Correlations Between Mini-Mental State Examination Score, Cerebrospinal Fluid Biomarkers, and Pathology Observed in Brain Biopsies of Patients With Normal-Pressure Hydrocephalus

Adila Elobeid, MD, Katarina Laurell, MD, PhD, Kristina Giuliana Cesarini, MD, PhD, and Irina Alafuzoff, MD, PhD

Abstract
Alzheimer disease (AD)–related pathology was assessed in cortical biopsy samples of 111 patients with idiopathic normal-pressure hydrocephalus. Alzheimer disease hallmark lesions—β-amyloid (Aβ) and hyperphosphorylated tau (HPtau)—were observed in 47% of subjects, a percentage consistent with that for whole-brain assessment reported postmortem in unselected cohorts. Higher-immunostained area fraction of AD pathology correlated with lower preoperative mini-mental state examination scores. Concomitant Aβ and HPtau pathology, reminiscent of that observed in patients with AD, was observed in 22% of study subjects. There was a significant correlation between Aβ-immunostained area fraction in tissue and Aβ42 (42-amino-acid form of Aβ) in cerebrospinal fluid (CSF). Levels of Aβ42 were significantly lower in CSF in subjects with concomitant Aβ and HPtau pathology compared with subjects lacking pathology. Moreover, a significant correlation between HPtau-immunostained area fraction and HPtau in CSF was noted. Both HPtau and total tau were significantly higher in CSF in subjects with concomitant Aβ and HPtau pathology compared with subjects lacking pathology. The 42-amino-acid form of Aβ (Aβ42) and HPtau in CSF were the most significant predictors of the presence of AD pathology in cortical biopsies. Long-term follow-up studies are warranted to assess whether all patients with idiopathic normal-pressure hydrocephalus with AD pathology progress to AD and to determine the pathologic substrate of idiopathic normal-pressure hydrocephalus.

Key Words: Amyloid β, Cerebrospinal fluid biomarkers, Cognitive status, Hyperphosphorylated tau, Normal-pressure hydrocephalus.

INTRODUCTION
For diagnosis of suspected Alzheimer disease (AD), 3 primary approaches are currently implemented: clinical assessment (including, among others, mini-mental state examination [MMSE]), assessment of cerebrospinal fluid (CSF) biomarkers, and imaging (positron emission tomography when possible) (1–7). Assessment of brain biopsy samples taken during life is not performed in subjects with suspected AD.

In subjects with suspected idiopathic normal-pressure hydrocephalus (iNPH), a brain biopsy sample can be obtained during ventriculoperitoneal shunt operation (8–13). Idiopathic normal-pressure hydrocephalus, first described by Adams et al (14) in 1965, is a syndrome of the classical triad of gait disturbance, dementia, and urinary incontinence, along with ventricular enlargement (14–16). Currently, there are no definitive neuropathologic hallmark lesions characterizing this condition (11).

In suspected iNPH, the impairments characterizing the condition are potentially reversible after ventriculoperitoneal shunt operation (13, 17, 18). However, the lack of clinical improvement after shunting has been suggested to be caused by the existence of concomitant neurodegenerative pathology (10). Several reports have indeed indicated that, at shunting, a substantial number of subjects with iNPH displayed AD-related pathology in cortical biopsies (8–12).

Aggregation of Aβ42—the 42-amino-acid form of β-amyloid (Aβ)—in the neuropil and accumulation of hyperphosphorylated tau (HTau) in neurons are the primary hallmarks of AD (19–23). These lesions are readily visualized in brain tissue samples obtained at autopsy (23–25). In line with this, the core CSF biomarkers to be assessed in patients with AD are total tau, HPtau, and Aβ42 (26, 27). Clinical studies indicate that the values of these CSF biomarkers (Aβ42, total tau, and HPtau) reliably predict the conversion of mild cognitive impairment into AD, and subjects with the stable form of mild cognitive impairment are reliably identified (28, 29). Interestingly, AD/CSF biomarkers have also been used to predict surgical outcomes in patients with iNPH (30–33).

