TOWARDS PERSONALIZED AND PRECISION MEDICINE IN COGNITIVE DISORDERS: RULING OUT ALZHEIMER'S PATHOLOGY IN PATIENTS WITH CLINICAL SUSPICION OF ALZHEIMER'S DISEASE.

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Abstract
Clinical diagnosis of Alzheimer's disease (AD) may not be easy in everyday practice, either due to atypical presentations of AD, or due to amnestic-like presentations of non-AD cognitive disorders. We present 4 patients with clinical suspicion of AD presence, 2 of them with amnestic mild cognitive impairment, one with amnestic dementia and one with primary progressive aphasia of the logopenic type. The first three patients had significant atrophy of medial temporal lobe. However, classical cerebrospinal fluid (CSF) biomarkers, revealed a non-AD profile in all four. Classical CSF biomarkers may serve as a significant tool, helpful not only for confirming the presence of AD, but also for excluding AD in cases with such a clinical suspicion. This may be significant when new, disease—modifying treatments are considered for the treatment of such patients.

Keywords: Alzheimer's disease; cognitive disorders; dementia; vascular cognitive disorder; cerebrospinal fluid.

ΟΔΕΥΟΝΤΑΣ ΠΡΟΣ ΤΗΝ ΠΡΟΣΩΠΟΠΟΙΗΜΕΝΗ ΙΑΤΡΙΚΗ ΚΑΙ ΤΗΝ ΙΑΤΡΙΚΗ ΑΚΡΙΒΕΙΑΣ ΣΤΙΣ ΓΝΩΣΙΑΚΕΣ ΔΙΑΤΑΡΑΧΕΣ: ΑΠΟΚΛΕΙΣΜΟΣ ΤΗΣ ΠΑΘΟΛΟΓΙΑΣ ΝΟΣΟΥ ALZHEIMER ΣΕ ΑΣΘΕΝΕΙΣ ΜΕ ΚΛΙΝΙΚΗ ΥΠΟΨΙΑ ΤΗΣ ΝΟΣΟΥ.

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Περίληψη:
Η κλινική διάγνωση της νόσου Alzheimer μπορεί να αποτελεί πρόκληση στην καθ' ημέρα πράξη, είτε λόγω άτυπων εκδηλώσεων της ίδιας της νόσου, είτε λόγω εκδήλωσης άλλων, μη-Alzheimer γνωσιακών διαταραχών, με αμνησικό πρότυπο. Παρουσιάζουμε τέσσερις ασθενείς με κλινική υποψία νόσου Alzheimer, 2 εκ των οποίων με αμνησική ήπια γνωσιακή διαταραχή, ένας με αμνησικού τύπου άνοια και ένας με πρωτοπαθή προϊούσα αφασία λογοπενικού τύπου. Οι πρώτοι τρεις ασθενείς παρουσίαζαν σημαντική ατροφία του μέσου κροταφικού λοβού. Εντούτοις, οι κλασσικοί βιοδείκτες ΕΝΥ ανέδειξαν μη Alzheimer παθολογία και στους τέσσερις ασθενείς. Οι κλασσικοί βιοδείκτες ΕΝΥ μπορούν να λειτουργήσουν σαν ένα σημαντικό βοήθημα, όχι μόνο για την επικύρωση της παρουσίας παθολογίας νόσου Alzheimer αλλά ακόμη και για τον αποκλεισμό αυτής σε περιπτώσεις ασθενών με κλινική υποψία της νόσου, λαμβάνοντας ιδιαίτερα υπόψιν και την προοπτική χρήσης νέων, νοσοτροποποιητικών παραγόντων για τη θεραπεία αυτών των ασθενών.

