





Article

# Alzheimer's Disease CSF Biomarker Profiles in Idiopathic Normal Pressure Hydrocephalus

Salvatore Mazzeo <sup>1,2</sup> , Filippo Emiliani <sup>1</sup>, Silvia Bagnoli <sup>1</sup>, Sonia Padiglioni <sup>3,4</sup>, Lorenzo Maria Del Re <sup>5</sup>, Giulia Giacomucci <sup>1</sup> , Juri Balestrini <sup>1</sup>, Assunta Ingannato <sup>1</sup>, Valentina Moschini <sup>6</sup>, Carmen Morinelli <sup>6</sup>, Giulia Galdo <sup>1</sup>, Cristina Polito <sup>2</sup>, Camilla Ferrari <sup>1</sup>, Gastone Pansini <sup>7</sup>, Alessandro Della Puppa <sup>1</sup>, Sandro Sorbi <sup>1,2</sup>, Benedetta Nacmias <sup>1,2</sup>  and Valentina Bessi <sup>1,4,\*</sup> 

- <sup>1</sup> Department of Neuroscience, Psychology, Drug Research and Child Health, Careggi University Hospital, University of Florence, Largo Brambilla, 3, 50134 Florence, Italy; salvatore.mazzeo@unifi.it (S.M.); filippo.emiliani@unifi.it (F.E.); silvia.bagnoli@unifi.it (S.B.); giuliagiacomucci.md@gmail.com (G.G.); juri.balestrini@unifi.it (J.B.); assunta.ingannato@unifi.it (A.I.); giulia.galdo@unifi.it (G.G.); camilla.ferrari@unifi.it (C.F.); alessandro.dellapuppa@unifi.it (A.D.P.); sandro.sorbi@unifi.it (S.S.); benedetta.nacmias@unifi.it (B.N.)
- <sup>2</sup> IRCCS Fondazione Don Carlo Gnocchi, 50143 Florence, Italy; cristina.polito@unifi.it
- <sup>3</sup> Regional Referral Centre for Relational Criticalities, 50139 Florence, Italy; sonia\_padiglioni@libero.it
- <sup>4</sup> Research and Innovation Centre for Dementia-CRIDEM, Careggi University Hospital, 50134 Florence, Italy
- <sup>5</sup> Research Unit of Medicine Ageing, Department of Experimental and Clinical Medicine, University of Florence, 50121 Florence, Italy; lorenzomaria.delre@unifi.it
- <sup>6</sup> Dipartimento Neuromuscolo-Scheletrico e Degli Organi di Senso, Careggi University Hospital, 50134 Florence, Italy; valentina.moschini02@gmail.com (V.M.); carmen.morinelli@studenti.unipd.it (C.M.)
- <sup>7</sup> Neurosurgery Unit, Careggi University Hospital, Largo Giovanni Alessandro Brambilla 3, 50134 Florence, Italy; gastonepansini@gmail.com
- \* Correspondence: valentina.bessi@unifi.it; Tel.: +39-05-7948660; Fax: +39-05-7947484



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**Abstract:** Patients with idiopathic normal pressure hydrocephalus (iNPH) frequently show pathologic CSF  $A\beta_{42}$  levels, comparable with Alzheimer's Disease (AD). Nevertheless, the clinical meaning of these findings has not been fully explained. We aimed to assess the role of AD CSF biomarkers ( $A\beta_{42}$ ,  $A\beta_{42}/A\beta_{40}$ , p-tau, t-tau) in iNPH. To this purpose, we enrolled 44 patients diagnosed with iNPH and 101 with AD. All the patients underwent CSF sampling. We compared CSF biomarker levels in iNPH and AD:  $A\beta_{42}$  levels were not different between iNPH and AD, while  $A\beta_{42}/A\beta_{40}$ , p-tau, and t-tau were significantly different and showed excellent accuracy in distinguishing iNPH and AD. A multiple logistic regression analysis showed that  $A\beta_{42}/A\beta_{40}$  was the variable that most contributed to differentiating the two groups. Furthermore, iNPH patients with positive  $A\beta_{42}/A\beta_{40}$  had higher p-tau and t-tau than iNPH patients with negative  $A\beta_{42}/A\beta_{40}$ . Those iNPH patients who showed cognitive impairment had lower  $A\beta_{42}/A\beta_{40}$  and higher p-tau than patients without cognitive impairment. We concluded that positive CSF  $A\beta_{42}$  with negative  $A\beta_{42}/A\beta_{40}$ , p-tau, and t-tau is a typical CSF profile of iNPH. On the contrary, positive  $A\beta_{42}/A\beta_{40}$  in iNPH patients, especially when associated with positive p-tau, may lead to suspicion of a coexistent AD pathology.

**Keywords:** Alzheimer's Disease; idiopathic normal pressure hydrocephalus; cerebrospinal fluid; biomarkers; cognitive impairment

## 1. Introduction

Idiopathic normal pressure hydrocephalus (iNPH) is the most common form of hydrocephalus in adults with an estimated prevalence of 5.9% in patients over 80 years [1]. It is characterized by a classical triad of symptoms (cognitive disturbances, balance/gait impairment, and urinary incontinence), in the presence of a communicating hydrocephalus and a normal opening pressure upon lumbar puncture [2]. It is crucial to diagnose this condition early because 70–80% of patients show clinical improvement following ventricular-peritoneal shunt insertion [3]. Nevertheless, iNPH diagnosis could be challenging because

the typical clinical triad is neither sensitive (it is present in <60% of patients [4,5]), nor specific, as clinical features of iNPH are shared with other cognitive and movement disorders, such as Alzheimer's Disease (AD), vascular dementia, Parkinson's disease, and atypical parkinsonian syndromes [6–9]. Moreover, cerebrospinal fluid (CSF) concentration of  $A\beta_{42}$  (one of the AD core biomarkers, whose reduction reflects  $A\beta$  amyloid plaques deposition in the brain [10]) was widely shown to be reduced also in iNPH [11]. In iNPH, the reduction of  $A\beta_{42}$  seems not to be related to  $A\beta$  amyloid plaques deposition, but to downregulation of  $A\beta$  production due to periventricular hypometabolism [12] and to the impairment of glymphatic clearance mechanisms and CSF turnover [13,14].

However, concomitant AD neuropathologic changes have been frequently seen in brain biopsies or in postmortem neuropathological examinations of patients with clinical diagnoses of iNPH [15–17]. Furthermore, the presence of AD pathology in iNPH was found to be associated with neuropsychiatric symptoms and behavior changes [18–21], as well as response to ventricular-peritoneal shunt [22–26]. Due to this evidence, a CSF AD biomarker analysis may have diagnostic and prognostic value, influencing the management of iNPH patients.

