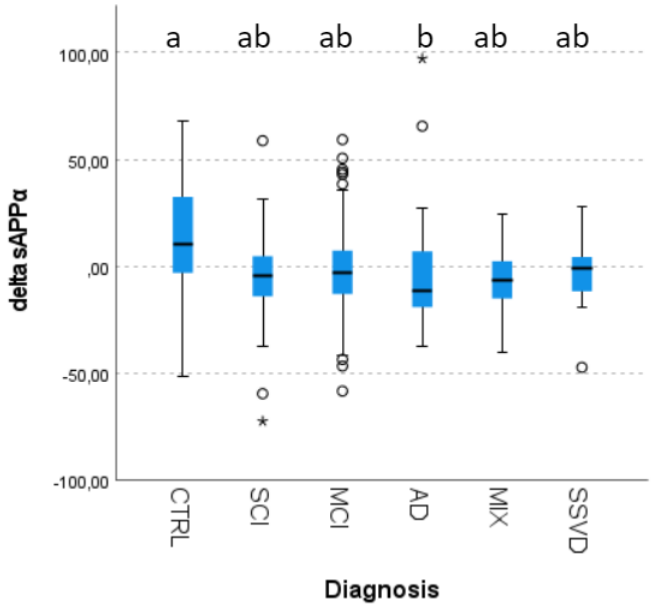


Figure 11: Graph showing the delta values in percentage between baseline and year 2 for the biomarker variable sAPPα.

Interestingly, the cognitive scores showed more prominent changes over the two years. For MMSE (Figure 12(A)), the changes in MMSE were similar for CTRL, SCI and MCI, while the disease groups AD, MIX and SSVD had similar reductions in MMSE.

Figure 12(B) shows that memory did not change in CTRL, SCI, and SSVD patients, but there was a significant difference between those groups and the AD group.

There were also indications that speed and executive functions differed between specific patient groups, such as significant differences in changes of TMT-A time between SCI and AD (Figure 12(C)) and significant differences between controls and MCI patients with regard to change in TMT-B time (Figure 12(D)).

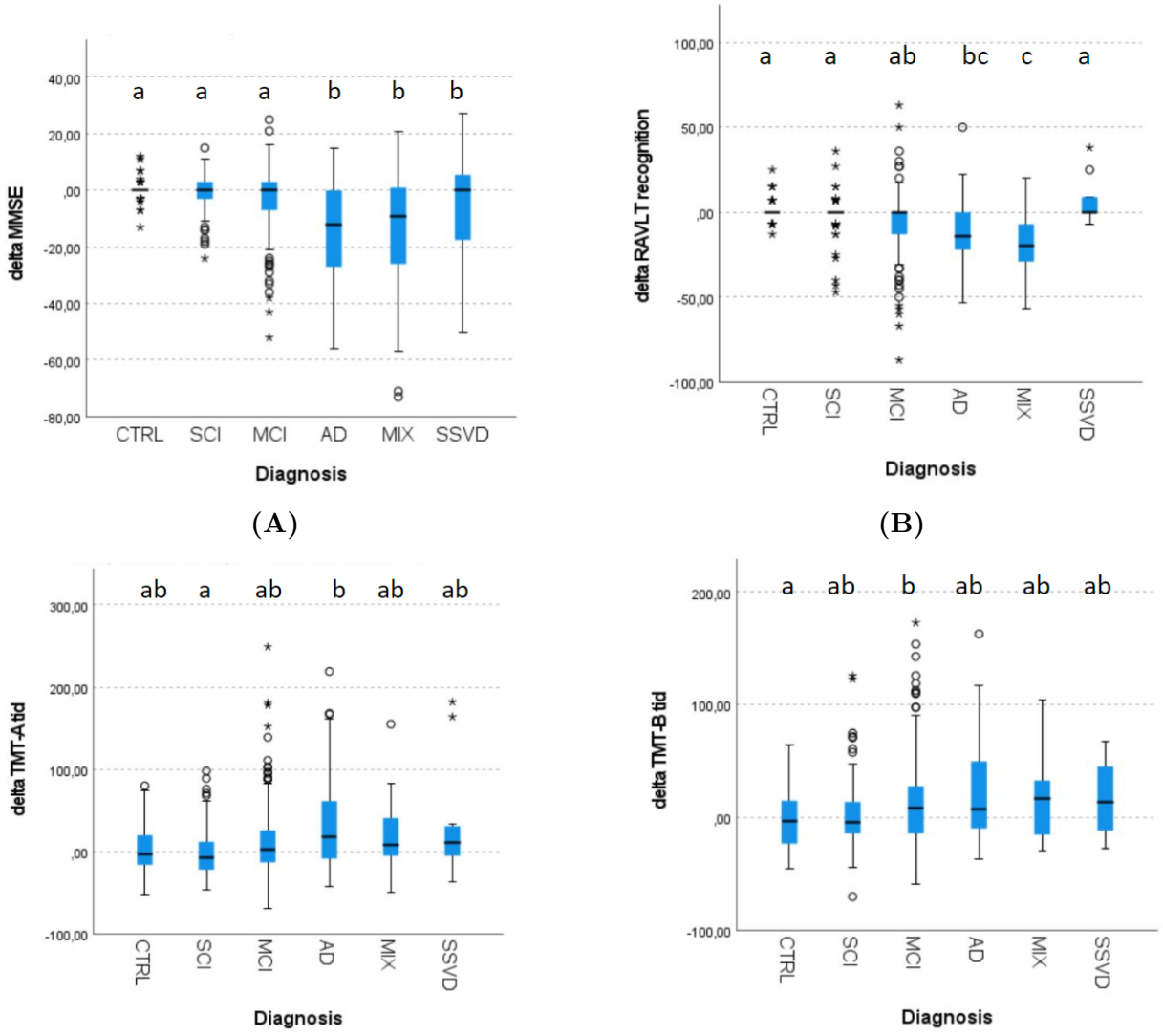


Figure 12: Graphs showing the delta values in percentage between baseline and year 2 for the cognitive variables **(A)**MMSE scores, **(B)**RAVLT scores, **(C)**TMT-A completion times and **(D)**TMT-B completion times.

3.6 Linear regression of delta values

Since the APP metabolites sAPPα/β have not previously been investigated longitudinally in this cohort, we decided to explore their delta values further using linear regression in the whole cohort (A.32-A.43).

A.32 shows a slight decrease in delta sAPPα as age increased in the cohort. This indicates that the potential pathological decrease in the biomarker mainly happens in older people.

In A.33, it can be seen that education has minimal effect on delta sAPPα, showing a minimal decrease in delta as education increases. This indicates that education cannot protect against a reduction of sAPPα.

In A.34, those with higher percentage increase in sAPPα had slightly higher MMSE scores, supporting the cognitively protective effects of sAPPα. This is in contrast to the data in A.35 where those with decreasing deltas of sAPPα had slightly higher RAVLT scores, than those that had increasing deltas which cannot be explained.

Changes from baseline to year 2 seem to minimally effect the changes of TMT-A and B times, which is seen in Figure 65 and 66, respectively.

For sAPPβ, the changes are almost identical to sAPPα, which is seen in A.38-A.43.

4 Conclusion

With the analysis of the biomarkers, is it possible to observe disease progression- When looking at the biomarkers individually, we can observe changes in the severity of the disease depending on the levels of the biomarker. When looking at Aβ 1-42 individually, it is noted that low levels of Aβ 1-42 is associated with a decrease in cognition. We could see an association of Aβ 1-42 with impairment in the general cognition, because of the decrease in MMSE scores, as well as impairment in memory as RAVLT scores are getting lower, and also worse speed and attention with the increasing TMT A and B test completion times. This could indicate that Aβ 1-42 is strongly associated with neurodegeneration.
Concerning T-tau and P-tau, we could observe that a high amount of those biomarkers were associated deterioration in general cognition with lowering of MMSE scores, impairment in memory with decrease in RAVLT scores, and also worsening in speed and attention with rising TMT A and B test completion times. So T-tau and P-tau are also biomarkers that indicate neurodegeneration.

The new biomarker sAPPα and sAPPβ seemed to be associated with neurodegeneration as well. Low values tended to be associated with impairment in general cognition with lower MMSE scores, memory impairment in memory with lower RAVLT scores and slightly worse speed and attention, as TMT A and B test completion times get higher, but at a more stable

rate compared to the previous. So it seems that these biomarkers could indicate neurodegeneration to some degree, but as they are relatively new in the biomarker field, not much research has been conducted on them.

The last biomarker, albumin ratio, indicate neurodegeneration with high and rising ratios. High albumin ratios were associated with general cognition impairment with lower MMSE scores, lower RAVLT scores which means worse memory, but the effect on speed and attention seemed to be minimalistic, almost non existent. So it seems that albumin ratio is associated the general cognition and memory, but has almost no association with speed and attention. These results were a bit unexpected, as patients with SSVD generally have high albumin ratios, and they usually have worse TMT A and B times. It it suggested that soluble APPα and APPβ, together with albumin ratio are correlated to worse TMT A and B times, but this could not be correlated to in this study. This may be because of the small sample size of 30, and a bigger sample size could show different results.

Regarding the linear regression analysis, it is important to note that MMSE - Abeta, MMSE - T-tau and RAVLT - T-tau had R-squared values between 0.1-0.3, and is considered a "very weak effect", meaning that variables might not be correlated. But, it is generally said that high values of R-squared are not always good for the regression model and that low values of R-squared are not always bad for the regression model.

5 Discussion

5.1 What is a good biomarker?

When comparing the healthy controls with the SCI group, significant differences could not be found, which means that even though the SCI group has self-reported issues with cognition, and even problems noticed by family and friends, both the biomarker analysis and the cognition tests showed no significant difference. They had similar biomarker values and performed similarly good on the cognition test. This indicates that the subjective evaluation of cognition could be better than the available clinical test.

But as we move over focus to the MCI group, and compare them to the healthy controls, significant differences start to emerge. The analysis show that that there is significant differences in the AD disease markers, which include Aβ 1-42, T-tau and P-tau. Progression of disease is observed in these markers. As the person is getting sicker, these values change change, where Aβ 1-42 will decrease in the direction towards the same levels as AD patients have. The T-tau and P-tau will also increase towards disease levels. Significant difference is also be seen in cognition. The general cognition MMSE scores is getting lower, the RAVLT memory scores are also lower, and the times for the TMT A and B test are getting higher. This indicates a decline in cognition.

When the patient is already diagnosed with with a disease, how are the biomarkers separated then? When comparing AD patients to those with mixed dementia (AD + SSVD), no significant differences in biomarkers and cognition test can be observed. The difference however, can be observed via MRI, where patients diagnosed with SSVD have significantly more white matter hyperintensities, which would then also be seen in the patients with mixed dementia.

To distinguish between AD and SSVD is a much more simpler task, as they have significant differences the biomarkers Aβ 1-42, T-tau, P-tau, albumin ratio and sAPPβ. The only marker with no difference was sAPPα. For the cognition, only the RAVLT test was significantly different, where the SSVD patients seem to score higher on RAVLT memory test, which corresponds to the reduced memory impairment in SSVD. By using these variables, one could easily separate distinguish the diseases when diagnosing the patient. An MRI evaluation could also contribute the process to increase accuracy.

Then to separate patients with mixed dementia to those with only SSVD, it was observed that there were significant differences in the disease markers, the Aβ 1-42, T-tau, P-tau and albumin ratio. The SSVD patients had lower Aβ 1-42 values and higher T-tau and P-tau values. That may be because those markers are more associated to AD.

How does follow up time and patient age affect biomarkers and cognition? When looking at the linear regression analysis for the baseline data in the whole population, a trend can be observed. It seem that as in all patient groups, majority of the biomarkers go towards the state that is considered worse, to the amount where the person gets "sicker". It can be seen even in the controls and the SCI group, which are considered healthy, that their values are worsening. This is to be expected as deterioration with increasing age is normal. Because of that, to perform accurate measurements, biomarker values and cognition scores would need to be adjusted to age. But when looking at the linear regression analysis for sAPPα/β and albumin ratio in the whole population, we could observe that they were more stable but increased slightly as age increases, however when looking at the delta values i.e, the difference between baseline and year 2, there seem to be no association with increasing age and level of soluble sAPPα/β. This means that the variable would not need to be adjusted to age and could indicate a more stable biomarker for evaluation of neurodegenerative diseases. As mentioned, the disease markers go towards the "worse" state, and it can be the reason why

the cognition becomes worse with time and age. But, when looking at the regression analysis in the healthy controls only, the analysis showed that the MMSE scores declined slightly but were stable, and the RAVLT scores were stable as well. The worsening could be seen in the speed and attention, as the TMT A and B test completion times increased with age even for the healthy controls. One thing to note is that the controls are extremely "healthy" and performed tests to ensure that they were very healthy, which could affect the results. Randomly selected people from all the population could show other results. Generally, it seems that when the memory is affected negatively somehow, there seems to be a indication of disease, but when speed and attention is affected negativity, it could be solely because of the increasing age. Also, there seems to be a point where the biomarkers seem to not get any worse, but the cognition seems to worsen with time. For example, comparing the baseline and year 2 data in Aβ 1-42 for AD patients, where not much difference is observed, but the cognition is declining. It may be because of the 2 year difference only and could show other results with more distant time points, or it may be that the biomarker has hit "rock bottom" and it is not possible to get any worse biologically, but the cognition is on a downwards spiral.

Another interesting thing to study was the effect of education and what potential protection it could have. When looking at the regression analysis comparing cognition to years of education, there seemed to be a trend where people that had longer education were cognitively healthier, and showed have better general cognition with higher MMSE scores and better speed and attention with lower TMT A and B test completion times. However, education did not seem to protect verbal memory, seen in RAVLT scores.

