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REVIEW

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Required techniques and useful molecular markers in the neuropathologic diagnosis of neurodegenerative diseases

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Abstract Modern neuropathologic methods and molecular biology have lead to increased understanding of neurodegenerative disorders and biologically based classifications of these disorders. The purpose of this review is to discuss neuropathologic methods that are useful in the characterization of neurodegenerative disorders, with emphasis on disorders of late life that present with dementia or movement disorders. A diagnostic algorithm is suggested for neuropathologic evaluation of neurodegenerative disorders. The importance of clinical information is emphasized in arriving at the most precise and meaningful neuropathologic diagnosis.

Keywords Amyloid · Dementia · Immunohistochemistry · Parkinsonism · Tau

Introduction to staining methods for neurodegenerative disorders

Modern neuropathologic methods and molecular biology have lead to increased understanding of neurodegenerative disorders. Increasingly, the classification of these disorders is based upon fundamental biologic attributes. For example, a modern neuropathologic classification of frontotemporal degeneration, a disorder that has great clinical and pathologic heterogeneity, incorporates not only traditional histopathologic features, but also molecular and biochemical characteristics of brain changes [77]. The purpose of this review is to discuss neuropathologic methods that are useful in the characterization of neurodegenerative disorders, with emphasis on disorders of late life that present with dementia or a movement disorder (reviewed in [17]). Discussion of neuromuscular degenerative disorders and developmental heredodegenerative disorders is beyond the scope of the present review.

Traditional histologic stains were developed empirically without understanding of their biologic basis to selectively differentiate particular tissue elements. Subsequent studies have determined the biochemical basis of the selectivity of some of these stains, but for the most part they remain pragmatic methodologies without a firm scientific basis. Among the exceptions to this rule are the Bodian silver stain, which has been shown to be specific to neurofilament protein [61] and the Gallyas silver stain, which is specific to tau protein [33].

Antibody-based staining methods have become routine in most diagnostic neuropathology laboratories. Immunohistochemical methods provide increasingly specific and biologically meaningful stains. They also offer the promise of greater standardization, although there still remain many factors that influence the quality of the immunohistochemical results, including tissue fixation and processing, antigen retrieval methods, antibody titers and specificity, as well as antibody detection methods [57, 69].

Histochemical detection of Alzheimer-type pathology

The most common of the neurodegenerative disorders is Alzheimer's disease (AD), which is characterized by two major histopathologic lesions—senile plaques (SP) and neurofibrillary tangles (NFT). While there are many additional histopathologic manifestations of AD, such as amyloid angiopathy and granulovacuolar degeneration, SP and NFT have the greatest diagnostic relevance (reviewed in [23]). SP are complicated lesions composed of extracellular amyloid deposits, as well as dystrophic neuronal processes and reactive glia. NFT, on the other hand, are simpler lesions composed of intracellular fibrillar proteinaceous aggregates. Amyloid in aging and AD is derived from an amyloid precursor protein (APP)

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by proteolytic processing [68]. The protein deposited in tissue is heterogeneous in composition, including various forms with N-terminal modifications and peptides with heterogeneous C termini.

Amyloid deposits are also found in non-Alzheimer disorders, including transmissible spongiform encephalopathies [25] and familial British dementia (FBD) [63]. In the former the deposited protein is prion protein (PrP), while ABri is deposited in FBD and a related disorder referred to as familial Danish dementia (FDD). In these disorders amyloid deposits are often accompanied by NFT, which may sometimes make their differentiation from AD difficult. Fortunately for diagnostic neuropathologists, these are rare disorders. An index of suspicion is needed in all atypical cases of AD to catch these rare cases. The morphology (e.g., kuru, cotton-wool or multi-centric plaques) and distribution of the amyloid deposits (e.g., many plaques in the cerebellum) can sometimes be a clue that the amyloid is not Alzheimer related. A positive family history, atypical clinical features and rapid clinical course are less reliable clues since many cases of AD may also be familial and have atypical clinical features. Confirmation of these rare disorders may require submission of material to laboratories that specialize in these disorders, since reagents may not be commercially available, especially antibodies to ABri. On the other hand, there are reliable commercial antibodies for detection of PrP, such as 3F4 [43], but the number of cases in which application of this immunostain would be needed may limit the practically of developing this method for routine diagnostic purposes for some laboratories that have low volume of neurodegenerative brains for diagnostic evaluation.