Nevertheless, CSF  $A\beta_{42}$  measurement is influenced by interindividual physiological differences in amyloid processing [27] and false negatives are common also in patients with AD, as well as false positives in patients with other neurodegenerative diseases and healthy individuals [28]. Measurement of the  $A\beta_{40}$  and  $A\beta_{42}/A\beta_{40}$  ratio have been proposed to overcome this limit [28].  $A\beta_{40}$  is the most abundant  $A\beta$  peptide and is less likely to aggregate than  $A\beta_{42}$ . In AD, the reduced levels of  $A\beta_{42}$  are associated with slightly increased or steady levels of  $A\beta_{40}$  [29]. Therefore, the  $A\beta_{42}/A\beta_{40}$  ratio is lower in AD than in healthy controls and showed higher accuracy as compared to  $A\beta_{42}$  in distinguishing AD from other neurodegenerative diseases [28]. In contrast, in iNPH patients, the CSF levels of all the amyloid precursor protein (APP) fragments ( $A\beta_{38}$ ,  $A\beta_{40}$ ,  $A\beta_{42}$ , sAPP $\alpha$ , and sAPP $\beta$ ) are decreased compared to controls [11,12,30]. Hence, we speculated that while  $A\beta_{42}$  levels are reduced in the CSF of iNPH patients,  $A\beta_{42}/A\beta_{40}$  may be normal and could be the key factor in interpreting results of CSF biomarkers in iNPH. In this study, we aimed to test our hypothesis by assessing the accuracy of each CSF biomarker in distinguishing iNPH and AD patients to define a typical biomarker profile of iNPH.

## 2. Materials and Methods

### 2.1. Participants and Clinical Assessment

We included 44 patients diagnosed with iNPH according to international guidelines [7] and 101 patients diagnosed with AD according to NIA-AA criteria [31]. All of the patients were consecutively referred to the Centre for Alzheimer's Disease and Adult Cognitive Disorders and Neurology Unit of Careggi Hospital in Florence for CSF collection between December 2018 and March 2022.

All patients in the iNPH group had ventricular enlargement associated with a patent Sylvian aqueduct and the absence of a macroscopic obstruction of CSF flow, lack of cortical atrophy, presence of periventricular water content, and an increased callosal angle in the coronal plane [32]. Patients in this group were not suffering from any other neurological, psychiatric, or medical conditions that could potentially explain their presenting symptoms.

We excluded AD patients with a history of head injury, other neurological and/or systemic diseases, major depression, and alcoholism or other substance abuse.

All of the patients underwent a comprehensive familial and clinical history, general and neurological examination, extensive neuropsychological investigation, brain magnetic resonance imaging (MRI) or brain Computed Tomography (TC), and lumbar puncture for CSF collection. Patients diagnosed with iNPH underwent a CSF tap test, a procedure that improves the diagnostic accuracy of iNPH and could predict a favorable response to CSF shunt surgery [33].

The local ethics committee approved the protocol of the study. All participants gave written informed consent to participate in the study.

## 2.2. CSF Tap Test

The iNPH patients underwent baseline evaluation of gait/balance and cognitive function within 24 h before the lumbar puncture. The baseline evaluation included: Short Physical Performance Battery [34], Mini-Mental State Examination [MMSE] [35], Frontal Assessment Battery [36], Phonemic Fluency Test, and Trail-making Test [37]. The lumbar puncture was performed at 9.00 a.m. with removal of 30–50 mL of CSF. The gait/balance assessment (Short Physical Performance Battery) was repeated 6, 24, and 48 h after the CSF subtraction. The neuropsychological examination (Phonemic Fluency test and Trail Making Test) was repeated 6, 24, and 48 h after the CSF subtraction.

## 2.3. CSF Biomarkers Analysis

The CSF samples collected by the lumbar puncture were immediately centrifuged and stored at  $-80\text{ }^{\circ}\text{C}$  until performing the analysis.  $A\beta_{42}$ ,  $A\beta_{42}/A\beta_{40}$  ratio, t-tau, and p-tau have been measured using a chemiluminescent enzyme immunoassay (CLEIA) analyzer LUMIPULSE G600 (Fujirebio, Tokyo, Japan). Cut-off values for CSF were determined by following Fujirebio guidelines (Diagnostic sensitivity and specificity using clinical diagnosis and follow-up golden standard, 19 November 2018 [38]):  $A\beta_{42} > 670\text{ pg/mL}$ ,  $A\beta_{42}/A\beta_{40}$  ratio  $> 0.062$ , t-tau  $< 400\text{ pg/mL}$  and p-tau  $< 60\text{ pg/mL}$ . Patients were rated as  $A\beta_{42}^{+}$  or  $A\beta_{42}^{-}$  and  $A\beta_{42}/A\beta_{40}^{+}$  and  $A\beta_{42}/A\beta_{40}^{-}$  if the  $A\beta_{42}$  and  $A\beta_{42}/A\beta_{40}$  were lower or higher than the cut-off values, respectively. Patients were rated as  $T^{+}$  or  $T^{-}$  and  $N^{+}$  or  $N^{-}$  if the CSF p-tau and t-tau concentrations were higher or lower than cut-off values, respectively [39].

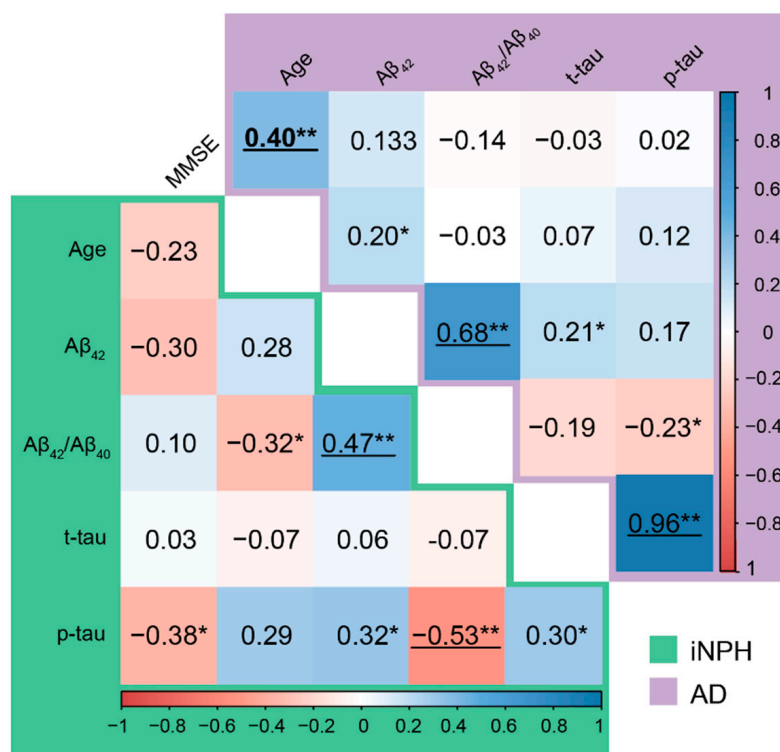
## 2.4. Statistical Analysis

Patient groups were characterized using means and standard deviations (SD). We tested for normality in the distribution of the data using the Kolmogorov–Smirnov test. Depending on the distribution of the data, we used *t*-tests or non-parametric Mann–Whitney *U* tests for between-groups comparisons and Pearson's *r* or Spearman's  $\rho$  for correlations. We used chi-square tests to compare categorical data. We calculated the size effect with Cohen's *d* for normally distributed numeric measures,  $\eta^2$  for Mann–Whitney *U* Test, and Cramer's *V* for categorical data. Receiver operating characteristic (ROC) analyses were performed to evaluate the ability of CSF biomarkers to distinguish between iNPH and AD. Youden's method was used to detect the best cut-off value and accuracy, sensitivity, and specificity. We used binomial logistic regression to ascertain the contribution of each biomarker in distinguishing iNPH and AD. Bonferroni correction was applied to correct for multiple comparisons. All statistical analyses were performed with SPSS software v.25 (SPSS Inc., Chicago, IL, USA) and the computing environment R 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria, 2013).

