RELIABILITY OF ABETA 42/40 RATIO RELATED TO LEVELS OF CSF ALBUMIN IN ALZHEIMER DISEASE PATIENTS

S. Toniolo[1], G. Sancesario[1], A. Cerri[2] , G.M. Sancesario[3].

[1]Dept of Neurosciences, University Tor Vergata, Rome, Italy, [2]Dept of Experimental Medicine and Surgery, University Tor Vergata, Rome, [3]Dept. of clinical and behavioural Neurology, Santa Lucia Foundation, Rome.

Introduction:

The cerebrospinal fluid (CSF) biomarkers T-Tau, P-tau and Aβ42 are currently used for the diagnosis of Alzheimer's disease. The T-Tau/Aβ42 ratio is considered the most useful index of AD pathology, though a wide range of combination of biomakers has been assessed through time. Nevertheless, since there is a significant heterogeneity in CSF Aβ42, the Aβ40 has recently been included as a biomarker of AD pathology, since the selective decrease in Aβ42 levels compared to constant or even elevated Aβ40 seems to be more specific for AD pathology. However, it is not completely understood whether the content of albumin in CSF affects the CSF Aβ peptide levels. Actually, human serum albumin (HSA) binds 95% of Aβ peptides in blood and inhibits Aβ fibrillization at micromolar levels in CSF, significantly increasing the lag time and decreasing the total amount of fibrils produced, and it's proven that the the amount of amyloid fibers generated directly correlates to the proportion of Aβ not competitively bound to albumin.

Aim:

The aim of our study was to assess whether the content of albumin in serum and CSF affects the CSF Aβ peptide levels, as shown by *in vitro* experiments. We compared the levels of CSF AD biomarkers (T-tau, P-tau, Aβ42, Aβ40) and their ratio with levels of CSF and serum proteins in three groups of patients (AD, Dementia not due to AD and MCI) to assess the reliability of Aβ40 and the Aβ42/40 ratio in clinical practice.

Materials and methods:

We enrolled a total of 174 patients that where followed in the Neurological Clinic of the Tor Vergata General Hospital of Rome between 2014 and 2016. In our population sample 56 patients met the diagnostic criteria for AD, 93 patients were affected by dementia not due to AD and 25 have been diagnosed as MCI patients. The three groups were equally sex and age matched. The non-AD dementia group consisted of patients with probable frontotemporal lobar degeneration (FTLD) (n =4), OSAS (n =6), amyloidosis (n =2), PSP (n =4), SLA with severe cognitive impairment (n = 3), probable dementia with Lewy bodies (DLB) (n =1), normal pressure hydrocephalus (NPH) (n =2), ADPD patients (n = 11), MSA (n = 2), epileptic patients with moderate-severe cognitive impairment (n = 4), CJD (N = 1), CBD (n = 2), Vascular dementia (n = 6), Pseudodepressive dementia (N = 1), encephalitis (n = 2), and other neurodegenerative diseases (Fahr syndrome, Kufor rabek disease, Anti GAD syndrome, Friedreich's ataxia). All patients underwent a complete neurological examination, MRI scan and CSF analysis. We analyzed the values of total CSF protein, CSF and serum Albumin, CSF index, serum and CSF IgG, Albumin CSF/serum ratio. The neuropsychological assessment included MMSE, Rey Auditory verbal learning test, Rey complex figure immediate and delayed recall, Raven's progressive Matrices, Stroop test, Verbal fluency test. Dementia due to AD was defined according to the criteria of 2007 by the International Working Group for New Research Criteria for the Diagnosis of AD. The diagnosis of MCI was made according to the Petersen criteria. The Diagnosis of FTD was made according to the Rascovsky criteria, the National Institute of Neurological Disorders and Stroke (NINDS) was used for vascular dementia, the Mc Keith criteria for DLB patients.

CSF sampling: In the AD patients, CSF T-Tau, P-Tau, Aβ42 and Aβ40 levels concentration were determined using a sandwich ELISA (Innotest hTAU-Ag, Innogenetics, Gent, Belgium). The first 12 mL of CSF was collected in a polypropylene tube, then directly transported to the local laboratory for centrifugation at 2000 × g at +4° C for 10 minutes.

Statistical analysis: The data are expressed as the mean ± standard deviation. The statistical analysis was conducted with SPSS 16.0 software (IBM, Somers, NY, USA) using a one-way analysis of variance (ANOVA) followed by Bonferroni post-hoc tests.

