Review

Circulating biomarkers of protein oxidation for Alzheimer disease: Expectations within limits

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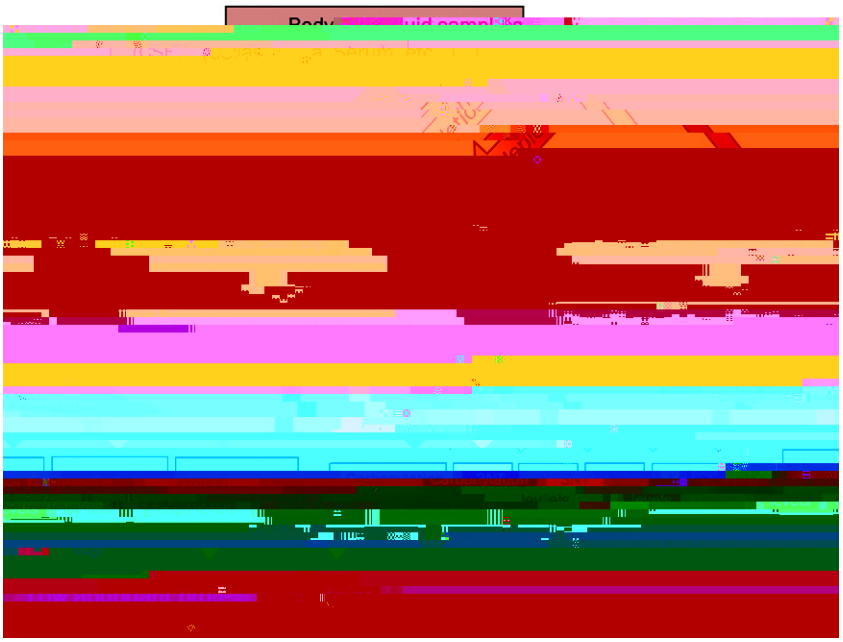
Alzheimer disease (AD), the most common dementing disorder, is a multifactorial disease with complex etiology. Among different hypotheses proposed for AD one of the most corroborated is the "oxidative stress hypothesis". Although recent studies extensively demonstrated the specific oxidative modification of selected

substantial cell loss. Thus, therapies are initiated only after diagnosis; their modest benefit, in part, may be explained by the fact that some irreversible brain damage already has occurred by the time dementia is recognized [11].

Current AD biomarkers are two characteristic proteins, β-amyloid and tau protein, which are assayed in cerebrospinal fl

are then combined, fractionated by nanoLC to generate reporter ions that show up at low mass-to-charge (m/z) values in the analysis by tandem mass spectrometry. Database searching, for the fragmentation data of the peptides, results in the identifi



Scheme 1. Representative scheme for analysis of biological fluids. Non-depleted samples are analyzed for total oxidation markers (protein carbonyls, 3NT, HNE, APPO and free thiol groups). Samples undergoing high abundant protein depletion are analyzed for potential biomarker discovery by redox proteomics approach (protein carbonyls, 3NT and HNE).

oxidative damage represent a satisfactory in vivo biomarkers in an experimental model of oxidative injury to rat liver [70]. Of these, MDA, F2-IsoPs and 8-OHdG were acceptable quantitative in vivo biomarkers of oxidative damage.

In the past years the analysis of body fluid composition by proteomics and redox proteomics has been performed by several groups [71–79] However, as previously stated, the discovery and validation of protein biomarkers from body fluids is disadvantaged by the enormous difference in quantity between the high and the low abundant proteins with a dynamic range of about 12 orders of magnitude. Serum albumin, the most abundant circulating protein, represents about two-thirds of the entire protein content of plasma, and with IgG and few other high-abundance proteins constitute greater than 90% of total protein mass, interfering with the detection of lower-abundance proteins that might represent the biologically interesting population [80,81]. Depletion of abundant plasma proteins prior to analysis is a common strategy for increasing the number of proteins detectable with mass spectrometry [82–84]. There are several methods for removing proteins based on their biochemical and biophysical features, such as molecular weight, hydrophobicity and isoelectric point [85–87]. Among these techniques, the most common ones rely on antibody-based retention of a chosen set of the most abundant proteins [88]. Commercial removal kits are available, and the clearing of high-abundant proteins ranging from 2 to 20 allowing the depletion of about 85–95% of the total protein content depending by the number of high-abundant protein considered. Several comparative studies concerning the various depletion approaches show improved quality of proteomics separation and quantitation applied to biological fluids after removal of high-abundant proteins [82,83,89]. Fig. 1 shows a 2D gel of human plasma after depletion of albumin and IgG (Panel a) and depletion of 14 high abundant protein (Agilent) (Panel b) obtained in our laboratory. By following this approach, we were able to successfully identify an increased number of proteins after depletion of albumin and IgG in amniotic fluid from women carrying Down syndrome fetus compared with healthy controls [90]. However the downsides of any depletion step is represented by the probability of removing some low-abundance proteins along with the abundant species as well as removing proteins non-covalently bound to albumin.

