

Amyloid- β and Hyperphosphorylated Tau in the Prediction of Alzheimer's Disease

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Abstract

Brain amyloid- β (A β) deposition is widely believed to be an early event in Alzheimer's disease (AD). A β plaques, along with intracellular neurofibrillary tangles, which largely comprise hyperphosphorylated tau (HP τ), are considered to be neuropathological hallmarks of AD. Brain biopsies from living subjects could be used to evaluate these findings. It is possible to obtain biopsies from patients with presumed normal pressure hydrocephalus during diagnostic or shunt procedures. The presence of both A β and HP τ in frontal cortex biopsy samples seems to be a strong indicator of AD with high specificity (98 %) but rather low sensitivity (36 %). A β alone is associated with AD and exhibits high sensitivity (87 %) but lower specificity (69 %), indicating that many patients with A β only may also develop clinical dementia other than AD. Although a definite diagnosis of AD can only be confirmed at autopsy, brain biopsies may be useful for identification of novel markers of AD in a research context. In addition, when available, brain biopsy can be a useful diagnostic aid and validate non-invasive techniques in the diagnosis of dementia.

Keywords

Brain biopsy, amyloid- β , hyperphosphorylated tau, immunohistochemistry, Alzheimer's disease, normal pressure hydrocephalus

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Neuropathological Findings of Alzheimer's Disease

Accumulation of amyloid- β (A β) together with hyperphosphorylated tau (HP τ) in the brain in predetermined neuroanatomical regions, are considered to be diagnostic hallmarks of Alzheimer's disease (AD).^{1,2} A neuropathological assessment of the brain at autopsy has been the only method available to confirm A β and HP τ deposits and reach the definite diagnosis of Alzheimer's disease.^{2,3} A β aggregation is first noted in the cerebral cortex,³ and HP τ is first seen in the olfactory bulb and hippocampal formation. Neurofibrillary tangles (HP τ) but not A β burden correlate with the degree of cognitive impairment and could be present even in cognitively unimpaired subjects.^{4,5} The association of neuropathological findings and clinical dementia is related with age so that the clinical significance is stronger in younger subjects.⁶

Criteria for the diagnosis of AD are evolving, with emphasis on early clinical recognition and multimodal evaluation using advanced imaging methods and biological markers.^{7,8} Clinical dementia of AD is usually preceded by deterioration of episodic memory, and a correct diagnosis at the mild cognitive impairment (prodromal AD) phase or even pre-clinically would lengthen the window for potential mechanism-based interventions to inhibit AD pathogenesis.^{9,10} Advanced magnetic resonance imaging (MRI) analyses^{11,12} and cerebrospinal fluid

(CSF) markers, including A β and tau protein isoforms^{13–15} are being validated as early diagnostic markers of AD. Positron emission tomography (PET) is another potential option for detecting brain pathology. Now followed by several other agents, Pittsburgh Compound-B (PIB) was the first potential tracer of A β depositions in the brain.^{16,17} and PIB uptake in PET has been shown to correlate with A β in preceding frontal cortical biopsy samples.¹⁸ Tau-specific imaging modalities are also coming. In the future the prediction of the risk of AD could ideally be performed in the initial phase of the pathological cascade, i.e. far before any clinical symptoms to optimise the effect of potential treatment modalities. Combined risk factor evaluation with biomarkers such as apolipoprotein E (APOE) genotype and CSF analysis could potentially predict the future AD with notable accuracy.¹⁰

Brain Biopsy in the Diagnosis of Dementia

Brain biopsy has hardly ever been included in the diagnosis of AD or other forms of dementia¹⁹ owing to lack of disease-modifying treatment and fear of complications. The predictive value of potential surrogate markers for AD in brain biopsy samples obtained at the prodromal phase were only scarcely verified¹⁹ until recently.²⁰ There are very few studies where the progression of degenerative changes seen in a brain biopsy obtained during life were followed up by an autopsy. Warren et al.¹⁹ summarised results of biopsy findings reported

in close to 20 publications including approximately 600 patients, most with dementia. Contrary to these 20 published reports on the brain biopsy taken during life, post mortem validations of these findings are sparse. Warren et al.¹⁹ reported that out of their own 90 consecutive cerebral biopsies obtained from patients with dementia, only 10 subjects had undergone post-mortem neuropathological examination. The agreement rate between biopsy and autopsy findings in these 10 cases was 40 %.

