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Research Article

Early Diagnosis of Alzheimer's Disease by a Synergistic Evaluation of Free Copper Ion in the Serum and SPECT Analysis

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Abstract

In this report, eight patients have been analyzed in order to demonstrate how a combination of two *in vitro* and *in vivo* assays might allow an early diagnosis of Alzheimer's disease (AD). For all these subjects, the measurements of free copper ion in the serum by C4D test and brain perfusion by SPECT have been reported. The MRI did not evidence anomalies while a free copper value > 2.3 μM combined with a hypoperfusion in target areas led to the identification of AD.

Keywords: Alzheimer's disease; Early diagnosis; Free copper ion; SPECT; Biomarkers; C4D test

Introduction

Alzheimer's disease (AD) and Vascular Dementia (VD) are the most common forms of dementia among the aging population. AD diagnosis is corroborated by neuropathological features such as senile plaques and neurofibrillary tangles, structural imaging, cerebrospinal fluid biomarkers, and amyloid PET imaging; VD diagnosis depends predominantly on the structural MRI findings and it is really difficult to discriminate VD from AD. Normally VD and AD in their first step are diagnosed as Mild Cognitive Impairment (MCI) and elements to differentiate VD from AD have not been already disclosed.

To date, the diagnosis of these pathologies is still late and there is an urgent need to identify biomarkers involved in an early stage of AD and VD and a new strategy to early diagnose these disorders.

Aim of this study is to demonstrate, through eight case reports, how a combination of two technologies, *in vivo* and *in vitro*, can be able to early diagnose AD and can be a useful tool for differentiating VD from AD.

Circulating biomarker and SPECT studies

Here we present a combined strategy by an *in vitro* assay, C4D test, and an *in vivo* analysis such as SPECT.

C4D test is a new assay that has been carried out and patented to measure the level of free copper in the serum of subjects [1,2]. Copper (Cu^{++}) is an essential cofactor of several proteins and enzymes and is involved as catalyst in redox reactions in many pathways such as cellular growth and metabolism, energy generation, oxygen transportation, hematopoiesis and signal transduction [3]. Copper is generally circulating bound to specific proteins such as ceruloplasmin (for 85-95%) and the amount not bound called '*free copper*' or labile copper can become dangerous if exceeds the physiological level. The free copper represents the 5-15% of total serum copper and it is released by the liver into general circulation. The toxicity of free copper is widely reported in the pathogenesis of some diseases such as Wilson Disease (WD) a rare autosomal recessive disorder [4], Alzheimer's disease (AD) [3,5] and Parkinson's disease (PD) [6]. It has been postulated that β-amyloid plaques are able to entrap metals within the plaques. Therefore, since high level of free copper ion in the serum correlates with the possibility to develop AD early stage, this ion is a useful circulating biomarker to early diagnose the disorder. Therefore, test C4D is useful to directly measure the free copper.

SPECT analysis is a technique useful to identify regional hypoperfusion in cerebrovascular disorder and neurodegenerative diseases. Indeed, since brain perfusion SPECT allows to estimate the cerebral blood flow, this technique can identify vascular etiologies and abnormal brain function in specific area of grey matter [7]. Moreover, as brain functional impairment usually precedes structural modification, SPECT identifies cerebral abnormalities before imaging techniques such as magnetic resonance imaging (MRI) or computed tomography (CT). With respect to PET technique, SPECT displays a higher temporal resolution and it is easier to be performed. Moreover, SPECT can be useful to confirm the diagnosis when structural neuroimaging findings are not able to definitively explain neurologic symptoms and it also can be used to characterize brain disorders. The radiotracer used in brain SPECT analysis is the ^{99m}Tc-hexamethyl propylene amine oxime (HMPAO). The advantage of this radiotracer is that it reaches the peak brain activity within 2 minutes after injection and since there is no redistribution, the radiotracer uptake remains unchanged for 2 h. Therefore, HMPAO can be injected into the patient also out of Nuclear Medicine Facility and the images can be acquired later. This is an advantage with respect to PET and MRI analyses [8]. HMPAO allows the clinical identification of the hypoperfused brain areas. The combined information from SPECT imaging and copper value by C4D test can lead to an early diagnosis of AD and also to discriminate AD from VD in their onset.