## 3. Results

### 3.1. Description of the Sample

At onset, all the patients in the iNPH group had balance/gait impairment, 33 (75.00%) had cognitive impairment, and 31 (70.45%) had urinary incontinence. Twenty-three patients (52.27%) had the complete triad, while three patients (6.82%) only had balance/gait impairment. Thirteen out of 44 iNPH patients (29.55%) experienced an improvement in their gait after the CSF tap test, while 31 patients (70.45%) did not. Figure 1 shows correlations among the demographic features and CSF biomarkers in the iNPH and AD groups (Figure 1). Both in AD and iNPH,  $A\beta_{42}/A\beta_{40}$  correlated with  $A\beta_{42}$  and p-tau correlated with t-tau. In the iNPH group  $A\beta_{42}/A\beta_{40}$  also correlated with p-tau. In AD patients, MMSE was significantly correlated with age.



**Figure 1.** Correlation matrix. Values quoted in the correlation matrix are Pearson’s *r* correlation coefficients. Statistical significance received a Bonferroni adjustment and was accepted at  $p < 0.01$  (significant correlations were reported as underlined characters). Color maps represent Pearson’s *r* correlation coefficients. \*  $p < 0.05$ , \*\*  $p < 0.01$ .

### 3.2. Comparison between iNPH and AD Groups

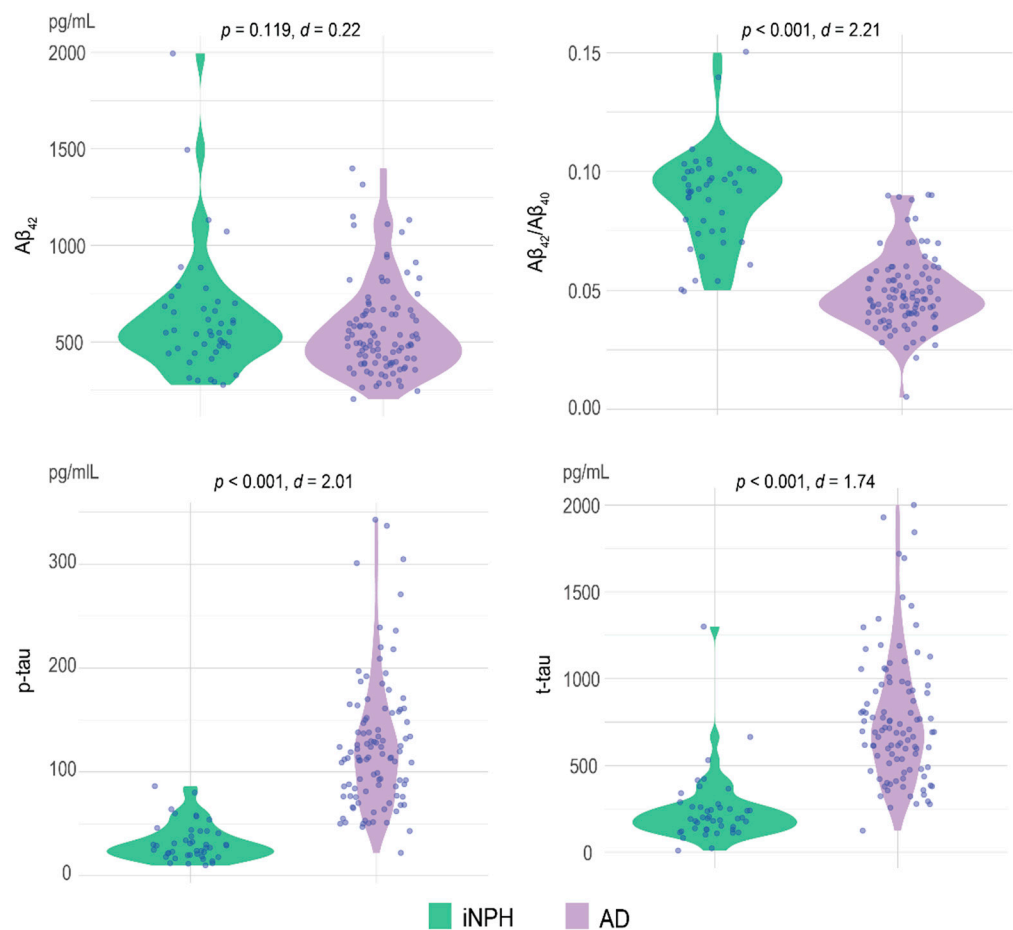
The iNPH patients were older than the AD ( $p = 0.044$ , Cohen’s  $d = -0.001$ ) and had a lower frequency of *APOE*  $\epsilon 4^+$  ( $\chi^2 = 5.74$ ,  $p = 0.017$ ), but these differences were not statistically significant when adjusted for multiple comparisons (accepted at  $p < 0.003$ ). There were no differences in years of education ( $p = 0.519$ , Cohen’s  $d = -0.135$ ). AD patients had lower mean MMSE scores than iNPH patients (24.25 [SD = 4.02] vs. 19.57 [SD = 4.85],  $p < 0.001$ , Cohen’s  $d = 1.05$ ). There were no differences in Aβ<sub>42</sub> concentration between iNPH and AD (624.89 [316.30.82] pg/mL vs. 563.49 [236.941] pg/mL,  $p = 0.119$ ,  $d = 0.22$ ), while the Aβ<sub>42</sub>/Aβ<sub>40</sub> ratios were significantly higher in iNPH than in AD (0.09 [0.02] vs. 0.04 [0.02],  $p < 0.001$ ,  $d = 2.21$ ). Patients in the iNPH group also had lower p-tau (32.13 [17.85] pg/mL vs. 125.52 [63.09] pg/mL,  $p < 0.001$ ,  $d = 2.01$ ) and t-tau (242.66 [205.59] pg/mL vs. 768.79 [374.22] pg/mL,  $p < 0.001$ ,  $d = 1.74$ ) concentrations than patients in the AD group (Table 1, Figure 2).

Thirty-one out of 44 iNPH patients and 79 out of 101 AD patients had positive Aβ<sub>42</sub> (CSF Aβ<sub>42</sub> concentrations below the cut-off value), with no difference in distribution between groups (70.45% [95% C.I. = 56.97:83.94] vs. 78.22% [95% C.I. 70.17:86.27],  $\chi^2 = 1.01$ ,  $p = 0.315$ ,  $V = 0.083$ ). On the opposite, proportions of a positive Aβ<sub>42</sub>/Aβ<sub>40</sub> ratio, (13.64% [95% C.I. = 3.50:23.78] vs. 93.07% [95% C.I. = 88.12:98.02],  $\chi^2 = 90.35$ ,  $p < 0.001$ ,  $V = 0.79$ ), p-tau (6.82% [95% C.I. = 0:14.27] vs. 88.12% [95% C.I. 81.81:94.43%],  $\chi^2 = 87.35$ ,  $p < 0.001$ ,  $V = 0.78$ ) and t-tau (11.36 [95% C.I. = 1.99:20.74] vs. 84.16% [95% C.I. = 77.04:91.28],  $\chi^2 = 68.98$ ,  $p < 0.001$ ,  $V = 0.69$ ) concentrations were significantly lower in the iNPH patients compared to the AD group (Table 1, Figure 3).