5.2 Future research

Future research should focus on better understanding the biological roles of the new biomarkers sAPPα and sAPPβ to find out how those biomarkers are related to the pathological processes and what effects they have on cognition. Those studies could be combined with the examination of different brain regions affected by cognitive disease and how they correlate with these biomarkers.

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A Appendix A - Results

A.1 Age

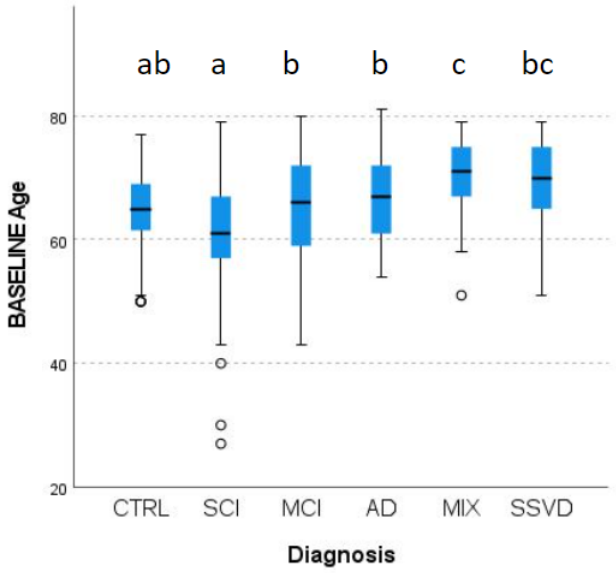

Figure A1: Graph showing how peoples age in the groups.

A.2 Education

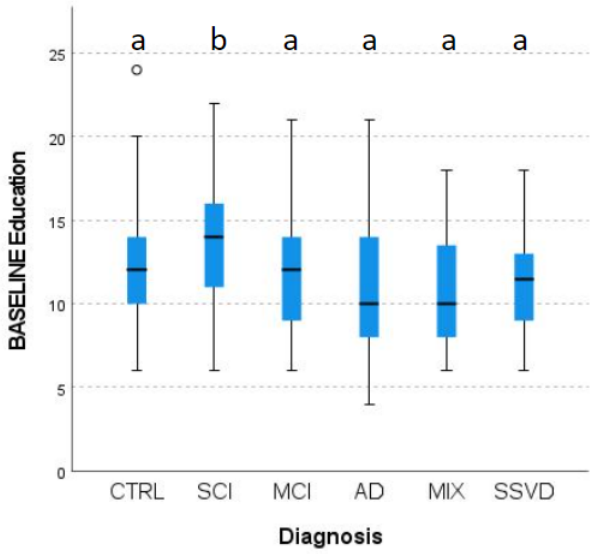

Figure A2: A graph showing years of education in the groups.

A.3 Age - P-Tau

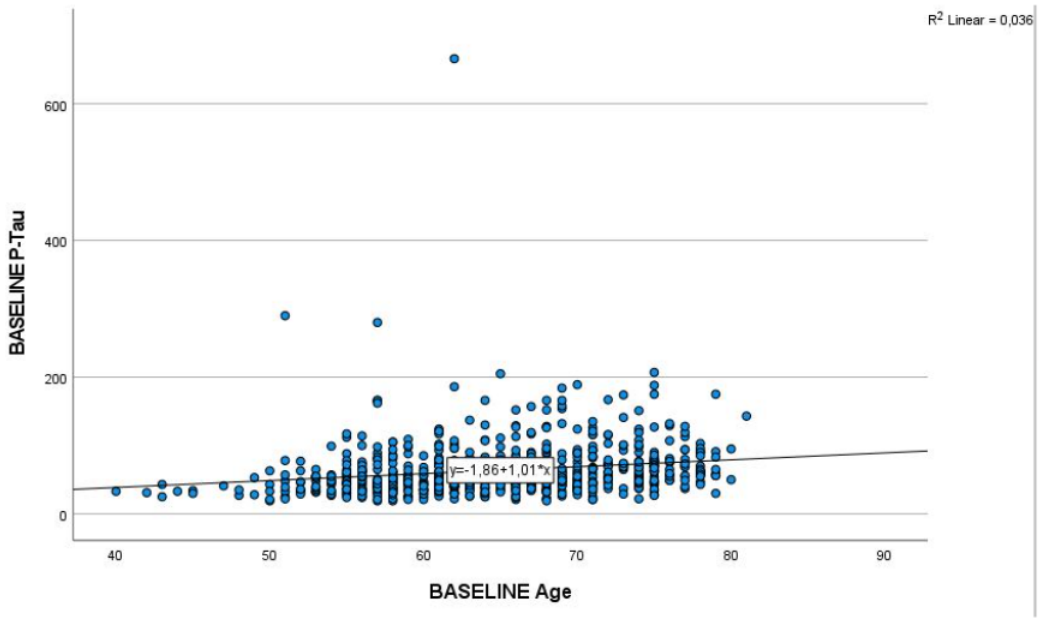

Figure A3: Linear regression analysis graph showing how increasing age is affecting the biomarker variable P-tau.

A.4 Age - Albumin ratio

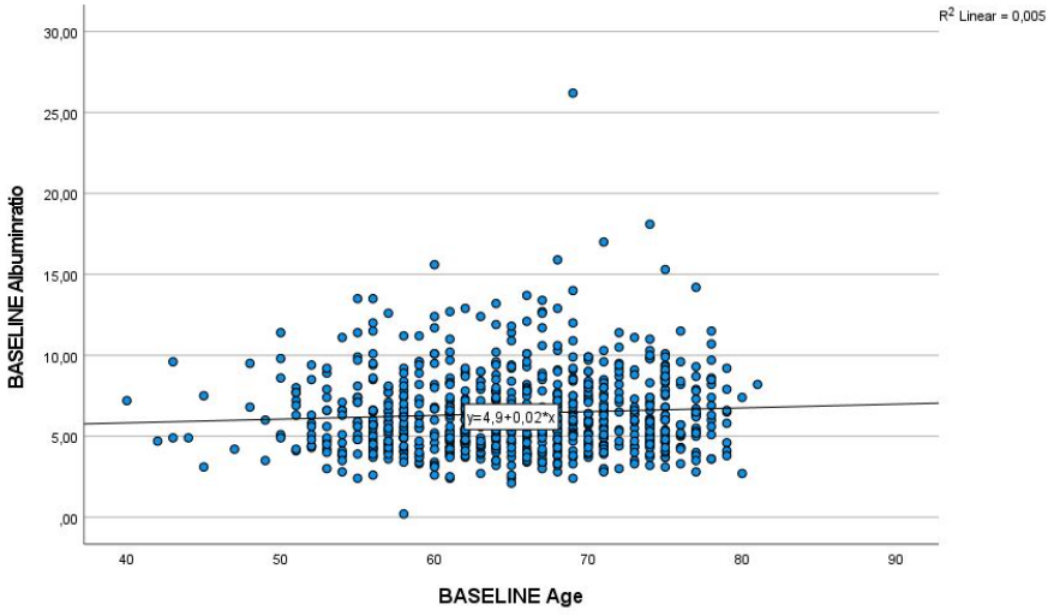

Figure A4: Linear regression analysis graph showing how increasing age is affecting the biomarker variable albumin ratio.

A.5 Age - sAPPα

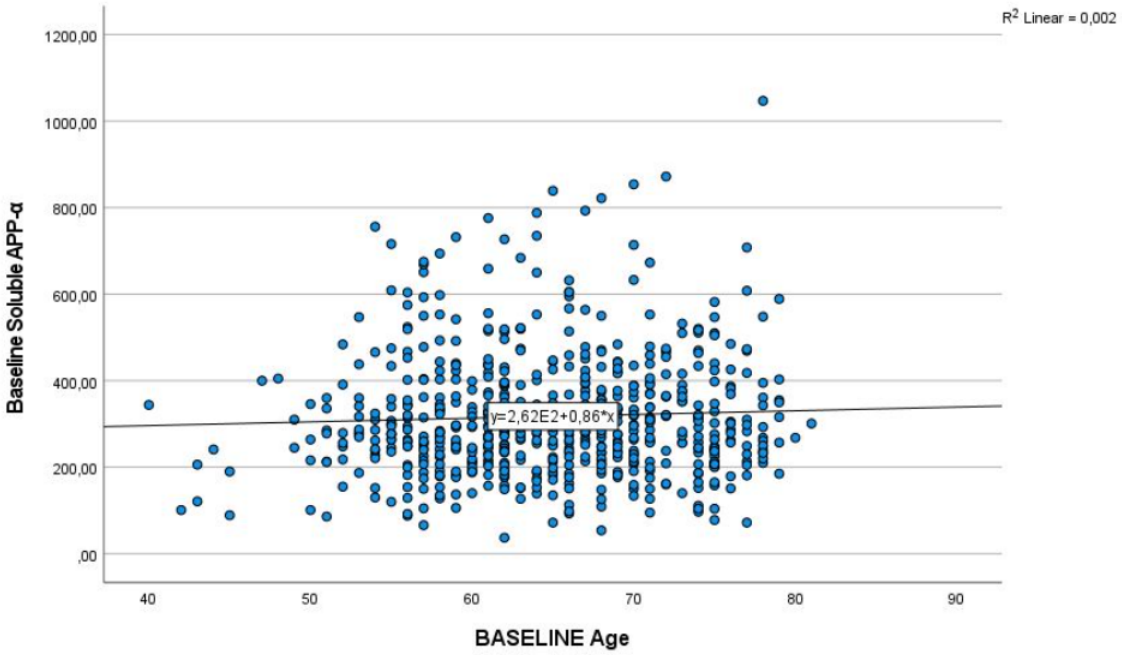

Figure A5: Linear regression analysis graph showing how increasing age is affecting the biomarker variable sAPPα.

A.6 Age - sAPPβ

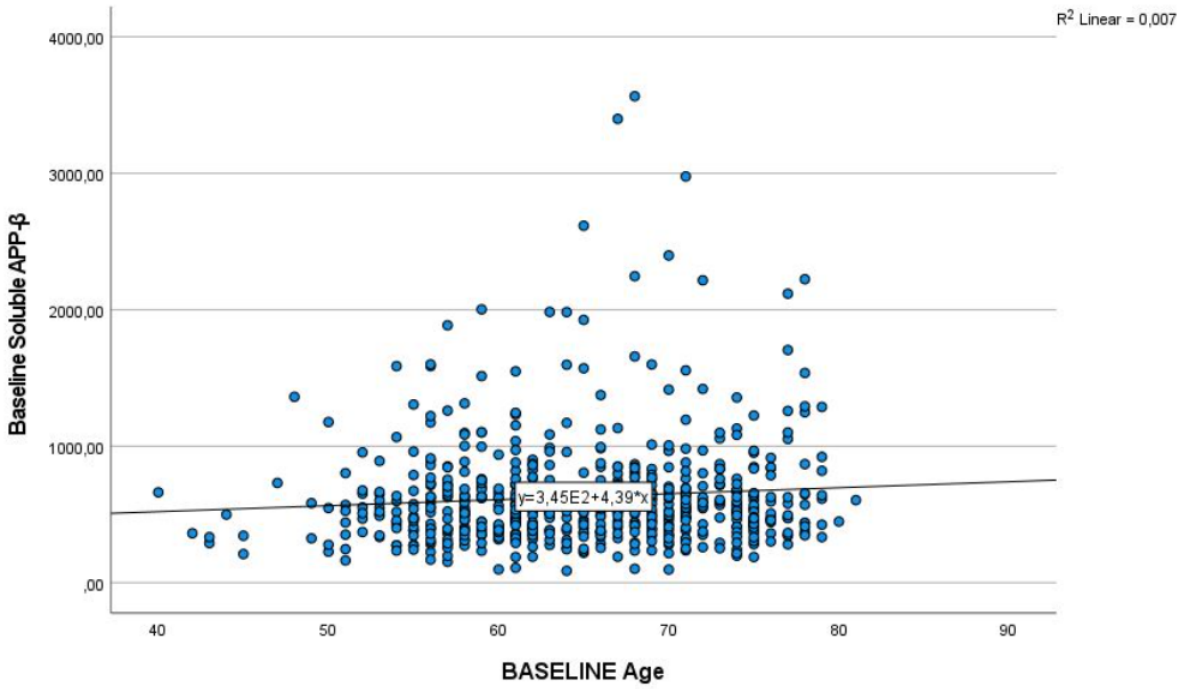

Figure A6: Linear regression analysis graph showing how increasing age is affecting the biomarker variable sAPPβ.