The early descriptions of key histopathologic features of AD were based upon silver stains, and these stains and modifications thereof are still used routinely in diagnostic neuropathology [56]. Early on, it was also recognized that amyloid stains were effective at detecting Alzheimer-type pathology, as well [67]. Among the amyloid stains, thioflavin-S fluorescence microscopy is a particularly sensitive method that is useful to study AD, albeit it requires evaluation of slides with a fluorescence microscope, which may not be readily available or convenient to use in some diagnostic laboratories.

Some silver stains and amyloid stains detect both SP and NFT, while others are more selective for one or the other. Thioflavin-S reveals SP, NFT as well as amyloid angiopathy. The Bielschowsky silver stain is widely used to detect Alzheimer-type pathology [45, 85], but it must be emphasized that there is considerable heterogeneity in the appearance of the Bielschowsky stain depending upon the particular laboratory in which it is performed and local modifications to the protocol. There is even variability within the same laboratory depending upon a number of very difficult to control staining conditions, such as storage time of the silver solutions. The Bielschowsky stain in most laboratories detects both SP and NFT. Some variations detect SP better than NFT, however. The Campbell-Switzer silver stain more reliably detects SP than the Bielschowsky stain, and it also permits detection of NFT and Lewy bodies [4, 53]. The Bodian stain is a reliable method for detecting NFT, but it is not a sensitive method for SP, detecting only the neuritic element of SP [45]. The Gallyas silver stain is a sensitive and relatively specific stain for NFT, with minimal staining of SP [7].

The most specific of the amyloid stains is the Congo-red stain when viewed with polarizing filters; however, Cong red is not a very sensitive stain for Alzheimer-type pathology [67, 84]. Sensitivity can be improved by viewing Congo red with fluorescence filters, but this methodology is not widely used, and the increased sensitivity comes at the expense of less specificity [2]. Thioflavin-S fluorescence microscopy is a more sensitive amyloid stain than Congo red, but it is also less specific [67]. Nevertheless, it permits detection of NFT and SP, including diffuse amyloid deposits. Lewy bodies are also weakly stained with thioflavin-S [62].

NFT are detected in a host of neurodegenerative diseases in addition to AD [47] and these lesions are variably detected with amyloid stains. In some disorders neurofibrillary and glial lesions may be completely negative with amyloid stains and even with some routinely used silver stains (e.g., Bielschowsky stain). Immunohistochemistry and newer silver stains, especially the Gallyas stain, are preferable methods to study non-Alzheimer degenerative disorders [21]. It is worth reiterating that amyloid stains and some commonly used silver stains must be used with caution for the detection of neurofibrillary pathology in non-Alzheimer degenerative disorders [78].

Other amyloid stains that can be used to visualize lesions in AD, such as Alcian blue and crystal violet stains, are not recommended for primary diagnostic purposes. Another fluorescence method that has been used to detect Alzheimer-type pathology is thiazin red [55]. The experience with this stain is far less than with thioflavin-S, but it appears to have the some of the same strengths and weaknesses as thioflavin-S [78]. Thioflavine T, which may be useful to detect systemic amyloid, is not recommended as a method to evaluate Alzheimer-type pathology [67].

Immunohistochemical detection of Alzheimer-type pathology

Biochemical analyses have demonstrated heterogeneity of the amyloid peptides (Aβ peptides) in AD brains [26, 44], and immunohistochemical studies have also demonstrated heterogeneity in composition of amyloid in SP [15, 36, 75]. Thus, the choice of antibody reagents for immunodetection of Aβ affects the number and types of SP that are detected. While a polyvalent, polyclonal antibody raised to amyloid extracted from AD brains would be theoretically superior to a monoclonal antibody that is specific to a single epitope of Aβ, a monoclonal antibody can be made widely available to many

laboratories, which offers the possibility of greater inter-laboratory standardization. Unfortunately, there is no consensus as to which Aβ antibody should be used for diagnostic purposes. (A useful resource for finding commercial antibodies that are useful for diagnostic evaluation of neurodegenerative disorders is the AlzForum web site [30].)