The objectives of the current study were to assess the incidence of AD-related alterations in CSF and cortical biopsy samples and to assess correlations between cognitive status assessed with MMSE, routinely used AD/CSF biomarkers, and neuropathologic hallmark lesions of AD observed in frontal cortical biopsy samples of an unselected cohort of subjects with a clinical diagnosis of iNPH. The latter was performed to determine whether the 3 methods applied here are comparable.
MATERIALS AND METHODS

During a 4-year period (2010–2013), the Pathology Department at Uppsala University Hospital (UUH) received frontal cortical biopsy samples from 163 subjects with a diagnosis of hydrocephalus (Table 1). One hundred forty-nine subjects were clinically diagnosed as having iNPH and consequently underwent a ventriculoperitoneal shunt operation at UUH. Obstructive and secondary hydrocephalus cases were not included in the present study (Table 1). Idiopathic normal-pressure hydrocephalus cases were assessed by a multidisciplinary iNPH team at UUH (including a neurologist, a neurosurgeon, a trained physiotherapist, and an occupational therapist) following standardized protocol. One hundred eleven subjects with complete clinical and laboratory data were needed for the present study (Table 1). The subjects gave their consent for the use of their tissue samples, and the study was authorized by the regional Ethics Committee of Uppsala, Sweden (2013/176).

Clinical Information

Clinical data included sex, age, and preoperative MMSE score (Table 1). Subjects were divided into 2 groups: unimpaired (i.e. lacking overt signs of cognitive impairment; subjects with MMSE scores ≥24) and demented (subjects with MMSE scores ≤23) (5).

CSF Analysis

Lumbar CSF samples were collected before surgery. Levels of Aβ42, total tau, and HPtau in CSF were assessed using commercial ELISA kits following the manufacturer’s protocol (Table 1).

Brain Samples

During shunt operation, biopsy samples from the frontal cortex were obtained using a disposable biopsy needle (Elekta Instrument AB, Innsbruck, Austria) (Fig. 1). Tissue samples were fixed in 10% formalin for 24 hours and embedded in paraffin. Consecutive 7-µm-thick paraffin wax–embedded sections were placed on SuperFrost Plus slides (Gerhard Menzel GmbH, Braunschweig, Germany) for further handling. The total area of needle biopsy (including gray matter and white matter) on a histologic slide measured approximately 20 to 30 mm².

Histology and Immunohistochemistry

We performed hematoxylin-and-eosin staining and immunohistochemistry using antibodies to Aβ protein (6F/3D, M0872; dilution 1:100, pretreatment with 80% formic acid for 1 hour; Dako, Glostrup, Denmark) and HPtau protein (AT8, MN1020; dilution 1:500; Thermo Scientific, Waltham, MA). Dako Autostainer plus was implemented and Dako EnVision FLEX detection system was used subsequently for visualization of staining results according to the manufacturer’s instructions. Sections were assessed using light microscopy.

Assessment Strategies

Semiquantitative assessment of Aβ aggregates and HPtau pathology was performed. Results were reported dichotomized (yes/no) and semiquantitatively in 3 levels: absent, sparse, or moderate/extensive (Table 2). Thereafter, we produced digital images of all slides with immunoreactivity (IR). Aperio image analysis (Leica Biosystems, Buffalo Grove, IL)–positive pixel count (version 9.1) was used for quantification of Aβ and HPtau load. The positive pixel count was applied selectively on the gray matter region in each slide. Total surface area of gray matter was measured in square millimeters. Gray matter area with IR (i.e. Aβ and HPtau positivity) was estimated in square millimeters. Results are reported as “immunostained area fraction” (i.e. area with IR / total gray matter area assessed × 100%).

TABLE 1. Selection and Characteristics of Patients Studied (n = 111)

