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## Title Page

Title : The standardization of cerebrospinal fluid markers and neuropathological diagnoses brings to light the frequent complexity of concomitant pathology in Alzheimer's disease : the next challenge for biochemical markers?

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## Abstract

During the last two decades, neuropathological examination of the brain has evolved both technically and scientifically. The increasing use of immunohistochemistry to detect protein aggregates paralleled a better understanding of neuroanatomical progression of protein deposition. As a consequence, an international effort was achieved to standardize hyperphosphorylated-Tau (phospho-TAU),  $\beta$ Amyloid (A $\beta$ ), alpha syncuclein (alpha-syn), phosphorylated transactive response DNA-binding protein 43 (phospho-TDP43) and vascular pathology detection. Meanwhile harmonized staging systems emerged in order to increase inter rater reproducibility. Therefore, a refined definition of Alzheimer's disease was recommended., a clearer picture of the neuropathological lesions diversity emerged secondarily to the systematic assessment of concomitant pathology highlighting finally a low rate of pure AD pathology.. This brings new challenges to laboratory medicine in the field of cerebrospinal fluid (CSF) markers of Alzheimer's disease : how to further validate total Tau, phospho-TAU, A $\beta$ 40 and A $\beta$ 42 and new marker level cut-offs while autopsy rates are declining?

# Article

## 1. Introduction

During the last decade, tremendous improvements of neuropathological diagnosis took place thanks to an international combining both a standardization of diagnostic techniques and harmonization of diagnostic criteria. Many studies integrated a new paradigm, namely inter-rater agreement in a multi-centric setting, this issue being a major focus of BrainNet Europe network since 2004 and 2015. The progression of protein aggregates in degenerative diseases is now well described leading to a better systematic evaluation of comorbidity in degenerative diseases including vascular diseases. Despite this, only few autopsy cohorts were financed worldwide. The decline of autopsy rates and as a consequence the decline of clinical, biological and pathological correlation is a fact. Many clinical trials of Alzheimer's disease (AD) failed but that didn't limit the decline of autopsy rates. Moreover, few new markers were integrated in neuropathological diagnosis that still relies principally on the detection of protein aggregates. Now that cerebrospinal fluid (CSF) markers of AD (total Tau, phospho-TAU, A $\beta$ 40 and A $\beta$ 42) achieved a good standardization thanks to thorough studies of pre-analytical and analytical confounding factors, laboratory medicine faces a new challenge : how to further validate CSF markers in patients with concomitant pathologies while autopsy rates are declining. Lessons learned from the input provided by 10 years of systematic biopsy in neuro-oncology showed the benefit of a systematic pathological assessment of lesions and raise the question of the potential role of surgical biopsy in degenerative diseases diagnosis.

## 2. Post-mortem examination of brain tissue : from clinical quality insurance to research

Post-mortem examination of the brain is at the interface between neurology, clinical chemistry, radiology and neuropathology. On one hand it can provide a complete assessment of frequent, well described brain pathologies. Through a complete neuropathological report, clinicians, biochemists, and radiologists will have an additional objective set of data to make further correlations with their own observations. On the other hand, it preserves tissues and stores them for future research. This gives new opportunities to researchers to assess how their findings from more fundamental models apply to human disease and uncover more subtle physiopathogenic

changes than protein aggregates. Despite the decline of autopsy rates many centers or networks of brain banks such as Brain Net Europe Consortium managed to structure harmonized regulations and sampling. In order to make tissues of the highest quality available across countries and let researchers publish results compliant with every country and every type of journal ethical regulations [1-5].

Similarly to other kinds of tissue banking, extrinsic quality criteria such as freezing interval time was first examined. Studies from post-mortem material in a multi centric setting among BrainNet Europe consortium or from individual center found out that additional extrinsic criteria specific to brain bank could be useful such as post-mortem delay, length and severity of agonal state, agonal hypoxia, type of freezing procedure, or pH of the brain at time of sampling. However, the misconception that post-mortem material is not a good fit for biochemical research is still common[6,7].

Many studies provided clues that DNA and RNA quality remained high and suitable for genetic studies including microarray technology[8,9].