Given that Aβ undergoes N-terminal modifications (e.g., pyroglutamate at residue-3 of Aβ [36]) and the fact that there is C-terminal heterogeneity (Aβ40 and Aβ42, being the most abundant species [26]), an all-purpose antibody for diagnostic purposes should recognize a relatively central domain of Aβ. Several commercial antibodies fit this description, including clones 6F/3D (residues 8–17) and 4G8 (residues 17–24). Others have epitopes closer to the N terminus, such as 6E10 (residues 1–17) and BAM10 (residues 1–10), and may not be as sensitive at detecting diffuse amyloid deposits. Immunostaining for Aβ is also influenced by tissue fixation and antigen retrieval. In most cases the tissue should be pre-treated with formic acid to uncover ''masked'' epitopes. It should be noted that immunostaining for Aβ may detect more lesions than histologic methods, and this should be taken into diagnostic consideration. This is particularly true for histologic methods that recognize only fibrillary secondary structure; since some Aβ-immunoreactive deposits may not be composed of fibrillar deposits (i.e., pre-amyloid) [73].

NFT are composed of fibrillar aggregates of the microtubule-associated protein tau. Similar to Aβ, immunohistochemical methods for tau protein have the theoretical possibility of greater standardization, but again there is no universally agreed upon antibody. There are many commercial antibodies to tau protein, and many of the most useful antibodies for diagnostic neuropathology detect phosphorylated epitopes of tau protein. Widely used commercial antibodies to phospho-tau epitopes include AT8, AT100, AT270 and AT180. Non-commercial phospho-tau antibodies that are also widely used include PHF-1, CP13 and AD2. Widely used antibodies to non-phosphorylated epitopes in tau (such as Tau-2) are more problematic and not recommended for routine neuropathologic diagnostic use of neurodegenerative disorders. In particular, Tau-2 has been shown to detect glial cells (probably microglia) in normal tissue [79] that may lead to the mistaken perception of a non-Alzheimer degenerative disease process, where abnormal tau protein is present in both neuronal and glial lesions.

Immunohistochemistry for tau in neurodegenerative tauopathies

While NFT are essential to the diagnosis of AD, they are not specific to AD and are found in a variety of other neurodegenerative disorders [47]. After the discovery that mutations in the tau gene could give rise to primary neurodegenerative disorders [31], the diagnostic significance of neurofibrillary lesions gained undisputed credence. Such disorders have come to be known as the ''tauopathies'' [47]. It is increasingly recognized that the neuropathology of the primary tauopathies involves glia as well as neurons. The most common of the primary tauopathies include progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), argyrophilic grain disease (AGD), postencephalitic parkinsonism, Parkinson-dementia complex of Guam and Pick's disease (PiD). The most common genetically determined tauopathy is referred to as frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) [24]. The tau gene is located on chromosome 17, but mutations have not been detected in the tau gene in all cases of FTDP-17 [64].

Glial lesions in the tauopathies are heterogeneous and are found in both astrocytes and oligodendrocytes. In CBD the characteristic lesion in affected cortical areas is the ''astrocytic plaque'', while in PSP ''tufted astrocytes'' are often numerous in motor cortex and basal ganglia [16]. Oligodendroglial ''coiled bodies'', which were first described in AGD [5], are found in most of the tauopathies and are relatively nonspecific. Tau-positive argyrophilic astrocytes in the subpial and perivascular space at the base of the brain and around the temporal horn of the lateral ventricle are referred to as ''thorn-shaped astrocytes''; they also lack diagnostic specificity, increasing in frequency with age [66].

Tau undergoes alternative splicing of exons 2, 3 and 10 to generate six major protein isoforms in the adult brain. Splicing is developmentally regulated, with only three of the six isoforms found in fetal brain. Particular tau splice variants have been shown to preferentially accumulate in pathological lesions of the neurodegenerative tauopathies [10]. For example, splice variants generated from mRNA that contains exon 10 (so-called ''4R tau'' since exon 10 encodes a conserved repeat region in the microtubule binding domain of tau generating tau with four repeats) accumulate within neuronal and glial lesions in PSP, CBD and AGD. These disorders have been referred to as the ''4R tauopathies'' [76]. In contrast, tau with three repeats (3R tau), which is generated by splice variants lacking exon 10, preferentially accumulate in neuronal lesions in PiD [10]. In AD the tau is composed of approximately equal amounts of 3R and 4R tau. The recognition that particular tau isoforms accumulate in the tauopathies has been based upon biochemical studies; however, recently several research laboratories have developed monoclonal antibodies that are specific to either 3R tau or 4R tau [14, 35]. Application of these antibodies to neurodegenerative disorders offers promise for more specific classification of cellular pathology. It has become clear that even in a disorder with predominance of 3R tau such as PiD [86], immunocytochemistry with 4R tau antibodies may reveal lesions, particularly glial lesions, that contain 4R tau [29]. In AD extracellular NFT show preferential immunostaining for 3R tau, while the ear-

liest neuronal lesions, so-called ''pre-tangles'', are often immunoreactive for 4R tau.