A.7 Age - MMSE

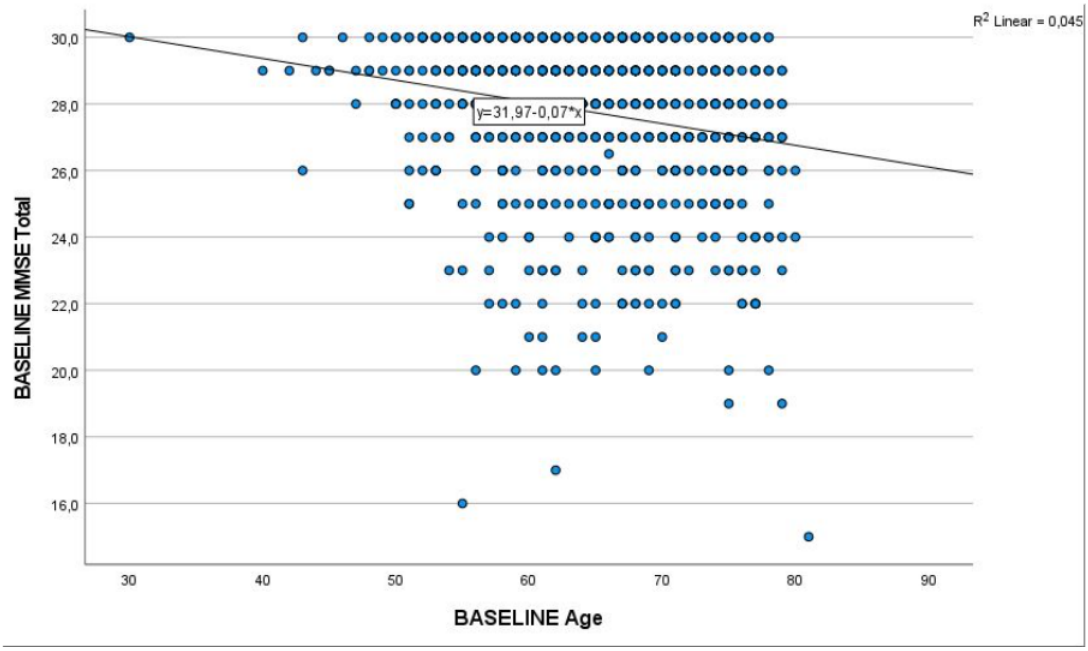

Figure A7: Linear regression analysis graph showing how increasing age is affecting the cognitive variable MMSE.

A.8 Age - RAVLT

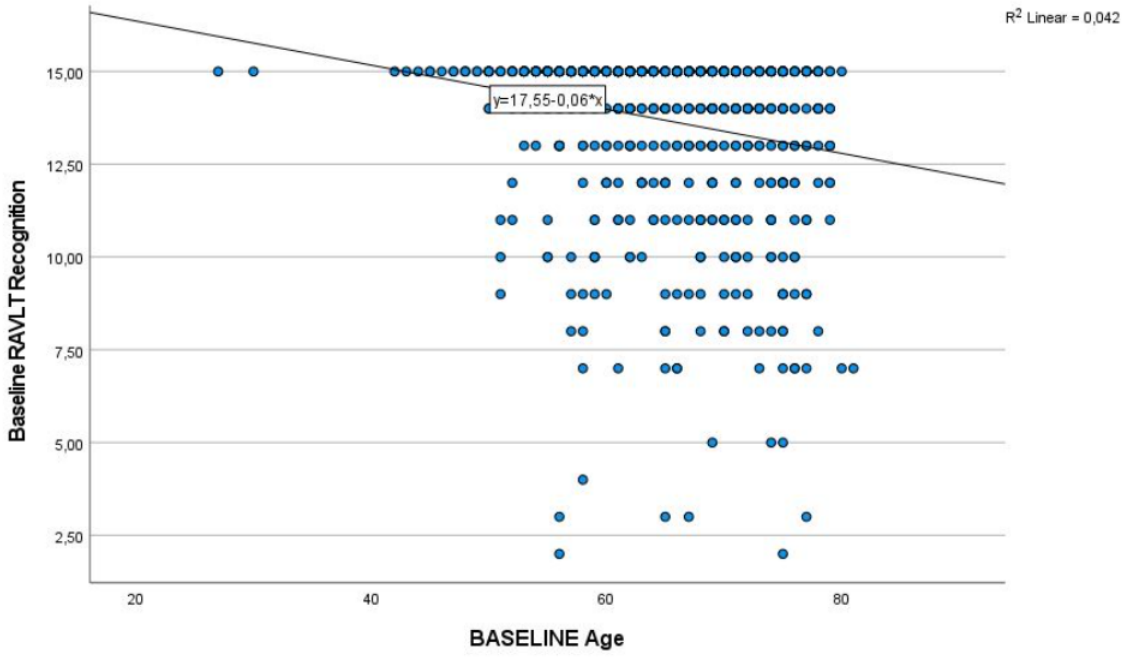

Figure A8: Linear regression analysis graph showing how increasing age is affecting the cognitive variable RAVLT.

A.9 Age - TMT A

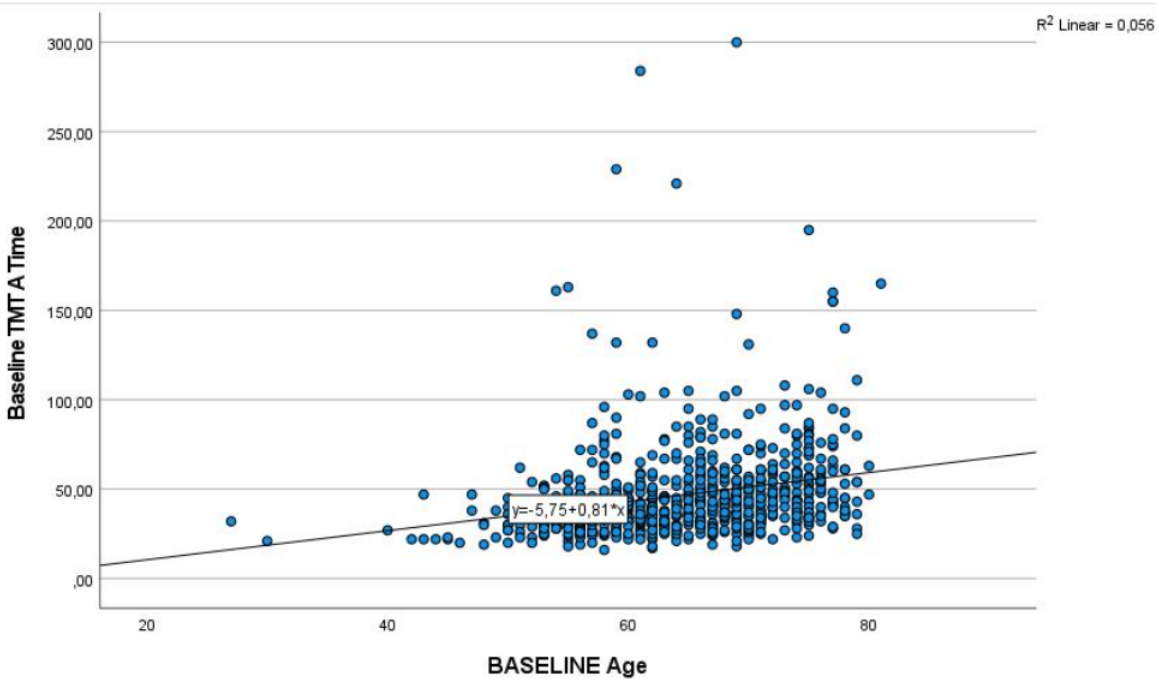

Figure A9: Linear regression analysis graph showing how increasing age is affecting the cognitive variable TMT-A.

A.10 Age - TMT B

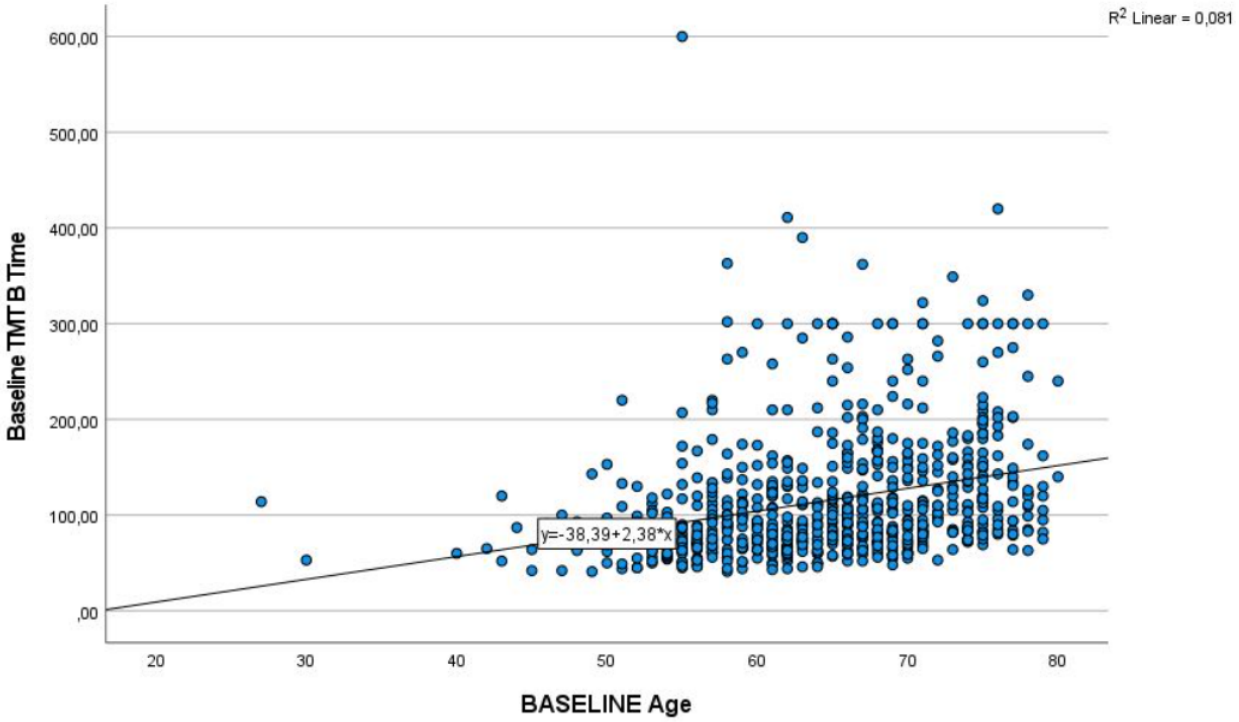

Figure A10: Linear regression analysis graph showing how increasing age is affecting the cognitive variable TMT-B.

A.11 MMSE - Albumin ratio

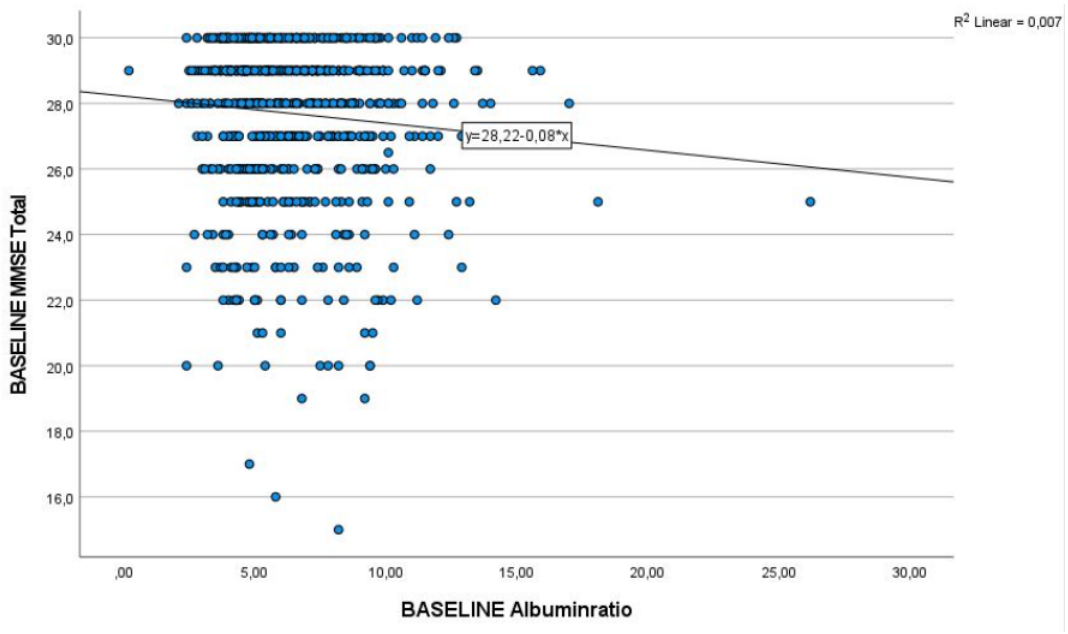

Figure A11: Linear regression analysis graph how increasing albumin ratio is affecting the cognitive variable MMSE.

A.12 MMSE - sAPPα

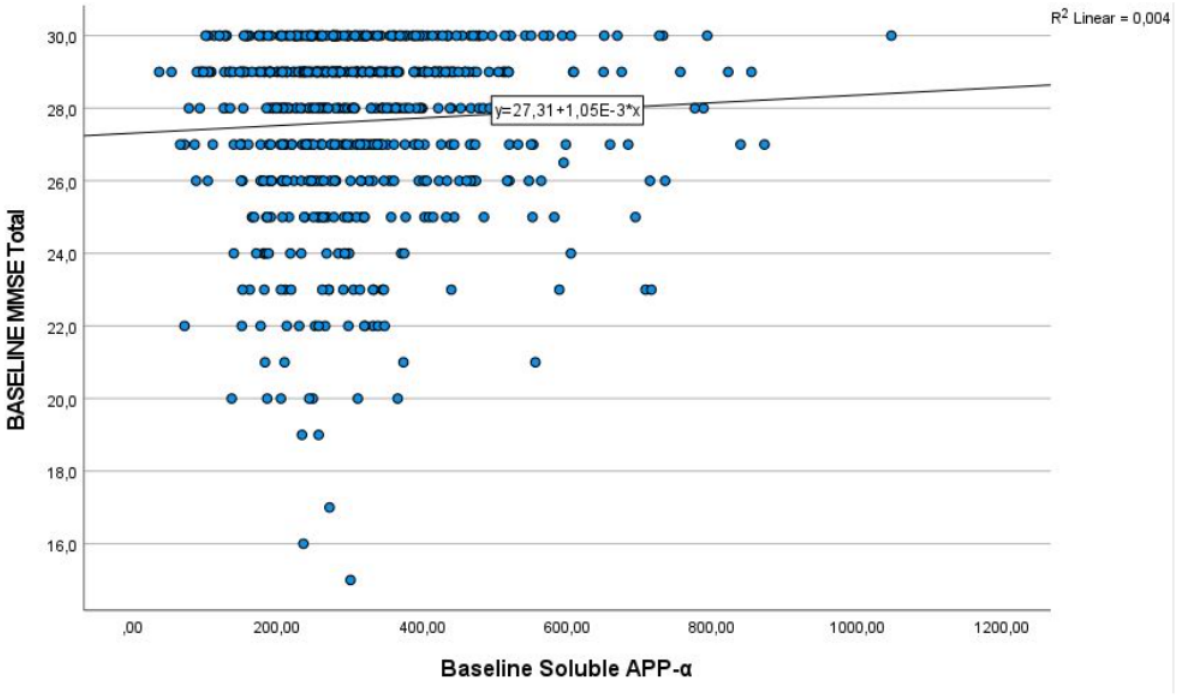

Figure A12: Linear regression analysis graph how increasing sAPPα is affecting the cognitive variable MMSE.

