Molecular Neurobiology (2019) 56:5792–5798
https://doi.org/10.1007/s12035-019-1501-x

SNAP-25 in Serum Is Carried by Exosomes of Neuronal Origin and Is a Potential Biomarker of Alzheimer's Disease

Cristina Agliardi[1] · Franca R. Guerini[1] · Milena Zanzottera[1] · Anna Bianchi[1] · Raffaello Nemni[1,2] · Mario Clerici[1,2]

Received: 12 December 2018 / Accepted: 18 January 2019 / Published online: 24 January 2019

Abstract

A loss of synaptic density and connectivity is observed in multiple brain regions of Alzheimer's disease (AD) patients, resulting in a reduced expression of synaptic proteins such as SNAP-25 (synaptosomal-associated-protein-25). SNAP-25 alterations thus could be an index of the degree of synaptic degeneration in the central nervous system (CNS). We isolated from serum of both AD patients and healthy controls (HC) a population of neuron-derived exosomes (NDEs) and measured the concentrations of SNAP-25 contained in such NDEs. The levels of SNAP-25 carried by NDEs were reduced in AD patients (mean 459.05 ng/ml, SD 146.35 ng/ml) compared to HC (mean 686.42 ng/ml, SD 204.08 ng/ml) ($p < 0.001$). As a further confirmation of these results, ROC (receiver operating characteristic) analyses indicated that the level of SNAP-25 carried by NDEs has the power to discriminate between AD and HC (AUC = 0.826, sensitivity = 87.5%, specificity = 70.6%, $p < 0.0001$, cut-off value 587.07 ng/ml). Notably, a correlation between the levels of SNAP-25 carried by NDEs and levels and cognitive status measured by MMSE score ($r = 0.465$, 95% CI 0.11 to 0.714, $p = 0.01$) was detected. This is the first report of SNAP-25 measurement in serum. These data suggest that NDE-carried SNAP-25 could be an effective and accessible biomarker that reflects synapses integrity in the brain.

Keywords Alzheimer's disease · SNAP-25 · Exosomes · Biomarker · Peripheral · Synaptic proteins

Introduction

Alzheimer's disease (AD) is the most common type of dementia and accounts for 50–70% of prevalent neurodegenerative dementia cases [1]. AD is characterised by the formation of β-amyloid (Aβ) plaques and neurofibrillary tangles in the brain parenchyma; this results in the loss of synapses and neurons which is observed in the early phases of AD and throughout the disease progression [2]. Notably, animal models of the early phases of AD have demonstrated that soluble Aβ oligomers cause loss of presynaptic proteins and synaptic dysfunction [3, 4]. As a consequence, the first and most evident clinical symptom of AD is a progressive loss of cognitive abilities with short-term memory impairment.

In the central nervous system (CNS), SNAP-25 (synaptosomal-associated protein-25), an essential component of the SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors) complex, is a marker of functional synapses. SNARE complex proteins mediate synaptic communication by initiating the docking of synaptic vesicles to the presynaptic membrane in neurons [5]. The interaction between SNAP-25, syntaxin 1, and synaptobrevin or VAMP (vescicle-associated membrane protein) allows neuronal exocytosis. The observation that a reduction of synaptic proteins could be a biomarker, indicating the degree of synaptic degeneration [6, 7], has prompted interest in detecting synaptic proteins in human biological fluid samples. SNAP-25 was first identified and measured in CSF in 1998 [8]. Subsequent analysed showed that SNAP-25 concentrations in CSF are significantly correlated with Parkinson's disease (PD) in a disease specific manner and with cognitive and motor symptom severity [9]. Other results suggested that measurement of SNAP-25 in CSF from AD patients using affinity purification and quantitative mass spectrometry could

✉ Cristina Agliardi
cagliardi@dongnocchi.it

[1] Laboratory of Molecular Medicine and Biotechnology, IRCCS Fondazione Don Carlo Gnocchi, Via Capecelatro 66, 20148 Milan, Italy

[2] Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy

be a useful CSF biomarker in the differential diagnosis of patients with AD and prodromal AD [7].

The early pathological impairments that characterize neurodegenerative diseases such as AD and PD are asymptomatic, and there is the urgent need of new biomarkers both for early identification and longitudinal follow-up of future cases [10, 11]. SNAP-25 demonstrated to be a potential good candidate biomarker of neurodegeneration, but CSF-based biomarkers are invasive and cannot be used on a large scale. Thus, we wondered if it was possible to detect and measure in peripheral blood SNAP-25 protein of neural origin. Our hypothesis was that SNAP-25 could be carried in peripheral blood by extracellular vesicles (EVs) and, in particular, by neuron-derived exosomes (NDEs).