The specificity of brain tissue, the different physiopathological processes ongoing in aging patients, the influence of neuronal loss and pre analytical events challenged existing reference genes commonly used. Multiple centric micro-array studies found out new reference genes, more robust to this peculiar model such as XPNPEP1 and AARS [10,11]

Different kinds of freezing procedures were tested and their influence on the quality and yields of molecules of interest as well as on morphological characteristics was assessed[5]. Therefore, post-mortem brain material has already proven to be an effective model for extensive fields of research even though, specific targets such as microRNAs stability need further validation for individual projects. An interesting, understated strategy for that kind of validation would be to simulate post-mortem delay in laboratory settings as suggested in previous post-mortem RNA and protein degradation studies[6,12]. Besides tissue quality criteria, the quality of neuropathological annotations, i.e., the quality of neuropathological assessment of the brain is of the highest importance.

### 3. Toward a standardized assessment of post-mortem brain

During the last two decades, the concept of propagation of misfolded proteins such as hyperphosphorylated Tau (phosphoTAU),  $\beta$ -amyloid (A $\beta$ ), alpha-synuclein (alpha-syn), and phosphorylated transactive response DNA-binding protein 43 (phosphoTDP43) was extensively implemented in neuropathological diagnosis. Indeed, the hypothesis that protein deposits progressed in a predictable pattern first emerged in 1961 from observations of Lewy bodies in Parkinson's disease. A more precise description of aggregation patterns came from the usage of immunohistochemistry (IHC) with specific primary antibodies[13,14]

At the beginning of the first period, poor inter rater agreements were observed in the assessment of mainstream lesions such as A $\beta$  plaques and phospho-TAU neurofibrillary tangles (NFTs). This situation urged the neuropathologists' community to improve the reliability of techniques used, re-evaluate recommendations for sampling and grading scheme in order to reach the highest standards as possible[15,16].

#### 3.1. Technical improvement in the detection of elementary lesions was the first major step.

Indeed, many assessment strategies relied on unspecific histochemical stains such as Gallyas, Bielschowsky, alkaline Congo red, crystal violet method and thioflavin S and / or immunohistochemical techniques as recommended by former NIA-Reagan or CERAD (Consortium to Establish a Registry for Alzheimer's Disease) previous criteria, using various histochemical staining methods, primary antibodies, pre-treatments and staining procedures[13,17]. It appeared that immunohistochemical techniques were the most sensitive. and provided a higher level of quality insurance[15]. In order to cope with technical methods heterogeneity used among neuropathology centers and their consequences on neuropathological assessment, Tissue Micro Array (TMA) based strategies were used. These TMA consisted in a standardized operation based on embedding in single blocks many samples of various patients from different centers . Sections from these blocks were stained using each center methods.. Their assessment by multiple trained neuropathologists produced an extensive amount of data from which analysis methods (combination of fixation / staining or antibody / pretreatment) that provided the best results were determined[15].

In addition, studies showed that higher levels of reproducibility among centers were obtained with immunohistochemistry techniques using specific antibodies against selected epitopes and staining methods for phospho-TAU, A $\beta$ , alpha-syn, TDP43, phospho-TDP43, ubiquitin, and p62. With the automation of immunohistochemistry, these techniques are now even easier to ensure quality than histochemistry staining[15,18-20]

Further interests in pre analytical influence on immunohistochemical lesion detection showed that the fixation time of brain tissue and the fixation method itself could heavily influence the sensitivity of immunohistochemical techniques. Brain hemispheres are often fixed for 30 days. Some centers store slices in formalin with sampling paraffin blocks for future studies. A systematic early sampling of blocks and thorough traceability of fixation process are of course highly recommended. However, it has also been shown that antigen retrieval methods greatly influenced the results on long term stored samples in formalin. The benefit of 80% formic acid combined or not with heat in pH 6 or pH9 buffer as antigen retrieval method was established, not only for  $\beta$ -amyloid detection, but also for Ubiquitin, p62 and alpha syn. In some cases no acceptable results were obtained on material after long term storage in formalin using antibodies to 3 and 4 repeat isoforms of Tau as well as clone KM51 antibody to alpha-syn. Interestingly, new antibodies, more robust to fixation time have been efficiently developed such as 5G4 clone against alpha syn[21,22].

### 3.2. Degenerative diseases staging

Degenerative disease staging for each kind of protein deposit is based on complex but well defined progression patterns across brain areas. Improving the reliability of staging strategies implied a standardization of sampling for any neuropathological investigation of the brain, regardless of the clinical context.