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Patients With Hydrocephalus (N = 163)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Clinical Diagnosis of iNPH</td>
</tr>
<tr>
<td></td>
<td>Clinical, CSF, and Biopsy Data Available (n = 111)</td>
</tr>
<tr>
<td>Age at biopsy, mean (SE), years</td>
<td>75.7 (1.2)</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td>Male</td>
</tr>
<tr>
<td></td>
<td>Female</td>
</tr>
<tr>
<td>Preoperative MMSE score, median (SE), years</td>
<td>22.1 (1.5)</td>
</tr>
<tr>
<td>Subjects with MMSE scores ≥23, n (%)</td>
<td>58 (52)</td>
</tr>
<tr>
<td>Subjects with MMSE scores ≥24, n (%)</td>
<td>53 (48)</td>
</tr>
<tr>
<td>CSF Aβ42 (Aβ), mean (SE)</td>
<td>596.5 (22.7)</td>
</tr>
<tr>
<td>CSF HPtau, mean (SE)</td>
<td>34.5 (1.6)</td>
</tr>
<tr>
<td>CSF total tau, mean (SE)</td>
<td>233.1 (15.9)</td>
</tr>
<tr>
<td>Surface area of gray matter, mean (SE), mm²</td>
<td>9.5 (1.8)</td>
</tr>
<tr>
<td>Subjects with IR at biopsy dichotomized (+/-), n (%)</td>
<td>59.5 (53)</td>
</tr>
<tr>
<td>Aβ+/HPtau+</td>
<td>24 (22)</td>
</tr>
<tr>
<td>Aβ+/HPtau-</td>
<td>24 (22)</td>
</tr>
<tr>
<td>Aβ-/HPtau+</td>
<td>4 (3)</td>
</tr>
</tbody>
</table>

*Cases not analyzed in the present study.
(+/-) IR absent; (+) IR present; NA, not available.
IBM SPSS statistics (version 22) was used for statistical analyses. Mann-Whitney U test and Kruskal-Wallis H test with Bonferroni adjustment were used to determine significant differences between groups. For assessment of correlations, Pearson correlation coefficients were calculated. Logistic regression analysis was performed to ascertain the effects of age, sex, MMSE score, and AD/CSF biomarkers on the likelihood that subjects would display AD pathologic changes in cortical biopsies. Cerebrospinal fluid HPtau/A\textsubscript{42} cutoff values for differentiating between patients with cortical AD-related pathology and those without biopsy IR were determined by receiver operating characteristic analysis.

RESULTS

The study included 111 subjects (67 men and 44 women) with a mean (SE) age at surgery of 75.7 (1.2) years (Table 1).

Biopsy Findings

The mean surface area of the gray matter assessed was approximately 9.5 mm\textsuperscript{2}, representing approximately 38% of the entire biopsy sample. Representative photomicrographs of different biopsy samples with various types and extent of IR pathology are presented in Figure 2. Beta-amyloid aggregates were observed in 48 of 111 biopsies (44%), HPtau pathology was observed in 28 of 111 biopsies (25%), and concomitant Aβ and HPtau pathology was observed in 24 of 111 biopsies (22%) among patients with iNPH. Sparse Aβ-IR was observed in 19 subjects, and the extent corresponded with a mean (SE) immunostained area fraction of 0.34 (0.05). Moderate to severe Aβ-IR was observed in 29 subjects, and the extent corresponded with a mean (SE) immunostained area fraction of 3.37 (0.4) (Table 3). In 15 of 28 subjects with HPtau pathology, IR lesions were already observed at low (100×) magnification (moderate/severe). Beta-amyloid- and HPtau-immunostained area fractions correlated with semiquantitative measurements of Aβ and HPtau in cortical biopsies ($r = 0.7$, $p = 0.0001$ and $r = 0.6$, $p = 0.0001$, respectively). A significant correlation between Aβ- and HPtau-immunostained area fractions ($r = 0.5$, $p = 0.0001$) was also noted.

CSF and Biopsy Findings

Cerebrospinal fluid levels of Aβ42 and HPtau are summarized in Tables 1 to 3. We observed a significant difference in CSF AD biomarkers, including CSF HPtau/Aβ42 ratio,
TABLE 2