A.13 MMSE - sAPPβ

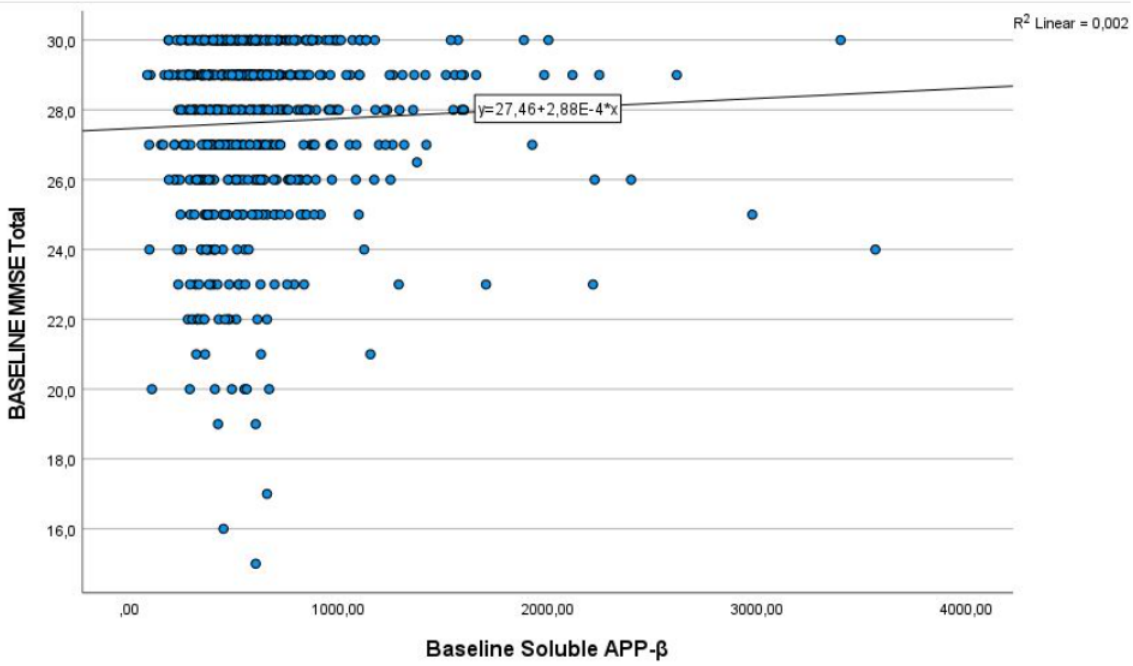

Figure A13: Linear regression analysis graph how increasing sAPPβ is affecting the cognitive variable MMSE.

A.14 RAVLT - sAPPα

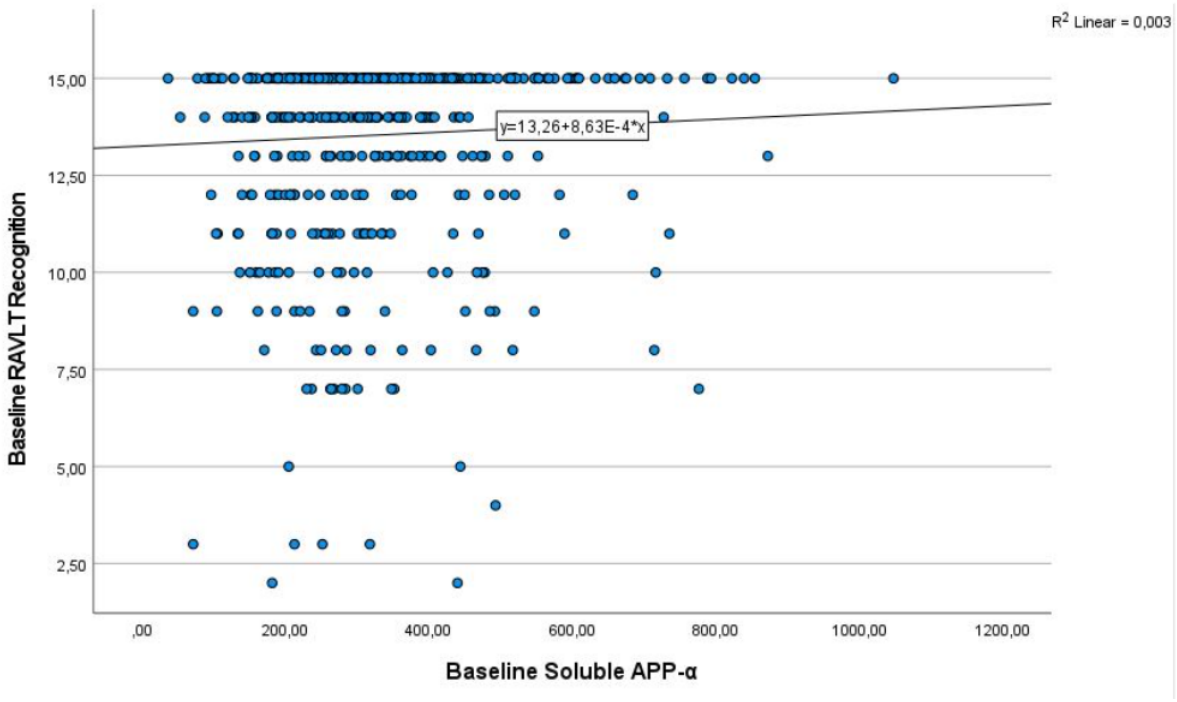

Figure A14: Linear regression analysis graph how increasing sAPPα is affecting the cognitive variable RAVLT.

A.15 RAVLT - sAPPβ

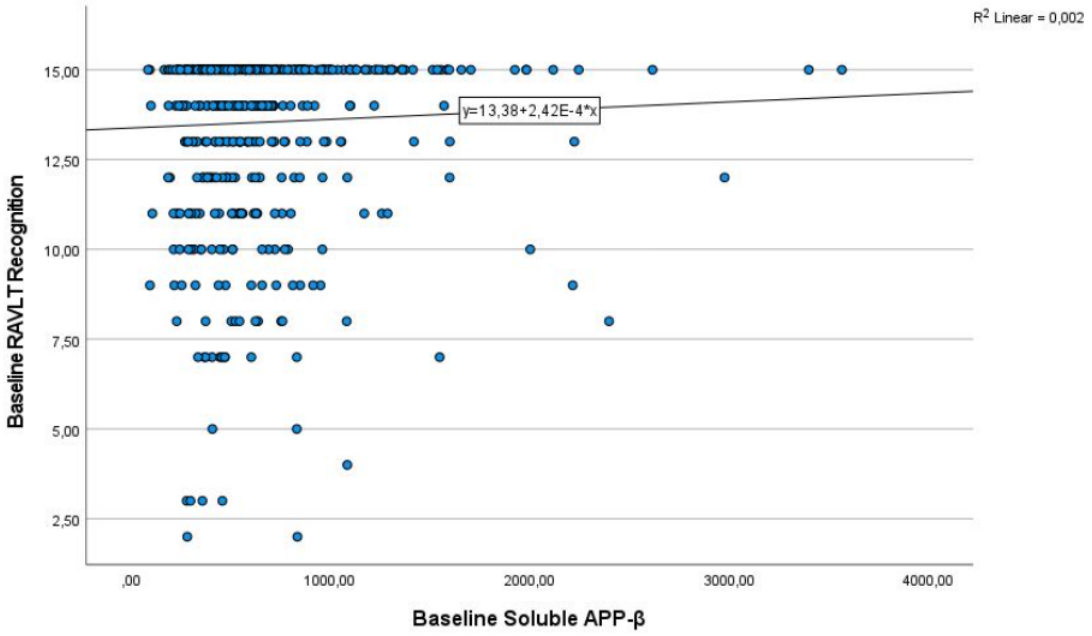

Figure A15: Linear regression analysis graph how increasing sAPPβ is affecting the cognitive variable RAVLT.

A.16 TMT-A - Aβ 1-42

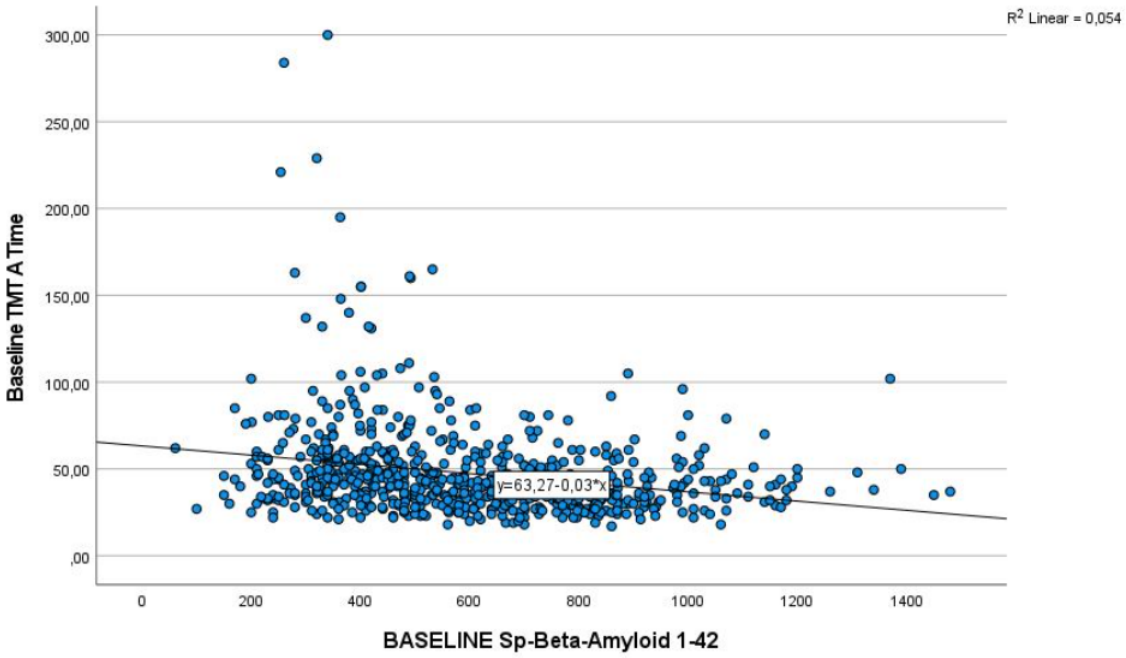

Figure A16: Linear regression analysis graph how increasing Aβ 1-42 is affecting the cognitive variable TMT-A.

A.17 TMT-A - T-tau

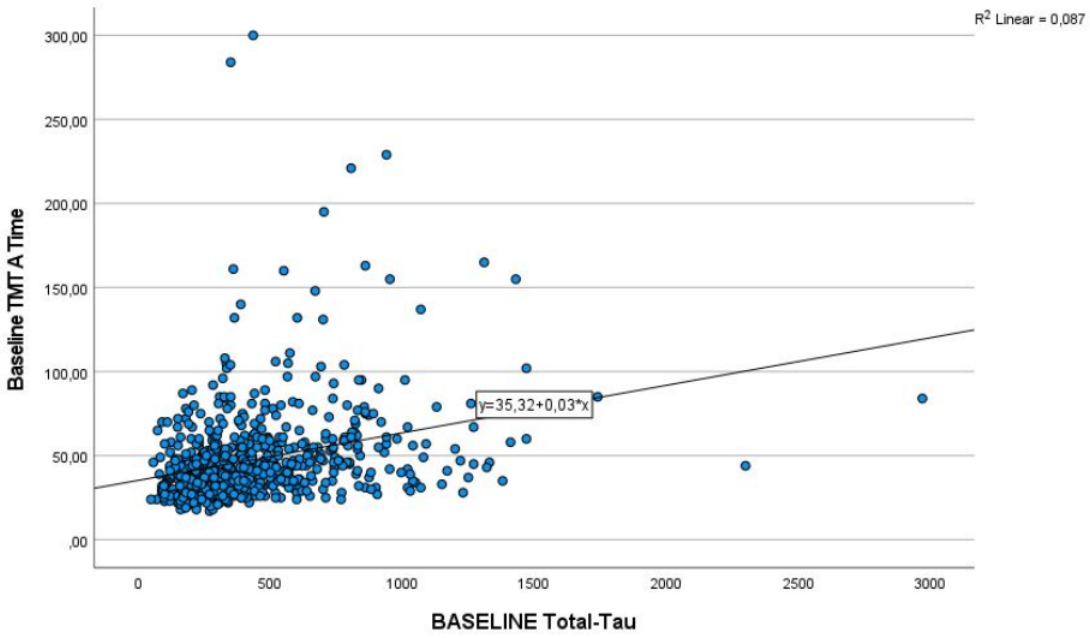

Figure A17: Linear regression analysis graph how increasing T-tau is affecting the cognitive variable TMT-A.