Exosomes are nano-sized (30–100 nm diameter) spherical particles surrounded by a phospholipid bilayer and loaded with a variety of bioactive molecules as protein and nucleic acids from donor cells. They participate in intercellular communication and are found in biologic fluids such as blood, urine, saliva, seminal fluid, ascites, amniotic liquid, synovial fluid, breast milk and CSF [12]. Exosomes are released as a result of the fusion of the multivesicular bodies (MVBs) with the plasma membrane [13, 14]; their composition is a consequence of their formation as well as the mechanisms of their secretion. Accumulating evidence shows that exosomes, because of their size, can cross the blood–brain barrier (BBB) in both directions [15–17]. As an example, glioblastoma specific mRNA was found in sera of patients and was suggested to be a biomarker in this disease. [18, 19].

We verified whether SNAP-25-containing NDEs could be identified in peripheral blood and, if that was the case, whether such SNAP-25-contaning NDEs could be used as a biomarker of synaptic loss in central nervous system (CNS) and, as a consequence, of AD.

Methods

Sample Collection and Study Population

Twenty-five patients with a diagnosis of AD were enrolled at the Neurologic Rehabilitation Unit of the IRCCS Don Carlo Gnocchi Foundation in Milano. All AD patients underwent complete medical and neurological evaluation, laboratory analysis, CT (computerized tomography) scan or MRI (magnetic resonance imaging), and other investigations (e.g., EEG (electroencephalography), SPECT (single-photon emission computed tomography) scan, CSF examination) to exclude reversible causes of dementia. The clinical diagnosis of AD was performed according to the NINCDS-ADRDA work group criteria [20] and the DSM-IV-TR [21]. Neuropsychological evaluation and psychometric assessment were performed with a Neuropsychological Battery that included the following: Mini Mental State Examination (MMSE), Digit Span Forward and Backward, Logical Memory and Paired Associated Words Tests, Token Test, supra Span Corsi Block Tapping Test, Verbal Fluency Tasks, Raven Colored Matrices, the Rey Complex Figure and Clinical Dementia Rating Scale (CDR) [22, 23]; the study conformed to the ethical principles of the Helsinki Declaration.

Seventeen healthy controls (HC) were recruited among relatives of AD patients; they underwent MRI (magnetic resonance imaging) scans that excluded the presence of any neurodegenerative pathology and in particular AD. Subject characteristics are summarized in Table 1.

The Ethical Committee of the Don C. Gnocchi Foundation IRCCS approved the study (Prot. N°10/2018/CE_FdG/SA); all the participants when possible, or patients' legal guardians when it was not, gave informed consent.

Ten millilitres of whole blood were collected from each subject participating at the study following standard procedures using a serum separator tube (SST II Advance, BD Vacutainer®). Samples were allowed to clot for 1 h at room temperature and then were centrifuged for 10 min at 3500 g. After centrifugation samples were aliquoted and stored at − 80 °C until use.

Total Exosomes and NDE Isolation from Peripheral Blood

Total exosomes were isolated from frozen sera samples with ExoQuick® (System Biosciences, LCC, USA) according to manufacturer's instructions and as previously described [24].

Table 1 Characteristics of patients and control subjects

			Age (years)	MMSE score (corrected)	Disease duration
Diagnosis	Total (*n*)	M/F (*n*)	Mean ± SEM (SD)	Mean ± SEM (SD)	Mean ± SEM
AD	24	8/16	77.67 ± 1.40 (6.84)	21.91 ± 0.91* (4.09)	4.50 ± 0.87 (2.87)
HC	17	4/13	76.47 ± 1.49 (6.16)	28.73 ± 0.43* (1.20)	NA

The significance of difference between cognitive state (MMSE) values (*$p < 0.0001$) was calculated by an unpaired Student's *t* test for AD vs HC

M male, *F* female, *SEM* standard error of the mean, *SD* standard deviation, *NA* not applicable

NDEs were isolated as previously described [25] starting from total exosomes isolation with ExoQuick® followed by immunoprecipitation with biotinylated L1CAM (CD171) antibody. L1CAM is a neuronal surface marker; it was selected as target for immunoprecipitation due to its high and relatively specific expression in neural tissue and because previous research demonstrated its high expression on exosomes derived from cultured neurons [26].