Λέξεις ευρετηρίου: Νόσος Αλτσχάιμερ,νοητικές διαταραχές, άνοια, ανοσοαγγειακή νοητική διαταραχή,εγκεφαλονωτιαίο υγρό

1. Introduction

Alzheimer's disease (AD) is the most common cause of cognitive decline and it can be diagnosed either in the mild cognitive impairment (MCI) or in the dementia stage by the use of clinically based criteria [1,2]. However, atypical presentations of AD may occur [3] including, among others, frontal or posterior presentations and primary progressive aphasia (PPA) of the logopenic type [4]. In the presence of such atypical presentations, in early disease, in the community and in the presence of comorbidities, it is long known that clinical diagnostic accuracy may drop substantially [5] and, up to 39% of patients in which a non-AD diagnosis was given during life, will prove to have AD at autopsy [6]. The opposite is also true and, up to 30% of patients diagnosed with AD, will prove to have a non-AD pathology at neuropathological examination [7]. Thus, in vivo clinical diagnosis of AD is probabilistic and postmortem verification (or ruling out) remains the gold standard for final diagnosis. Primary age-related tauopathy (PART) [8] and limbic-predominant age-related TDP-43 encephalopathy (LATE) [9] are two pathologies which may present in the elderly as cognitive decline of the hippocampal amnestic type or "AD-type" dementia.

During the last decade, the 3 "established" or "classical" cerebrospinal fluid (CSF) biomarkers for AD have been incorporated in diagnostic criteria/guidelines [1,3] and classification systems [10]: (a) Amyloid-β peptide with 42 amino acids ($A\beta_{42}$) which is decreased in AD, is considered as a marker of amyloid plaque pathology [11], (b) tau protein phosphorylated to a threonine residue at position 181 ($\tau_{P\text{-}181}$) which is increased in AD, is considered as a marker of tangle formation [12] and (c) total tau protein (τ_T) which is increased in AD is a nonspecific marker of neuronal and/or axonal degeneration [13]. The $A\beta_{42}/A\beta_{40}$ ratio may be preferred to $A\beta_{42}$ alone since it seems to perform diagnostically better than the latter [14]. With a sensitivity and specificity at the level of ≥ 90% [3], they are useful in identifying the "AD neurochemical fingerprint" in atypical [15–18] or mixed [18–20] cases.

The aim of the present study is to describe a series of patients with a clinical presentation suggestive of AD, in which, however, CSF biomarkers ruled out the "AD neurochemical fingerprint", indicating a non-AD pathology.

2. Patients and Methods

2.1. Patients

Patients presented here were examined at the 2nd Department of Neurology ("Attikon" Hospital). They had cognitive impairment with presentation typical for, or highly suggestive of AD. Initially, history, neurological and complete physical examination were recorded routinely. Secondary causes including thyroid disease, B12 deficiency, neurosyphilis, brain tumor, subdural hematoma or normal pressure hydrocephalus were excluded. Initial clinical diagnosis was performed according to widely accepted criteria for MCI or dementia due to AD [1,2], for PPA [21], for Vascular cognitive impairment (VCI) [22] and for the behavioral variant of Frontotemporal Dementia (FTDbv) [23]. A written informed consent was obtained for all cases. The study had the approval of the Bioethics Committee (157/16-03-2021) and the Scientific Board (A13/07-04-2021) of "Attikon" Hospital and was conducted according to the ethical guidelines of the 1964 Declaration of Helsinki.

2.2. Neuropsychological testing

Following history and clinical examination a battery of neuropsychological tests was used, as routinely performed in our department. Global tests for assessment of cognition and activities of daily living included the Addenbrooke's Cognitive Examination-Revised version (ACE-R), the Mini Mental State Examination (MMSE), the Clinical Dementia Rating (CDR, both sum of boxes and overall score) and the Instrumental Activities of Daily Living (IADL) [24-27]. Brief tests for memory (free and cued recall), frontal function, visuospatial skills and depression included the 5-words memory test [28], the Frontal Assessment Battery (FAB) [29], the CLOX (1 and 2) [30] and the short version of the Geriatric Depression Scale (GDS) [31], respectively.