A.18 TMT-A - P-tau

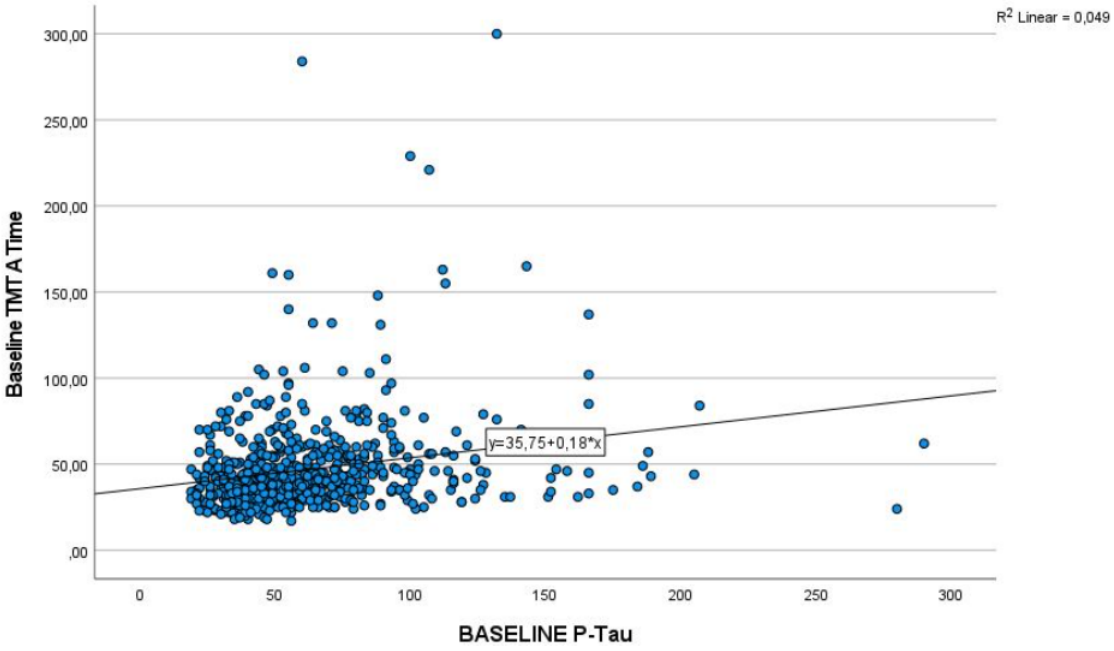

Figure A18: Linear regression analysis graph how increasing P-tau is affecting the cognitive variable TMT-A.

A.19 TMT-A - Albumin ratio

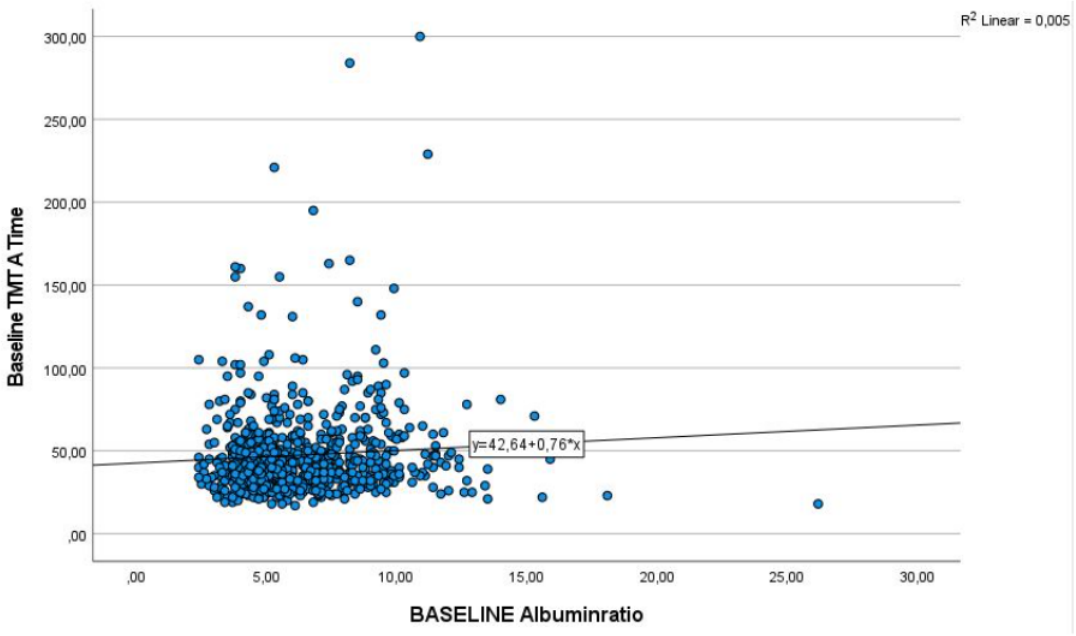

Figure A19: Linear regression analysis graph how increasing albumin ratio is affecting the cognitive variable TMT-A.

A.20 TMT-A - sAPPα

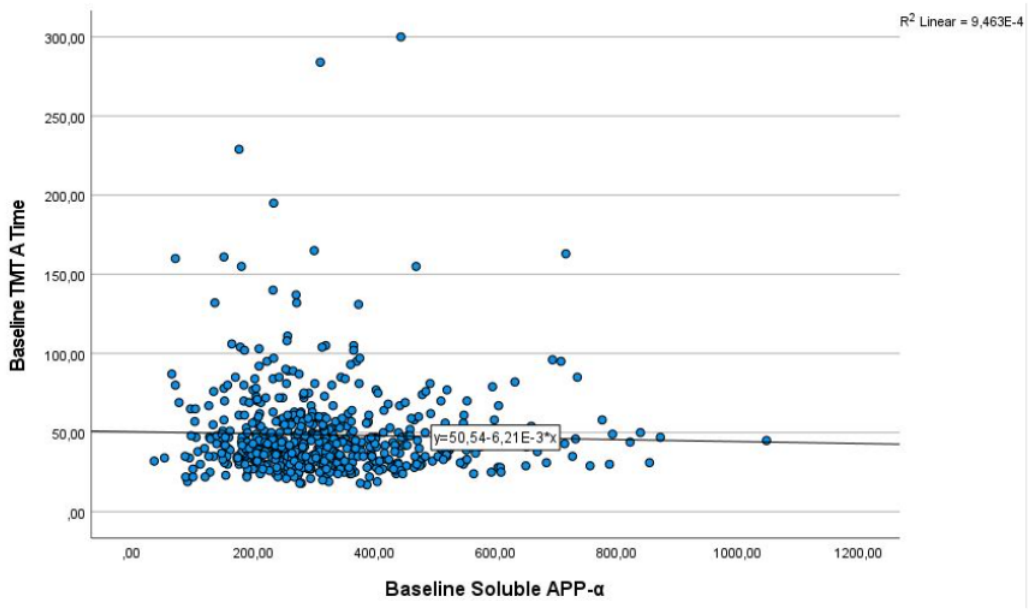

Figure A20: Linear regression analysis graph how increasing sAPPα is affecting the cognitive variable TMT-A.

A.21 TMT-A - sAPPβ

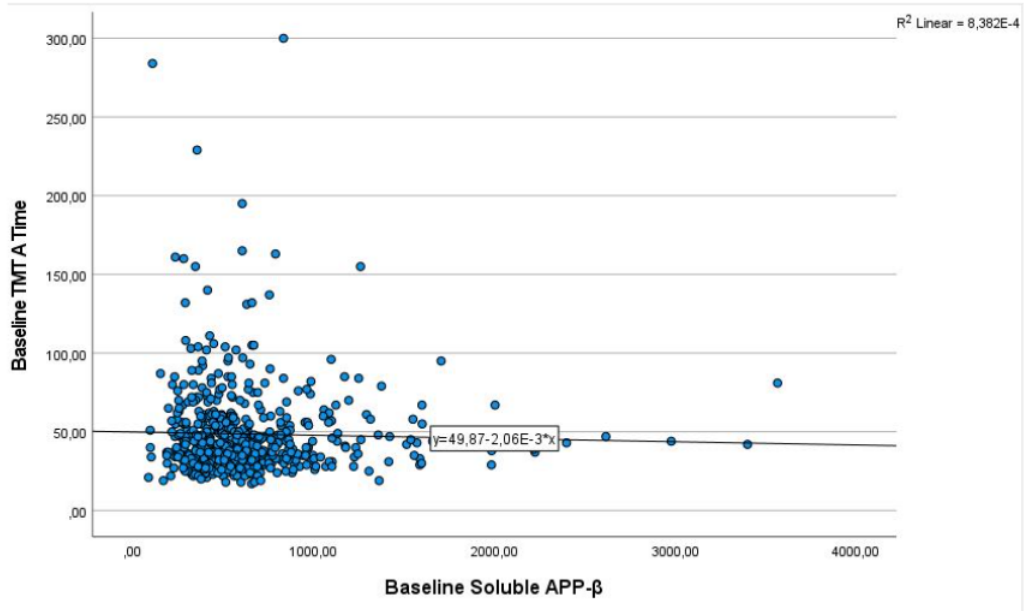

Figure A21: Linear regression analysis graph how increasing sAPPβ is affecting the cognitive variable TMT-A.

A.22 TMT-B - Aβ 1-42

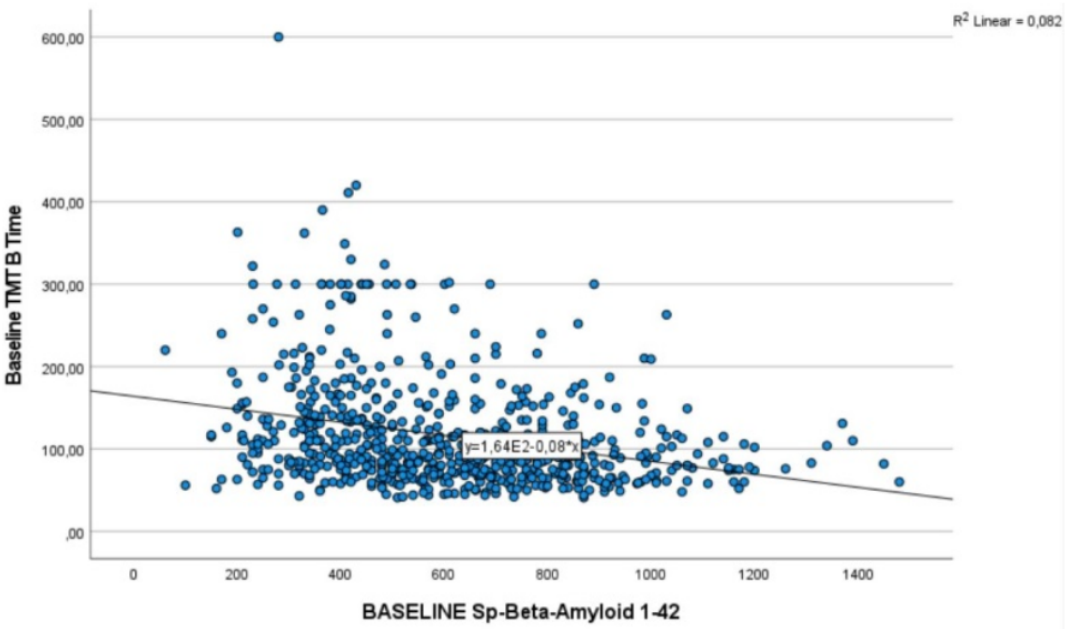

Figure A22: Linear regression analysis graph how increasing Aβ 1-42 is affecting the cognitive variable TMT-B.

A.23 TMT-B - T-tau

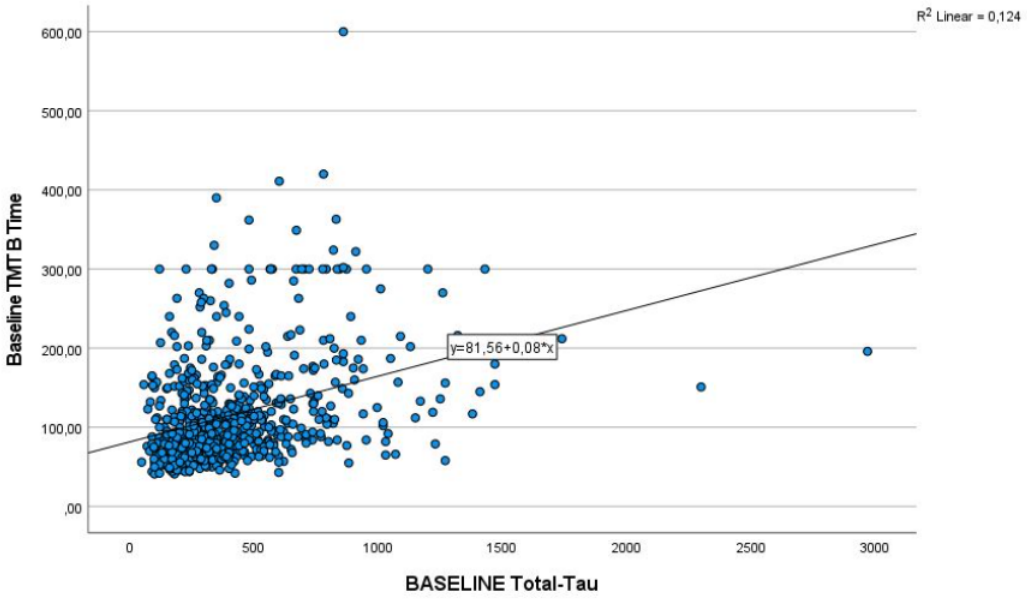

Figure A23: Linear regression analysis graph how increasing T-tau is affecting the cognitive variable TMT-B.