Protein Isolation and Western Blot Analysis

Proteins were extracted from (1) total sera samples, (2) total exosomes extracted from sera, (3) total exosomes extraction supernatants, (4) NDEs and (5) NDEs extraction supernatants samples with M-PER™ reagent (Thermo Fisher Scientific, USA) according to manufacturer's instructions, adding a cocktail of protease and phosphatase inhibitors. Protein concentrations were determined with a Qubit™ fluorometer (Thermo Fisher Scientific, USA). Samples were stored at − 80 °C until use. Proteins extracted from NDEs were separated by SDS-PAGE using Mini PROTEAN® TGX™ precast gels (Bio-Rad, USA) and automatically transferred to PVDF (polyvinylidene difluoride) membranes with Trans-Blot® instrument (Bio-Rad, USA). The membranes were blocked (5% non-fat dry milk in Tris-buffered saline containing 0.1% Tween 20), then incubated with primary mouse monoclonal antibodies against SNAP-25 (1:1000; SNAP-25 monoclonal antibody (SP12), Invitrogen), TSG101 (1:1000; TSG101 monoclonal antibody (4A10), Thermo Fisher Scientific, USA), Alix (1:1000; Alix monoclonal antibody (3A9), Thermo Fisher Scientific, USA) and CD171 (1:1000; CD171 Monoclonal Antibody (eBio5G3 (5G3), eBioscience™) at room temperature for 2 h or 4 °C overnight and followed by incubation with a secondary anti mouse HRP-conjugated antibody for 1 h 45 min (1:20,000) at room temperature. The membranes were developed with Clarity Max Western ECL Substrate (Bio-Rad, USA) and imaged by ChemiDoc™ Gel Imaging System (Bio-Rad, USA).

Measurement of SNAP-25 Carried by NDEs by ELISA

Protein extracts from NDEs were further analysed using Human SNAP-25 sandwich enzyme-linked immunosorbent assay (ELISA) kits (LS-F17747, LifeSpan BioSciences, Inc., USA). A 1:10 dilution of NDE protein extracts obtained from an initial volume of 500 µl of serum was used. Samples were incubated in the plates coated with SNAP-25 antibody for 2 h at 37 °C. A biotin-conjugated detection antibody was then added, which binds to the captured antigen. Unbound detection antibody was washed away. An avidin-horseradish peroxidase (HRP) conjugate was then added which binds to the biotin. After washing, a TMB substrate was then added which reacts with the HRP enzyme resulting in colour development. A sulphuric acid stop solution was then used to terminate colour development reaction, and then, the optical density (OD) of the well was measured at a wavelength of 450 nm via an absorbance microplate reader (SunRise™, TECAN, Switzerland). The OD of the samples was compared with the OD standard curve generated using known antigen concentrations.

Statistical Analysis

Shapiro–Wilks tests showed that SNAP-25 concentration data were normally distributed in both AD and HC groups. A one-way ANOVA test was performed to compare NDEs' SNAP-25 levels between groups and logistic regression was applied considering the condition of illness as dependent variable and NDEs' SNAP-25 concentrations and gender as covariates. The discriminatory ability of NDEs' SNAP-25 concentration was presented by using receiver operating characteristic (ROC) analyses with calculation of the area under the curve (AUC), sensitivity and specificity as well as 95% confidence interval (CI). The relationship between MMSE cognitive scores and SNAP-25 levels was determined using Pearson correlation coefficient (r) with 95% CI and linear regression analyses. In all cases, differences were considered statistically significant when $p \leq 0.05$. MedCalc® software (MedCalc®, 14.10.2, Belgium) was used for all statistical analyses.

Results

Peripheral SNAP-25 Is Carried by NDEs

Serum of AD patients and HC was analysed to verify whether it would have been possible to detect the presence of SNAP-25. Results showed that this was indeed the case as SNAP-25 could be identified in sera of both groups of individuals (Fig. 1a).

The origin of the SNAP-25 detected in sera was analysed next. In particular, we examined the possibility that SNAP-25 could be carried form the CNS to peripheral blood by exosomes that originated in neural cells. To this end, the presence of SNAP-25 was analysed in total exosomes proteins extracts, in proteins extracts from L1CAM-expressing NDEs and in protein extract of total exosomes depleted of NDEs. Results clearly showed that SNAP-25 was detectable in total exosomes and in NDEs but not in total exosomes depleted of NDEs either in AD patients or in HC (Fig. 1b), clearly indicating that SNAP-25 in serum is carried by NDEs.

Peripheral SNAP-25 Levels Are Lower in AD Patients Compared to HC

Once the origin of SNAP-25 in sera was understood, SNAP-25 concentration in NDEs was measured by ELISA in 24 AD