Results:

Outline of the population sample:

	AD	Non AD dementia	MCI
Number	56	93	25
Age	71,80 (+/- 6,5)	69,14 (+/- 8,5)	70 (+/- 8,7)
Sex M:F	27:29	55: 38	10:15
MMSE	22,15 (+/- 5,5)	23,53 (+/- 4,5)	26,76 (+/- 2,2)
T-Tau	717,95 (+/- 425,93)	350,98 (+/- 282,7)	226,48 (+/- 91,2)
P-tau	87,41 (+/-41,94)	44,01 (+/- 5,6)	35,84 (+/- 14,16)
Abeta 42	436,05 (+/-117,9)	699,17 (+/-260,8)	645,60 (+/-231,8)
Abeta 40	11669,95 (+/- 4099,8)	10468,44 (+/- 4899,5)	9325,72 (+/- 4051,6)
T-tau/Abeta42	1,77 (+/- 1,2)	0,60 (+/- 0,76)	0,38 (+/- 0,16)
Abeta 42/40	0,41 (+/- 0,2)	0,74 (+/- 0,29)	0,75 (+/- 0,25)
CSF Protein	47,46 (+/- 15,2)	52,68 (+/- 23,6)	43,14 (+/- 11,8)
CSF albumin	27,33 (+/- 11,8)	31,07 (+/- 15,6)	25,73 (+/- 10,2)
CSF IgG	3,02 (+/- 1,3)	3,77 (+/- 2,7)	2,49 (+/- 0,9)
CSF index	0,47 (+/- 0,4)	0,5 (+/- 0,9)	0,49 (+/- 0,6)
Serum IgG	986,2 (+/- 250)	956,8 (+/- 218)	863,2 (+/- 218)
Serum Albumin	4088,75 (+/- 408)	4052,37 (+/- 493)	4192,8 (+/- 442)
Albumin ratio	6,6 (+/- 2,6)	7,98 (+/- 4,4)	6,17 (+/- 2,4)

The AD patients showed a higher T-tau level (717,95) vs Non AD dementia (350,98) and MCI (226,48), a higher P-tau level (87,41 vs 44,01 of Non AD dementia, and 35,84 of MCI), a lower Aβ42 level (436,05), vs Non AD dementia group (699,17) and MCI (639,25).
The T-tau/Aβ42 ratio was significantly higher (1.77 vs 0,60 and 0,377) and Aβ42/40 ratio was significantly lower in AD patients (0,41 vs 0,74 in Non AD dementia patients and 0,75 in MCI patients).
Aβ40 did not show significantly different values between the three groups. Serum protein, CSF and serum albumin, CSF Index, serum IgG levels and Albumin ratio were comparable between the three groups.
The mean age of the AD group was 71,80 (+/- 6,5), 69,14 (+/- 8,5) in Non AD dementia group, 70 (+/- 8,7) in MCI patients. MMSE values were 22,15 (+/- 5,5) in AD patients, 23,53 (+/- 4,5) in Non AD dementia group, 26,76 (+/- 2,2) in MCI patients.

AD CSF biomarkers vs CSF and Serum Protein biomarkers

		CSF Protein	CSF Albumin	CSF IgG	CSF index	Serum IGG	Serum Albumin	Albumin ratio
T-tau	Pearson	0,062	0,055	0,044	-0,144	0,036	-0,018	0,046
	p-value	0,414	0,467	0,561	0,056	0,637	0,806	0,542
P-tau	Pearson	-0,084	-0,118	-0,142	-0,132	0,04	0,038	-0,143
	p-value	0,264	0,113	0,06	0,079	0,598	0,617	0,056
Aβ40	Pearson	-0,137	-0,194	-0,175	-0,096	0,05	-0,021	-0,189
	p-value	0,068	0,009	0,02	0,204	0,512	0,784	0,011
Aβ42	Pearson	-0,037	-0,051	-0,027	0,099	0,032	0,059	-0,052
	p-value	0,628	0,494	0,724	0,188	0,674	0,43	0,491
Aβ 42/40	Pearson	0,133	0,170	0,161	0,213	-0,07	0,091	0,159
	p-value	0,078	0,022	0,032	0,004	0,352	0,224	0,033
T-tau/ Aβ42	Pearson	0,047	0,032	0,029	-0,147	0,032	-0,011	0,017
	p-value	0,534	0,672	0,705	0,05	0,675	0,887	0,816

We found no correlation between AD CSF biomarkers and level of CSF protein, Albumin in CSF and serum, Albumin ratio, CSF index, Serum and CSF IgG.