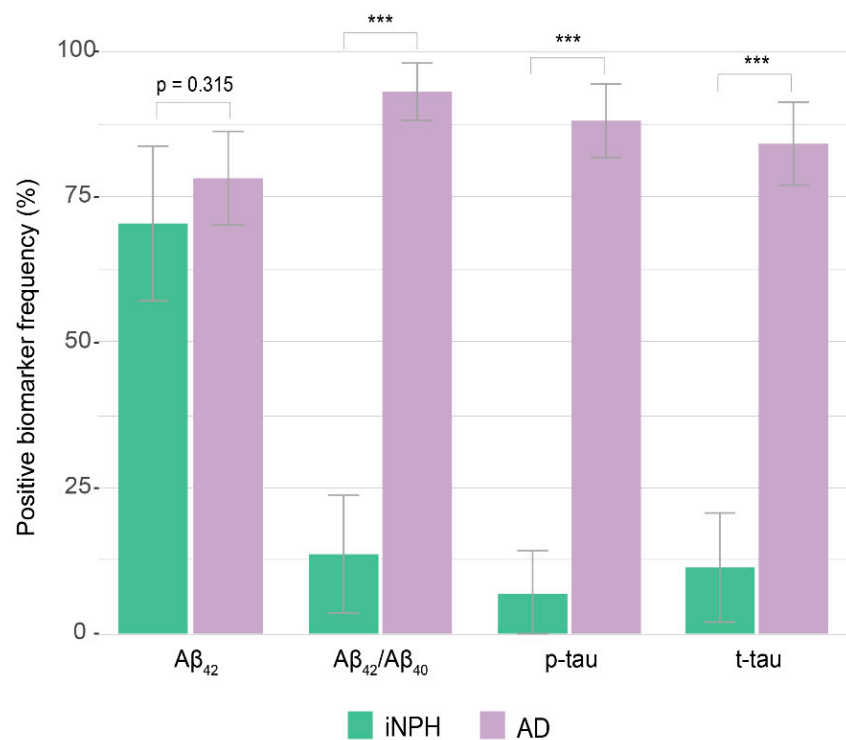
**Table 1.** Comparison of demographic variables and CSF biomarker values between iNPH and AD patients.

	iNPH	AD
N	44	101
Age, mean (SD)	73.92 (7.42)	71.28 (6.83)
Sex (women/men)	26/18	50/51
Years of education, mean (SD)	9.71 (3.94)	9.98 (4.21)
APOE ε4+, % (95 CI %)	24.14 (8.56:39.71)	49.45 (39.18:59.72)
MMSE, mean (SD)	24.25 (4.02) <sup>a</sup>	19.57 (4.85) <sup>a</sup>
Aβ <sub>42</sub> (pg/mL), mean (SD)	624.89 (316.30)	563.49 (236.94)
Aβ <sub>42</sub> /Aβ <sub>40</sub> , mean (SD)	0.09 (0.02) <sup>b</sup>	0.04 (0.02) <sup>b</sup>
p-tau (pg/mL), mean (SD)	32.13 (17.85) <sup>c</sup>	125.52 (63.09) <sup>c</sup>
t-tau (pg/mL), mean (SD)	242.66 (205.57) <sup>d</sup>	768.79 (374.22) <sup>d</sup>
Aβ <sub>42</sub> <sup>+</sup> , % (95% C.I.)	70.45 (56.97:83.94)	78.22 (70.17:86.27)
Aβ <sub>42</sub> /Aβ <sub>40</sub> <sup>+</sup> , % (95% C.I.)	13.64 (3.50:23.78) <sup>e</sup>	93.07 (88.12:98.02) <sup>e</sup>
T <sup>+</sup> , % (95% C.I.)	6.82 (0:14.27) <sup>f</sup>	88.12 (81.81:94.43) <sup>f</sup>
N <sup>+</sup> , % (95% C.I.)	11.36 (1.99:20.74) <sup>g</sup>	84.16 (77.04:91.28) <sup>g</sup>

Values quoted in the table are mean (±SD) or n (%). Statistical significance received a Bonferroni adjustment and was accepted at  $p < 0.003$ . <sup>a</sup>  $p < 0.001$ ,  $d = 1.05$ ; <sup>b</sup>  $p < 0.001$ ,  $d = 2.21$ ; <sup>c</sup>  $p < 0.001$ ,  $d = 2.01$ ; <sup>d</sup>  $p < 0.001$ ,  $d = 1.74$ ; <sup>e</sup>  $\chi^2 = 90.35$ ,  $p < 0.001$ ,  $V = 0.79$ ; <sup>f</sup>  $\chi^2 = 87.35$ ,  $p < 0.001$ ,  $V = 0.78$ ; <sup>g</sup>  $\chi^2 = 68.98$ ,  $p < 0.001$ ,  $V = 0.69$ .



**Figure 2.** Comparison of the CSF biomarker concentrations and Aβ<sub>42</sub>/Aβ<sub>40</sub> ratio between iNPH and AD. Values quoted on the y-axis indicate the CSF concentration (expressed as pg/mL) for Aβ<sub>42</sub>, p-tau, t-tau, and the value of the ratio for the Aβ<sub>42</sub>/Aβ<sub>40</sub>.  $p$ -values and Cohen's  $d$  are reported. Statistical significance received a Bonferroni adjustment and was accepted at  $p < 0.003$ .



**Figure 3.** Relative frequencies of positive Aβ<sub>42</sub>, Aβ<sub>42</sub>/Aβ<sub>40</sub>, p-tau, and t-tau in iNPH and AD. Statistical significance received a Bonferroni adjustment and was accepted at  $p < 0.003$ . \*\*\*  $p < 0.001$ .