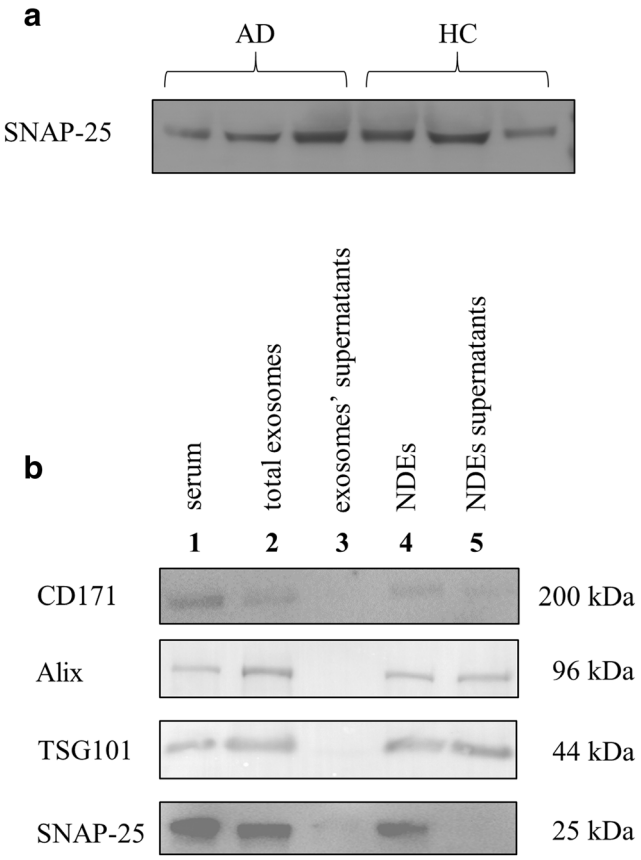

Fig. 1 Western blots. **a** SNAP-25 in sera of AD patients and HC. Representative result obtained in three AD patients and three HC is shown. **b** Lane 1: protein extracts from serum; lane 2: protein extracts from total exosomes; lane 3: protein extracts from total exosomes isolation supernatant; lane 4: protein extracts from exosomes of neuronal origin (NDEs); lane 5: protein extracts from NDE's isolation supernatant. CD171 was detected in serum, total exosomes, NDEs and slightly in NDEs supernatants; Alix and TSG101 were detected in serum, total exosomes, NDEs and in NDEs supernatants; SNAP-25 was visible in serum, total exosomes and in NDEs

patients and 17 HC. Results showed that SNAP-25 concentrations in NDEs resulted normally distributed in both AD patients and HC and were significantly reduced in AD patients (mean 459.05 ng/ml; SD 146.35 ng/ml; range 165.66–729.82 ng/ml) compared to HC (mean 686.42 ng/ml; SD 204.08 ng/ml; range 337.16–1135.48 ng/ml) ($p < 0.001$). These results are shown in Fig. 2.

Notably, the NDE concentration of SNAP-25 did not correlate with age ($p = 0.124$, Pearson correlation coefficient $r = -0.244$ (95% CI −0.514–0.068) even when possible correlations were sought in the two analysed groups separately (data not shown). A logistic regression was performed next considering the presence/absence of a diagnosis of AD as the dependent variable and NDEs' SNAP-25 concentration and gender as covariates. The model confirmed the previous results. Thus, a correlation between NDEs' SNAP-25 concentration and AD was observed ($p = 0.003$), but no correlations could be

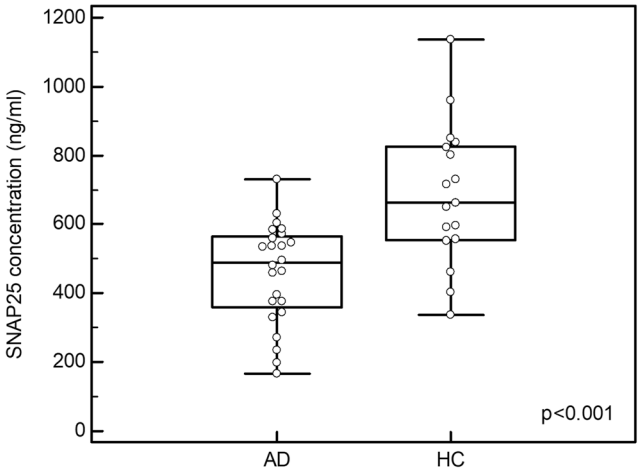

Fig. 2 Distribution of SNAP-25 protein levels in neuronal-derived exosomes extracted from serum in Alzheimer's disease (AD) patients and healthy controls (HC). Each point in a frame depicts the value for a single subject (24 AD patients and 17 HC subjects); the horizontal line represents the median value

detected between NDEs' SNAP-25 concentration and gender ($p = 0.468$) (overall model fit $\chi^2 = 15{,}783$; $p = 0.0004$).

A ROC analysis was finally performed in order to assess the diagnostic value of NDEs' SNAP-25 concentration; the performance was found to be good (AUC = 0.826; sensitivity = 87.5%, specificity = 70.6%; $p < 0.0001$) (Fig. 2). ROC analyses identified a cut-off value for SNAP-25 of 587.07 ng/ml (Fig. 3).

Peripheral SNAP-25 Levels Correlate with MMSE

Age and education MMSE-corrected scores were available for 20 AD patients and 8 HC. As per definition, mean MMSE score in AD patients was significantly lower than that observed in HC ($p < 0.0001$) (Table 1). The existence of possible correlations between NDEs' SNAP-25 concentration and MMSE cognitive scores was analysed next. Results showed that MMSE scores are indeed positively correlated with NDEs' SNAP-25 concentration in the overall group of AD and HC individuals analysed ($r = 0.465$, 95% CI 0.11 to 0.714, $p = 0.01$, Pearson correlation) (Fig. 4). The correlation between SNAP-25 levels in NDEs from AD patients and disease duration, instead, failed to reach statistical significance (data not shown).