#### 3.2.1. Harmonization of brain sampling

Selected anatomical areas included regions displaying specific protein deposits or pathological changes that captured the highest percentage of cases for each stage of any given degenerative disease. Optimally, these anatomical areas had to be recognizable under microscope. This minimal set of regions should be assessed in each brain that is sampled in a brain bank independently of the clinical presentation or the age of the subject [16,23,24]. Furthermore, the

recent evolution of the understanding of vascular contributions to cognitive impairment led the neuropathologists' community to draw guidelines for a more systematic assessment of cardiovascular pathology. Indeed, cardiovascular pathology tends to affect selected brain regions even without any macroscopic change and a systematic microscopic assessment was required. Hence, a minimal set of anatomical areas sampling and techniques to address this specific issue had to be defined[24-27]

In the setting of brain banking, a common strategy is to randomly select half a brain for frozen storage while the other hemisphere is dissected for neuropathological diagnosis. This could be a limitation on the evaluation of certain asymmetric diseases. Thus, minimal neurological clinical data should be taken into account to avoid selection bias and underevaluation of neuropathological lesions. Asymmetry of lesion load is often obvious in vascular pathology. However it has also been shown to happen in degenerative pathology such as phosphoTDP43, Tau and Fus deposits in the behavioral variant of fronto-temporal dementia[28].

#### 3.2.2. Improvement of grading schemes and evaluation of their reliability

Once the best techniques to detect protein aggregates were determined, difficulties to apply previous staging schemes were evidenced. For example, the dramatic improvement of A $\beta$  detection after optimized pre-treatment using formic acid combined with anti A $\beta$  4G8 clone showed how numerous diffuse A $\beta$  plaques were and how difficult the distinction between a diffuse plaque from a neuritic plaque cut at its periphery was. Any attempt to apply semi quantitative assessments such as the one recommended by former CERAD for neuritic A $\beta$  plaques led to poor inter-rater agreements[17,29].

Similar observations were done applying consensus guidelines of Parkinson's disease (McKeith staging and Braak staging), dementia with Lewy-bodies (DLB) and multiple system atrophy (MSA), semiquantitative rating of alpha-syn positive structures led to poorer inter-rater agreements than a dichotomized assessment of their presence or absence in a determined area. Therefore, future assessment methodology of most common protein alterations had to take into account that limitation. BrainNet Europe reported that a reliable assessment of the extent of alpha-syn protein

alteration could be carried out following acknowledged consensus criteria after exclusion of semiquantitative rating from McKeith and Braak staging[19,20,30,31].

phosphoTAU neurofibrillary tangles (NFTs) neuroanatomical progression was first described using 2 large hemisphere 100 $\mu$ m sections at the level of anterior hippocampus and at the level of striate area and peristriate region. Further studies showed that the evaluation of the progression of NFTs' pathology could be achieved using 7 $\mu$ m thick sections of 4 selected areas embedded in standard pathology blocks: occipital cortex including calcarine fissure; temporal cortex including middle temporal gyrus and at least a part of superior temporal gyrus; anterior hippocampus at the level of uncus and posterior hippocampus at the level of lateral geniculate nucleus. This 6 step staging system proved to be reliable even in an inter laboratory setting. [13,32,33]. This set of samples also allows the identification of less common tauopathies with associated phospho-Tau glial cell pathology. Of course, their assessment requires anti phospho-Tau staining of additional regions such as striatum and thalamus. The use of antibodies specific of 3 repeat (3R) or 4 repeat (4R) Tau isoforms gives additional information about the underlying pathology, i.e. AD (a mix of 3R and 4R pTAU) being biochemically distinguishable from other forms of fronto temporal dementias: Pick Disease (PD) (with 3R phospho-TAU only), progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD) and argyrophilic grain disease, (AGD) (with 4R phospho-Tau only)[34,35].

The assessment of A $\beta$  was based on quantitative or semi-quantitative counts of neuritic plaques in selected cortical areas. Specific antibodies to A $\beta$  were developed as early as neuritic plaques were described in 1985. However, histochemical silver stains remained widely used. In 2000, the description of the sequential deposition of plaques throughout the central nervous system as proposed in Thal & Braak staging challenged previous staging systems. Inter laboratory trials showed that dichotomized assessments of regional distribution of lesions brought the highest levels of agreement as compared to quantitative or semi-quantitative approaches using 6 paraffin blocks (frontal cortex, occipital cortex, hippocampus, basal forebrain, midbrain and cerebellum). A similar approach was successfully established for vascular amyloid angiopathy using the same samples[36-38].