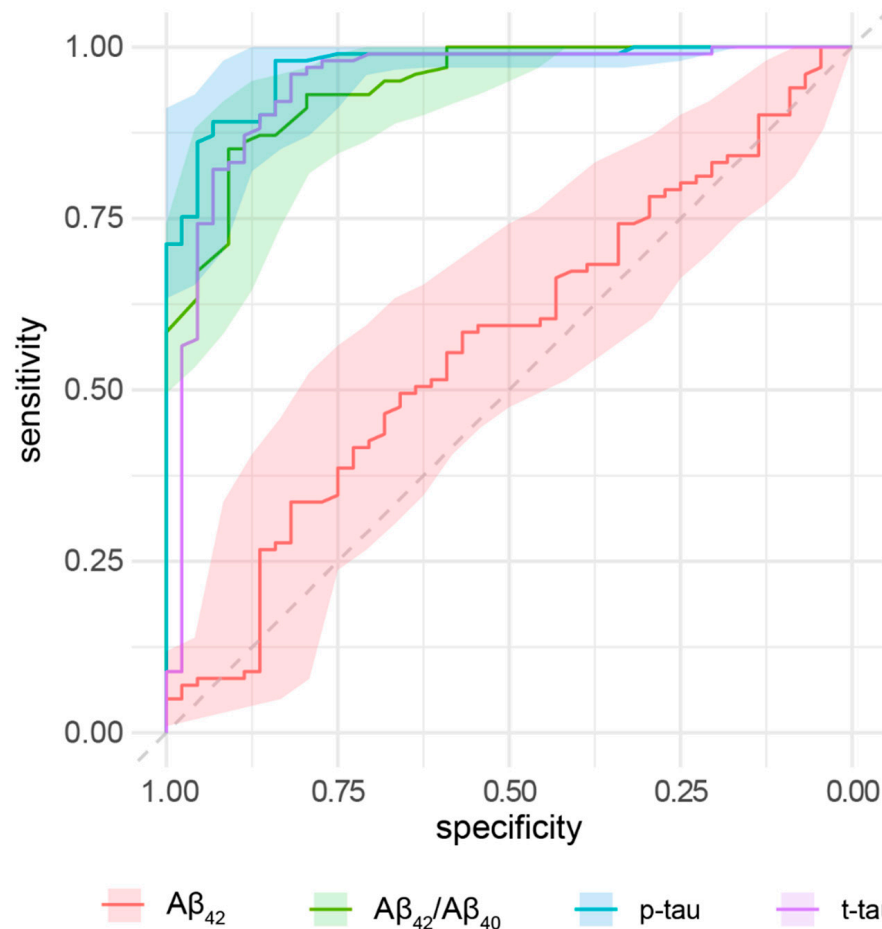
### 3.3. CSF Biomarkers Accuracy in Distinguishing between iNPH and AD

Table 2 summarizes the area under the curve (AUC), accuracy, sensitivity, and specificity of each CSF biomarker in distinguishing iNPH and AD patients. We identified the cut-off values by Youden’s method. The Aβ<sub>42</sub>/Aβ<sub>40</sub> ratio, p-tau, and t-tau showed very high accuracy without differences between the three biomarkers, as showed by the intersection of the 95% confidence intervals. Aβ<sub>42</sub> was not able to distinguish between iNPH and AD (Figure 4).

**Table 2.** CSF biomarker accuracy.

	Cut-Off	AUC	Accuracy, % (CI.95%)	Sensitivity, % (CI.95%)	Specificity, % (CI.95%)
Aβ <sub>42</sub>	776.34	0.567	64.14 (56.33:71.95)	84.16 (78.22:90.10)	18.18 (11.90:24.46)
Aβ <sub>42</sub> /Aβ <sub>40</sub>	0.068	0.943	86.21 (80.60:91.82)	87.13 (81.68:92.58)	84.09 (78.14:90.04)
p-tau	65.24	0.969	88.28 (83.04:93.52)	95.45 (92.06:98.84)	85.15 (79.36:90.94)
t-tau	509.09	0.941	80.69 (74.27:87.11)	93.18 (89.08:97.28)	75.25 (68.23:82.27)

Cut-off values were estimated by Youden’s method. Area under the curve (AUC), accuracy, sensitivity, and specificity for each biomarker are reported. Accuracy, sensitivity, and specificity are expressed as percentages (95% C.I.).



**Figure 4.** ROC curves for the accuracy of  $A\beta_{42}$ ,  $A\beta_{42}/A\beta_{40}$ , p-tau, and t-tau in distinguishing iNPH and AD. Colored shapes indicate 95% C.I.

### 3.4. Logistic Regression Models

To ascertain the effect of each biomarker in discriminating between iNPH and AD adjusting for age, we performed a multivariate logistic regression model including age and CSF biomarker concentrations as independent variables. The regression model was statistically significant ( $\chi^2 = 137.19$ ,  $p < 0.001$ ). The model explained 86.53% (Nagelkerke  $R^2$ ) of the variance in progression. The accuracy of the model in distinguishing between AD and iNPH was 93.79% (95% C.I. = 89.86:97.72) (sensitivity = 96.04% [95% C.I. = 92.87:99.21], specificity = 88.64% [95% C.I. = 83.47:93.81]). The  $A\beta_{42}/A\beta_{40}$  ratio was shown as the only variable which significantly contributed to the model, independent of confounding factors ( $B = -128.38$ , S.E. = 47.83,  $p = 0.007$ ) (Table 3).

We performed the same analysis considering CSF biomarkers after dichotomization according to cut-off values (Table 3). The regression model was statistically significant ( $\chi^2 = 128.67$ ,  $p < 0.001$ ). The model explained 83.21% (Nagelkerke  $R^2$ ) of the variance in progression. Age ( $B = -0.15$ , S.E. = 0.06,  $p = 0.013$ ),  $A\beta_{42}/A\beta_{40}$  ( $B = 4.37$ , S.E. = 1.06,  $p < 0.001$ ) and p-tau ( $B = 5.05$ , S.E. = 1.58,  $p = 0.001$ ) significantly contributed to the model (Table 3).

**Table 3.** Multivariate logistic regression models.

	<b>B</b>	<b>S.E.</b>	<b>p</b>	<b>OR</b>	<b>95% C.I.</b>	
					<b>Lower</b>	<b>Upper</b>
CSF biomarkers (quantitative values)						
Age	−0.18	0.08	0.024	0.83	0.71	0.98
Aβ <sub>42</sub>	0.01	0.00	0.081	1.00	0.99	1.01
<b>Aβ<sub>42</sub>/Aβ<sub>40</sub></b>	<b>−128.38</b>	<b>47.83</b>	<b>0.007</b>	<b>2.013 × 10<sup>−31</sup></b>	<b>3.44 × 10<sup>−97</sup></b>	<b>9.03 × 10<sup>−8</sup></b>
p-tau	0.04	0.03	0.193	1.04	0.98	1.09
t-tau	0.00	0.00	0.347	1.07	.99	1.01
χ <sup>2</sup> = 137.19, p < 0.001, Nagelkerke R <sup>2</sup> = 86.53%						
CSF biomarkers (dichotomized values)						
Age	−0.15	0.06	0.013	0.86	0.76	0.97
A (Aβ <sub>42</sub> <sup>+</sup> )	−2.06	1.21	0.092	0.13	0.01	1.35
<b>A (Aβ<sub>42</sub>/Aβ<sub>40</sub><sup>+</sup>)</b>	<b>4.37</b>	<b>1.06</b>	<b>&lt;0.001</b>	<b>79.20</b>	<b>9.89</b>	<b>643.36</b>
<b>T<sup>+</sup></b>	<b>5.05</b>	<b>1.58</b>	<b>0.001</b>	<b>155.78</b>	<b>7.09</b>	<b>3420.53</b>
N <sup>+</sup>	−1.28	1.46	0.381	0.278	0.02	4.86
χ <sup>2</sup> = 128.67, p < 0.001, Nagelkerke R <sup>2</sup> = 83.21%						

Regression Coefficients (B), Standard errors (S.E), p-value (p), Odds Ratio (OR), and 95% Confidence Intervals (95% C.I.) for covariates included in the logistic regression models are reported. Statistical significance received a Bonferroni adjustment and being accepted at the p < 0.01, highlighted in bold.