A.24 TMT-B - P-tau

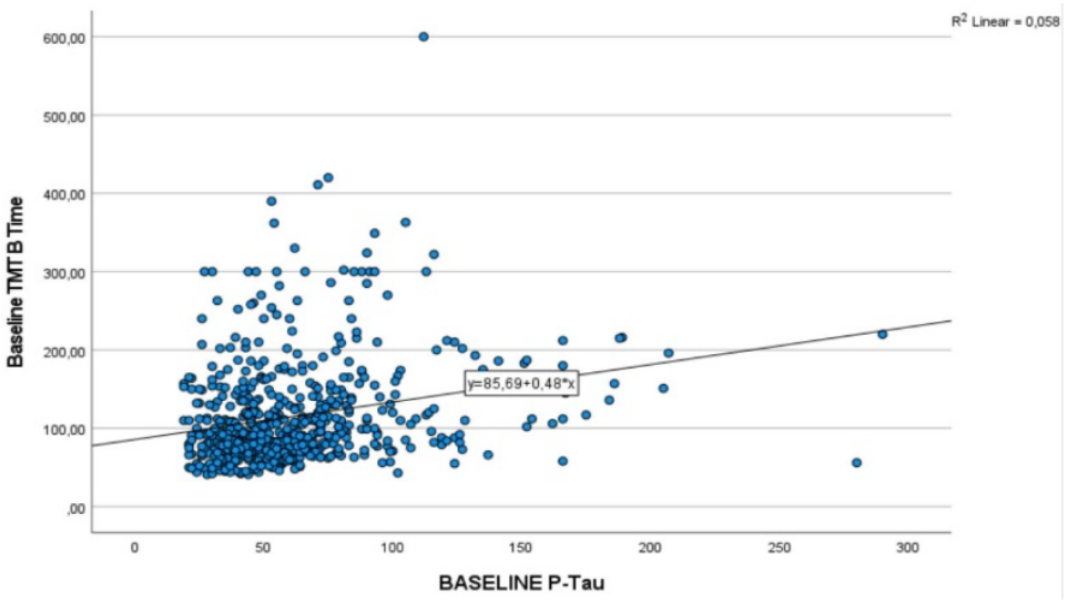

Figure A24: Linear regression analysis graph how increasing P-tau is affecting the cognitive variable TMT-B.

A.25 TMT-B - sAPPα

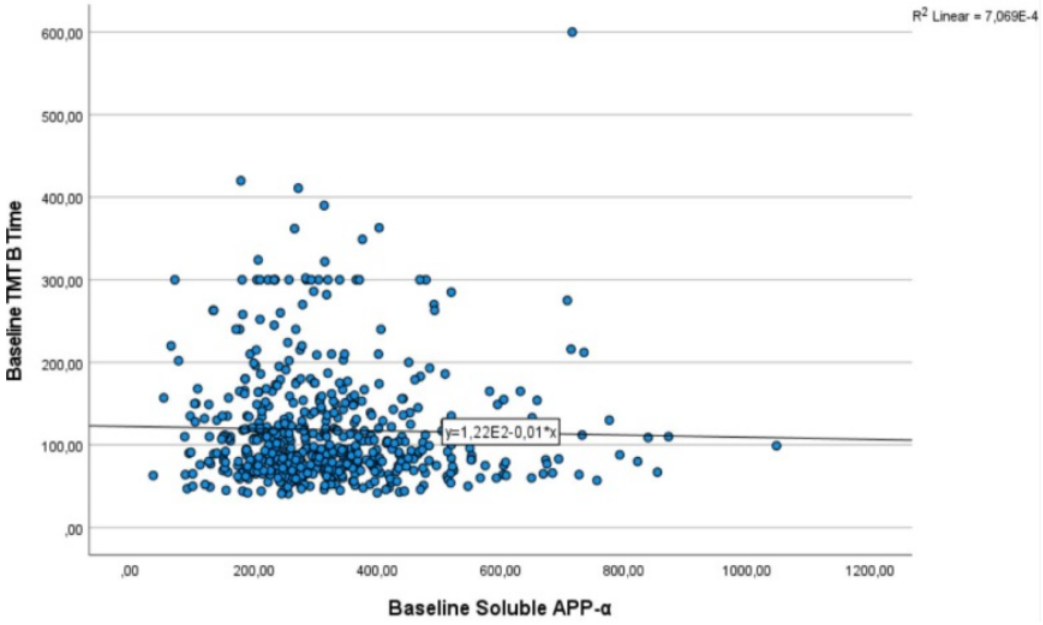

Figure A25: Linear regression analysis graph how increasing sAPPα is affecting the cognitive variable TMT-B.

A.26 TMT-B - sAPPβ

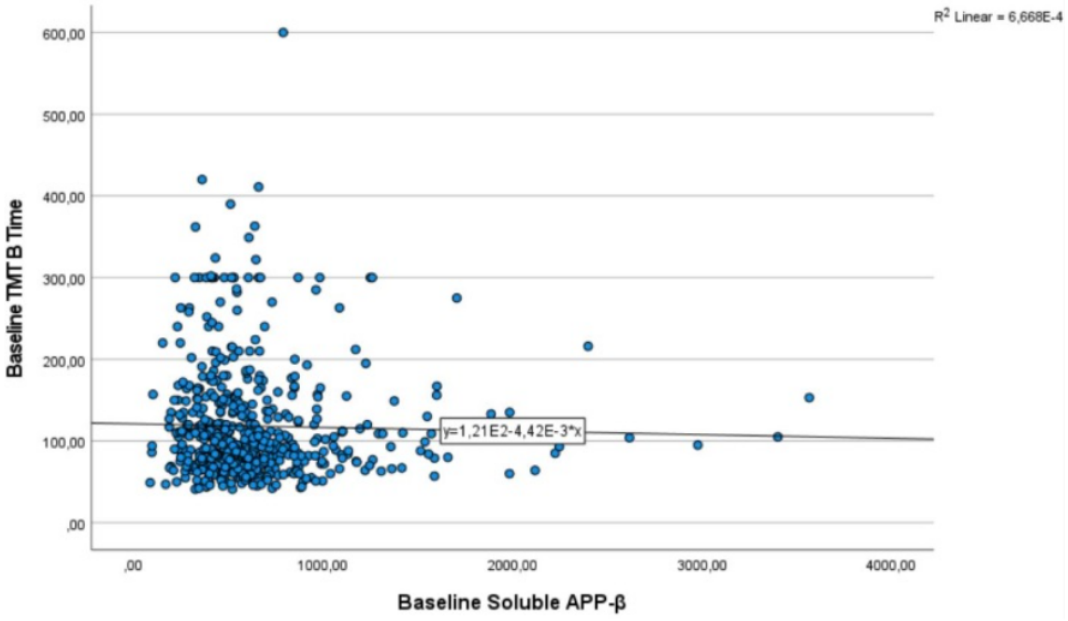

Figure A26: Linear regression analysis graph how increasing sAPPβ is affecting the cognitive variable TMT-B.

A.27 Delta Aβ 1-42

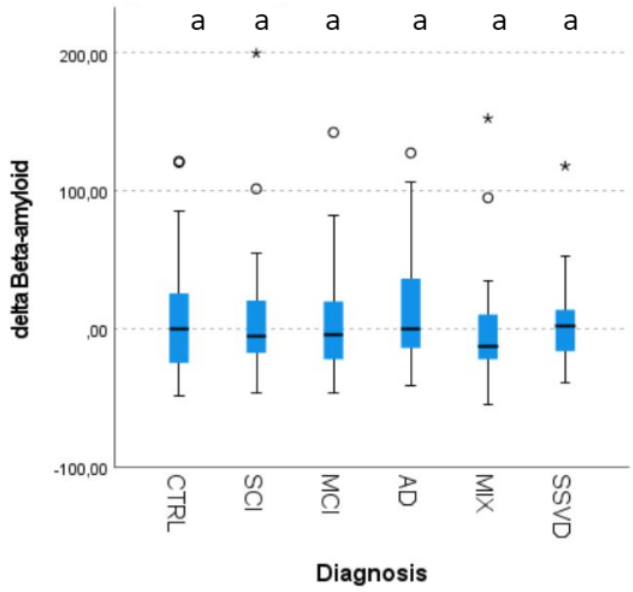

Figure A27: Graph showing the delta value in percentage between baseline and year 2 for the biomarker variable Aβ 1-42.

A.28 Delta T-tau

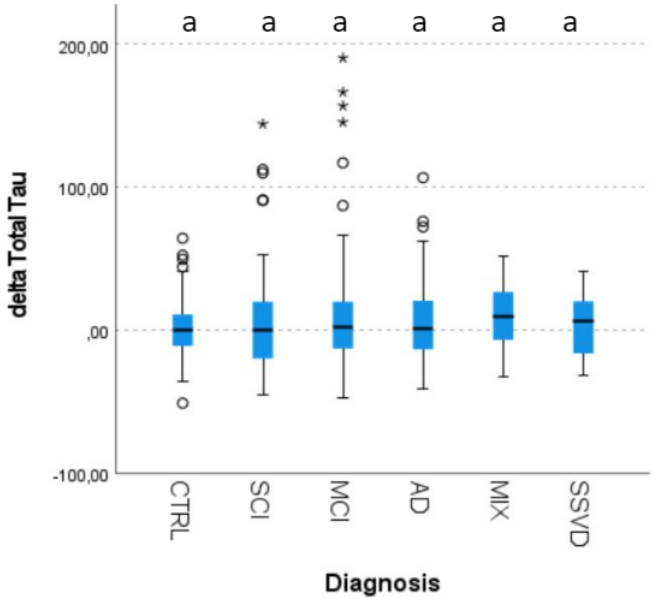

Figure A28: Graph showing the delta value in percentage between baseline and year 2 for the biomarker variable T-tau.

A.29 Delta P-tau

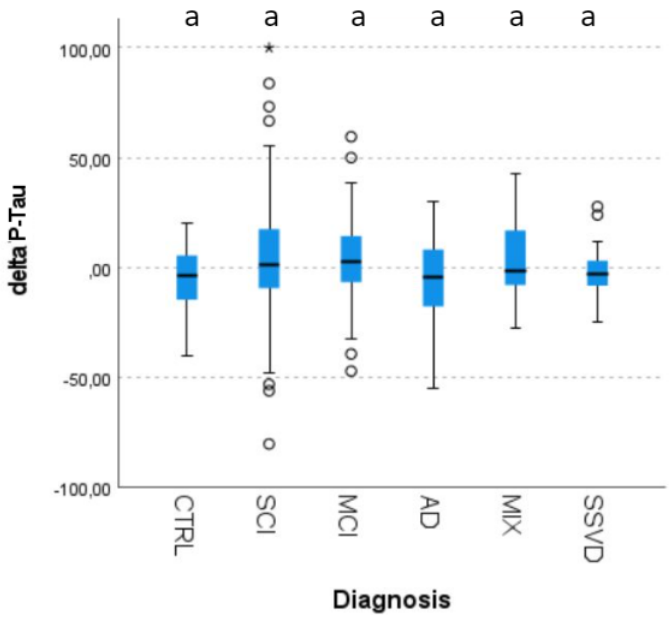

Figure A29: Graph showing the delta value in percentage between baseline and year 2 for the biomarker variable P-tau.

A.30 Delta albumin ratio

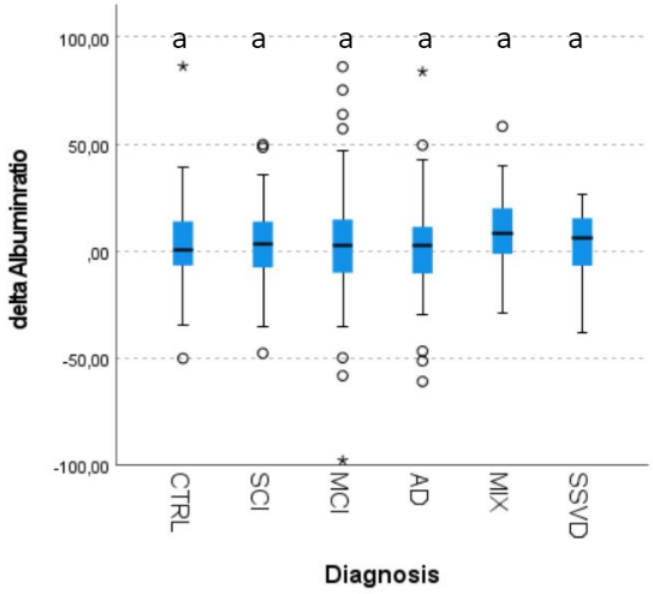

Figure A30: Graph showing the delta value in percentage between baseline and year 2 for the biomarker variable albumin ratio.

A.31 Delta sAPPβ

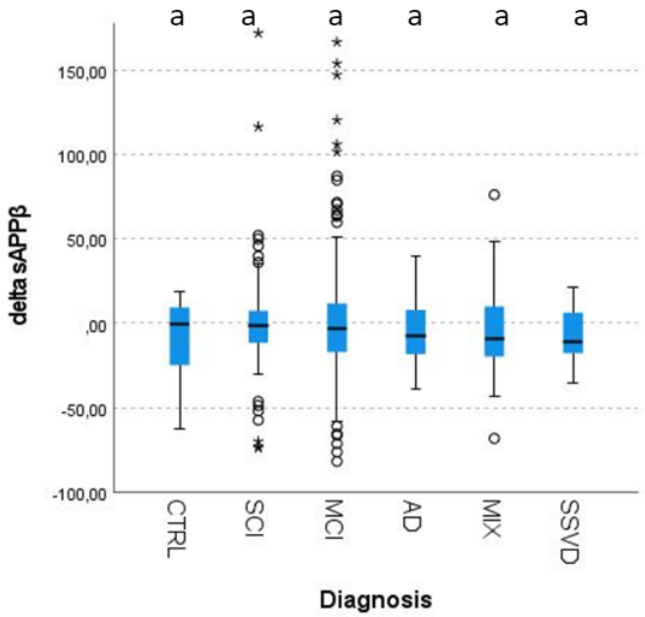

Figure A31: Graph showing the delta value in percentage between baseline and year 2 for the biomarker variable sAPPβ.