Discussion

A loss of synaptic density and of synaptic connectivity is consistently observed in multiple brain regions of AD patients in the early phases of AD and throughout disease progression [2]; this results in alterations of presynaptic terminal proteins, such as SNAP-25 [27]. With first experiments, we were able

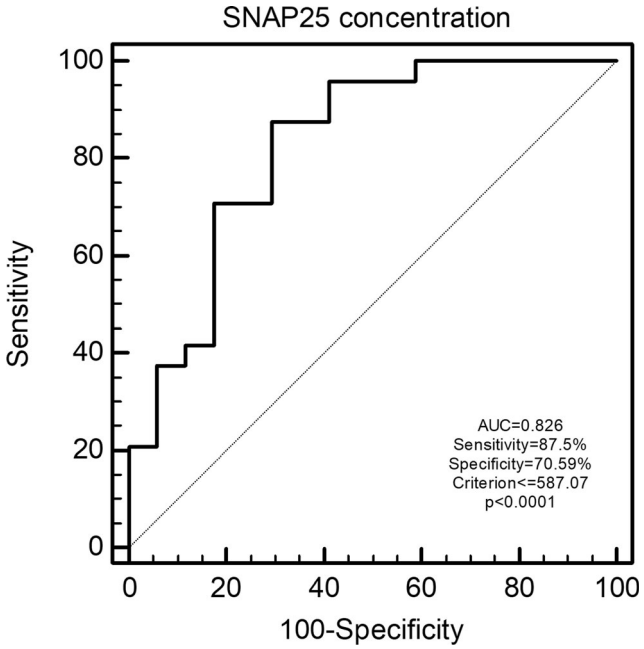

Fig. 3 ROC analysis of SNAP-25 concentrations in serum exosomes of neural origin as candidate biomarker for Alzheimer's disease (AD). ROC receiver operating characteristic, AUC area under curve

to detect SNAP-25 in serum of both AD patients and HC subjects. Because SNAP-25 is normally not expressed in blood cells, our hypothesis was that peripheral SNAP-25 could derive from exosomes originated in CNS that cross the brain–blood barrier (BBB) to end up in peripheral blood. There is in fact accumulating evidence showing that exosomes can cross the BBB in both directions [15, 16, 18]. Thus, (1) astrocyte-derived exosomes were isolated from peripheral blood [28] and (2) brain exosomes were shown to be present in the peripheral blood of genetically modified animals [25].

Results herein confirm that SNAP-25 can be detected in sera and indicate that SNAP-25 is transported in serum inside

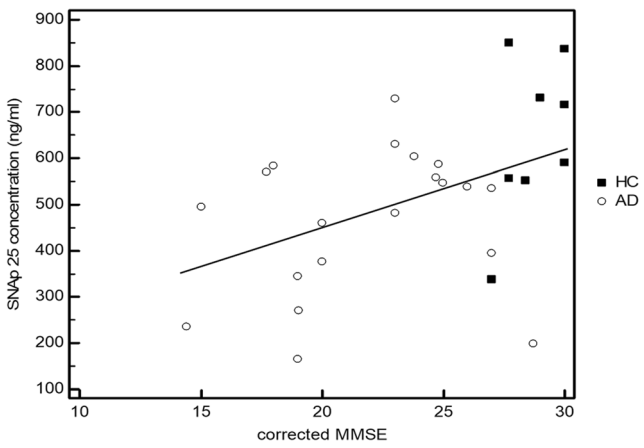

Fig. 4 Scatter diagram of the significant correlation between SNAP-25 carried by peripheral exosomes of neural origin and age- and education-corrected MMSE scores ($r = 0.465$, 95% CI 0.11 to 0.714, $p = 0.01$, Pearson correlation)

exosomes of neuronal origin. Notably, experiments conducted with protein extracts from such exosomes showed that mean SNAP-25 levels were lower in AD compared to HC. Moreover, SNAP-25 concentration correlated with cognitive impairment, as measured by MMSE score, suggesting that measurement of SNAP-25 serum concentration could be an easy accessible marker to follow progression of cognitive decline in AD. Notably, whereas serum concentration of other synaptic proteins, including synaptotagmin, synaptopodin, synaptophysin, neurogranin and GAP43, had been shown to be reduced in NDEs of AD patients compared to HC [29], this is the first description of an alteration of SNAP-25, an essential component of the SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors) complex, in peripheral blood.

SNAP-25 was first described to be present in CSF by Thompson and collaborators [8]. Later, the group of Brinkmalm, using a sophisticated method that combines affinity purification and mass spectrometry, purified SNAP-25 fragments in CSF and observed that the concentration of such fragments was significantly increased in the CSF of AD patients compared to controls [7]. Even more recently, Öhrfelt et al. confirmed that higher levels of SNAP-25 are present in AD CSF [30]. Moreover, very recent results have also shown that (1) CSF SNAP-25 concentration and the SNAP-25/Aβ42 ratio are increased in the early clinical stage of AD, (2) SNAP-25 CSF concentration is significantly higher in AD patients who carry APOE ε4 compared to non-carriers and to individuals with a diagnosis of mild cognitive impairment (MCI) [31] and (3) SNAP-25 CSF concentration associates with MMSE and Aβ and tau CSF concentration [32]. Autopsy results, finally, showed that SNAP-25 protein is significantly reduced in brain samples of AD patients compared to controls [33–35].