Methods to detect Lewy bodies

Lewy bodies are hyaline cytoplasmic neuronal inclusions that are the histopathologic hallmark of Parkinson's disease (PD), the second most common neurodegenerative disorder after AD. Lewy bodies are also found in the brains of individuals who in life had clinical presentations quite distinct from PD, such as senile psychosis, dementia or autonomic failure. The distribution of Lewy bodies alone does not permit accurate prediction of the clinical presentation. Thus, an accurate clinicopathologic diagnosis of the Lewy body disorders at the present time requires clinical information [54]. In the absence of clinical information a descriptive diagnosis is all that is possible with certainty.

While Lewy bodies can be detected with routine histologic methods, such as hematoxylin and eosin (HE) stains, these methods greatly underestimate the density and distribution of Lewy bodies and related lesions, in particular neuritic pathology (so-called ''Lewy neurites'') [8]. Some silver stains have been modified to permit detection of Lewy bodies, and the Campbell-Switzer silver stain is one such method. Lewy bodies are also weakly stained with fluorescence methods for amyloid such as thioflavin-S and thiazin red.

Immunohistochemistry for α-synuclein is the preferred method for detecting Lewy bodies [59]. α-Synuclein is a member of a family of homologous proteins, including β- and γ-synuclein, but for the purpose of this discussion synuclein refers only to α-synuclein [12]. There are a number of commercial monoclonal antibodies to synuclein, including Syn-1 and LB509, that are useful for diagnostic immunohistochemistry. In addition to the particular antibody used, antigen retrieval methods have been shown to be particularly important in detection of pathologic forms of synuclein. For example, formic acid and protease pretreatment of tissue sections enhances the signal-to-noise ratio [28, 74]. This is related to the fact that synuclein is normally abundant in synaptic termini, and immunostaining of normal tissue for synuclein reveals extensive fine punctate immunoreactivity of gray matter. The aforementioned pretreatments abolish much or all of the normal synuclein and permit selective visualization of pathologic synuclein. Biochemical studies have demonstrated that this property is similar to the protease resistance that characterizes prion disorders [41].

Before the recognition that antibodies to synuclein specifically labeled Lewy bodies [34, 71], other immunohistochemical methods were employed to study Lewy body disease (LBD), particularly ubiquitin immunohistochemistry [48]. Ubiquitin is preferable to routine histologic methods for analysis of LBD, but it has a number of problems. In particular, for cases with concurrent AD, ubiquitin immunohistochemistry is problematic because it also labels neurofibrillary pathology. When this occurs in neurons that are vulnerable to both NFT and Lewy bodies, such as neurons in the limbic lobe and the amygdala [65], this can easily lead to misinterpretation of NFT as Lewy bodies. To circumvent this problem double immunolabeling for ubiquitin and tau has been used in some laboratories, but this is a method that is time consuming, expensive and difficult to standardize.

Ubiquitin immunohistochemistry

Ubiquitin is a small heat shock protein that is involved in a number of physiologic processes, most notably energy-dependent degradation of denatured or abnormal proteins through the proteasome [60]. Abnormal proteins destined for degradation by the proteasome have polyubiquitin chains conjugated to their peptide backbone. Ubiquitin is also conjugated to proteins through other acceptor sites without generating a polyubiquitin chain, and these ubiquitinated proteins are not targeted to the proteasome for degradation. Finally, ubiquitin is present as non-conjugated molecules in the cytosol of cells. Thus, antibodies to ubiquitin can detect free, conjugated or both free and conjugated ubiquitin. Even conjugated ubiquitin is heterogeneous with respect to its functional significance. Some antibodies to ubiquitin may be specific to the polyubiquitin chain, which would theoretically be the best type of antibody for diagnostic neuropathologic purposes [37].