A.32 Delta sAPPα - Age

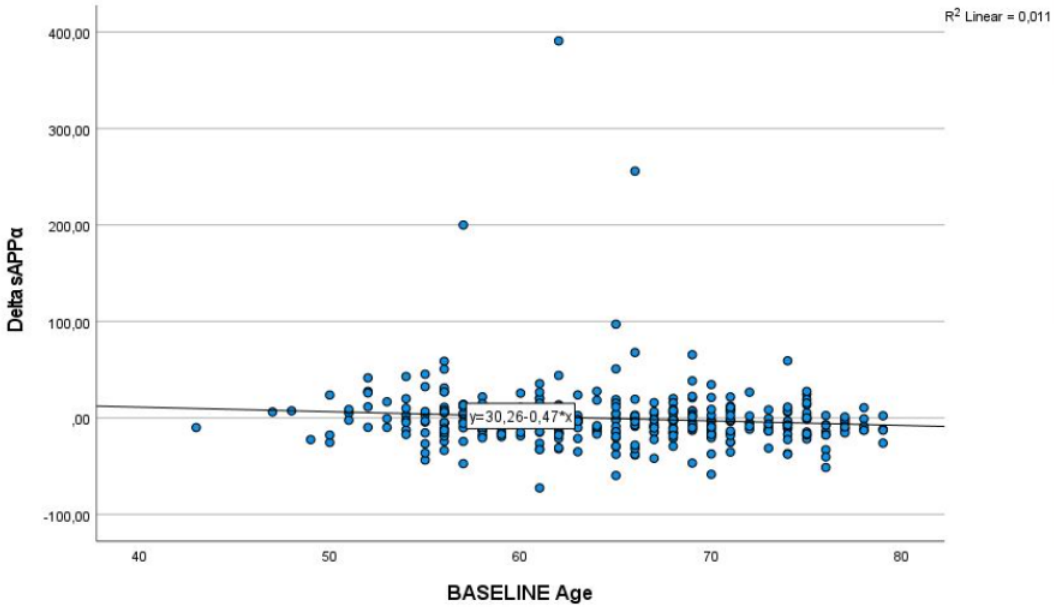

Figure A32: Linear regression analysis graph showing how the delta values for sAPPα changes with increasing age.

A.33 Delta sAPPα - Education

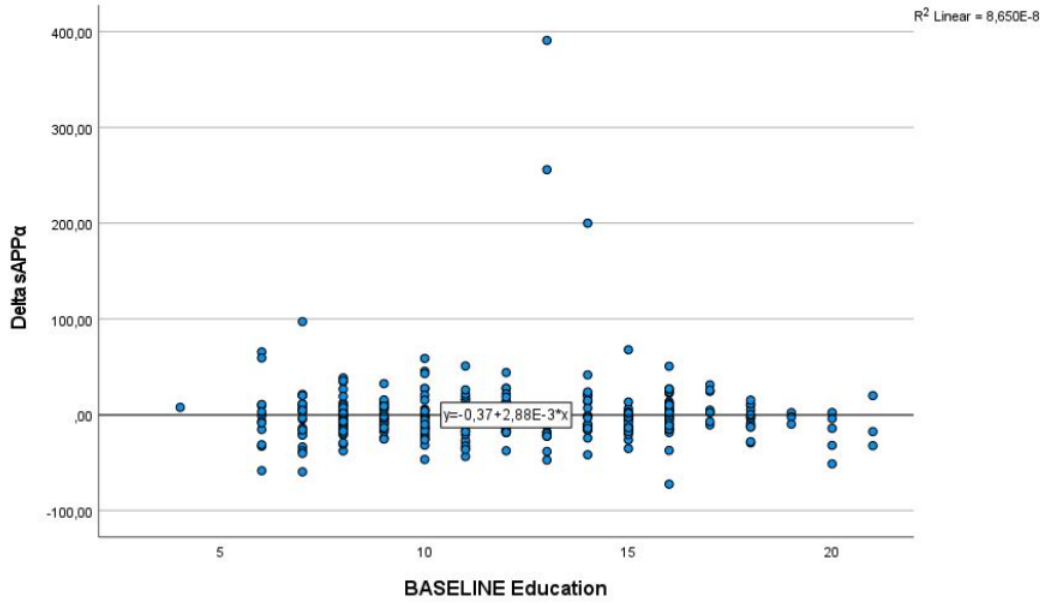

Figure A33: Linear regression analysis graph showing how the delta values for sAPPα changes with longer education.

A.34 Delta sAPPα - MMSE

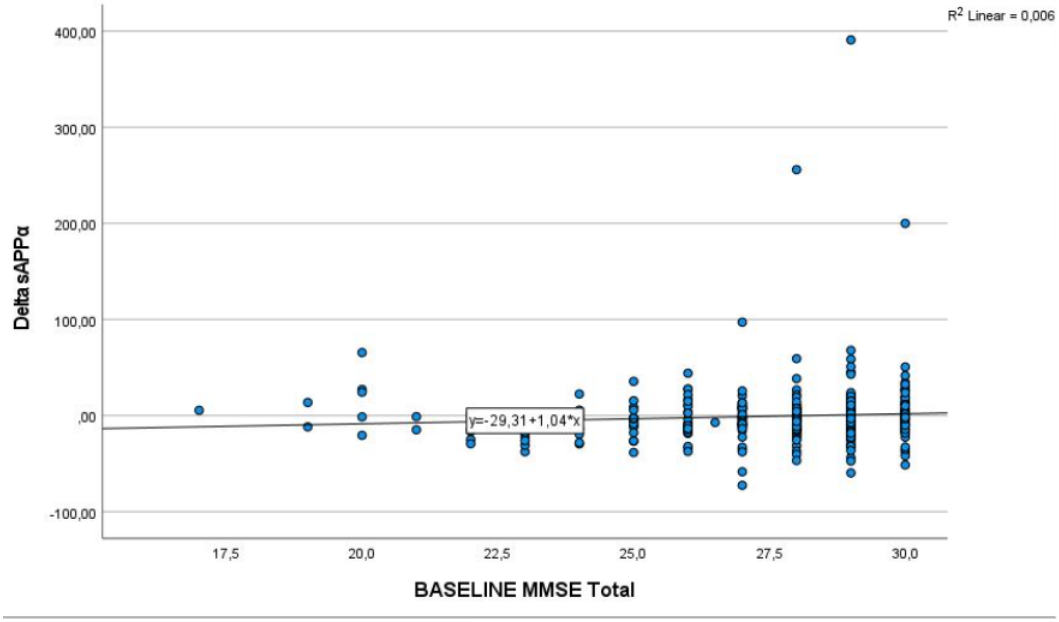

Figure A34: Linear regression analysis graph showing how MMSE scores change with increasing delta values for sAPPα.

A.35 Delta sAPPα - RAVLT

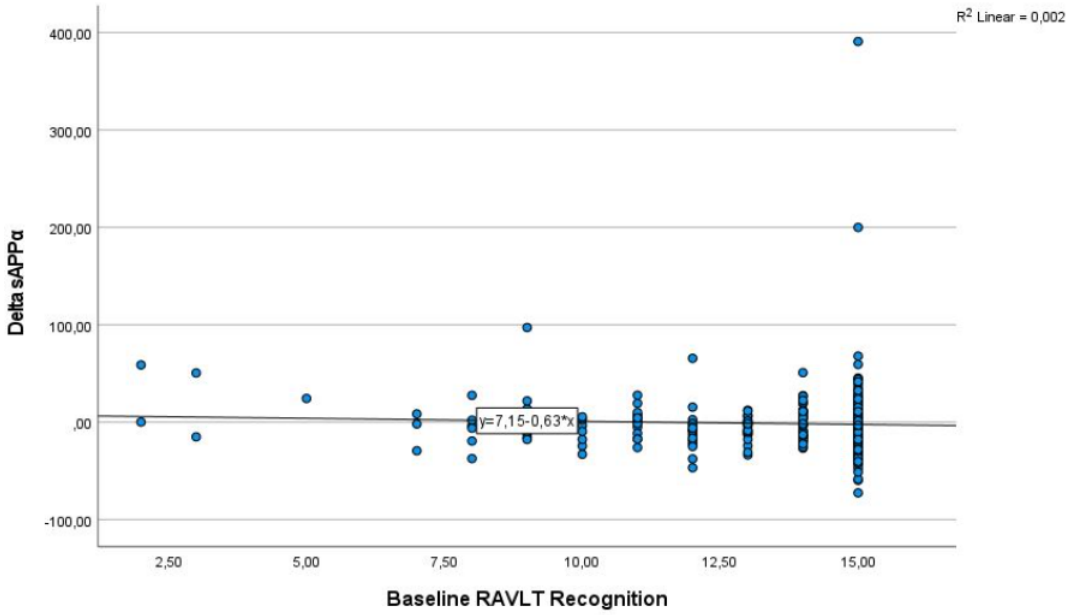

Figure A35: Linear regression analysis graph showing how RAVLT scores change with increasing delta values for sAPPα.

A.36 Delta sAPPα - TMT-A

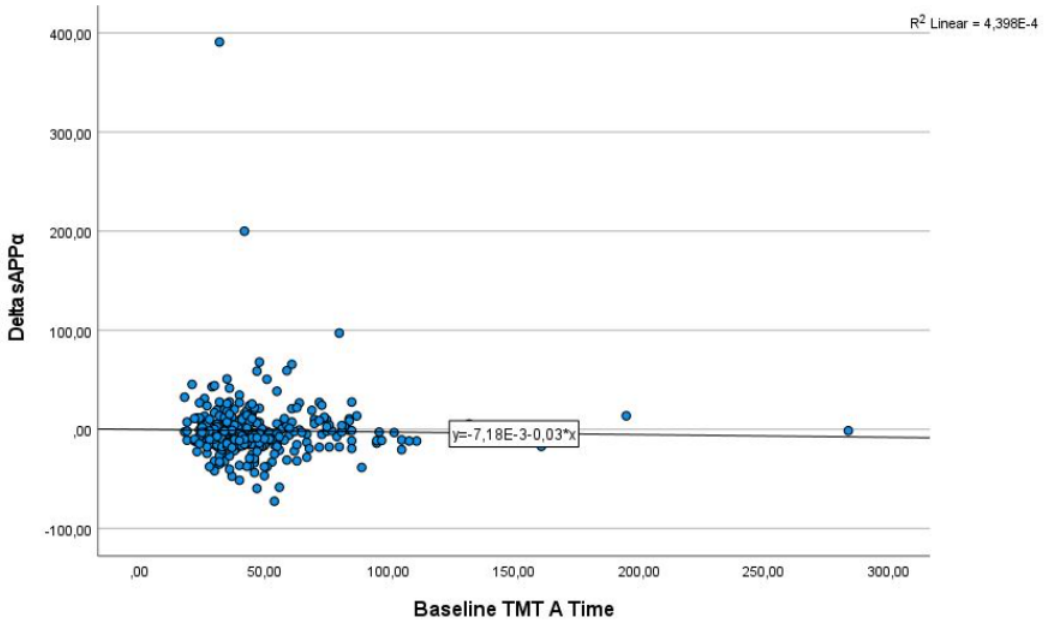

Figure A36: Linear regression analysis graph showing how TMT-A completion times change with increasing delta values for sAPPα.

A.37 Delta sAPPα - TMT-B

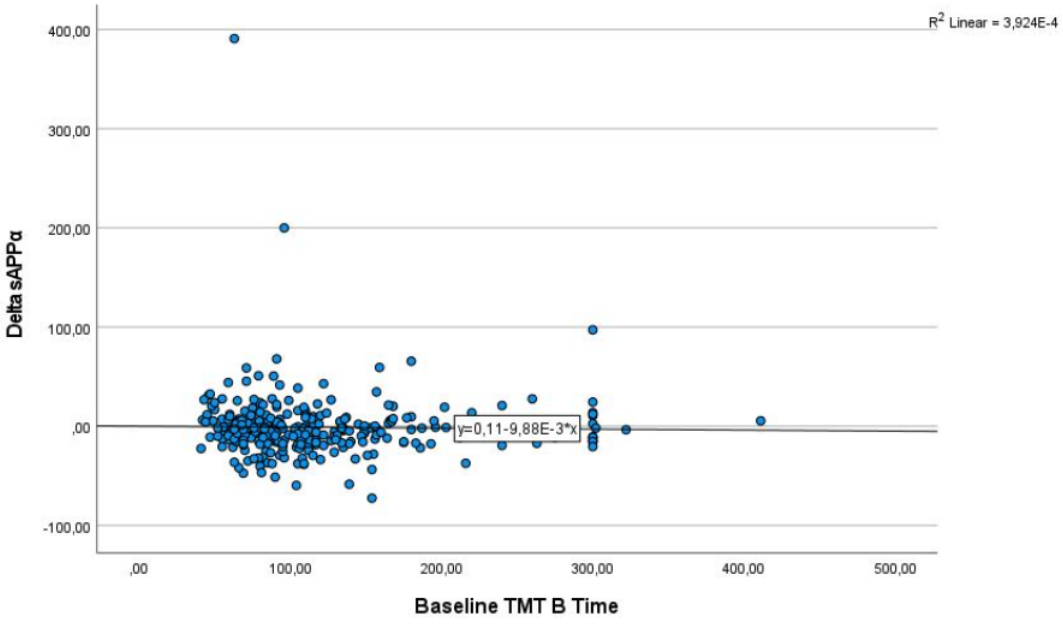

Figure A37: Linear regression analysis graph showing how TMT-B completion times change with increasing delta values for sAPPα.