Results herein, thus, are in accordance with those showing that the concentration of other synaptic proteins is reduced in sera of AD [29], as well as those deriving from autopsy analyses evidence, but are in contrast with data indicating that the CSF concentration of SNAP-25 is increased in AD. Notably, a similar dichotomy between sera and CSF is also present when other synaptic proteins, e.g. neurogranin, are measured. Thus, whereas the concentration of this protein is reduced in plasma NDEs of AD, its concentration is increased in CSF of the same patients compared to the values detected in controls [6, 36–38].

A possible way to explain these discrepancies is that, whereas the SNAP-25 detected in peripheral blood is contained in NDEs that generate from active synapses in the brain and are actively released by neurons, SNAP-25 in the CSF would result from the continuous leakage of protein from various brain areas into the brain interstitial fluid that clears into the CSF [39]. If this is the case, the measurement of NDE-contained SNAP-25 in serum is a direct reflection of the synaptic loss that characterizes the progression of AD.

Because SNAP-25 is expressed not only in brain tissues but also, for example, in pancreatic endocrine β-cells where it regulates insulin secretion [40], the measurement of SNAP-25 transported from CNS to blood by NDEs instead of measuring total SNAP-25 in the CSF is a way to have a mirror of what happens in the brain. It is also to be said that L1CAM, the marker used for NDEs enrichment, is highly expressed in neurons, but it is also expressed at low levels in other cell types: for this reason, there is the need to discover cellular surface biomarkers that are cell-specific in order to find unique signatures of peripheral exosomes subpopulations.

A limitation of this study is the relativity small sample size; it is our intent (1) to confirm the results in a larger cohort which could also include MCI (mild cognitive impairment) patients; (2) to test SNAP-25 concentrations in NDEs of patients with other non-AD dementias, including vascular dementia, in order to evaluate the specificity and prognostic value of NDEs' SNAP-25 in AD diagnosis; and (3) to test SNAP-25 levels in NDEs as AD biomarker in prospective longitudinal studies designed to delineate the clinical course of cognitively normal subjects with altered NDE profiles to test if it may possible to identify high-risk subjects early in the preclinical stage. If confirmed, these findings could be important for earlier diagnosis and also, for example, to assess the progression of the disease and to monitor drug effects.

In conclusion, we investigated a possible new strategy to study synaptic pathology in AD by measuring the presynaptic protein SNAP-25 levels in peripheral exosomes of neuronal origin. The technique can be extended both to other pathologies of CNS and also to evaluate other molecules.

AD, as other neurodegenerative diseases such as PD, has an insidious course with a long preclinical phase; the early pathological changes are often asymptomatic, and there is the urgent need to find valid biomarkers of pathology that should be non-invasive as CSF-based biomarkers and less expensive than MRI and PET.

Funding Information This work was supported by Italian Ministry of Health (Ricerca Corrente 2018).

Compliance with Ethical Standards

The study conformed to the ethical principles of the Helsinki Declaration. The Ethical Committee of the Don C. Gnocchi Foundation IRCCS approved the study (Prot. N°10/2018/CE_FdG/SA); all the participants when possible, or patients' legal guardians when it was not, gave informed consent.