Given the diversity of ubiquitin moieties in the cell, it is not surprising that there is considerable heterogeneity in ubiquitin immunohistochemistry. While ubiquitin immunohistochemistry is an important tool for neuropathology, it must also be acknowledged that immunoreagents to ubiquitin for routine diagnostic neuropathology produce varied results. A monoclonal antibody that has proven to be consistent and sensitive for routine diagnostic immunohistochemistry is UBI-1 (MAB1510).

It is important to point out that ubiquitin immunohistochemistry reveals a diversity of age-related lesions in the human brain [20]. Since most of the neurodegenerative disorders under consideration occur in mid-to-late life, the presence of age-related ubiquitin immunoreactive structures is expected and should not be misinterpreted as pathologic. The most prevalent of these is so-called granular degeneration of myelin, which is a punctate and granular immunoreactivity in the myelin sheath (Fig. 1A). It is widespread in myelinated fibers throughout the brain and spinal cord and increases with age. Another of the age-related ubiquitin-immunoreactive structures in the aged brain are spherical granular structures in the cortex, especially on the limbic cortices and the amygdala (Fig. 1B). While some studies have suggested that these may be dystrophic neuronal processes, this is not firmly established and at least one study suggested that they may be with

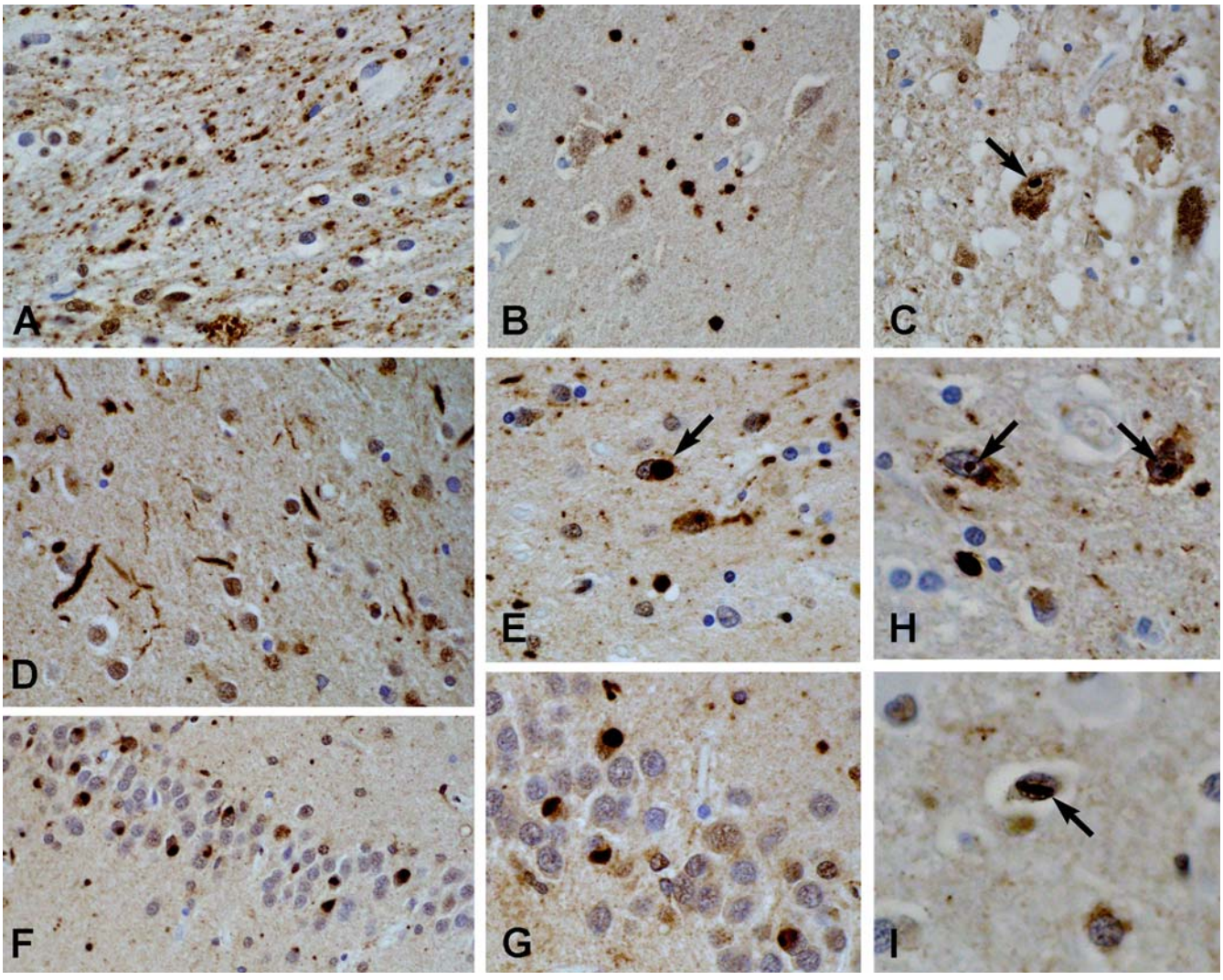


Fig. 1 Ubiquitin immunoperoxidase stain in a normal elderly individual (**A**–**C**) and in FTLD-U (**D**–**I**). Cerebral white matter with extensive granular degeneration of myelin (**A**); dystrophic neurites in the superficial layers of entorhinal cortex (**B**); Marinesco body (*arrow*) in the substantia nigra (**C**); curvilinear and spherical dystrophic neurites in the caudate nucleus (**D**); neuronal cytoplasmic inclusions in dentate fascia (**E**, **G**); neuronal cytoplasmic inclusions in caudate nucleus (**F**); neuronal intranuclear inclusions (*arrows*) in caudate nucleus (**H**, **I**) (*FTLD-U* frontotemporal lobar degeneration with ubiquitin-immunoreactive neuronal inclusions in the cortex, hippocampus and striatum)

astrocytic processes. Occasionally, ubiquitin immunoreactivity is detected in the nucleus, which may be related to the fact that some nuclear proteins, such as histone, may be ubiquitinated. Marinesco bodies, age-related spherical intranuclear inclusions found most often in neurons of the substantia nigra, are also ubiquitin immunoreactive (Fig. 1C). These should not be interpreted as intranuclear inclusions