Alphas-synuclein pathology neuroanatomical progression along pigmented nuclei and cortices was suggested in the mid 20th century. Therefore, the two classifications commonly used (Mc Keith

staging in the late 90's and Braak staging in the early 2000) implemented neuroanatomical based staging of the disease, from its first stages in medulla oblongata to cortex. In inter-laboratory settings, similarly to A $\beta$  evaluation, regional dichotomized assessment proved to be much more robust than the semiquantitative approach[14,30,39,40].

Before 2006, ubiquitin-positive, phospho-Tau and alpha-syn negative inclusions were the hallmarks of frontotemporal lobar degeneration (FTLD) with ubiquitin-positive inclusions and amyotrophic lateral sclerosis (ALS). In 2006, it was discovered that a large subset of these patients had a common alteration of Transactive DNA-Binding Protein 43 (TDP43) expression that was associated to a hyperphosphorylation and ubiquitination. This common pathologic process linked these two diseases. The detection of cytoplasmic perikaryal inclusions or long aggregates in dendrites according to McKenzie / Neumann staging was shown to be efficiently detected in inter-laboratory trials from BrainNet Europe[20,41]. However, distinct patterns of TDP43 sequential progression were shown in large series of patients with either FLTD or ALS[42-44]. In AD, pTDP43 was found and a 5-step-classification was proposed before it evolved to a VI-step staging system after evaluation of more regions[45,46].

### 3.2.3. Consequences on International recommendations on AD staging

The systematic use and the standardization of IHC techniques combined to the strength brought by dichotomized assessment of the topographical distribution of lesions led to establish new diagnostic criteria of AD as recommended by the 2012 National Institute of Aging and Alzheimer's Association. The novelty of this staging system was to combine the information of phospho-Tau neuritic process density (B), A $\beta$  neuritic plaques distribution (A) and density (C) in a common, synthetic ABC score. This system provides 4 levels of probability that observed AD related pathology was the cause of a patient's dementia : None, Low, Intermediate and High (Table 1)[47,48].

### 3.2.4. Consequences on aging-related patterns recognition

Tau-related pathology in aging brain has now been extensively described. Interestingly both neuronal and glial patterns were identified. Primary Age-Related Tauopathy (PART) is defined by neuronal phospho-TAU positive NFTs pathology with a Braak distribution from stage I to IV but

lacking concomitant A $\beta$  pathology[49]. Aging-related tau astroglipathy (ARTAG) is a term that refers to a morphological spectrum of astroglial pathology detected by anti Tau immunohistochemistry, especially with anti-Tau phosphorylation-dependent and 4R isoform-specific antibodies. ARTAG is generally observed in patients over 60 years old. Anatomical distribution of ARTAG extends from subpial and subependymal location to perivascular areas, subcortical white matter and gray matter[50-54].

A systematic assessment of cognitively unimpaired patient showed that additional immunoreactivity for phospho-TDP43, alpha-syn and A $\beta$  was detected in a proportion of patients that increased with age : from 24% at 60 year old to 32% at 80-89 years old[52].

#### 4. What to expect from post-mortem neuropathological examination of AD and related degenerative disorders

##### 4.1. Data collected and Conclusion of the report

Neuropathological reports have now evolved to a systematic assessment of the extent of lesions for each kind of common pathogenic process. Morphological features are revealed by the combination of a minimal set of histochemical staining with an increasingly large panel of IHC techniques. General pathology changes such as cardiovascular pathology are best evaluated using hematein & eosin (HE) with saffron (HES), Masson's Trichrome stain and periodic acid schiff reagent (PAS). Myelin is commonly assessed using luxol fast blue (LFB). Bielschowsky and Gallyas Silver stains tend to be replaced by the use of specific immunostaining against phospho-Tau, R3 and R4 phospho-TAU isoforms, A $\beta$ , alpha-syn, p62, phospho-TDP43, prion protein and ubiquitin. Additional optional immunohistochemical staining may be prescribed after a first evaluation on the basis of neuropathological lesions observed, such as glial markers (GFAP, Aquaporin 4, Olig2...), neuronal markers NeuN, NeuF, SMI32..), microglial cell activation and macrophages (CD68, CD163), other immune cells staining (CD3, CD20..). Hence, the neuropathology report is getting increasingly complex and may be a source of misunderstanding between neuropathologists and medical doctors or scientists that are not familiar enough with pathology terms or clones/antibodies terminology.