<table>
<thead>
<tr>
<th>Group</th>
<th>MMSE score ≥ 24</th>
<th>MMSE score ≤ 23</th>
<th>No IR</th>
<th>Aβ-IR</th>
<th>HPTau-IR</th>
<th>Aβ-HPtau/Aβ-IR</th>
<th>Aβ-HPtau/Aβ^+</th>
<th>HPTau-IR</th>
<th>HPTau/Aβ-IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Patients</td>
<td>53</td>
<td>59</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>12.2(2.7)</td>
<td>12.2(2.7)</td>
<td>16.28</td>
<td>2.04</td>
</tr>
<tr>
<td>Age, Mean (SE), Years</td>
<td>75.4(1.0)</td>
<td>75.2(1.0)</td>
<td>75.4(1.0)</td>
<td>75.2(1.0)</td>
<td>75.3(1.5)</td>
<td>75.3(1.5)</td>
<td>75.3(1.5)</td>
<td>75.3(1.5)</td>
<td>75.3(1.5)</td>
</tr>
<tr>
<td>Male/Female</td>
<td>62/38</td>
<td>59/41</td>
<td>62/38</td>
<td>62/38</td>
<td>59/41</td>
<td>12.2(2.7)</td>
<td>12.2(2.7)</td>
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<td>5</td>
<td>5</td>
<td>4</td>
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<td>12.2(2.7)</td>
<td>16.28</td>
<td>2.04</td>
</tr>
<tr>
<td>Age, Mean (SE), Years</td>
<td>75.6(0.9)</td>
<td>75.2(1.0)</td>
<td>75.6(0.9)</td>
<td>75.2(1.0)</td>
<td>75.5(1.1)</td>
<td>75.5(1.1)</td>
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<td>62/38</td>
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<td>16.28</td>
<td>2.04</td>
</tr>
<tr>
<td>Age, Mean (SE), Years</td>
<td>75.6(0.9)</td>
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<td>12.2(2.7)</td>
<td>16.28</td>
<td>2.04</td>
</tr>
</tbody>
</table>

MMSE Score and Other Assessed Variables

The mean (SE) preoperative MMSE score was 22.1 (0.5) (Table 1). There was no significant difference in MMSE score between subjects lacking AD-related IR and subjects displaying IR pathology (p = 0.07), or between subjects with sparse Aβ-IR pathology and subjects with moderate/severe Aβ-IR pathology (p = 0.06) (Tables 2, 3).

Fifty-eight of 111 subjects (52%) had MMSE scores of 23 or lower. Cerebrospinal fluid Aβ42 levels were significantly lower in these subjects compared with subjects with MMSE scores of 24 or higher (Mann-Whitney U test, p < 0.005). When all 111 subjects were included, there was a significant correlation between MMSE score and Aβ in CSF (r = 0.3, p = 0.001). When 53 subjects with MMSE scores of 24 or higher were included, the correlation between HPTau in CSF and age became stronger (r = 0.41, p = 0.002); the correlation was lost when 58 subjects with MMSE scores of 23 or lower were tested (r = 0.13, p = 0.34).

When all cases were included, a significant correlation between MMSE scores and Aβ- or HPTau-immunostained area fraction was noted (r = −0.23, p = 0.01 and r = −0.25, p = 0.01, respectively). There was a significant correlation between Aβ-immunostained area fraction and Aβ42 in CSF (r = −0.32, p = 0.001) and between HPTau-immunostained area fraction and HPTau in CSF (r = 0.32, p = 0.001) (Fig. 4). The correlation between Aβ-immunostained area fraction and Aβ42 in CSF (r = −0.34, p = 0.01) and between HPTau-immunostained area fraction and HPTau in CSF (r = 0.33, p = 0.02) became less significant when the 53 subjects with MMSE scores of 24 or higher were included in the analysis. By contrast, when the 58 subjects with MMSE scores of 23 or lower were included, the correlation between Aβ- and HPTau-immunostained area fraction became stronger (r = 0.6, p = 0.0001). The correlations between Aβ-immunostained area fraction and Aβ42 in CSF (r = −0.28, p = 0.04) and between HPTau-stained area fraction and HPTau in CSF (r = 0.4, p = 0.005) became less significant. The immunostained area fraction of Aβ and HPTau were lower but did not reach significant difference in subjects with MMSE scores of 24 or higher (mean [SE], 0.6 [0.2] and 0.2 [0.1], respectively) compared with subjects with MMSE scores of 23 or lower (mean [SE], 1.2 [0.3] and 1.2 [0.4], respectively).

Logistic Regression Analyses

Logistic regression analysis was performed to ascertain the effects of age, sex, MMSE score, and AD/CSF biomarkers...
on the likelihood that subjects would display AD pathologic changes in cortical biopsy. The logistic regression analysis showed that Aβ and HPtau in CSF (p = 0.003 and p = 0.004, respectively) were the most significant predictors of the presence of AD pathology in cortical biopsy. Increase in HPtau in CSF was associated with an increased likelihood of exhibiting AD-related pathology in cortical biopsy (odds ratio [OR], 1.091; confidence interval [CI], 1.03–1.16), whereas increase in Aβ in CSF was associated with a reduction in the likelihood of exhibiting AD-related neuropathologic changes at biopsy (OR, 0.99; CI, 0.99–0.99). The logistic regression model was significant ($\chi^2 = 30.73$, $p = 0.00$). The model explained 32% (Nagelkerke $R^2$) of the variance in IR in cortical biopsy and correctly classified 75% of cases.

