Neurovascular Dysfunction in Alzheimer's Disease: Assessment of Cerebral Vasoreactivity by Ultrasound Techniques and Evaluation of Endothelial Biomarkers

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Aims of the study

- To assess the cerebral vasomotor reactivity in patients with Alzheimer's Disease (AD) by using a Transcranial Doppler Ultrasound (TCD) study;

- To assess the endothelial dysfunction in patients with AD by investigating the circulating angiogenic cells such as endothelial progenitor cells (EPCs) [1];

- To evaluate if the changes in both circulating angiogenic cells and cerebral vasomotor reactivity can influence the cognitive decline.

Matherials and methods:

We recruited thirty-five AD subjects, matched for age, sex and education to seventeen healthy control subjects. All the subjects underwent brain MRI, Neuropsychological evaluation and Carotid Duplex Ultrasonography. The TCD study is an ultrasound technique used for the indirect estimation of changes in cerebral blood flow (CBF). We used the trans-temporal acoustic window to evaluate the mean flow velocity of the middle cerebral artery (MCAFV). Cerebrovascular reactivity to hypercapnia was evaluated by means of the breath-holding index (BHI). Ten AD subjects underwent to a follow up TCD study after one year. Endothelial function was evaluated in twenty-four AD subjects and ten healthy control subjects, according to the level of EPCs. Peripheral blood EPCs was counted by flow cytometry from venous blood samples. We used two cellular markers to identify peripheral EPCs because CD34 recognizes cells of endothelial lineage, whereas CD133 primarily detects immature EPCs that are highly capable of differentiating into endothelial cell. Three populations of cells were analyzed: CD34+, CD133+ and double-stained cells (CD34+ and CD133+), and expressed as numbers of positive cells in each population. We also analyzed VEGF (pg/ml), IL-18 (pg/ml), IL-18BPa (pg/ml) IL-18 free (pg/ml).

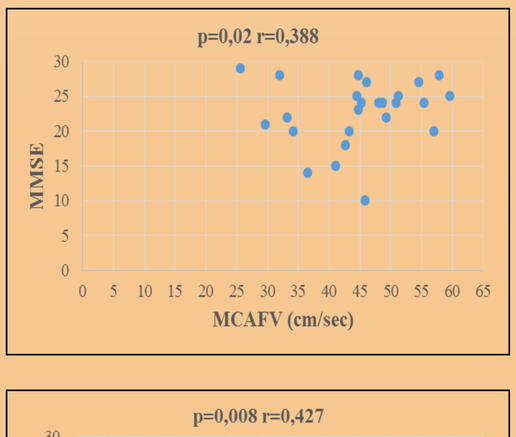
	AD group (n=35) mean ± SD	Control group (n=17) mean ± SD	P values
MCAFV (cm/s)	44,9 ± 9	52 ± 9,4	**0,03**
BHI	1 ± 0,3	1,3 ± 0,3	**0,02**

Table 1

Figure 1

Results:

Both MCAFV and BHI values were significantly lower in AD subject than in healthy control (44,9 ± 9 vs 52 ± 9,4, p=0,03 and 1 ± 0,3 vs 1,3 ± 0,3, p=0,02) **(Table 1, Figure 1**). A statistically significant correlation was found between MCAFV and BHI values and MMSE scores (p= 0,02 r=0,388 and p=0,03 r=0,360) (**Figure 2**), especially when we corrected the values for age and education (p=0,008, r=0,427 and p=0,02 r=0,383) (**Figure 3**). In follow up group we found MCAFV values significantly lower than the base line group (48,55 ± 7,9 vs 39,64 ± 8,27 p=0,039) but only a positive trend of correlation for BHI values (1,00 ± 0,32 vs 0,83 ± 0,25 p=0,28) **(Table 2)**. We found that subjects with AD had lower CD34+CD133+ EPCs counts than controls (51,4 ±14,6 vs 63,9 ± 18,25 p=0,01) (**Table 3**), but a lower CD34+CD133+ EPCs number was not associated with lower MMSE (p=0,32 r=0,183) **(Figure 4**). No other significant correlation was found considering the cellular markers. The level of circulating CD34+ cells has not been found to be correlated with MCAFV and BHI in AD subjects.



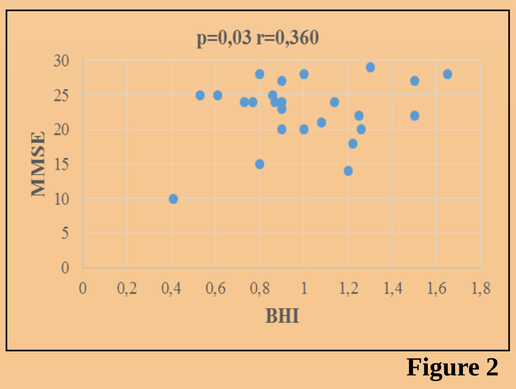
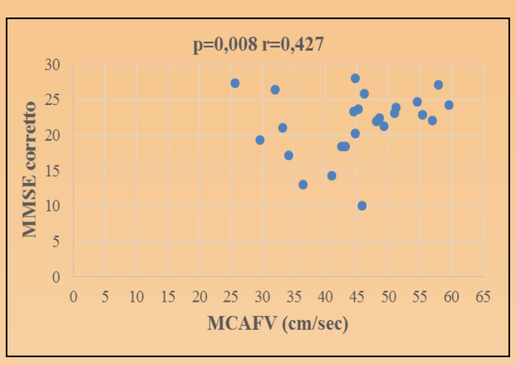