As a result of the extensive improvement of staging systems described above, the overall findings of the neuropathological assessment of each brain could be conveniently summarized in tables

such as the one suggested in table n°2. This would help neurologists, biochemists and radiologists in charge of a patient, understand at first glance the level of complexity and the load of the pathology at time of death. It would help them understand eventual atypical aspects of the evolution, response to treatment, CSF biomarkers results or radiologic changes. *In fine*, this better understanding of the pathology would help them give clearer explanations to the family about the patient's disease.

As far as post-mortem neuropathology report would also serve as brain bank sample annotation, this kind of summarized information would also be helpful to import directly staining scores in the brain bank databases. This will minimize the risk of errors or misunderstandings from biobank staff in charge of those tasks, who may not have required training to fully understand a common neuropathology report.

#### 4.2. Better evaluation of comorbidity in AD

The systematic evaluation of concomitant pathology using more precise and standardized criteria has been extensively applied in autopsy series. This gave rise to comparable studies that converged to the same result: pure AD is seen in a minority of cases. Most commonly found comorbidities with AD are phospho-TDP43 pathology, alpha-syn pathology and vascular pathologies. Interestingly, those figures are prone to change because of a better sampling and a wider systematic assessment of these lesions such as phospho-TDP43 pathology associated with AD[45,46].

AD can be associated with alpha-syn pathology in 16 to 25% of cases. Observations describe alterations that resemble lewy bodies (LBs) or more pleomorphic cytoplasmic inclusions. Two patterns of alpha-syn deposits are seen : cases with deposits following Braak / Mc Keith neuro anatomical progression and cases with pathology restricted to amygdala (24%) without brainstem pathology (amygdala predominant pathology). However, the clinical significance of amygdala predominant cases remains unknown : indeed, studies with detailed clinical follow up data available did not show further differences for extra pyramidal signs between these patients and pure AD patients [55-58]. AD and phospho-TDP43 pathology can be associated in 30% to 57% of brains. As previously described the evaluation of phospho-TDP43 pathology strategy has faced

major changes. From the rough idea of having 2 distinct patterns of phospho-TDP43 deposits called Limbic (limited to the entorhinal cortex and dentate gyrus of hippocampus) and Diffuse (widespread and involves occipito-temporal Cortex and inferior temporal gyri), a more subtle description of phospho-TDP43 is now accepted as described above. phospho-TDP43 pathology load increases with age, correlates with cognitive decline and coexists with hippocampal sclerosis[45,46,59].

Finally, AD is associated to vascular pathology in up to 44% of brains with AD. Vascular pathology divides in small-vessel disease (28%), severe leukoencephalopathy (12%), severe amyloid angiopathy (10%) and large-vessel infarct (2%) [24,26,58,60]. Hence in every day routine diagnosis, comorbidity is at risk of increasing variations in clinical course, cognitive decline, CSF marker values and radiological picture [61].

#### 5. Cerebrospinal fluid (CSF) markers in AD

A major challenge for neurologists is to identify the cause of cognitive impairment as early as possible. This is a fundamental step of any therapeutic strategy to prevent or slow down the course of the disease. CSF markers have become a mainstream tool to explore the potential pathogeny of patients with mild cognitive impairment (MCI). The determination of total Tau (t-Tau), Tau phosphorylated at threonine 181 (phospho-Tau) and  $\beta$ -amyloid peptides (A $\beta$ 42 A $\beta$ 40 has now become a major tool for this purpose, these markers being considered as core biomarkers of AD. A $\beta$  peptides are cleaved from the amyloid precursor protein (APP) by  $\beta$ - and  $\gamma$ -secretase enzymes and these peptides present multiple forms, ranging from 15 to 16 to up to 43 amino acids in length . A $\beta$  can be detected in the CSF at high concentrations, the most abundant being A $\beta$ 40 (80%). . Hence, measuring A $\beta$ 40 peptide reflects the total amyloid load. A $\beta$ 42 appears essential to initiate A $\beta$  aggregation, and is considered central to the amyloid cascade hypothesis of AD [62].

Similarly, phospho-Tau (included in NFTs) is also detectable in CSF and reflects both the formation and spreading of NFTs in the brain[63-66].