Total tau in CSF (p = 0.08; OR, 0.99; CI, 0.99–1.00), sex (p = 0.23; OR, 1.7; CI, 0.7–4.29), MMSE score (p = 0.44; OR, 0.96; CI, 0.88–1.06), and age (p = 0.77; OR, 1.02; CI, 0.95–1.09) were not significant predictors of the presence of AD pathology in cortical biopsy.

Receiver operating characteristic analysis showed that the optimal CSF HPtau/Aβ42 cutoff value for identifying a patient with cortical AD-related pathology at biopsy (vs those lacking the pathology) was 0.06 (area under the curve, 0.75; sensitivity, 62%; specificity, 86%). A significant correlation between CSF HPtau/Aβ42 and MMSE score ($r = -0.4$, p = 0.001), Aβ-immunostained area fraction ($r = 0.35$, p = 0.0001), or HPtau-immunostained area fraction ($r = 0.35$, p = 0.0001) was observed (Fig. 5).

**DISCUSSION**

Assessment of AD-related CSF biomarkers and MMSE scores is commonly used when assessing patients with iNPH; thus, numerous reports have summarized these observations...
(30, 32–40). However, relatively fewer studies have reported brain pathology findings from cortical biopsy samples obtained during shunt surgery (8, 9, 31, 41–44). In the present study, a cohort of 111 subjects with iNPH (identified by the multidisciplinary iNPH team at UUH) was available. We assessed cognitive impairment using MMSE scores, AD/CSF biomarkers, and AD-related hallmark lesions in cortical biopsies assessed by immunohistochemical stains. This was performed to assess the incidence of AD-related alterations in iNPH and to investigate correlations between these 3 parameters in a clinical setting when assessing cognitive impairment.

We noted a significant correlation between age of patients and HPTau in CSF, a finding in line with a previous study by Miyajima et al (32) that included 46 patients with iNPH aged 60 to 87 years. Moreover, in line with several earlier reports, we did not find any significant correlation between age of subjects and CSF levels of Aβ42 (30, 32, 35, 38, 44).

Fifty-two of 111 study subjects (47%) displayed AD-related hallmark lesions in cortical biopsy. The observation that nearly 50% of patients with iNPH displayed AD-related hallmark lesions in cortical biopsy samples obtained during shunting is in line with some previously reported results that included 118, 56, or 182 patients with iNPH (8, 9, 42). A higher incidence (67%) and a lower incidence (25%) have also been reported (31, 41). The differing results are probably attributable to smaller sample sizes (i.e. 37 and 28 patients with iNPH, respectively) (31, 41). Another significant issue that should be taken into consideration is the small size of cortical brain samples. In general, these needle-biopsy samples measured only a few millimeters in size, thus representing only a small fraction of the brain tissue assessed while undergoing routine neuropathologic investigation. Here, we assessed 1 tiny cortical biopsy that is less than 10% of an ordinary tissue block compared with 16 to 17 (or more) tissue blocks obtained from different brain regions that are generally assessed for neuropathologic diagnosis.

Twenty-two percent (24 of 111) of the patients with iNPH assessed here displayed concomitant Aβ and HPTau-IR in cortical biopsy. This result is in line with the findings of Seppälä et al (42), who reported that 18% of their cases displayed concomitant Aβ and HPTau pathology in cortical biopsy. Later in 2014, Pyykkö et al (43) reported in a clinical follow-up study of 63 patients with iNPH that 16% of subjects developed AD; moreover, 50% of these subjects had shown concomitant Aβ and HPTau pathology in cortical biopsy samples obtained up to 6 years earlier during shunt operation. Thus, concomitant Aβ and HPTau pathology is observed in a subset of subjects with iNPH; up to 50% of these subjects seem to develop AD during follow-up. These previous reports suggested that around 10% of subjects in our cohort would eventually develop AD. Thus, follow-up studies are indeed warranted to investigate the long-term outcomes of AD development in a subset of iNPH. This indicates that neuropathologic assessment of brain biopsy samples obtained from subjects with suspected iNPH can be helpful in predicting their long-term outcomes.