4. Biomarkers of protein oxidation: from the brain to the periphery

Several studies highlight the involvement of OS in the pathogenesis of AD, by identifying specific proteins oxidatively modified in different brain regions [91]. A significant increase in protein carbonyls

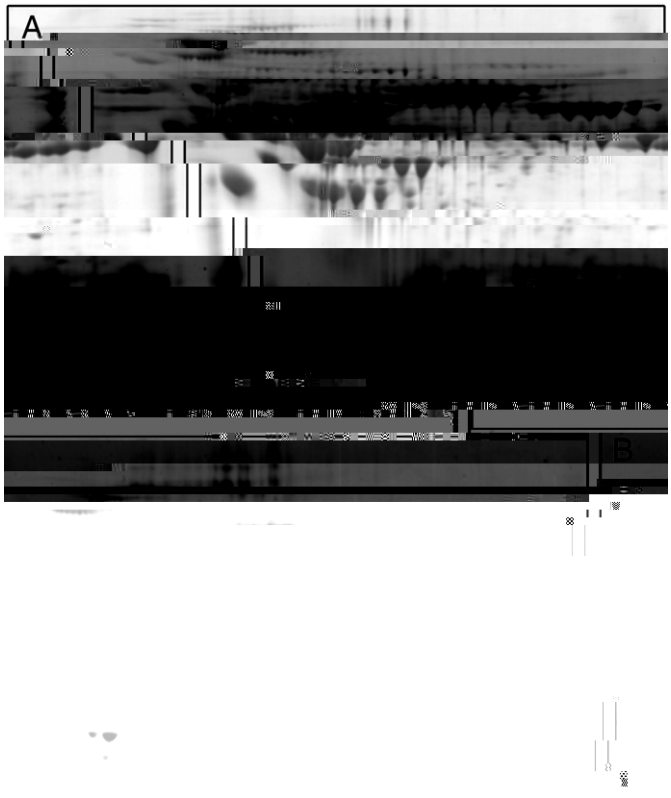


Fig. 1. Two-dimensional electrophoresis gel analysis of human plasma samples depleted with: A ProteoPrep blue Albumin & IgG depletion kit (Sigma-Aldrich); B Human 14 Multiple Affinity Removal System Spin Cartridge (Agilent).

5.1. Protein oxidation in CSF

Considering that AD is a “brain” disease, CSF is the most proximal to CNS and logical source to find any biomarker directly related to the pathology. Therefore, composition of CSF partially reflects cerebral metabolic changes and enables screening of ongoing pathophysiological process in brain [77]. The first report on protein oxidation in CSF samples was from Tohgi et al. [29] who demonstrated that 3-nitrotyrosine moderately but significantly increased with advancing age, and showed a remarkable increase in patients with AD. As the free tyrosine concentration did not decrease, the increase in 3-nitrotyrosine with age or associated with AD did not appear to be directly related to an increase in free-nitrated tyrosines. Rather, the increased 3-nitrotyrosine was likely due to an increase in nitrated tyrosines in proteins or increased degradation of 3-nitrotyrosine-

endogenous antioxidant enzymes such as glutathione peroxidase, glutathione reductase and superoxide dismutase were also evaluated. The total antioxidant plasmatic status of the patients with AD both in light-moderate phase and in advanced phase was lower than in the controls. No signifi

possibility. CSF biomarkers combined with MRI measurements of medial temporal lobe atrophy have been reported to increase the accuracy of AD diagnosis [11]. In fact only a combination of different markers could, most likely, offer a certain diagnosis and be able to capture all aspects of the disease.

Our laboratory is presently performing the analysis of both CSF and plasma samples from MCI and AD living patients, by proteomics and redox proteomics approach coupled with the use of prefractionation methods, in the effort to find potential markers of peripheral damage, which could contribute to a more accurate diagnosis and prognosis.

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