CSF AD biomarkers correlate with cortical brain biopsy findings. CSF A $\beta$  42 decreases result mainly from a reduction of its cerebral clearance. Consequently, its reduction was considered to reflect A $\beta$ 42 deposition in plaques. It is likely, however, that other pathological processes will also

lead to lower CSF A $\beta$ 42 levels. Indeed, low concentrations of CSF A $\beta$ 42 were also detected in Creutzfeldt-Jakob disease (CJD) patients without amyloid plaques at autopsy, in patients with various neurological infectious diseases (neuroborreliosis, bacterial meningitis and opportunistic infections in HIV), in amyloid angiopathy or in patients with normal pressure hydrocephaly. However those different conditions do not alter similarly A $\beta$ 42 and A $\beta$ 40 peptides. Hence, dosing both peptides in CSF to calculate the A $\beta$ 42/A $\beta$ 40 ratio seems to bring more specific informations regarding AD status[65,67,68]. In addition, the amyloidopathy observed with positive electron tomography (PET) was better correlated to CSF A $\beta$ 42/A $\beta$ 40 ratio than to A $\beta$ 42 alone[69].

However, many studies showed high variations in the values measured from one center to another and even in single centers. This variability in diagnosis accuracy was as high as 2 to 3 folds between the lowest and highest cut-off values in inter-laboratory settings across Europe. This variability was mainly reported to pre-analytical confounders and to analytical variability. Indeed, traumatic sampling with more than 5000 red cells/ $\mu$ L and a proteinorachia > 2.5 g/L induces significant variations (>10%) on total Tau (t-Tau) and A $\beta$ 42 values. The different tubes used to collect CSF show also high variations in their ability to adsorb A $\beta$  peptides. The comparison of different tube references in-inter laboratory settings showed a dramatic reduction of the coefficient of variation (CV) between laboratories when using the same tube with low adsorption. Studies of additional potential pre-analytical cofounders didn't prove any significant effect concerning patients' fasting, the time of sampling, CSF gradient and the diameter of the needle. Among major recommendations for the management of pre-analytical cofounders, a thorough tracking of each step is recommended using a form successively filed by neurologists, local laboratory staff (from the patient's hospital) and from the central laboratory staff in charge of the CSF A $\beta$  and Tau analyses[70-75].

Analytical variability can be assessed through external quality assessment programs in inter-laboratory settings. It appeared that ELISA assay providers themselves might be a cause of variations between centers. More precisely, differences of antibodies provided within the kit, differences in calibrators and calibration models, compliance of users to kit instructions led to establish reference methods that could be controlled using certified reference material (CRM). The

automation of ELISA processes also led to important decreases in within-run variability from 6% to 3% (own center, mono centric data)[74,76-78].

In the context of the BIOMARKPD (JPND) consortium, the collaboration between academic and industrial teams resulted in a significant decrease of biomarkers between-run variability. For example, inter-laboratory analytical variability decreased from 30% to 15% between 2011 and 2017 for the determination of A $\beta$ 42 peptide (Worldwide EEQ for AD biomarkers, organized by The Salhgrenska Academy at University of Gotheburg, Molndal, Sweden). Finally, we started a new era in the standardization of CSF biomarkers thanks to certified material of reference for A $\beta$  peptides and new immunoassays proposed in random-access platforms, paving the way to a dramatic reduction of the variability for these different assays. In our laboratory, within-run variability range from 0.6% to 1% for the 4 biomarkers using this technology (personal data). Although the preliminary results on reproducibility seem encouraging, further studies are needed to identify a possible batch-to-batch variability[79].

Now that CSF collection and analyses seem to be more standardized, it is time to inquire about concomitant pathologies, this question remaining poorly investigated. Indeed, medical conditions such as neuro-inflammation, vascular pathology, hydrocephaly and copathologies (phospho-TDP43, alpha-syn and phospho-Tau) are prone to influence the results of CSF markers dosage in ranges that need to be further studied[61,80]. Furthermore, the use of etiological biomarkers could improve the diagnosis of other neurodegenerative diseases and then co-pathologies. In this context, studies have been conducted to detect in CSF the aggregated proteins observed in cerebral tissues. As an example, CSF total alpha-synuclein was decreased in Parkinson's disease and Dementia with Lewy-bodies compared to Alzheimer's disease and control patients. Conversely, oligomeric forms, considered as toxic species of alpha-syn, were increased in case of synucleinopathy [81]. Similarly, efforts were made to quantify TDP43 and phospho-TDP43 in CSF of patients presenting FTLN and/or ALS. Despite some promising preliminary results, more studies need to be performed to validate these biomarkers in clinical practice [82].