In the current study, we observed a significant correlation between Aβ-immunostained area fraction and CSF/Aβ42 level. This result was confirmed by logistic regression analysis. In a study including 182 subjects with iNPH, where 55 subjects presented with available data needed, comparable results were reported (42). In line with previous reports, CSF/Aβ42 levels were significantly lower in our 24 subjects with concomitant Aβ and HPTau pathology compared with 59 subjects lacking IR in biopsies (42). This observation is in agreement with postmortem studies reporting a correlation between CSF/Aβ42 and AD-related pathology (i.e. Aβ and HPTau pathology) (45, 46).

We found a significant correlation between HPTau-immunostained area fraction and CSF/HPTau. This result was confirmed by logistic regression analysis. Both HPTau and total tau in CSF were significantly higher in subjects with concomitant Aβ and HPTau pathology in biopsies compared with subjects lacking IR pathology, in agreement with findings reported for postmortem studies of patients with AD (46, 47). Interestingly, we noted a significant difference in CSF HPTau/Aβ42 ratio between subjects with sparse Aβ-IR pathology and those with moderate/severe Aβ-IR pathology.
Furthermore, we also noted a near-significant difference (p = 0.06) in MMSE scores between subjects with sparse Aβ-IR pathology and those with moderate/severe Aβ-IR pathology. Thus, focal markers (biopsy findings) seem to be in line with global disease markers (CSF HPtau/Aβ42 ratio and MMSE scores).

Mini-mental state examination scores correlated with biopsy findings (i.e. lower MMSE scores corresponded with higher-immunostained area fraction of IR pathology). In line with this, Golomb et al (9) reported that patients with iNPH who displayed AD-related pathology in cortical biopsies were more cognitively impaired than those lacking AD-related pathology. When subjects with sparse Aβ-IR pathology were compared with those displaying moderate/severe Aβ-IR pathology, MMSE scores were lower in the latter group, but the difference did not reach statistical significance (p = 0.06).

Both iNPH and AD occur in the same age group, with similar complaints of memory impairment. In clinical practice, differential diagnosis for a subset of AD and iNPH is challenging. In 2003, Silverberg et al (48) even proposed that AD and iNPH are manifestations of CSF circulatory alteration (i.e. impaired CSF production and turnover). They suggested that AD develops when Aβ clearance is altered (causing accumulation of Aβ) and that iNPH develops when CSF outflow resistance predominates. They also added that a hybrid of AD and iNPH may exist in some subjects. Thus, the 2 disorders may represent 2 ends of the spectrum of circulatory failure (48). The observation of AD-related pathology in brain biopsies in patients with iNPH might also be explained by the age-related increase in the prevalence of Aβ and HPtau pathology in the brain of demented and nondemented subjects, which has been reported by several investigators (49–52).

FIGURE 3. Scatter plots of Aβ 1–42 (Aβ42) in CSF in relation to MMSE score (A), HPtau in CSF in relation to MMSE score (B), Aβ in CSF in relation to age (C), HPtau in CSF in relation to age (D), and Aβ42 in CSF in relation to HPtau in CSF (E). Correlation coefficients and p values were determined using Pearson correlation. Cases are identified by sex.
These observations are in line with the current study of patients with iNPH (Aβ in 44% and HPtau in 25%). Interestingly, cortical Aβ aggregates and cortical HPtau (Braak stages III–VI) were observed in 43% and 31% of unselected post-mortem materials, respectively, from 534 subjects who had been assessed during a more than 5-year period within the same catchment area (namely, UUH; unpublished data). These observations indicate that the incidence of cortical Aβ and HPtau in patients with iNPH seems to be in line with the age-matched unselected cohort. This observation and previous publications certainly support long-term follow-up studies of shunt-operated patients with iNPH.

One of the strengths of the current study is the considerably large cohort of patients with iNPH included, avoiding significant selection bias. Furthermore, each subject was assessed by a team of professionals following detailed standardized protocol.