2.3. Neuroimaging

A routine 1.5 or 3T brain magnetic resonance imaging (MRI) scan was available for all patients, including 3D T1W sequences, suitable for assessing cortical and central atrophy. Medial Temporal lobe Atrophy (MTA) was assessed according to the Medial Temporal Atrophyvisual scale [32]. The recently introduced Entorhinal Cortex Atrophy Score (ERICA) was also determined at the level of the mammillary bodies [33,34].

2.4. Lumbar puncture and CSF biomarker measurements

According to widely accepted recommendations on standardized operative procedures for CSF biomarkers [35], lumbar puncture was performed using a standard, 21-22G, Quincke type needle, at the L4-L5 interspace, at 9-12 a.m. In brief, CSF was collected in 6 polypropylene tubes, as described elsewhere [17]. The 1st and 2nd tubes (1 ml each) were used for routine CSF cytology and biochemistry, respectively. The 3rd tube (2 ml) was used for oligoclonal bands and IgG index determinations. The following 2 tubes (5 ml each) were used for biomarker determinations. The last tube (~2 ml) was used for syphilis serology or other tests according to clinical indications. All

CSF samples had < 500 red blood cells/μL. The 2 tubes intended for CSF biomarker analysis, were immediately centrifuged (2000g×15 min), aliquoted in polypropylene tubes (1 ml each) and finally stored at –80°C. Aliquots were thawed only once, just before analysis, which was performed within 3 months of storage.

Classical CSF biomarkers ($A\beta_{42}$, $A\beta_{40}$, $\tau_{P\text{-}181}$ and τ_T) were measured in a Euroimmun Analyzer I (Euroimmun, Lübeck, Germany), in duplicate, with double sandwich enzyme-linked immunosorbent assay (ELISA) by commercially available kits [EUROIMMUN Beta-Amyloid (1- 42) ELISA, EUROIMMUN Beta-Amyloid (1- 40) ELISA, EUROIMMUN pTau(181) ELISA and EUROIMMUN Total-Tau ELISA respectively, Euroimmun, Lübeck, Germany], according to manufacturer's instructions and by the use of 4-parameter logistic curves as described elsewhere[16]. All procedures were performed under a stable temperature (21 ± 2 °C) and quality control samples (both in-house and provided by the manufacturer) were used in each run. The inter- and intra-assay coefficients of variation were both < 7% for all biomarkers. All assays were performed at the Unit of Neurochemistry and Biological Markers of the 1st Department of Neurology ("Eginition" Hospital) and, according to the cut-off values of our laboratory, biomarker concentrations were considered abnormal when $A\beta_{42}$<480 pg/ml, $A\beta_{42}/A\beta_{40}$< 0.094, $\tau_{P\text{-}181}$>60 pg/ml and τ_T>400 pg/ml, $\tau_{P\text{-}181}/A\beta_{42}$> 0.205 and $\tau_T/A\beta_{42}$> 0.710 [16,17].

Based on CSF biomarker concentrations and the presence or absence of atrophy on structural neuroimaging, the profile of each patient was determined according to the AT(N) classification system [10], as already described and diagrammatically illustrated elsewhere [17]. The CSF AD profile ("neurochemical fingerprint") was defined as decreased $A\beta_{42}$ or decreased $A\beta_{42}/A\beta_{40}$ and increased $\tau_{P\text{-}181}$ and thus, compatible with the $A^+T^+(N)^+$ or $A^+T^+(N)^-$ profiles [10]. On the contrary, the $A^-T^-(N)^+$ or $A^-T^+(N)^+$ profiles were considered compatible with non-AD pathology [10].

Genotyping of ***APOE*** was performed at the Department of Clinical Biochemistry of "Attikon" Hospital. Genomic DNA was extracted from 200 μl of blood using the "High Pure PCR Template Kit" (Roche, Mannheim, Germany). For the amplification of the ***APOE*** gene, 30 ng of genomic DNA was amplified using a "real-time qPCR kit" (TIB MolBiol, Berlin, Germany) in the "Light Cycler PCR" platform (Roche, Mannheim, Germany).