References

1. Winblad B, Amouyel P, Andrieu S, Ballard C, Brayne C, Brodaty H, Cedazo-Minguez A, Dubois B et al (2016) Defeating Alzheimer's disease and other dementias: a priority for European science and society. Lancet Neurol 15(5):455–532. https://doi.org/10.1016/S1474-4422(16)00062-4
2. Masliah E, Mallory M, Alford M, DeTeresa R, Hansen LA, McKeel DW Jr, Morris JC (2001) Altered expression of synaptic proteins occurs early during progression of Alzheimer's disease. Neurology 56(1):127–129
3. Busche MA, Eichhoff G, Adelsberger H, Abramowski D, Wiederhold KH, Haass C, Staufenbiel M, Konnerth A et al (2008) Clusters of hyperactive neurons near amyloid plaques in a mouse model of Alzheimer's disease. Science 321(5896):1686–1689. https://doi.org/10.1126/science.1162844
4. Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, Brett FM, Farrell MA et al (2008) Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. Nat Med 14(8):837–842. https://doi.org/10.1038/nm1782
5. Jahn R (1999) Recycling of synaptic vesicle membrane within nerve terminals. Brain Res Bull 50(5–6):313–314
6. Kvartsberg H, Duits FH, Ingelsson M, Andreasen N, Öhrfelt A, Andersson K, Brinkmalm G, Lannfelt L et al (2015) Cerebrospinal fluid levels of the synaptic protein neurogranin correlates with cognitive decline in prodromal Alzheimer's disease. Alzheimers Dement 11(10):1180–1190. https://doi.org/10.1016/j.jalz.2014.10.009
7. Brinkmalm A, Brinkmalm G, Honer WG, Frölich L, Hausner L, Minthon L, Hansson O, Wallin A et al (2014) SNAP-25 is a promising novel cerebrospinal fluid biomarker for synapse degeneration in Alzheimer's disease. Mol Neurodegener 9:53. https://doi.org/10.1186/1750-1326-9-53
8. Thompson PM, Rosenberger C, Holt S et al (1998) Measuring synaptosomal associated protein-25 kDa in human cerebral spinal fluid. J Psychiatr Res 32(5):297–300
9. Bereczki E, Bogstedt A, Höglund K, Tsitsi P, Brodin L, Ballard C, Svenningsson P, Aarsland D (2017) Synaptic proteins in CSF relate to Parkinson's disease stage markers. NPJ Parkinsons Dis 3:7. https://doi.org/10.1038/s41531-017-0008-2
10. Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, Iwatsubo T, Jack CR Jr et al (2011) Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 7(3):280–292. https://doi.org/10.1016/j.jalz.2011.03.003
11. Lashley T, Schott JM, Weston P, Murray CE, Wellington H, Keshavan A, et al (2018) Molecular biomarkers of Alzheimer's disease: progress and prospects. Dis Model Mech 11(5). doi: https://doi.org/10.1242/dmm.031781.
12. Urbanelli L, Magini A, Buratta S, Brozzi A, Sagini K, Polchi A, Tancini B, Emiliani C (2013) Signaling pathways in exosomes biogenesis, secretion and fate. Genes (Basel) 4(2):152–170. https://doi.org/10.3390/genes4020152
13. Harding C, Heuser J, Stahl P (1983) Receptor-mediated endocytosis of transferrin and recycling of the transferrin receptor in rat reticulocytes. J Cell Biol 97(2):329–339
14. Bellingham SA, Guo BB, Coleman BM, Hill AF (2012) Exosomes: vehicles for the transfer of toxic proteins associated with neurodegenerative diseases? Front Physiol 3:124. https://doi.org/10.3389/fphys.2012.00124
15. Wiklander OP, Nordin JZ, O'Loughlin A, Gustafsson Y, Corso G, Mäger I et al (2015) Extracellular vesicle in vivo biodistribution is