## 6. How to reconnect neuropathology to clinical and laboratory medicine

Refining the early diagnosis of AD and other dementias is facing the incredibly complex extent of concomitant pathology observed in post-mortem series. As a consequence of the decline of autopsy rates, the lack of neuropathological assessment is increasing the disconnection between neuropathology findings and clinics or laboratory medicine. This situation slows down the validation of refined cut-off that would better take into account associated disorders to AD. Furthermore, diagnostic strategies still rely on markers discovered more than 30 years ago. Phospho-Tau and its isoforms were described between 1975 and 1986. In 1986, it was reported that Tau protein was a constituent of the paired helical filaments that were observed in neurons in AD [83-87]. Specific anti-A $\beta$  antibody was synthesized from isolates of brain samples of patients suffering from cerebral amyloid angiopathy in 1985. Immunoreactivity against neuritic plaques was shown the same year[88]. Its biochemical characteristics was described decades ago[89].

Novel candidate markers are proposed by different groups, for example, to differentiate AD from FTLN or to diagnose AD from plasma samples. However these groups struggle to get further validation of their findings because of the lack of fully characterized cohorts of patients[90-94].

Strikingly, diagnoses in the field of degenerative diseases and in the field of brain tumors took opposite directions. While the neuropathologists and neurologists/neurochemists' correlations decreased in the field of degenerative diseases, a systematic surgical biopsy approach including elderly patients in the field of brain tumors emerged. This allowed the validation at a fast pace of many new tumor subtype markers, including molecular biology markers in a trans-disciplinary approach that led to the concept of pathological and molecular integrated diagnoses promoted by WHO 2016 classification. Hence some entities such as oligodendroglioma or astrocytoma diagnoses are better defined pathologically and on the molecular level. New therapeutic schemes are now available for elderly patients with glioblastoma such as short-course radiation plus Temozolomide. This last trial was conducted in series of patients enrolled above 65 years of age (65-90), with systematic neuropathological and molecular characterization of their GBM. Many of these patients would not have been biopsied 10 years ago in many centers because of the lack of proven treatment efficiency. However, it has been now demonstrated that those patients benefit

from treatment compared to supportive care alone without significant deterioration of quality of life [95,96]

Therefore, beside the importance of maintaining the highest levels of autopsy rates in AD through donor programs, the question of adding surgical brain needle biopsy as an additional diagnostic tool has to be considered. Contrary to brain tumors which constitute an obvious target for the surgeon, making a biopsy in the setting of degenerative diseases would require a precise strategy based on the knowledge of proteinopathies anatomical distribution. This should be elaborated in a trans-disciplinary setting in order to combine already available clinical, biochemical, and radiological information. Both cortex and white matter should be sampled. Multiple teams have already started this approach and proposed decision making algorithms. Surgical biopsy is often indicated in the context of specific conditions that may constitute the cause of a patient's dementia and that could potentially be treated. These conditions include inflammatory diseases, infections or neoplasia not otherwise diagnosed. In that setting, surgical samples have to be large enough to evaluate grey and white matter pathology. Furthermore, sterile, frozen samples would be of the highest interest to facilitate in situ diagnosis of infectious diseases or autoimmune diseases using immunofluorescence techniques, improve molecular detection of infectious agents and improve the sensitivity of clonality assays in lymphoproliferative disorders[97].

Normal-Pressure Hydrocephalus is another interesting clinical condition in which the value of frontal lobe needle biopsy has been scrutinized recently. .Indeed, the symptoms of the classical triad (gait disturbance, dementia, and urinary incontinence, along with ventricular enlargement) can decrease after ventriculo-peritoneal shunt operation. During the procedure, it has been shown that a frontal lobe needle biopsy is easy to obtain and bring useful information about underlying A $\beta$  and Phospho-Tau proteinopathies. Based on this, patients with Normal-Pressure Hydrocephalus and AD-related pathology in cortical biopsy would probably benefit from referral to specialized memory clinics for further clinical assessment and care. Systematic CSF sampling of those patients helped further correlations between Phospho-Tau and A $\beta$  pathologies and cognitive status[65,97,98]. Interestingly, recent reports show how extensive immunohistochemical studies can be conducted on such small biopsies including detailed studies of A $\beta$ 1-42 and A $\beta$ 1-40 relative expressions, neuronal markers expression, inflammation markers and further degenerative disease markers

such as Phospho-TDP43 and Alpha-syn deposits. These data would constitute a welcome addition to PET and imaging results.