3. Results

All 4 reported patients had normal routine CSF cytology and biochemistry, normal IgG index and absence of oligoclonal bands, or any indication of neurosyphilis. Their demographic, clinical, neuropsychological and CSF neurochemical data are summarized in Table 1.

3.1. Patient 1

A 69 years-old male reported difficulty with recent memory during the last 3 years. He frequently repeated the same questions and needed to keep memos, but otherwise led an independent life, with no difficulty in every-day living. No family history of cognitive disorder was reported. The clinical picture was attributed to "depression" by a psychiatrist and mirtazapine 30 mg every night at bedtime was prescribed with no effect. Clinical and neurological examination was essentially normal. Neuropsychological examination revealed a hippocampal amnestic pattern, while a frontal component was also present. Neuroimaging (Figure 1a) revealed parietal cortical atrophy and involvement of the medial temporal lobe with an Medial Temporal Atrophygrade of at least 2 and an ERICA score of 2, indicating significant entorhinal cortex atrophy, which has been suggested to be compatible with AD [33,34]. A few, mild white matter lesions were observed, which were considered insignificant. The clinical picture was considered compatible with MCI due to AD [1]. However, CSF biomarkers revealed abnormal levels of only $\tau_{P\text{-}181}$ with no amyloid positivity. Thus, his AT(N) profile was $A^-T^+(N)^+$ (neurodegeneration was positive due to atrophy), indicating non-AD pathological change [10]. He was heterozygote for the ε2 allele of ***APOE***.

3.2. Patient 2

This is an 83 years-old male with a history of hypertension under losartan and hydrochlorothiazide. He reported difficulty with recent memory during the last 1.5 year. He keeps memos and frequently repeats the same questions, but otherwise he can perform relatively complex tasks such as handling money and banking and there was no difficulty with activities of everyday living. No family history of cognitive disorder was reported. Clinical examination revealed indifferent plantar responses but was otherwise normal. Neuropsychological examination revealed a hippocampal amnestic pattern, while a frontal component was also present. Neuroimaging (Figure 1b) revealed significant load of white mater lesions. Frontal and parietal cortical atrophy was also present together with involvement of the medial temporal lobe, with an Medial Temporal Atrophygrade of at least 2 and an ERICA score of 2, indicating significant entorhinal cortex atrophy, which has been suggested to be compatible with AD [33,34]. The clinical picture was considered compatible with MCI due to AD [1], with subcortical small vessel disease (SSVD). However, CSF analysis revealed normal levels of all 3 classical biomarkers. Thus, his AT(N) profile was $A^-T^-(N)^+$ (neurodegeneration was positive due to atrophy),

Table 1. Demographic, clinical, imaging and neurochemical data of the 4 patients.

	Patient 1	**Patient 2**	**Patient 3**	**Patient 4**
Gender	male	male	male	female
Age (years)	69	83	53	63
Education (years)	6	6	16	15
Disease duration (years)	3	1.5	5	2
ACE-R [24]	73/100	65/100	71/100	76/100
MMSE [25]	26/30	25/30	22/30	26/30
CDR sum of boxes [26]	3	1.5	6	0.5
CDR overall [26]	0.5	0.5	1	0.5
IADL [27]	8/8	8/8	3/8	8/8
5-words delayed recall [28]	2+0/5	3+0/5	0+0/5	4+1/5
FAB [29]	11/18	12/18	16/18	11/18
CLOX1 [30]	9/15	10/15	10/15	9/15
CLOX2 [30]	15/15	13/15	14/15	13/15
GDS [31]	8/15	1/15	8/15	2/15
MTA grade [32]	2-3	3	4	0
ERICA score [33]	2	2	3	1
Vascular component (MRI)	Minimal	Significant	No	No
Initial clinical diagnosis	MCI due to AD	MCI due to AD plus SSVD	Dementia due to AD	PPA logopenic, probably due to AD
APOE	ε2 / ε3	ε2 / ε2	Not available	ε3 / ε3
$A\beta_{42}$ (pg/ml) (abnormal < 480)	830	1310	612	1102
$A\beta_{40}$ (pg/ml)	6657	7169	2892	7118
$A\beta_{42}/A\beta_{40}$ (abnormal < 0.094)	0.125	0.183	0.211	0.155
$\tau_{P\text{-}181}$ (pg/ml) (abnormal > 60)	62.9 ↑	54.7	19.6	75.7 ↑
τ_T (pg/ml) (abnormal > 400)	332	388	203	582 ↑
$\tau_{P\text{-}181}/A\beta_{42}$	0.076	0.042	0.032	0.069
$\tau_T/A\beta_{42}$	0.400	0.296	0.332	0.528
AT(N) profile [10]	$A^-T^+(N)^+$	$A^-T^-(N)^+$	$A^-T^-(N)^+$	$A^-T^+(N)^+$
Final diagnosis	Non-AD (tauopathy?)	VCI alone or mixed with non-AD pathology	Non-AD (FTLD?)	Non-AD (FTLD-tau?)