determined by cell source, route of administration and targeting. J Extracell Vesicles 4:26316. https://doi.org/10.3402/jev.v4.26316
16. Bala S, Csak T, Momen-Heravi F, Lippai D, Kodys K, Catalano D, Satishchandran A, Ambros V et al (2015) Biodistribution and function of extracellular miRNA-155 in mice. Sci Rep 5:10721. https://doi.org/10.1038/srep10721
17. Chen CC, Liu L, Ma F, Wong CW, Guo XE, Chacko JV, Farhoodi HP, Zhang SX et al (2016) Elucidation of exosome migration across the blood-brain barrier model in vitro. Cell Mol Bioeng 9(4):509–529. https://doi.org/10.1007/s12195-016-0458-3
18. Noerholm M, Balaj L, Limperg T, Salehi A, Zhu LD, Hochberg FH, Breakefield XO, Carter BS et al (2012) RNA expression patterns in serum microvesicles from patients with glioblastoma multiforme and controls. BMC Cancer 12:22. https://doi.org/10.1186/1471-2407-12-22
19. Chen WW, Balaj L, Liau LM, Samuels ML, Kotsopoulos SK, Maguire CA, LoGuidice L, Soto H et al (2013) BEAMing and droplet digital PCR analysis of mutant IDH1 mRNA in glioma patient serum and cerebrospinal fluid extracellular vesicles. Mol Ther Nucleic Acids 2:e109. https://doi.org/10.1038/mtna.2013.28.
20. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM (1984) Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Service Task Force on Alzheimer's disease. Neurology 34(7):939–944. https://doi.org/10.1212/WNL.34.7.939
21. American Psychiatric Association (2000) Diagnostic and statistical manual of mental disorders: DSM-IV-TR. American Psychiatric Association, Washington, DC
22. Folstein MF, Folstein SE, McHugh PR (1975) Mini-mental state. A practical method for grading the cognitive state of patients for the clinicians. J Psychiatr Res 2(3):189–198. https://doi.org/10.1016/0022-3956(75)90026-6
23. Hughes CP, Berg L, Danziger WL, Coben LA, Martin RL (1982) A new clinical scale for staging of dementia. Br J Psychiatry 140:566–572. https://doi.org/10.1192/bjp.140.6.566
24. Helwa I, Cai J, Drewry MD, Zimmerman A, Dinkins MB, Khaled ML, Seremwe M, Dismuke WM et al (2017) A comparative study of serum exosome isolation using differential ultracentrifugation and three commercial reagents. PLoS One 12(1):e0170628. https://doi.org/10.1371/journal.pone.0170628
25. Mustapic M, Eitan E, Werner JK Jr, Berkowitz ST, Lazaropoulos MP, Tran J, Goetzl EJ, Kapogiannis D (2017) Plasma extracellular vesicles enriched for neuronal origin: a potential window into brain pathologic processes. Front Neurosci 11:278. https://doi.org/10.3389/fnins.2017.00278
26. Fauré J, Lachenal G, Court M, Hirrlinger J, Chatellard-Causse C, Blot B, Grange J, Schoehn G et al (2006) Exosomes are released by cultured cortical neurones. Mol Cell Neurosci 31(4):642–648
27. Honer WG (2003) Pathology of presynaptic proteins in Alzheimer's disease: more than simple loss of terminals. Neurobiol Aging 24(8):1047–1062
28. Goetzl EJ, Mustapic M, Kapogiannis D, Eitan E, Lobach IV, Goetzl L, Schwartz JB, Miller BL (2016) Cargo proteins of plasma astrocyte-derived exosomes in Alzheimer's disease. FASEB J 30(11):3853–3859
29. Goetzl EJ, Kapogiannis D, Schwartz JB, Lobach IV, Goetzl L, Abner EL, Jicha GA, Karydas AM et al (2016) Decreased synaptic proteins in neuronal exosomes of frontotemporal dementia and Alzheimer's disease. FASEB J 30(12):4141–4148
30. Öhrfelt A, Brinkmalm A, Dumurgier J, Zetterberg H, Bouaziz-Amar E, Hugon J et al (2018) A novel ELISA for the measurement of cerebrospinal fluid SNAP-25 in patients with Alzheimer's disease. Neuroscience. https://doi.org/10.1016/j.neuroscience
31. Zhang H, Therriault J, Kang MS, Ng KP, Pascoal TA, Rosa-Neto P et al (2018) Cerebrospinal fluid synaptosomal-associated protein 25 is a key player in synaptic degeneration in mild cognitive impairment and Alzheimer's disease. Alzheimers Res Ther 10(1):80. https://doi.org/10.1186/s13195-018-0407-6
32. Wang S, Zhang J, Pan T, for Alzheimer's Disease Neuroimaging Initiative (2018) APOE ε4 is associated with higher levels of CSF SNAP-25 in prodromal Alzheimer's disease. Neurosci Lett 685:109–113. https://doi.org/10.1016/j.neulet.2018.08.029
33. Wakabayashi K, Honer WG, Masliah E (1994) Synapse alterations in the hippocampal-entorhinal formation in Alzheimer's disease with and without Lewy body disease. Brain Res 667(1):24–32
34. Shimohama S, Kamiya S, Taniguchi T, Akagawa K, Kimura J (1997) Differential involvement of synaptic vesicle and presynaptic plasma membrane proteins in Alzheimer's disease. Biochem Biophys Res Commun 236(2):239–242
35. Minger SL, Honer WG, Esiri MM, McDonald B, Keene J, Nicoll JA et al (2001) Synaptic pathology in prefrontal cortex is present only with severe dementia in Alzheimer disease. J Neuropathol Exp Neurol 60(10):929–936
36. Goetzl EJ, Boxer A, Schwartz JB, Abner EL, Petersen RC, Miller BL, Carlson OD, Mustapic M et al (2015) Low neural exosomal levels of cellular survival factors in Alzheimer's disease. Ann Clin Transl Neurol 2(7):769–773. https://doi.org/10.1002/acn3.211
37. Fiandaca MS, Kapogiannis D, Mapstone M, Boxer A, Eitan E, Schwartz JB et al (2015) Identification of preclinical Alzheimer's disease by a profile of pathogenic proteins in neurally derived blood exosomes: a case-control study. Alzheimers Dement 11(6):600–7.e1. https://doi.org/10.1016/j.jalz.2014.06.008
38. Kester MI, Teunissen CE, Crimmins DL, Herries EM, Ladenson JH, Scheltens P, van der Flier WM, Morris JC et al (2015) Neurogranin as a cerebrospinal fluid biomarker for synaptic loss in symptomatic Alzheimer disease. JAMA Neurol 72(11):1275–1280. https://doi.org/10.1001/jamaneurol.2015.1867
39. Wellington H, Paterson RW, Portelius E, Törnqvist U, Magdalinou N, Fox NC, Blennow K, Schott JM et al (2016) Increased CSF neurogranin concentration is specific to Alzheimer disease. Neurology 86(9):829–835. https://doi.org/10.1212/WNL.0000000000002423
40. Jahn R, Fasshauer D (2012) Molecular machines governing exocytosis of synaptic vesicles. Nature 490(7419):201–207. https://doi.org/10.1038/nature11320