An issue to be considered in this study is the limited size of the brain biopsy samples assessed, which represented only a small fraction of the whole brain. This might certainly lead to false-negative results. The frontal cortex is affected with Aβ at the early stages of AD (21, 25), whereas this region of the brain does not represent the most vulnerable region of neuronal degeneration; HPtau pathology is observed early on in subcortical regions and reaches the neocortex at Braak stages IV to VI (22, 23). The severity of cognitive impairment has been reported to correlate with burden and regional distribution of HPtau pathology; most subjects with dementia attributable to AD have reached Braak stages IV to VI (22, 23, 53). The incidence of AD-related neuropathologic changes in the brains of patients with iNPH is certainly underestimated because of the relatively small size of the tissue samples and the anatomic location of the biopsy (22, 23). Thus, a lack of pathology in this brain region does not exclude the presence of pathology in other more vulnerable brain regions (21–23). Another issue to be taken in consideration is the MMSE, which has been reported to have low sensitivity for detecting mild degrees of cognitive impairment (54); thus, some of our

![FIGURE 4. Scatter plots of Aβ-stained area fraction in cortical biopsies in relation to HPtau-immunostained area fraction in cortical biopsies (A), Aβ 1–42 (Aβ42) in CSF in relation to Aβ-stained area fraction in cortical biopsies (B), and HPtau levels in CSF in relation to HPtau-immunostained area fraction in cortical biopsies (C). (D) Error bar summarizing the distribution of the Aβ-immunostained area fraction within MMSE-based groups; each bar within each MMSE-based group is defined by sex distribution. (E) Error bar summarizing the distribution of HPtau-immunostained area fraction within MMSE-based groups; each bar within each MMSE group is defined by sex distribution. Mann-Whitney U test was used to determine significant differences between MMSE groups.](http://jnen.oxfordjournals.org/)

J Neuropathol Exp Neurol • Volume 74, Number 5, May 2015

AD Pathology in Normal-Pressure Hydrocephalus

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subjects with MMSE scores of 24 or higher might indeed have displayed mild cognitive impairment. To predict the existence of AD-related pathologic changes in the brain, we estimated that the optimal CSF HPTau/A42 cutoff value (applying receiver operating characteristic analysis) was 0.6. A similar study including 37 subjects with iNPH reported a value of 0.37 (31). This significant difference in results is probably attributable to differences in statistical methods (we used the Youden index), significant differences in sample size (this study: 111 vs 37 subjects), and selection bias (various percentages of subjects with AD-related pathology). In summary, assessment of AD pathology in patients with iNPH might be a useful diagnostic tool in explaining baseline cognitive scores and thus estimating the outcomes of cognitive status after shunt surgery. In line with this, previous reports suggested a noninvasive clinical assessment tool (i.e. positron emission tomography imaging) based on the reported significant association between Aβ in cortical biopsies and positron emission tomography imaging of Aβ (55).

In conclusion, our findings indicate that, when assessing subjects with iNPH, there is a significant correlation between MMSE scores, values of AD/CSF biomarkers, and existence of AD-related pathologic changes in frontal cortical biopsies. Beta-amyloid 1–42 and HPTau in CSF were the most significant predictors of the presence of AD-related pathology in cortical biopsies, and the HPTau/Aβ42 cutoff value for identifying patients with cortical AD pathology was 0.06. Furthermore, a significant difference in CSF HPTau/A42 cutoff value was noted when we compared subjects with sparse Aβ-IR pathology in cortical biopsy and subjects with moderate/severe Aβ-IR pathology in cortical biopsy.

An important observation here was the relatively similar incidence of cortical Aβ and HPTau pathology in patients with iNPH compared with an age-matched cohort of unselected autopsy cases. The results obtained from the assessment of focal tiny biopsy samples correlated with the observations made while assessing global alterations such as AD/CSF biomarkers. Based on this, patients with iNPH with AD-related pathology in cortical biopsy, particularly those with both Aβ and HPTau, would probably benefit from referral to specialized memory clinics for further clinical assessment and long-term follow-up studies, including postmortem evaluation. Furthermore, long-term follow-up studies of subjects with iNPH are certainly warranted to improve understanding of the substrate of this challenging disorder.

ACKNOWLEDGMENT

We thank the medical laboratory technologists Maud Salomonsson and Karin Ståxing for their skilled technical assistance, Statiscicon for assisting with statistical analyses, and Meena Strömquist for her critical reading of the manuscript.

REFERENCES