that are found in trinucleotide-repeat disorders, frontotemporal lobar degeneration (FTLD) (Fig. 1H, I) or multiple system atrophy (MSA). While age-related ubiquitin-immunoreactive structures are not indicative of a specific pathologic process, they can, however, be used to the advantage of the pathologist as an internal standard of the quality of the ubiquitin immunostain. If they are not present, the ubiquitin stain is faulty.

Neurofilament immunohistochemistry for neurodegenerative disorders

Neurofilament immunohistochemistry is a useful method to study axonal pathology in neurodegenerative disorders since neurofilament antibodies, particularly antibodies to phosphorylated neurofilament epitopes, specifically label axons [72]. This methodology is useful to demonstrate tract degeneration, which is common in motor neuron disorders such as amyotrophic lateral sclerosis or primary lateral sclerosis. Silver stains, such as Bodian's stain and the Bielschowsky stain, can also be used for this purpose, but they are less selective and specific.

Another use for neurofilament immunohistochemistry is detection of ballooned neurons [19]. The presence of ballooned neurons is a cytologic feature of a number of neurodegenerative disorders, especially the neurode-

generative tauopathies. They are common in CBD, AGD and PiD. As such, they cannot be considered a specific histopathologic change any more than are NFT. Ballooned neurons are characterized by swelling and vacuolation of neuronal perikarya and proximal dendrites, and are particularly frequent in pyramidal neurons in lower cortical layers. Phospho-neurofilament epitopes are normally most abundant in axonal neurofilaments and scarcely detected in the neurofilaments in the perikarya [72]; in ballooned neurons the expression of phospho-epitopes in neuronal perikarya is can be considered aberrant.

In addition to neurofilament immunohistochemistry, another sensitive method to detect ballooned neurons is immunostaining for the small heat shock protein, αB-crystallin [52]. While αB-crystallin is normally detected in a subpopulation of oligodendrocytes and astrocytes, it is not found in normal neurons, which makes this a more selective method for detecting ballooned neurons than neurofilament immunohistochemistry. There are many commercially available monoclonal antibodies to neurofilament (SMI-31 is a widely used monoclonal antibody to phospho-epitopes), but there are no high quality monoclonal antibodies to αB-crystallin that have been widely used in diagnostic immunohistochemistry. If this method is used it is likely that a polyclonal antibody will be used.