Normal Pressure Hydrocephalus – A Window for Brain Biopsy

Normal pressure hydrocephalus (NPH) is a damaging neurodegenerative disorder that can lead to dementia and typically presents with a clinical triad of symptoms including cognitive impairment, gait difficulty and urinary incontinence in various combinations.^{21,22} Brain imaging demonstrates dilated brain ventricles and obliterated cortical sulci. NPH may appear after brain insult such as subarachnoid haemorrhage, trauma or infection (secondary NPH [sNPH]) or without any known predisposing factors (idiopathic NPH [iNPH]).²¹ Contrary to other forms of dementia the symptoms can be alleviated by shunt surgery; however, the outcome of treatment varies considerably and is highly dependent on patient selection.²³⁻²⁵ Differentiation between NPH and AD can be difficult, especially when cognitive symptoms are atypical for AD, motor symptoms dominate and there is central atrophy in brain imaging. Furthermore, the differential diagnosis between NPH and vascular dementia (VaD) can be even more difficult.²⁶ Vascular changes in brain imaging are frequently seen concomitant pathology, similar to AD-related changes in brain biopsy.

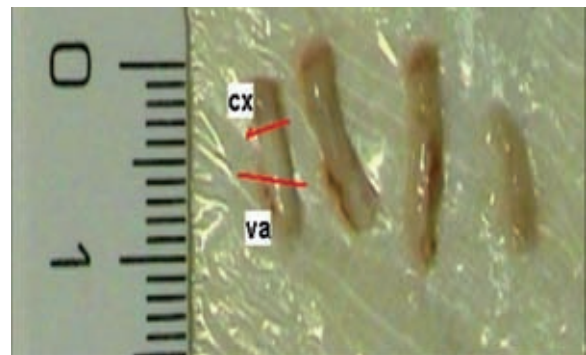
Currently, brain biopsy taken during shunt surgery or diagnostic procedures for NPH has only limited value in the differential diagnosis of NPH and VaD but offer an opportunity to assess the AD-related pathology in patients with signs of cognitive impairment or potential pre-clinical stage of AD. AD-related lesions are seen in up to 70 % of NPH patients depending on patient selection and staining methods used.

Since 1991 the diagnostic work-up of iNPH at Kuopio University Hospital (KUH) Neurosurgery Department has included sampling of a small right frontal cortical biopsy and 24-hour intracranial pressure (ICP) monitoring.^{18,20,27-29} Prior to placement of intra-ventricular catheter through a burr hole, cylindrical cortical brain biopsies were obtained with forceps (2–5 mm in diameter and 3–7 mm in length) and currently by standard disposable biopsy needle (biopsy dimensions 1–2 x 5–10 mm, see *Figure 1*). As of 2010 the KUH Neurosurgery NPH Registry (www.uef.fi/nph) kept records of over 600 consecutive patients evaluated for suspected NPH from a defined catchment population. The patients are actively followed-up and the clinical data are carefully re-evaluated by a neurologist sub-specialised in memory disorders to detect the development of cognitive impairment sufficient for the diagnosis of any form of dementia according to DSM-IV criteria.³⁰ The specific forms of dementia are diagnosed according to established criteria.³¹⁻³⁴

Brain Biopsy in the Diagnosis of Alzheimer's Disease

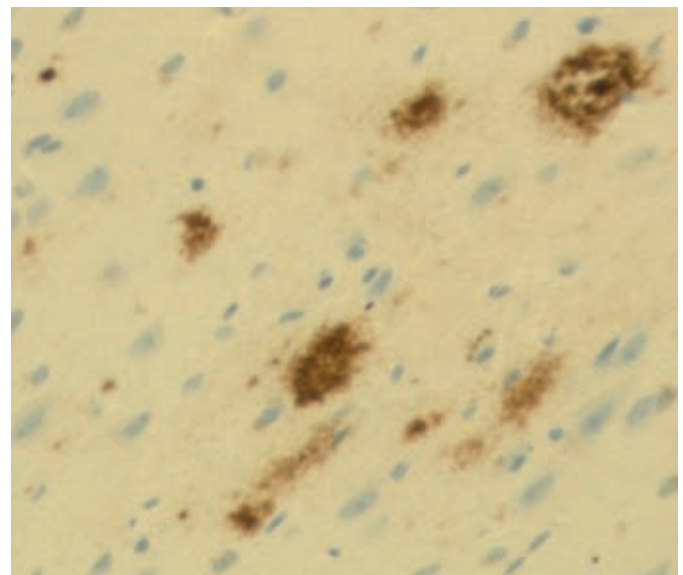
In our own recently published registry-based series²⁰ of 433 patients with immunostained frontal cortical samples, 42 had A β and HP τ , 144 had only A β and 247 had neither A β nor HP τ . Of the 433 patients with adequate follow-up data, 94 developed clinical AD during a median