### 3.5. Comparison between iNPH and AD with Positive Aβ<sub>42</sub>

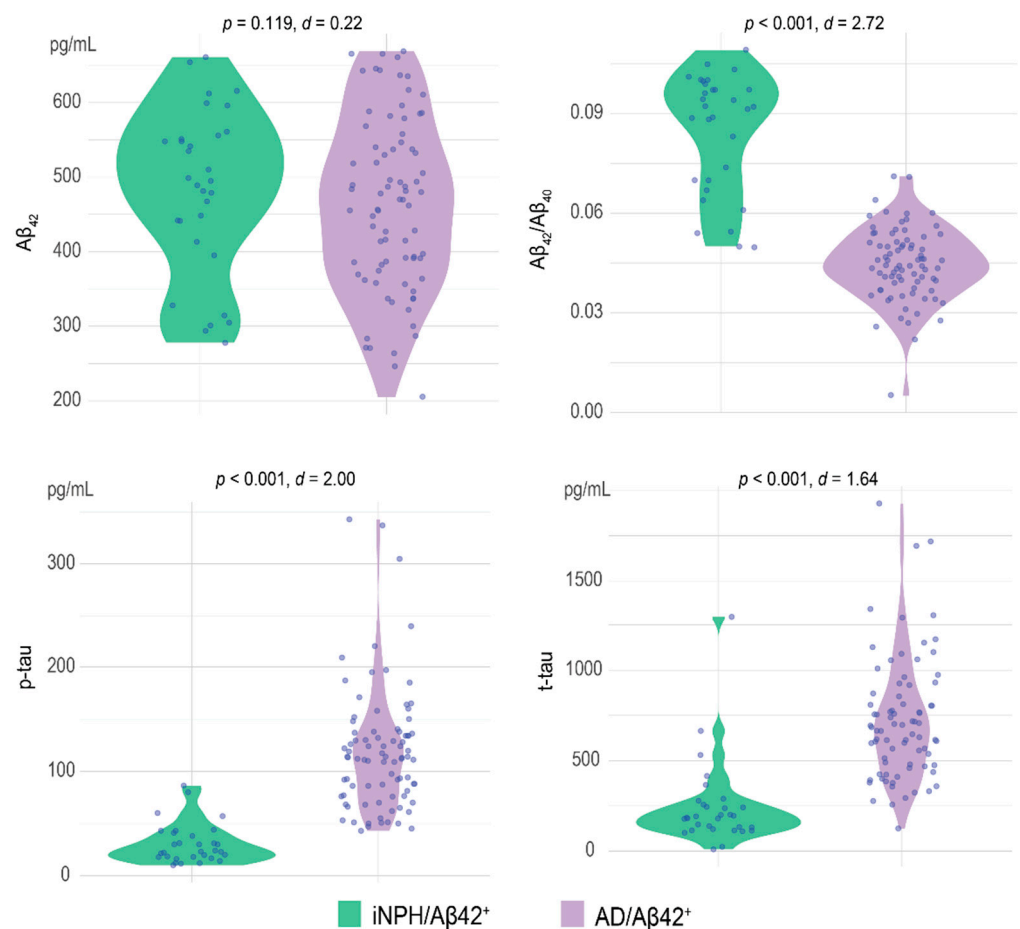
To explore the meaning of positive Aβ<sub>42</sub> in iNPH patients, we compared the AD and iNPH groups and considered only patients who had positive Aβ<sub>42</sub> (31 iNPH/Aβ<sub>42</sub><sup>+</sup> vs. 79 AD/Aβ<sub>42</sub><sup>+</sup>, Table 4). The iNPH/Aβ<sub>42</sub><sup>+</sup> had higher MMSE than AD/Aβ<sub>42</sub><sup>+</sup> (24.68 [3.43] vs. 19.53 [3.42], p < 0.001, d = 1.50). There was no difference in age. The lower frequency of APOE ε4 in iNPH than in AD/Aβ<sub>42</sub><sup>+</sup> patients was not statistically significant when adjusted for multiple comparison (23.81 [95% C.I. = 5.59:42.03], χ<sup>2</sup> = 4.52, p = 0.033, V = 0.22). The CSF biomarker concentrations (Aβ<sub>42</sub>/Aβ<sub>40</sub>, p-tau, and t-tau) were different between iNPH/Aβ<sub>42</sub><sup>+</sup> and AD/Aβ<sub>42</sub><sup>+</sup> (Figure 5). In particular among 31 iNPH/Aβ<sub>42</sub><sup>+</sup>, only six patients (19.35% [95% C.I. = 5.45:33.26]) had positive Aβ<sub>42</sub>/Aβ<sub>40</sub>, two patients (6.45% [95% C.I. = 0:15.10]) had positive p-tau, and four patients (12.90% [95% C.I. = 1.10:24.70]) had positive t-tau. In contrast, 76 out of 79 AD/Aβ<sub>42</sub><sup>+</sup> patients had positive Aβ<sub>42</sub>/Aβ<sub>40</sub> (96.20% [95% C.I. = 91.99:100]), 69 had positive p-tau (87.34% [95% C.I. = 80.01:94.67]), and 66 had positive t-tau (83.54% [95% C.I. = 75.37:91.72]).

**Table 4.** Comparison of demographic variables and CSF biomarker values between iNPH/Aβ<sub>42</sub><sup>+</sup> and AD/Aβ<sub>42</sub><sup>+</sup> patients.

	<b>iNPH/Aβ<sub>42</sub><sup>+</sup></b>	<b>AD/Aβ<sub>42</sub><sup>+</sup></b>
N	31	79
Age, mean (SD)	73.08 (8.24)	70.95 (6.97)
Sex (women/men)	19/12	37/42
Years of education, mean (SD)	9.73 (3.77)	10.21 (4.39)
APOE ε4 <sup>+</sup> , % (95 CI %)	23.81 (5.59:42.03)	50.00 (38.45:61.55)
MMSE, mean (SD)	24.68 (4.02) <sup>a</sup>	19.53 (3.42) <sup>a</sup>
Aβ <sub>42</sub> (pg/mL), mean (SD)	482.48 (110.44)	462.76 (116.18)
Aβ <sub>42</sub> /Aβ <sub>40</sub> , mean (SD)	0.08 (0.12) <sup>b</sup>	0.04 (0.01) <sup>b</sup>
p-tau (pg/mL), mean (SD)	30.44 (19.10) <sup>c</sup>	120.02 (60.25) <sup>c</sup>
t-tau (pg/mL), mean (SD)	244.74 (238.07) <sup>d</sup>	724.65 (338.935) <sup>d</sup>
Aβ <sub>42</sub> /Aβ <sub>40</sub> <sup>+</sup> , % (95% C.I.)	13.64 (3.50:23.78) <sup>e</sup>	93.07 (88.12:98.02) <sup>e</sup>
T <sup>+</sup> , % (95% C.I.)	6.82 (0:14.27) <sup>f</sup>	88.12 (81.81:94.43) <sup>f</sup>
N <sup>+</sup> , % (95% C.I.)	11.36 (1.99:20.74) <sup>g</sup>	84.16 (77.04:91.28) <sup>g</sup>

Values quoted in the table are mean (±SD) or n (%). Statistical significance received a Bonferroni adjustment and was accepted at p < 0.003). <sup>a</sup> p < 0.001, d = 1.50; <sup>b</sup> p < 0.001, d = 2.72; <sup>c</sup> p < 0.001, d = 2.00; <sup>d</sup> p < 0.001, d = 1.64; <sup>e</sup> χ<sup>2</sup> = 69.29, p < 0.001, V = 0.79; <sup>f</sup> χ<sup>2</sup> = 63.66, p < 0.001, V = 0.76; <sup>g</sup> χ<sup>2</sup> = 48.01, p < 0.001, V = 0.66.