Neurofilament antibodies, especially phospho-neurofilament antibodies, inconsistently detect NFT and most evidence suggests that this is probably due to cross-reactivity of neurofilament antibodies with phospho-tau. On the other hand, neurofilament antibodies are useful for detecting neuronal inclusions in a rare neurodegenerative disorder referred to as neurofilament inclusion disease (NFID) [39]. NFID most often presents as a frontal lobe dementia. A d9evelopmentally expressed form of neurofilament, α-internexin, has recently been shown to be even more specific for inclusions in NFID [11]. In the adult brain there is minimal expression of α-internexin so that the inclusion bodies are readily detected against a negative background. In contrast, axons as well as pathologic inclusion bodies are immunostained with neurofilament antibodies.

A diagnostic algorithm (Fig. 2)

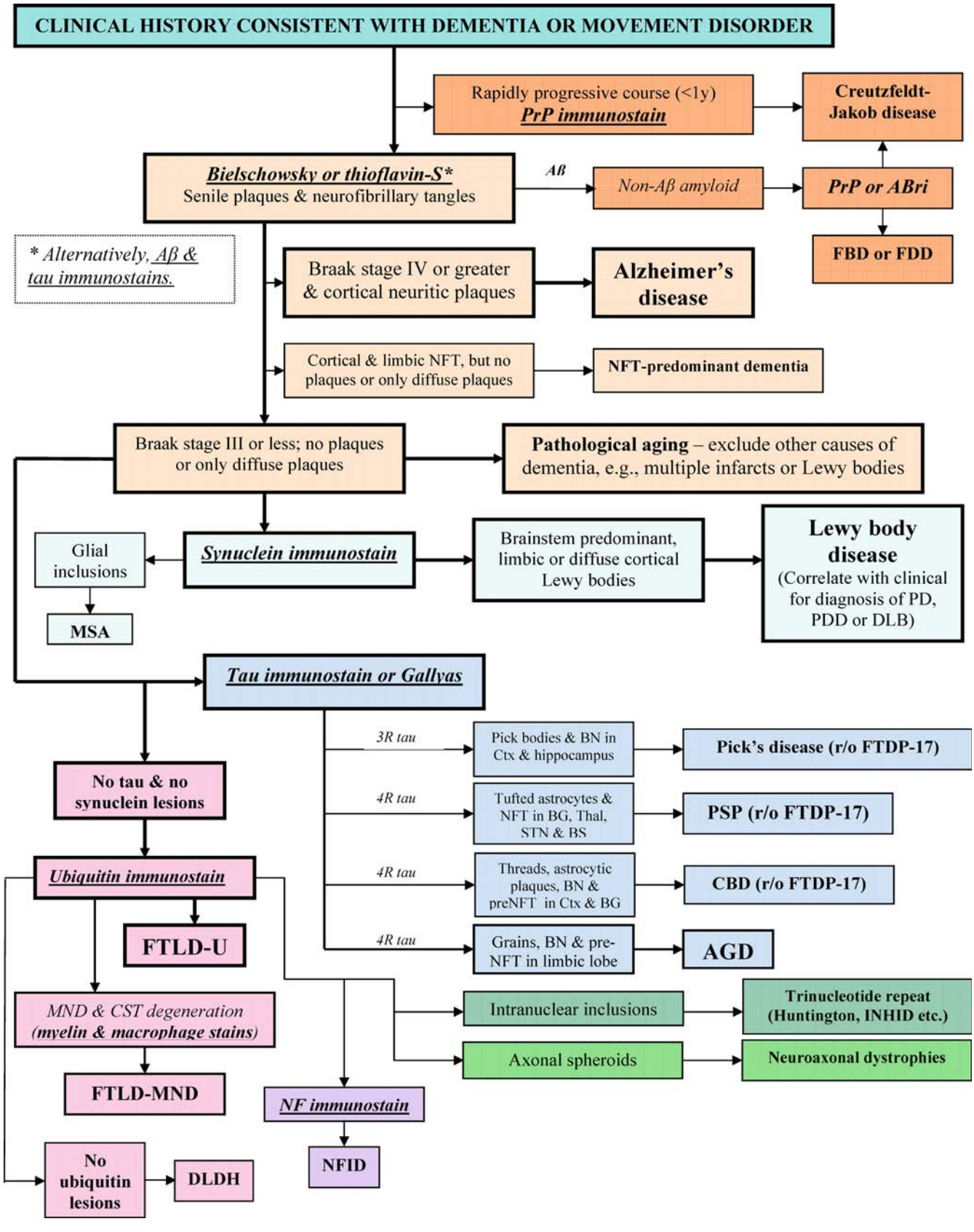
The starting point for neuropathologic evaluation of a brain with a neurodegenerative disorder should include clinical information that documents age of onset, disease duration, presence of family history and major neurologic features. The disease duration is particularly important in excluding cases that are potentially transmissible, such as Creutzfeldt-Jakob disease. The later often has a very short duration and a fulminant clinical course. Precautions need to be taken [9] and the diagnosis confirmed with immunohistochemical methods for prion protein (PrP) or immunochemical analyses of brain tissue.