A.38 Delta sAPPβ - Age

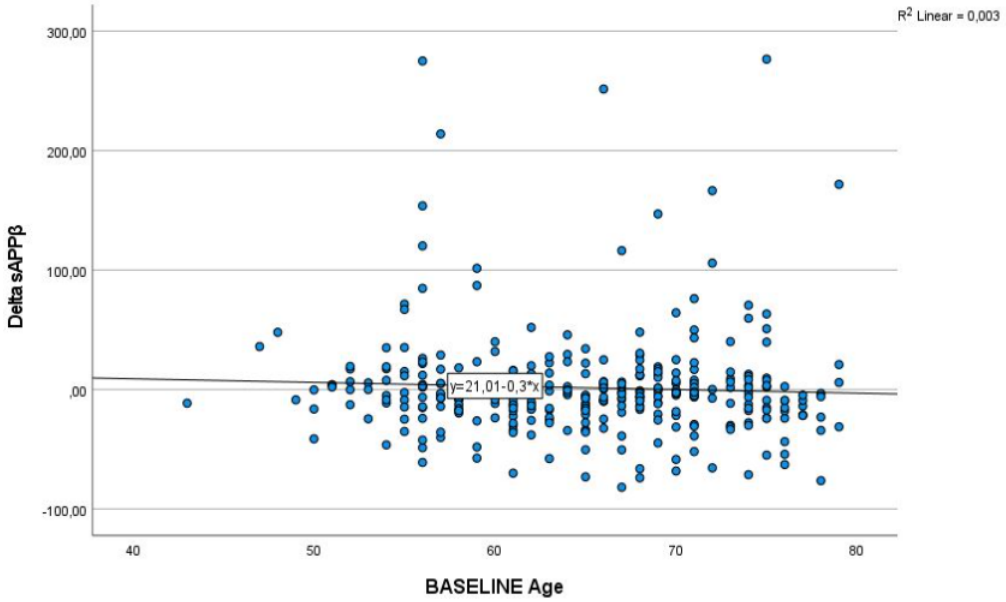

Figure A38: Linear regression analysis graph showing how the delta values for sAPPβ changes with increasing age.

A.39 Delta sAPPβ - Education

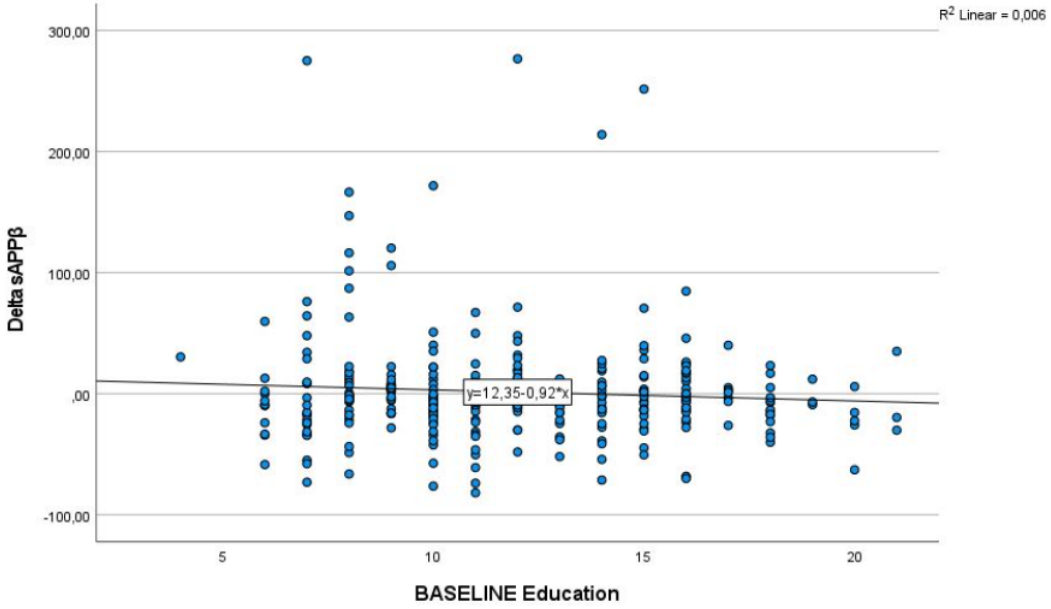

Figure A39: Linear regression analysis graph showing how the delta values for sAPPβ changes with longer education.

A.40 Delta sAPPβ - MMSE

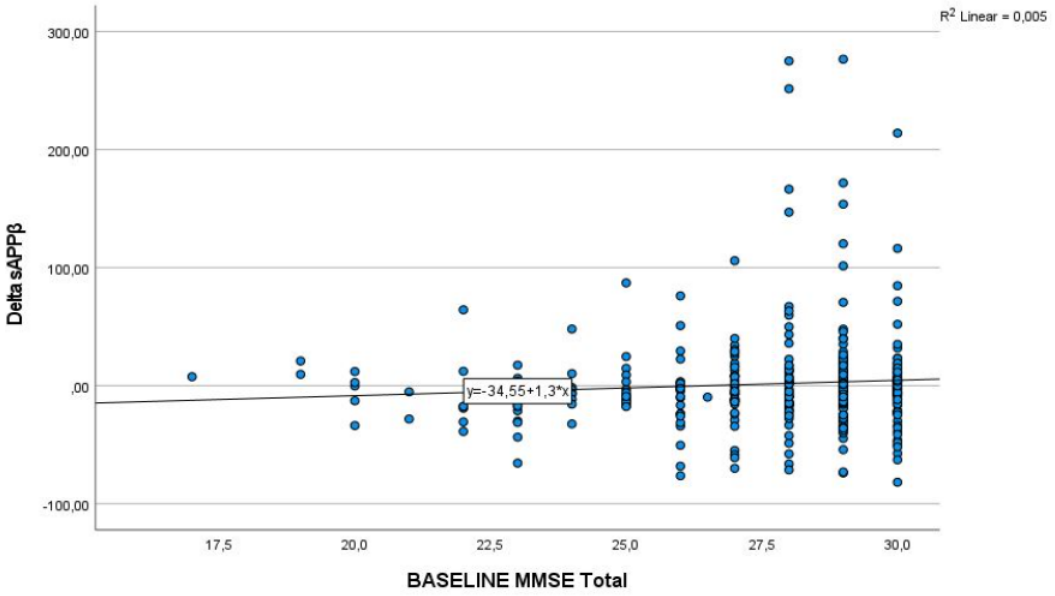

Figure A40: Linear regression analysis graph showing how MMSE scores change with increasing delta values for sAPPβ.

A.41 Delta sAPPβ - RAVLT

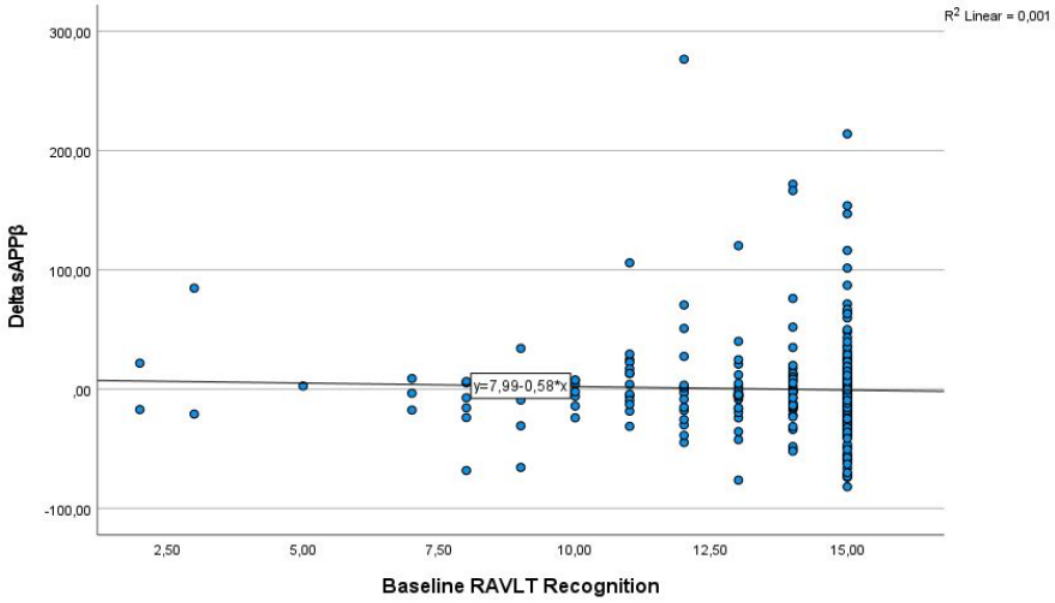

Figure A41: Linear regression analysis graph showing how RAVLT scores change with increasing delta values for sAPPβ.

A.42 Delta sAPPβ - TMT-A

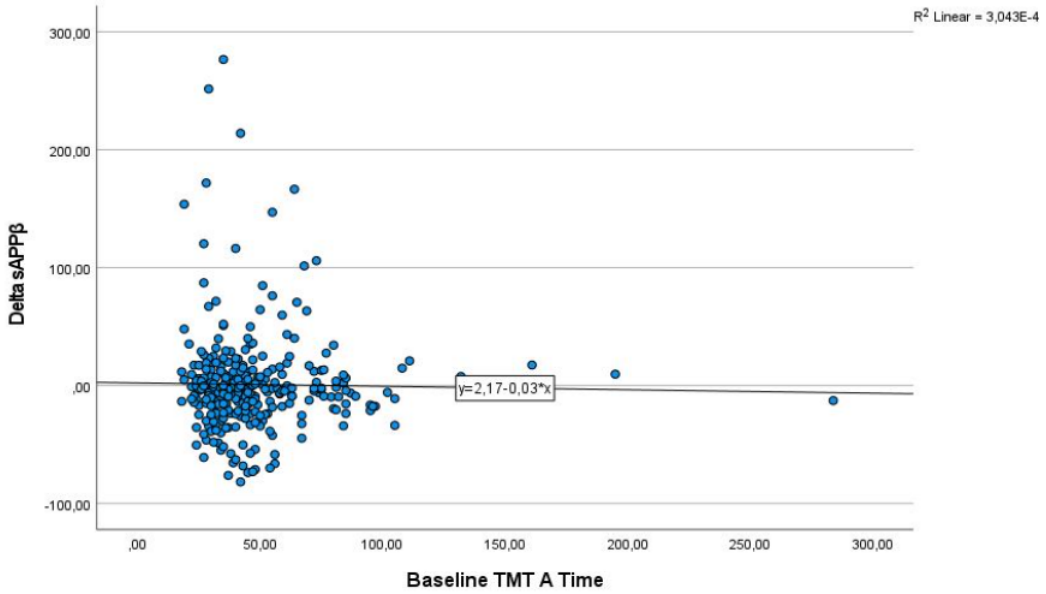

Figure A42: Linear regression analysis graph showing how TMT-A completion times change with increasing delta values for sAPPβ.

A.43 Delta sAPPβ - TMT-B

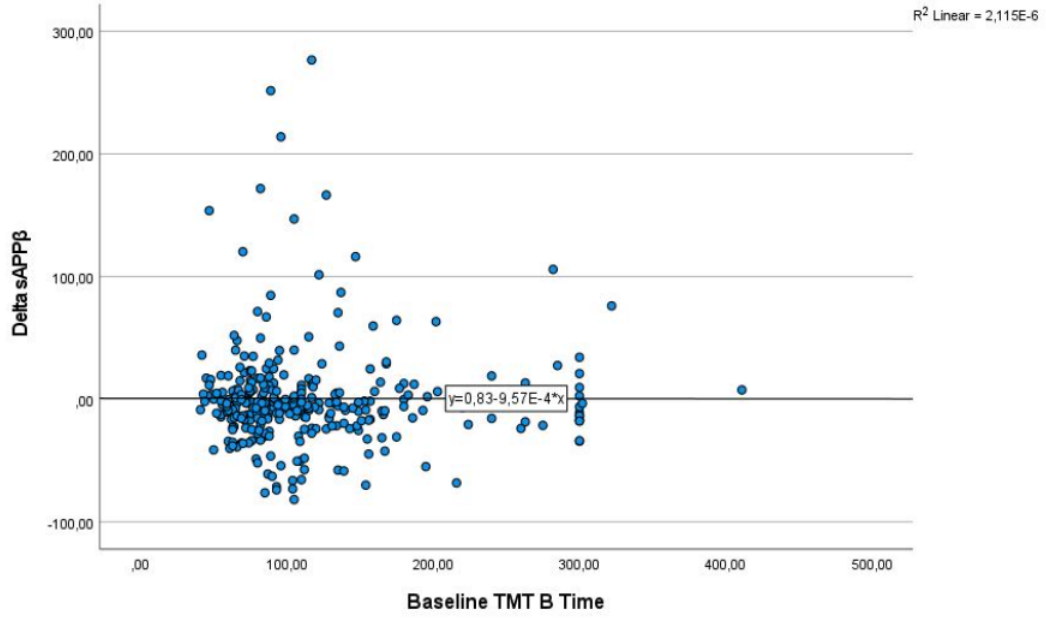

Figure A43: Linear regression analysis graph showing how TMT-B completion times change with increasing delta values for sAPPβ.