ACE-R: Addenbrooke's Cognitive Examination-Revised, MMSE: Mini Mental State Examination, CDR: Clinical Dementia Rating, IADL: Instrumental Activities of Daily Living, FAB: Frontal Assessment Battery, GDS: Geriatric Depression Scale, MRI: Magnetic Resonance Imaging, MCI: Mild Cognitive Impairment, SSVD subcortical small vessel disease, PPA: Primary Progressive Aphasia, FTLD: Frontotemporal Lobar Degeneration. ↑ Increased (abnormal) levels.

indicating non-AD pathological change [10]. He was homozygote for the ε2 allele of ***APOE***.

3.3. Patient 3

A 53 years-old male with no family history, developed difficulty with recent memory 5 years ago. Difficulty with complex tasks and orientation in place and time was gradually added, together with apathy. No inappropriate, perseverative or compulsive behavior, disinhibition, loss of empathy and sympathy or language disorder were reported. Some increase in appetite with weight gain (5 kg) was noted. At the time of examination, he was dependent to a significant degree and needed the care of his mother, at least partially. Neurological examination revealed brisk tendon reflexes symmetrically and

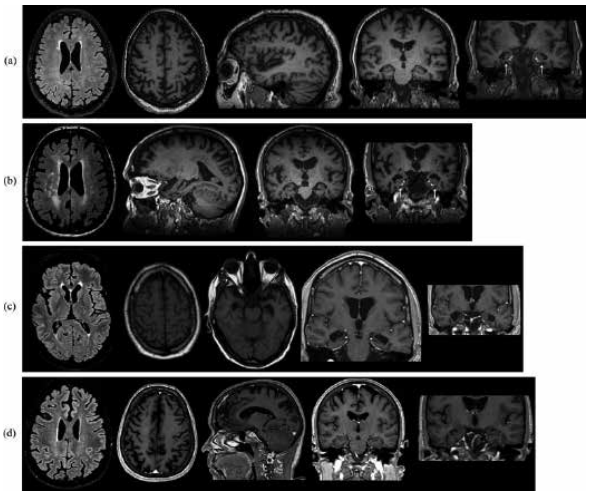

Figure 1. Magnetic resonance imaging of the 4 patients presented. (a) patient 1, (b) patient 2, (c) patient 3 and (d) patient 4. In (a) and (b): arrows indicate widening of the collateral sulcus and arrowheads indicate the "tentorial cleft sign", compatible with significant entorhinal cortex atrophy [33,34]

bilateral extensor plantar response, but no primitive reflexes. Neuropsychological examination showed a significant hippocampal amnestic disorder, while a frontal component was also present. Visuoconstructive abilities were preserved. Neuroimaging (Figure 1c) revealed significant atrophy of the hippocampal formation with an Medial Temporal Atrophygrade of 4 and an ERICA score of 3, indicating significant entorhinal cortex atrophy, which has been suggested to be compatible with AD [33,34]. Left anterior temporal atrophy was also observed. The clinical picture was considered compatible with dementia due to AD [2]. However, CSF analysis revealed normal levels of all 3 classical biomarkers. Thus, his AT(N) profile was $A^-T^-(N)^+$ (neurodegeneration was positive due to atrophy), indicating non-AD pathological change [10].