Materials and Methods

Eight patients have been recruited in accordance to the Declaration of Helsinki and firstly studied by MRI. All these patients referred amnesia episodes but MRI did not show clinical evidences probably

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Table 1: Psychological, *in vivo*, and *in vitro* evaluations of selected patients.

Patient	MMSE	NMRI	Hypoperfused area	C4D, μM	Diagnosis
1	24,7	nn	post-central bilateral	2.3	AD
2	25,4	nn	parieto-occipital and occipital	2.1	AD+VD
3	27,75	nn	superior parietal and post-central gyrus	2.2	AD+VD
4	NT	athropy	frontal lobes and superior and medial-brain temporal gyrus	2,0	VD
5	30	NT	temporal	2.5	AD
6	22,4	NT	temporal	2.3	AD
7	22,7	NT	temporal	2.6	AD
8	27,3	NT	frontal lobes and superior and medial-brain temporal gyrus	2.1	VD

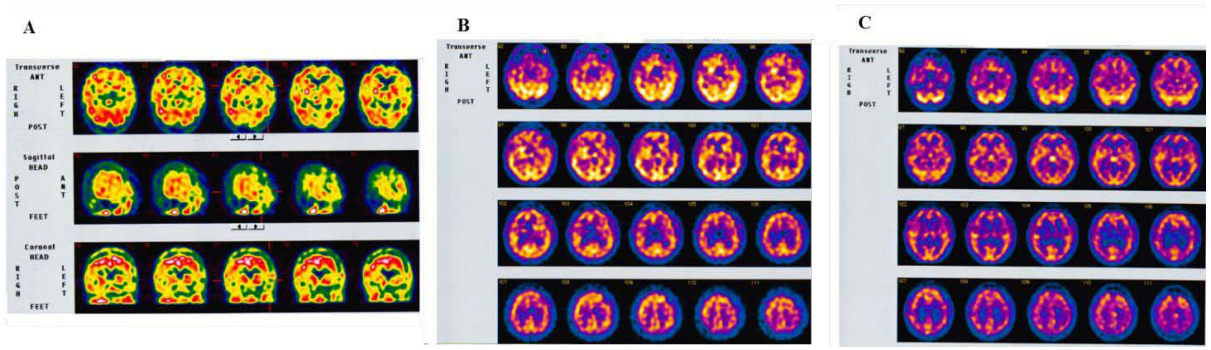

Figure 1: SPECT scan of patients **7** (**A**), **2** (**B**) and **3** (**C**).

because all the patients were in an early stage of the disorders. Thus, the same eight patients entered living lab project (code number: 5BQM3Y5) funded by Apulia Region and they were inserted into the following schedule:

- MMSE examination (Mini-Mental State Examination);
- Measurement of the free copper ion level in the serum by C4D test;
- Brain SPECT by HMPAO perfusion.

Patients displaying free copper ion concentration > 2 μM are considered positive to C4D test, since the cut off from several studies is 1.6-1.9 μM, while patients showing a value < 2 μM were estimated negative. All free copper concentrations are reported in Table 1 together with other parameters such as MMSE (Mini-Mental State Examination) that is the mostly used test by clinicians to diagnose dementia and its progression and severity. ADL and IADL are other tools useful to refer to daily self care activities of subjects (see supplementary materials). MMSE range is from 0 to 30; a score ranging from 26 to 30 is indicative of cognitive normality; a score ranging from 18 to 24 indicates a moderate to mild impairment while a score of 25 is borderline. A severe impairment of cognitive skills is represented by a score < 18 [9]. For all patients, MMSE, ADL and IADL did not identify a significant neuropathological condition and these values are available in (supplementary data).