Figure 2



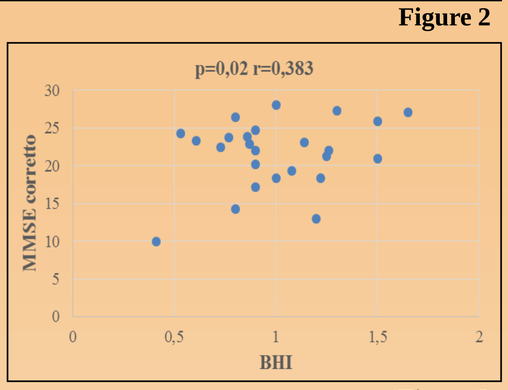


Figure 3

	AD patients baseline (n=35) mean ± SD	AD patients 1 year follow up (n=10) mean ± SD	P values
MCAFV (cm/s)	48,55 ± 7,9	39,64 ± 8,27	**0,039**
BHI	1,00 ± 0,32	0,83 ± 0,25	0,28

Table 2

	AD group (n=24) mean ± SD	Control group (n=10) mean ± SD	P values
CD34/ml	10948,4 ± 14475,8	2609,5 ± 3404,8	0,82
CD34/CD133/CD309 /ml	116,4 ± 122,4	71,24 ± 65,8	0,36
% total CD34	0,027 ± 0,02	0,029 ± 0,01	0,40
% CD34/CD133/CD309	3,2 ± 3	3,6 ± 2,9	0,38
% CD34/CD133/CD309/PBL	0,00063 ± 0,00064	0,001 ± 0,0007	0,21
% CD34/CD133	51,4 ±14,6	63,9 ± 18,25	**0,01**
VEGF (pg/ml)	378,4 ± 206,7	288,6 ±214,9	0,39
IL-18 (pg/ml)	227,1 ± 104,9	183,4 ± 46,4	0,25
IL-18BPa (pg/ml)	11511,6 ± 3511	10513,8 ± 1793,6	0,58
IL-18 free (pg/ml)	135,5 ± 53,3	115,4 ± 29,2	0,18

Table 3

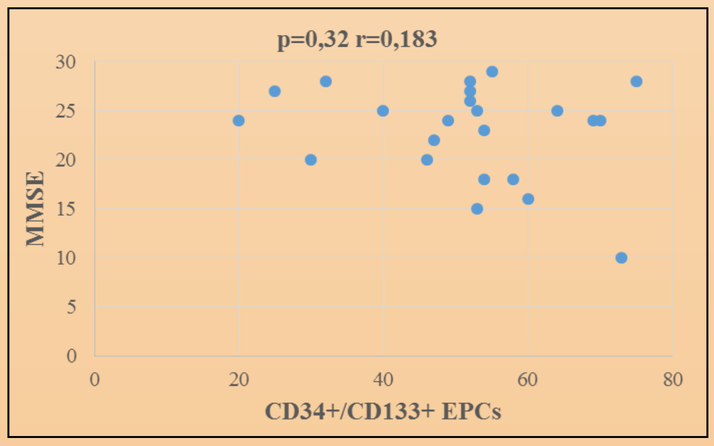


Figure 4

Conclusions:

- Our results confirm that TCD is a valuable method to study the hemodynamic changes in patients with AD, correlating with the severity of cognitive impairment;
- Impaired cerebral blood flow and alteration of cerebrovascular reactivity in AD may reflect increased arteriolar wall stiffness attributable to intrinsic anatomical changes [2];
- Evaluation of cerebral microvessel function may give us useful information for the identification of AD patients who are at significant risk of a rapid and pronounced progression of cognitive impairment [3];
- Further researches are needed to fully establish whether altered cerebral hemodynamics may be considered an independent factor in predicting cognitive decline or an effect of pathologic processes involved in AD [4] ;
- Our results provided evidence that patients with AD have reduced circulating EPCs, suggesting that an abnormal capacity to regenerate endothelium is associated with AD [1] ;
- According to recent literature, the absence of a significant correlation with cognitive decline suggests that CD34+ CD133+ EPCs as an endothelial biomarker is not valuable for the diagnosis and evaluation of cognitive evolution in AD [5] .

Limitations of the study:

- Small sample of AD subjects and healthy control subjects;
- TCD study is highly operator dependent, hampered by the 10 to 15% rate of inadequate acoustic windows and TCD BHI technique needs full cooperation from the subjects;
- Vascular endothelial biomarkers, as EPCs, may be too sensitive and not specific enough in older population.

References:

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