**Figure 5.** Comparison of CSF biomarker concentrations and  $A\beta_{42}/A\beta_{40}$  ratio between iNPH/ $A\beta_{42}^+$  and AD/ $A\beta_{42}^+$ . The values quoted on the y-axis indicate the CSF concentration (expressed as pg/mL) for  $A\beta_{42}$ , p-tau, and t-tau.

### 3.6. Features of iNPH Patients Classified According to $A\beta_{42}$ and $A\beta_{42}/A\beta_{40}$ Status

We classified iNPH patients according to  $A\beta_{42}$  status (30  $A\beta_{42}^+$  and 14  $A\beta_{42}^-$ ). We found no differences in the demographic features and the  $A\beta_{42}/A\beta_{40}$ , p-tau, and t-tau values. *APOE*  $\epsilon 4$  allele was not associated with positive  $A\beta_{42}$  status. There were no differences in proportion of cognitive impairment and urinary incontinence between  $A\beta_{42}^+$  and  $A\beta_{42}^-$ . When we compared patients according to  $A\beta_{42}/A\beta_{40}$  status (6  $A\beta_{42}/A\beta_{40}^+$  and 38  $A\beta_{42}/A\beta_{40}^-$ , Table 5), patients with positive  $A\beta_{42}/A\beta_{40}$  had higher p-tau (59.46 pg/mL [23.74] vs. 27.47 [11.81],  $p < 0.001$ ,  $d = 1.87$ ) than  $A\beta_{42}/A\beta_{40}^-$  patients. Furthermore,  $A\beta_{42}/A\beta_{40}^+$  had higher frequencies of positive p-tau (33.33% [95% C.I. = 0:71.05] vs. 2.63% [C.I. 95% = 0:7.72,  $\chi^2 = 7.68$ ,  $p = 0.006$ ,  $V = 0.41$ ) and t-tau (50.00% [95% C.I. = 9.99:90.01] vs. 5.26% [C.I. 95% = 0:12.36,  $\chi^2 = 10.29$ ,  $p = 0.001$ ,  $V = 0.48$ ) as compared to  $A\beta_{42}/A\beta_{40}^-$ . Notably, all the patients in the  $A\beta_{42}/A\beta_{40}^+$  group had positive  $A\beta_{42}$ , while four had negative p-tau. Only one patient with positive p-tau had negative  $A\beta_{42}/A\beta_{40}$ . Urinary incontinence was more frequent in the  $A\beta_{42}/A\beta_{40}^-$  group (76.32% [95% C.I. = 62.80:89.83] vs. 33.33% [95% C.I. 4.39:71.05],  $\chi^2 = 4.56$ ,  $p = 0.032$ ,  $V = 0.323$ ), while there were no differences in cognitive impairment between  $A\beta_{42}/A\beta_{40}^+$  and  $A\beta_{42}/A\beta_{40}^-$ . Particularly in the  $A\beta_{42}/A\beta_{40}^+$  group, all the patients had cognitive impairment, but only two out of the six had urinary incontinence.

**Table 5.** Comparison of demographic variables and CSF biomarkers values between iNPH  $A\beta_{42}/A\beta_{40}^-$  and iNPH  $A\beta_{42}/A\beta_{40}^+$  groups.

	iNPH $A\beta_{42}/A\beta_{40}^-$	iNPH $A\beta_{42}/A\beta_{40}^+$
N (%)	38 (86.36%)	6 (13.64%)
Age, mean (SD)	73.36 (7.21)	77.61 (8.61)
Sex (women/men)	24/14	2/4
Years of education, mean (SD)	9.54 (3.94)	10.60 (4.28)
APOE $\epsilon 4^+$ , % (95 CI %)	20.00 (4.32:35.68)	50.00 (1.00:99.00)
MMSE, mean (SD)	24.52 (4.14)	22.80 (3.35)
$A\beta_{42}$ (pg/mL), mean (SD)	644.66 (335.17)	499.67 (85.63)
p-tau (pg/mL), mean (SD)	27.47 (11.82) <sup>a</sup>	59.46 (23.74) <sup>a</sup>
t-tau (pg/mL), mean (SD)	222.29 (201.57)	371.67 (199.11)
A ( $A\beta_{42}^+$ ), % (95% C.I.)	65.79 (50.71:80.87)	100
T <sup>+</sup> , % (95% C.I.)	2.63 (0:7.71) <sup>b</sup>	33.33 (0:71.05) <sup>b</sup>
N <sup>+</sup> , % (95% C.I.)	5.26 (0:12.36) <sup>c</sup>	50.00 (9.99:90.01) <sup>c</sup>
Cognitive impairment, % (95% C.I.)	71.05 (56.63:85.47)	100
Urinary incontinence, % (95% C.I.)	76.32 (62.80:89.83) <sup>d</sup>	33.33 (4.39:71.05) <sup>d</sup>
Response to CSF tap test, % (95% C.I.)	28.95 (14.53:43.37)	33.33 (0:71.05)

Values quoted in the table are mean ( $\pm$ SD) or *n* (%). Statistical significance received a Bonferroni adjustment and being accepted at the  $p < 0.004$ . <sup>a</sup>  $p < 0.001$ ,  $d = 1.87$ ; <sup>b</sup>  $\chi^2 = 7.68$ ,  $p = 0.006$ ,  $V = 0.41$ ; <sup>c</sup>  $\chi^2 = 10.29$ ,  $p = 0.001$ ,  $V = 0.48$ ; <sup>d</sup>  $\chi^2 = 4.56$ ,  $p = 0.032$ ,  $V = 0.323$ .

### 3.7. Association between Clinical Features and CSF Biomarkers

The iNPH patients who showed cognitive impairment had lower  $A\beta_{42}/A\beta_{40}$  (0.087 [0.022] vs. 0.097 [0.009],  $p = 0.036$ ,  $d = 0.61$ ) and higher p-tau (35.48 [18.00] vs. 22.08 [13.62],  $p = 0.029$ ,  $d = 0.84$ ) than patients without cognitive impairment at onset. CSF biomarker concentrations were not associated with urinary incontinence, nor with response to CSF tap test.