3.4. Patient 4

A 63 years-old right-handed female reported a language impairment during the last 2 years. She had a relatively decreased fluency due to word-finding difficulty. No significant impairment in other cognitive domains was reported by her or her relatives. She was completely independent and she could still work as a fashion designer. The only difficulty in everyday life was due to the language difficulty. Clinical examination was essentially normal. On neuropsychological testing frequent stops in spontaneous speech were noted in an effort to find the appropriate words. Phonological errors were not infrequent and repetition was definitely impaired. Motor and grammatical/structural aspects of speech, single word comprehension and object knowledge were preserved. Some degree of difficulty in frontal tests was observed and, additionally, there was an impression of possible apraxia in mimicking hand or finger movements, but none of these was reflected in visuospatial ability, everyday life and professional aspects. Graphesthesia and stereognosis were normal. Neuroimaging (Figure 1d) revealed parietal cortical atrophy with preservation of medial temporal lobe. Some degree of medial frontal atrophy was also observed. The clinical diagnosis was that of logopenic variant PPA [21], with the notable imaging finding of predominantly right (and not left) parietal atrophy. Although AD is the most common underlying pathology in logopenic PPA [4], CSF biomarkers revealed abnormal levels of $\tau_{P\text{-}181}$ and τ_T with no amyloid positivity. Thus, her AT(N) profile was $A^-T^+(N)^+$, indicating non-AD pathological change [10]. She was homozygote for the ε3 allele of ***APOE***.

4. Discussion

In the present study we present a series of 4 cognitively impaired patients with a clinical presentation typical for or suggestive of AD as the underlying pathology. However, CSF biomarkers showed normal amyloid levels in the form of both $A\beta_{42}$ alone and the $A\beta_{42}/A\beta_{40}$ ratio, which is considered to better reflect brain amyloid status [14]. Thus, according to the AT(N) classification system, AD or Alzheimer's continuum was excluded in all four and a diagnosis of non-AD pathological change was supported [10].

The 1st patient was considered to suffer of MCI due to AD, since the neuropsychological impairment was compatible mainly with the hippocampal amnestic type and there was medial temporal atrophy especially involving the entorhinal cortex in a significant degree (ERICA score 2). This clinical and imaging presentation would be considered rather typical for AD. However, CSF biomarkers, revealed the $A^-T^+(N)^+$ profile which, despite of various controversies and possible overlapping pathologies [36,37], it could be suggestive of a tauopathy. The patient was in the senile age (onset of symptoms > 65 years), but not at an age > 80 years. However, PART [8], which is a 3- and 4-repeat tauopathy [38] is a tempting diagnosis, since it has been observed not only in the oldest old [39], but also in "younger" old patients [40].

A debate exists as to whether PART is a completely different entity or, somehow, it belongs to the Alzheimer's spectrum [37]. Indeed, patients with the A^-T^+ profile may share some clinical, neuropsychological and imaging similarities with those with the typical AD profile (A^+T^+) [40]. However, some studies identify significant differences between PART and AD, especially slower rates of cognitive decline [39,41], lower ***APOE***ε4 frequency [39–42] and higher ***APOE***ε2 frequency [39,42] in PART, suggesting that at least a subgroup of PART patients does not belong to the Alzheimer's spectrum. Our patient had the ***APOE***ε2 and no ε4

allele, which could offer some resistance to Aβ formation and thus to AD, as previously suggested [42]. The (co)existence in our patient of argyrophilic grain disease [38,43] or ageing-related tau astrogliopathy [44], (both 4-repeat tauopathies) could be alternative diagnostic possibilities, as well as coexistence of PART with LATE [9].