Despite the tremendous improvements in anesthesiology and in neurosurgery, neurologists in charge of patients with degenerative diseases hardly consider brain biopsy as an option because of the little therapeutic options available. However, systematic correlation of neuropathology data with CSF markers in the 2 aforementioned groups of patients would help to refine cut-offs of already known AD/CSF markers and also of future markers, provided that lumbar puncture and neuropsychological evaluation were planned before surgery[99].

## Tables

### 1. Table 1 :

Table 1 : National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease. A $\beta$  pathology evaluation is reflected in A and C Scores. **A Score** corresponds to a simplified 3-step equivalent of the 5-step Thal & al staging of A $\beta$  deposits originally published in 2002. Equivalence with the original scheme is reminded in parentheses. **C Score** corresponds to a 3-step scale evaluation of neuritic plaques density as described by CERAD in 1991: C/0, none, 1, sparse, 2, moderate, 3, frequent. Phospho-Tau positive NFTs distribution is reflected in **B Score**. It is a simplified 3-step scale derived from the 6-step Braak & Braak staging scheme. The equivalence with the original scheme is underlined by the roman figures in parenthesis. Likelihood that dementia was caused by AD pathology results of the combination of A, B and C scores : None (Very Light grey), Low (Light grey), Intermediate (Grey), High (Dark Grey)

Stage of Thal A $\beta$ pathology simplified (original) staging	CERAD A $\beta$ Neuritic Plaque density	Braak stage of pTAU NFT pathology simplified (original) staging				Likelihood that dementia was caused by AD pathology
		<b>B Score</b>				
<b>A Score</b>	<b>C Score</b>	B0	B1	B2	B3	
		0	(I-II)	(III-IV)	(V-VI)	
0 = none	0					None
1(1-2)	0-1					Low
	2-3					
2(3)	any score					Intermediate
3=4-5	0-1					High
	2-3					

1. Table 2 :

Table 2 : example of synthetic overview of lesions, degenerative pathology load after neuropathological post-mortem brain evaluation (AD : Alzheimer's Disease, NFTs Neurofibrillary tangles, A $\beta$  :  $\beta$  Amyloid, CAA : Cerebral amyloid angiopathy, ARTAG : Aging-related tau astrogliopathy, PART : Primary age related tauopathy)

Pathology	Score	Result
Alzheimer's disease related pathology	Tau NFTs Braak stage (1-6)	
	Phases of A $\beta$ deposit Thal (1-5)	
	ABC Score NiARI	
	Probability AD NIA-RI	
Amyloid angiopathy	A $\beta$ Thal stage (1-3)	
	Type of CAA (1=capil)	
Vascular Pathology and related lesions	Deramecourt Score	
	Cerebral Infarcts?(yes/No)	
	Lacuna	
	Hippocampic sclerosis?	
Alpha synuclein Pathology	Synuclein Braak stage (1-6)	
	McKeith stage (Brainstem, Limbic, Neocortical)	
	Amygdala predominant?	
DFT-TAU Pathology	Type	
	Grains argirophiles/Score Saito?	
DFT-TDP Pathology	Classification de McKenzie Neumann	
	Joseph score	
DFT-FUS Pathology	NIFID/BIBD/aFTL <sub>DU</sub>	
DFT- others Pathology		
Aging Pathology	ARTAG	
	PART	
	Other Aging related pathology?	

<b>PRION protein pathology?</b>		
<b>Multiple Sclerosis</b>	<b>Active Plaque?</b>	
<b>Other findings</b>	<b>Neoplastic pathology?</b>	
<b>Is this cas a control ?</b>		

## Figure legend

Figure :Neuropathological examination of post-mortem brain combined with brain banking is an interface between the clinical and the research fields. On the clinical side it is a valuable tool for pre-mortem clinical, biological and radiological quality insurance. On the research side, fully characterized and well preserved tissue material provides a valuable model for fundamental and translational research.

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