## 4. Discussion

Our first result confirmed that patients with iNPH have CSF  $A\beta_{42}$  concentrations comparable to AD and lower concentrations of p-tau and t-tau than AD, as widely described in previous studies [21,40–42]. In more detail, about 70% of patients in our series had positive CSF  $A\beta_{42}$ , while p-tau and t-tau were positive in 7% and 11% of patients, respectively, which is consistent with a previous report by Santangelo et al. [21]. In contrast, we showed that  $A\beta_{42}/A\beta_{40}$  was higher in iNPH than AD. Only 14% of iNPH patients had a  $A\beta_{42}/A\beta_{40}$  ratio in the pathologic range (below the cut-off value), compared to 93% in the AD group.

When we estimated the diagnostic values of each biomarker,  $A\beta_{42}/A\beta_{40}$ , p-tau, and t-tau had very good accuracy in distinguishing between iNPH and AD, with no significant differences between the biomarkers. The logistic regression analysis demonstrated that  $A\beta_{42}/A\beta_{40}$  and p-tau contributed to distinguishing iNPH patients from AD patients, in line with other works [43,44], with the notion that these biomarkers are more specific for AD than  $A\beta_{42}$  and t-tau [45,46]. Nevertheless, we would like to point out that, among iNPH patients with positive  $A\beta_{42}/A\beta_{40}$ , four had negative p-tau, while only one iNPH with positive p-tau had negative  $A\beta_{42}/A\beta_{40}$ . For the purposes of clinical practice, this remark mainly addresses to consider  $A\beta_{42}/A\beta_{40}$  to identify AD pathology in iNPH patients and to use p-tau as a support biomarker. Based on the evidence that p-tau and t-tau can be increased in iNPH in association with long disease duration [47] and poor response at the CSF tap test [48], other authors suggested that p-tau and t-tau biomarkers may be prognostic factors more than diagnostic tools [49]. We aim to test this hypothesis and clarify the role of p-tau and t-tau in further works with wider samples. Notably, the cut-off values identified in our sample by automatized method were consistent with the cut-off values adopted according to LUMIPULSE producer guidelines. This result suggests using

the same cut-off values adopted in clinical practice for the diagnosis of AD, as well as to distinguish iNPH from AD.

Even though several studies already showed that  $A\beta_{42}$  is lower in iNPH patients than in healthy controls, the discrepancy between  $A\beta_{42}$  and  $A\beta_{42}/A\beta_{40}$  was shown only by a few previous studies [43,50]. Our results confirm these reports and are in line with the evidence that in iNPH patients, the CSF levels of all the amyloid precursor protein (APP) fragments ( $A\beta_{38}$ ,  $A\beta_{40}$ ,  $A\beta_{42}$ , sAPP $\alpha$ , and sAPP $\beta$ ) are decreased compared to controls [11,12,30]. In contrast, the levels of  $A\beta_{42}$  in AD are associated with slightly increased or steady levels of  $A\beta_{40}$  [29]. Consequently, the ratio between  $A\beta_{42}$  and  $A\beta_{40}$  is supposed to be normal in iNPH patients without AD copathology, as reported by previous studies [43,50] and described in our sample. In particular, we showed that in 86% of iNPH patients with pathologic  $A\beta_{42}$ , the  $A\beta_{42}/A\beta_{40}$  ratio was normal, suggesting that in the majority of cases, the reduction of  $A\beta_{42}$  is associated with an equal reduction of  $A\beta_{40}$ . Furthermore, we showed that iNPH patients with positive  $A\beta_{42}$  had lower CSF p-tau and t-tau concentrations than AD patients. In particular, only four out of 31 iNPH patients with positive  $A\beta_{42}$  also had positive t-tau and two out of these patients had positive p-tau. This evidence may support the hypothesis that  $A\beta_{42}$  in iNPH patients does not indicate AD copathology.

The role of positive  $A\beta_{42}/A\beta_{40}$  in iNPH is more difficult to interpret. We found that iNPH patients with positive  $A\beta_{42}/A\beta_{40}$  had higher p-tau and t-tau concentrations than iNPH patients with negative  $A\beta_{42}/A\beta_{40}$ . In line with a previous result [43], we also found that iNPH patients who showed cognitive impairment had lower  $A\beta_{42}/A\beta_{40}$  and higher p-tau than patients without cognitive impairment. Moreover, 100% of iNPH patients with positive  $A\beta_{42}/A\beta_{40}$  also had positive  $A\beta_{42}$ , which might suggest that, in this group of patients, the low  $A\beta_{42}/A\beta_{40}$  ratio is associated with a higher reduction of  $A\beta_{42}$  than  $A\beta_{40}$ , as found in the AD patients [29]. Finally, we found that urinary incontinence was uncommon in iNPH with positive  $A\beta_{42}/A\beta_{40}$  (only two out of six), as most of the patients in this group showed only gait/balance disturbance and cognitive impairment, suggesting a possible misdiagnosis. Considering this evidence, positive  $A\beta_{42}/A\beta_{40}$  might indicate a coexistent AD pathology in patients affected by iNPH or a diagnosis of AD dementia.

This hypothesis is supported by many studies which demonstrated that the  $A\beta_{42}/A\beta_{40}$  ratio is more accurate than  $A\beta_{42}$  in identifying AD pathology [51,52].

If confirmed by further works, our results might suggest consideration of positive  $A\beta_{42}$  with negative  $A\beta_{42}/A\beta_{40}$  and p-tau as the typical CSF profile of iNPH. On the contrary, positive CSF  $A\beta_{42}/A\beta_{40}$  should lead to suspicion of an underlying AD pathology.

This study had some limitations: (i) the relatively small sample size, especially when we classified patients according to  $A\beta_{42}/A\beta_{40}$  status, which limits the impact of our conclusions; (ii) quantitative scores of gait/balance assessment are not available; (iii) data about gait/balance disturbance and urinary incontinence are not available for the AD group. However, we provided several pieces of evidence which may serve as starting points for future studies. As already stated, this is one of the first studies assessing  $A\beta_{42}/A\beta_{40}$  in differential diagnostics of iNPH. Moreover, despite the small sample size, this is the first study to have classified patients according to  $A\beta_{42}/A\beta_{40}$  status. Many studies divided iNPH patients according to their  $A\beta$  status, but did not distinguish between  $A\beta_{42}$  and  $A\beta_{42}/A\beta_{40}$ . As shown,  $A\beta_{42}$  could not be considered as an index of  $A\beta$  pathology in iNPH. On the other hand, considering only  $A\beta_{42}/A\beta_{40}$  allowed us to classify iNPH patients as carriers or non-carriers of  $A\beta$  pathology with a higher accuracy.

## 5. Conclusions

We showed that positive CSF  $A\beta_{42}$  with negative  $A\beta_{42}/A\beta_{40}$ , p-tau, and t-tau is a frequently encountered finding in iNPH and should be considered as a typical CSF profile of iNPH. On the other hand, positive  $A\beta_{42}/A\beta_{40}$  is uncommon in iNPH and is associated with a prevalent cognitive syndrome. Our results are in line with previous evidence and suggest that clinicians should not diagnose AD pathology in patients with iNPH

and isolated positive CSF A $\beta$ <sub>42</sub>. On the contrary, positive A $\beta$ <sub>42</sub>/A $\beta$ <sub>40</sub> in iNPH patients, especially when associated with positive p-tau, may lead to suspicion of a coexistent AD pathology or revision of the diagnosis of iNPH.

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