Results and Discussion

Patient **1** shows, by SPECT analysis, a significant hypoperfusion in brain post-central bilateral areas and free copper ion concentration was 2.3 μM. Patient **2** shows a decreased perfusion in the parieto-occipital and occipital areas and a borderline value of free copper ion (2.1 μM) was found. Patient **3** displays a decreased perfusion in the superior parietal cortex and in the post-central gyrus with free copper value of 2.2 μM. Patients **4** and **8** show a high flow reduction in both frontal lobes and in the superior and medial-brain temporal gyrus and a free copper value of 2 μM. The SPECT imaging for patients **5** and **7** displayed hypoperfusion in temporal areas of the brain and the highest values of free copper ion in the serum (2.5 μM for patient **5** and 2.6 μM for patient **7**). Patient **6** displays an hypoperfusion widely diffused in temporal brain areas and a free copper ion of 2.3 μM.

In Figure 1, SPECT scans identifying AD (A) and a mixed AD/VD condition (B,C) are reported. For each scan, trasverse, coronal and sagittal reconstruction are depicted. In Figure 1, A section, SPECT scans show hypoperfusion in the anterior third superior and medial temporal gyri; trasverse scans display a decreased flow in bilateral and temporal parietal areas (left) while coronal scans show hypoperfused temporal area (left). In Figure 1, B section, SPECT scans show hypoperfusion areas in both hemispheres; transverse scans display a decreased flow in the postcentral (left), parietal-occipital and occipital while coronal scan a reduction in the post-central (letf), supra marginal and temporal areas (left). In Figure 1, C section reports hypoperfused areas in the parietal superior cortex and in the post-central gyrus. Transverse scans do not display significative differences between the two hemispheres while coronal scans show a decreased flow in the pre and post-central areas (left).

The patients analyzed by MRI (data not shown) did not display significant evidences of neurological features due to neurodegeneration as demonstrated by a value of free copper ion in the serum pf patients around 2.3 μM and a brain SPECT scan.

Therefore, these results demonstrate that the combination of an *in vitro* test (measurement of free copper ion in the serum) and *in vivo* analyses (SPECT) could give a great opportunity to discriminate between AD and VD in subject where MRI is unable to detect difference in terms of volume of the target area involved in AD.

In conclusion, a free copper ion concentration could be a discriminative factor for detecting AD in its early step in association with SPECT analysis whereas VD displays a widely hypoperfusion and in the meantime it seems to be less sensitive than AD to ion copper increase.

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References

1. Patent WO2014/181215 A2.
2. Patent WO2014/071973 A1.
3. Pal A, Siotto M, Prasad R, Squitti R. Towards a unified vision of copper involvement in Alzheimer's disease: a review connecting basic, experimental, and clinical research. J Alzheimers Dis. 2015; 44: 343-354.
4. Bandmann O, Weiss KH, Kaler SG. Wilson's disease and other neurological copper disorders. Lancet Neurol. 2015; 14: 103-113.
5. Squitti R. Copper subtype of Alzheimer's disease (AD): meta-analyses, genetic studies and predictive value of non-ceruloplasmim copper in mild cognitive impairment conversion to full AD. J Trace Elem Med Biol. 2014; 28: 482-485.
6. Dusek P, Roos PM, Litwin T, Schneider SA, Flaten TP, Aaseth J. The neurotoxicity of iron, copper and manganese in Parkinson's and Wilson's diseases. J Trace Elem Med Biol. 2015; 31: 193-203.
7. Catafau AM. Brain SPECT in clinical practice. Part I: perfusion. J Nucl Med. 2001; 42: 259-271.
8. Elman S, Lewis DH. Principles of Brain Perfusion SPECT. PET and SPECT of Neurobiological Systems. 2014; 125-147.
9. Folstein MF, Folstein SE, McHugh PR. Mini-mental state. A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975; 12: 189–198.

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