But, is really the A^-T^+ profile (using $\tau_{P\text{-}181}$ for T) indicative of PART? It has been suggested that abnormal levels of $\tau_{P\text{-}181}$ may provide different information in cognitively impaired as compared to cognitively unimpaired subjects and in different clinical settings [45]. In a recent study, subjects with either the A^-T^+ or the A^-T^- profile had similar rates of cognitive decline and showed similar findings in the temporal lobes in Positron Emission Tomography for tau (tau-PET), suggesting that an elevated $\tau_{P\text{-}181}$ in the absence of Aβ abnormality may not necessarily reflect tangle formation, but may be related to altered CSF turnover/kinetics [46]. However, data on this field are few and conflicting.

The 2nd patient had significant subcortical small vessel disease (evident in neuroimaging). However, since (a) frontal dysfunction was not the prominent one, (b) he did not have significant apathy or depression, (c) he did not have any urinary symptoms such as frequency and/or urgency and (d) he did not suffer of any type of gait disorder, he did not fulfill the VASCOG criteria for predominant vascular etiology of cognitive impairment [22]. The hippocampal amnestic pattern in neuropsychological testing, combined with cortical and medial temporal/entorhinal cortex atrophy, lead to the impression of AD with concurrent SSVD. Biomarker levels in the CSF did not support this diagnosis and, the $A^-T^-(N)^+$ profile could be suggestive of VCI alone [1]. However, atrophy in the medial temporal lobe structures, raises the possibility of a concurrent neurodegenerative pathology. It seems that LATE may be the more plausible for this 83-year-old patient [9]. LATE is a distinct type of TDP-43 proteinopathy affecting the amygdala, the hippocampal formation including the entorhinal cortex and spreading to the temporal cortex, insula, orbitofrontal cortex and middle frontal gyrus [9]. It typically results in an amnestic syndrome which may gradually affect many cognitive domains. He did not show imaging characteristics of hippocampal sclerosis, but this is not required [9]. On the other hand, PART cannot be totally excluded, since not all cases of tauopathy are necessarily accompanied by increased CSF levels of $\tau_{P\text{-}181}$ [47]. Our patient had a $\tau_{P\text{-}181}/\tau_T$ ratio of 0.141; in our laboratory, values < 0.163 have been suggested as indicative of TDP-43 (and not tau) pathology in non-AD patients, but this is only speculative [15]. Both PART and LATE may coexist in the same patient [48]. Our patient was homozygote for the ***APOE***ε2 allele, but results on the ***APOE*** status in LATE are conflicting and the exact role of ε4 in cases of LATE without concomitant AD pathology remains to be elucidated [9]. Recent findings suggest that ***APOE***ε2 may have a protective role against multiple concurrent neurodegenerative pathologies [49], while it may exacerbate TDP-43 toxicity in the absence of concurrent AD pathology [50].

The 3rd patient developed an amnestic dementia at the presenium. Significant apathy was present, but this is observed in roughly 50% of patients with AD [51]. Mild hyperphagia with weight gain could be a red flag, but he did not fulfill even the criteria for possible FTDbv [23]. Thus, based on the amnestic neuropsychological profile and the significant atrophy of the hippocampal formation, he was initially considered as a presenile case of AD. Biomarker analysis revealed the $A^-T^-(N)^+$ profile. Frontotemporal lobar degeneration would be the most suitable diagnosis, given the asymmetric (left) anterior temporal lobe atrophy. Episodic memory disorder, despite being the hallmark of AD, it can be observed in FTDbv, sometimes causing diagnostic difficulties, especially in early cases [52]. Hippocampal atrophy can also be seen in some patients with FTD [53]. This patient's $\tau_{P\text{-}181}/\tau_T$ ratio was 0.097, which is very low for the cut-offs of our[15] and other laboratories [54], suggesting a possible TDP-43 pathology, but is insecure to theorize based solely on this ratio.