Bonferroni Post Hoc test:

	(I) diagnosis	(J) diagnosis	Mean Difference (I-J)	Std. Error	Sig.
T-Tau	1	2	366,97(*)	54,15	0
		3	491,47(*)	77,009	0
	2	1	-366,97(*)	54,15	0
		3	124,50	72,13	0,258
	3	1	-491,47(*)	77,009	0
		2	-124,50	72,13	0,258
P-tau	1	2	43,40(*)	4,87	0
		3	51,57(*)	6,93	0
	2	1	-43,40(*)	4,87	0
		3	8,17	6,49	0,629
	3	1	-51,57(*)	6,93	0
		2	-8,17	6,49	0,629
Aβ40	1	2	1201,51	768,17	0,359
		3	2344,23	1092,39	0,1
	2	1	-1201,51	768,17	0,359
		3	1142,72	1023,12	0,797
	3	1	-2344,23	1092,39	0,1
		2	-1142,72	1023,12	0,797
Aβ42	1	2	-263,12(*)	37,29	0
		3	-209,55(*)	53,03	0
	2	1	263,12(*)	37,29	0
		3	53,57	49,67	0,847
	3	1	209,55(*)	53,03	0
		2	-53,57	49,67	0,847
Aβ42/40	1	2	-,033(*)	0,00427	0
		3	-,034(*)	0,00608	0
	2	1	,033(*)	0,00427	0
		3	-0,00034	0,00569	1
	3	1	,0336(*)	0,00608	0
		2	0,00034	0,00569	1
T-tau/Aβ42	1	2	1,167(*)	0,152	0
		3	1,40(*)	0,216	0
	2	1	-1,17(*)	0,152	0
		3	0,227	0,203	0,794
	3	1	-1,40(*)	0,217	0
		2	-0,227	0,203	0,794

	(I) diagnosis	(J) diagnosis	Mean Difference (I-J)	Std. Error	Sig.
CSF Protein	1	2	-5,225	3,360	0,366
		3	4,319	4,759	1
	2	1	5,225	3,360	0,366
		3	9,5435	4,468	0,102
	3	1	-4,319	4,759	1
		2	-9,5435	4,468	0,102
CSF Albumin	1	2	-3,734	2,33	0,334
		3	1,606	3,318	1
	2	1	3,734	2,33	0,334
		3	5,34	3,108	0,263
	3	1	-1,606	3,318	1
		2	-5,34	3,108	0,263
CSF IgG	1	2	-0,752	0,364	0,12
		3	0,5296	0,523	0,939
	2	1	0,7524	0,364	0,12
		3	1,282(*)	0,4918	0,03
	3	1	-0,5296	0,5235	0,939
		2	-1,282(*)	0,4918	0,03
CSF index	1	2	-0,0227	0,0128	0,232
		3	-0,0201	0,0184	0,824
	2	1	0,0227	0,0128	0,232
		3	0,0026	0,0173	1
	3	1	0,0201	0,0184	0,824
		2	-0,0026	0,0173	1
Serum IgG	1	2	29,444	38,934	1
		3	123,006	55,928	0,088
	2	1	-29,445	38,934	1
		3	93,56	52,602	0,231
	3	1	-123,006	55,928	0,088
		2	-93,56	52,602	0,231
Serum Albumin	1	2	36,38	77,92	1
		3	-104,05	110,81	1
	2	1	-36,38	77,92	1
		3	-140,43	103,78	0,533
	3	1	104,05	110,81	1
		2	140,43	103,78	0,533
Albumin ratio	1	2	-1,382	0,621	0,082
		3	0,429	0,883	1
	2	1	1,382	0,621	0,082
		3	1,812	0,827	0,09
	3	1	-0,429	0,883	1

Tab: 1: AD, 2: Non AD dementia, 3 MCI.

Bonferroni post hoc test was applied between the three groups:
T-tau and P-tau were significantly higher in AD patients compared to patients with dementia not due to AD and vs MCI patients but were not able to distinguish between MCI patients and patients with dementia not due to AD. Aβ40 was comparable between the 3 groups. Aβ42, Aβ40 and the Abeta 42/40 ratio are able to distinguish between AD patients and patients with dementia not due to AD and vs MCI patients but are not able to discriminate between MCI patients and patients with dementia not due to AD.
Regarding the protein markers there is only a mild increase in CSF IgG in Non ad dementia patients compared to MCI patients, but with a p-value of 0.03. None of the the serum and CSF protein biomarkers are able to discriminate between the three groups of patients.

Discussion:

The rate at which fiber formation is nucleated for both Aβ40 and Aβ42 is significantly inhibited at physiological levels of albumin. *In vitro* evidence shows that the total concentration of fiber generated is reduced by HSA, and this suggests that HSA binds to Aβ molecules and traps them in a nonfibrillar form so that they are not available to form fibers. We wanted to investigate whether the CSF biomarkers and especially Aβ40 would be influenced by the variations of HSA. We found no correlation between values of serum and CSF protein compared to the AD CSF biomarkers, therefore we consider Aβ40 in CSF to be a stable marker which does not vary between different groups of patients and it's not influenced by valued of total proteins, HSA (as could be suggested by in vitro experiments) and CSF albumin *in vivo*. Therefore CSF Aβ40 and the Aβ42/40 ratio are useful in clinical practice and represent a reliable biomarker of AD pathology.

Referencies:

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