Figure 1: Cylindrical Brain Biopsy Specimens Taken by 14 Gauge Biopsy Needle



CX = cortex, VA = white matter.

Figure 2: Typical Extracellular Amyloid- β Plaques, Antibody 6F3D, magnification x100



follow-up of 4.4 years (up to 17 years). According to multivariate logistic regression analysis A β together with HP τ (odd ratio [OR] 68) and A β alone (OR 11) were independent risk factors for AD. In the prediction of AD, A β together with HP τ was specific (98 %) but rather insensitive (36 %), while A β alone had lower specificity (69 %) but higher sensitivity (87 %). The predictive value was notably higher in patients who were ≤ 65 years of age. In the absence of A β and HP τ only one of the 77 patients developed AD.²⁰

Of the 433 biopsied patients, 219 subjects with apparent NPH according to ICP measurement received a shunt, and 26 of them also finally developed AD. This indicates that NPH and AD are basically different entities, but there is some overlap.²⁰ In addition, when the diagnostic set-up of the patient otherwise indicates NPH, the pathological findings of the biopsy should not be used as an exclusion criteria for shunt but taken into consideration during follow-up, especially if cognitive status deteriorates in spite of a shunt. After all, with the most severe AD-related pathology, NPH or at least a positive response to a shunt, is unlikely.^{20,35}

We made the final clinical diagnosis according to all available medical data before and after the biopsy including the final cognitive status. It

Figure 3: Intraneuronal Hyperphosphorylated Tau, Antibody AT8, Magnification x200

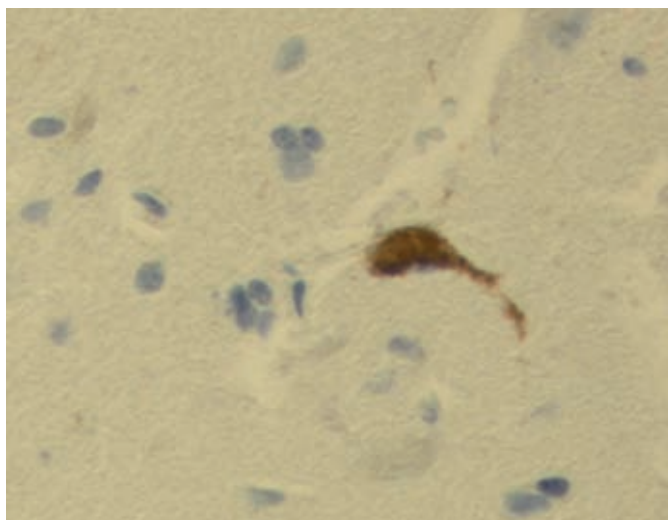
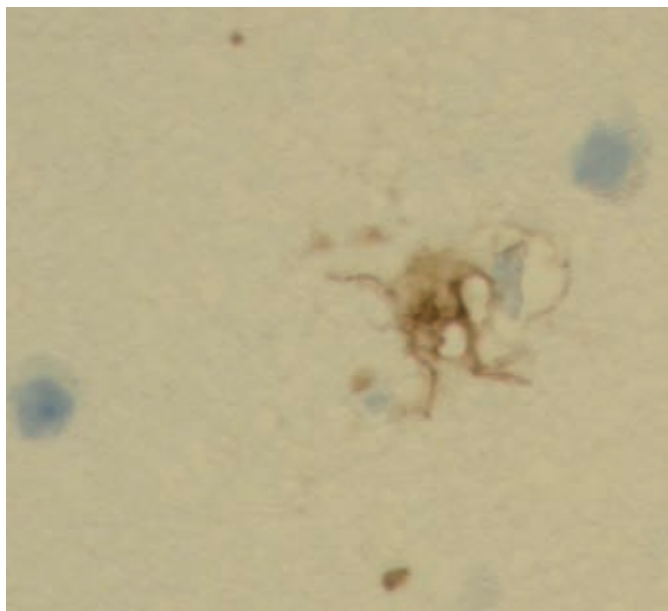


Figure 4: Positive Staining by Antibody p62, Magnification x400



should be noted that only a subset of patients with A β alone finally developed clinical AD. On the other hand, the median follow-up time was restricted to 4.4 years owing to substantial mortality and when the patients with clinical AD were excluded the A β alone or together with HP τ did not predict other types of dementia. These findings together support the concept of amyloid accumulation as preceding phenomenon of clinical AD.