The 4th patient was different. She developed a rather typical logopenic-type language disorder, which was the only cause of difficulty in everyday life and, with a duration of 2 years fulfills the clinical criteria of logopenic PPA [21]. The mild apraxic signs were observed only during examination, they were not accompanied by any apraxic symptoms in everyday life and such signs have been reported in logopenic PPA patients, especially those due to AD [55]. At least 50%–56% and probably 76% of logopenic PPA patients have AD pathology, based in pathological data[4,56,57]. Studies based on biomarkers show a percentage of about 75%–79% [15,55,58]. She was ***APOE*** ε3 homozygote, but it has been observed that the percentage of ***APOE*** ε4 carriers in PPA due to AD is not increased as compared to controls and is lower as compared to the amnestic phenotypes of AD [59]. Thus, for our patient, AD seemed to be the most probable diagnosis. However, CSF biomarker analysis revealed the $A^-T^+(N)^+$ profile, which may be suggestive of tauopathy. Pathological data suggest that, following AD, neurodegenerative disorders with decreasing order of frequency are FTLDTDP-43, FTLD-tau and a combination of the two [56]. Indeed, non-AD logopenic patients have been reported to suffer of tau [60], TDP-43 [61], or even Lewy body [62] pathology.

In all four patients the use of CSF biomarkers (together with atrophy in neuroimaging) as suggested by the AT(N) classification system excluded AD or

Alzheimer's spectrum as the cause of their disorder. However, the exact cause was only partially suspected in the presence of the $A^-T^+(N)^+$ profile, possibly pointing to tauopathy, but not clarifying which one. In the case of the $A^-T^-(N)^+$ profile no diagnosis of inclusion can be made. Other biomarkers may be useful in this context. Imaging biomarkers include both amyloid- and tau-PET may be of help as they may not only differentiate between AD and non-AD cases in hippocampal amnestic patients, but may detect tauopathy restricted to medial temporal lobes, which corresponds to PART [63]. A combination of MRI and PET may prove helpful in the identification of LATE[64]. Assessment of CSF TDP-43, either alone or in combination with τ_T and $\tau_{P\text{-}181}$ [65], may also be useful in identifying TDP-43 pathology in FTLD patients[66] and increased TDP-43 levels in astrocyte-derived extracellular vesicles in plasma may prove useful tool in identifying patients with LATE [67]. Plasma progranulin concentration may help in identifying FTLD patients with possible mutations in the ***GRN*** gene [68]. Neurofilament light protein (NfL, a nonspecific marker of neuronal/axonal injury) and neurogranin (a marker of synaptic dysfunction) may offer additional information in some patients [69]. Concentration of CSF α-synouclein, although traditionally thought as marker of synucleinopathy [70] is an emerging rather than an established biomarker, since data are conflicting, the various forms of α-synouclein may show differences among various neurodegenerative disorders [71] and methodological issues remain to be addressed [72]. Furthermore, recent evidence suggests that a-synuclein may be a biomarker of AD and it is involved in the pathogenesis of this disorder [73,74].

5. Conclusions

Classical CSF biomarkers may serve as a significant tool, helpful not only for confirming the presence of AD, but also for excluding AD in cases with such a clinical suspicion. Sometimes they may provide a clue for the underlying non-AD pathology and sometimes not. However, even the exclusion of AD presence in otherwise typical AD-like patients may be significant if and when new, disease—modifying treatments are considered for the treatment of such patients.

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Institutional Review Board Statement: The study had the approval of the Bioethics Committee and the Scientific Board of "Attikon" Hospital (approval numbers 157/16-03-2021 and A13/07-04-2021, respectively). It was performed according to the 1964 Declaration of Helsinki.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study and/or next of kin caregivers (depending on the severity of cognitive impairment).

Data Availability Statement: The data presented in this study are available upon request from the corresponding author. The data are not publicly available due to privacy restrictions.

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Conflicts of Interest: G.T., G.P.P., I.T, S.F., and A.B. are clinical investigators in the "EVOKE" and "EVOKE plus" trials of semaglutide for early Alzheimer's disease (NovoNordisk, NCT04777396 and NCT04777409 respectively). G.P.P received fees from Biogen International and from ITF Hellas, as a consultant of advisory boards. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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