A study using silver stain methods that are less sensitive than immunohistochemistry (IHC) techniques has reported that 35 of 56 patients (63 %) with cognitive impairment and suspected NPH displayed AD-related pathology in frontal brain biopsy, i.e., neuritic/diffuse plaques.³⁶ In another report 10 (26 %) out of 38 patients with NPH had AD-related tangles and plaques and 12 (43 %) of the 28 patients with adequate follow-up developed clinical AD.³⁷ These studies are not fully comparable owing to different patient selection criteria and methodology, but clearly indicate that AD-related

pathologies are frequent in patients with suspected NPH. In addition to these, vascular changes and non-specific findings such as gliosis and meningeal thickening, have been observed. It is noteworthy that by applying currently used techniques, no changes specific for NPH have been observed.

Despite the fact that biopsy samples are small in size and from only a single region of the brain, according to autopsy studies the frontal cortex is a fairly representative location to evaluate A β deposits in AD.⁴ However, HP τ depositions especially in the early phase may escape the small frontal cortical samples since they usually begin to accumulate in the medial temporal lobe and olfactory bulb. Still further studies correlating brain biopsy and post-mortem findings are needed to validate the clinical significance of the surgically obtained frontal cortical biopsy in diagnostics of dementia. From the 433 patients in our series, 253 died, but only 10 had had a full neuropathological examination of the brain. Three of these cases had displayed A β aggregates in the biopsy specimen and all of them displayed A β pathology later in the post mortem specimen. Interestingly, the final stage A β pathologies were quite prominent reaching Thal phases 4 to 5. Furthermore, all 10 cases displayed A β pathology in post mortem study and in two cases the A β pathology had reached Thal phase 3 even if nothing was seen at biopsy, although with nearly six years delay.³⁸

Methodological Aspects Biopsy Procedure

Standardised location for biopsy is essential. For detection of cortical AD pathology (A β) right (non-dominant) prefrontal area 2–3 cm from the midline in front of the coronal suture of the skull is considered to be ideal. This is also the standard puncture site of intraventricular catheters. Image guided (navigated) technique is preferable to optimise the site of interest if the standardised location is not used or lesion detectable by imaging methods is the primary target.

A standard 12 mm burr hole made under local anaesthesia and sedation allows large enough exposure to the cerebral cortex (an even smaller hole can be made with a biopsy needle). In case of a cortical vein in the site of the initial burr hole, an enlargement or even second burr hole might be needed. Avoidance of any injury to cortical veins is compulsory to avoid potential complications.

Biopsy can be performed using small forceps preferably with a small surgical (dura) knife avoiding the crushing of the tissue. The easiest way to obtain a standard biopsy is a cutting biopsy needle (at least 14 Gauge [G]) giving 1–2 x 5–10 mm cylindrical transcortical samples with underlying white matter (see *Figure 1*). When the primary indication of the surgical procedure is biopsy, e.g. in progressive dementia with an undefined origin, a larger sample is usually needed, by less invasive diagnostic methods .

Processing of the Sample

Ideally the fresh sample is transferred directly to the pathology laboratory with as short delay as possible (less than 60 minutes). Thus, the sample can be processed by a pathologist, divided into paraffin block and fresh frozen sample (cortex and white matter in different tubes). A formalin fixed (FF) sample alone is sufficient if only haematoxylin-eosin (HE), silver and IHC methods are going to be used. For special research purposes immediately (e.g. in an operating theatre) frozen samples might be considered.

Immunohistochemical Staining

Extracellular accumulation of A β could be divided into fleecy, diffuse and dense plaques (see *Figure 2*). For A β , several antibodies binding to different epitopes are available (e.g. 6F3D, M0872, Dako; dilution 1:100; pre-treatment 80 % formic acid for one hour). Non-specific labelling, especially if seen intracellularly, should be excluded using several antibodies if needed.³⁹ Fibrillar A β can be detected by congo red or thioflavin-S. Silver staining can be used for comparison of historical studies.

Intraneuronal hyperphosphorylated tau (HP τ , see *Figure 3*) can be stained by AT8 (3Br-3, Innogenetics; dilution 1:30). For differential diagnosis, p62 (3/p62 lck ligand, BD Bioscience) seems to be a sensitive marker of neurodegeneration (see *Figure 4*). In cases where brain biopsy is the primary indication for surgery in the diagnosis of dementia, see updated review by Schott et al. 2010⁴⁰ for an optimised protocol.

Histological Evaluation

The stained sections are primarily evaluated under light microscopy by a neuropathologist. Cellular or neuritic HP τ and fleecy, diffuse and dense A β aggregates are sought for within the whole sample and graded at least as present or absent.⁴¹⁻⁴³ Written expression of the findings is useful for clinical use. Semi-quantification (plaque count and/or automated analysis of the percentage area of pathological protein accumulation in the sample) can be useful, especially for research purposes.

Pathology Report

For clinical use, a pathology report should be prepared in relation to the patient's age and symptoms. The clinical picture should always be the leading point and the diagnosis of AD should never be stated solely based on biopsy findings. Fleecy A β without HP τ in a patient >80 years of age without notable cognitive symptoms can be in normal limits but a similar finding in those <60 years of age even without any cognitive symptoms may indicate a notable risk of future AD. Dense A β plaques with HP τ in neurons may confirm the diagnosis of AD if amnesic cognitive symptoms are present or indicate almost inevitable future AD.

Procedure-related Risks

In our own series of 468 patients,²⁰ the management mortality rate up to 12 months associated with the biopsy and 24-hour ICP monitoring was 0.6 % (three of 468). Two patients developed intracerebral haematoma and died at two and eight months, respectively. One case of fatal ischaemic stroke developed during ICP monitoring. One patient developed post-operative bacterial meningitis (0.2 %) but did not

deteriorate clinically. Importantly, these complications were not directly related with biopsy itself and could have happened without it. However, complication rates of over 10 % are reported, particularly when rather large samples including leptomeninges, cortex and white matter had been resected.¹⁹ In addition to haemorrhage and infection, seizures have also been reported.¹⁹

Future Perspectives

Clinical dementia of AD is usually preceded by deterioration of episodic memory, and a correct diagnosis at the mild cognitive impairment (prodromal AD) phase or even pre-clinically would lengthen the window for potential mechanism-based interventions to inhibit AD pathogenesis.^{9,10} Although the first Phase III trials of anti-amyloid agents have failed to indicate any clinical effect,⁴⁴ several potential agents, such as A β or tau aggregation inhibitors, A β vaccination or anti-A β antibodies, γ -secretase modulators, microtubule stabilisers and mitochondrial stabilisers, are under investigation.⁴⁵

The new biologically targeted therapies emphasise the early and specific diagnosis.⁴⁶ Brain biopsy is a potential option in the investigation of dementia when a specific diagnosis cannot be made by standard non-invasive means,¹⁹ and it can be used to validate less invasive markers.¹⁸ When available, brain biopsy may allow earlier diagnosis in specific cases and help in the stratification of the patients according to the inclusion and exclusion criteria of future clinical trials, i.e. known A β status when planning a treatment strategy affecting A β production and/or metabolism.²⁰ The sensitivity and specificity of A β and tau presented²⁰ are under the threshold requested to be considered biomarkers, and lower than sensitivity and specificity of the same proteins in cerebrospinal fluid. In that study the patient population was not originally typical subjects with suspected AD, instead it was a heterogeneous sample of various neurodegenerative disorders.

Imaging-targeted brain biopsies of a few millimetres can be obtained from non-eloquent areas with minimal risk of complications through a burr hole, under local or general anaesthesia, using a stereotactic frame temporarily fixed to the skull or non-rigid neuronavigational needle biopsy systems. Brain biopsy in early AD may open a research window to study the pathobiology of AD. Also the obtained data from biopsy and CSF samples may introduce new potential surrogate markers for AD.

In conclusion, the concomitant presence of A β and HP τ strongly indicate the presence or potential later development of clinical AD, the absence of A β and HP τ nearly exclude AD and the presence of A β alone indicates a notable risk of AD but should not be considered as inevitable AD. ■

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