Given the frequency of neurodegenerative disorders in mid-to-late adult life, when Alzheimer-type pathology is common, it is important that all degenerative disorders be screened for SP and NFT. The presence of pathologically confirmed AD does not preclude the possibility of a secondary disease process, most frequently LBD. As such, it is good practice to screen for Lewy body pathology, even in cases with advanced AD. Several studies have noted that the amygdala may be the best single brain region for screening for Lewy bodies [28]; however, it must be recognized that some cases of advanced AD may have Lewy bodies restricted to the amygdala [65]. Their presence in the amygdala does not guarantee that they will be found in brainstem nuclei that are vulnerable to Lewy bodies in PD.

Assessment of Alzheimer-type pathology can be performed economically with thioflavin-S fluorescence microscopy or at somewhat greater expense with silver stains, such as Bielschowsky, Campbell-Switzer, methenamine silver or other modifications of the silver staining technique. Immunohistochemistry for Aβ and tau is not necessary for routine diagnostic purposes, although such methods might eventually have their greatest utility in standardizing neuropathologic diagnoses across laboratories.

It is important in this assessment to determine the nature, density and distribution of neocortical SP and NFT. Modern diagnostic criteria for AD [32] employ a staging scheme for neurofibrillary pathology that was initially developed by Braak and Braak [6]. In this staging scheme clinically definite AD is associated with Braak stages V–VI. The likelihood that dementia is due to AD has intermediate probability when the Braak stage is III–IV, and there is low or no likelihood that dementia is due to AD in cases with Braak stage 0–II. Neuropathologic studies of prospectively followed individuals indicates that Braak stage III is associated with early memory impairment, but not mental status changes and definitely not with frank dementia [27]. Whenever evaluating the brain of an individual with clinical dementia and a Braak stage of III or less, it is important to exclude other pathologic processes, most often LBD and vascular-ischemic dementia, that might have contributed to cognitive impairment. The pathologic substrate of vascular-ischemic dementia is a topic of other reviews [38, 58, 81] and will not be discussed further, as the emphasis of the present discussion is on diagnostic methods for neurodegenerative disorders.

Synuclein immunostaining reveals not only Lewy bodies, but also oligodendroglial inclusions, so-called glial cytoplasmic inclusions [70], which are the histopathologic hallmark of MSA [46]. MSA presents with degeneration in the substantia nigra and posterior putamen (striatonigral degeneration) as well as pathology in the inferior olivary nucleus, pontine base and the cerebellum (olivopontocerebellar degeneration). Glial inclusions are also detected in sparse numbers in many cases of LBD [82] and some cases of MSA have intraneuronal synuclein-immunoreactive inclusions (espe-


CLINICAL HISTORY CONSISTENT WITH DEMENTIA OR MOVEMENT DISORDER
Rapidly progressive course (<1y)
PrP immunostain
Creutzfeldt-Jakob disease
Bielschowsky or thioflavin-S*
Senile plaques & neurofibrillary tangles
Aß
Non-Aß amyloid
PrP or ABri
FBD or FDD
* Alternatively, Aß & tau immunostains.
Braak stage IV or greater & cortical neuritic plaques
Alzheimer's disease
Cortical & limbic NFT, but no plaques or only diffuse plaques
NFT-predominant dementia
Braak stage III or less; no plaques or only diffuse plaques
Pathological aging – exclude other causes of dementia, e.g., multiple infarcts or Lewy bodies
Glial inclusions
Synuclein immunostain
Brainstem predominant, limbic or diffuse cortical Lewy bodies
Lewy body disease
(Correlate with clinical for diagnosis of PD, PDD or DLB)
MSA
Tau immunostain or Gallyas
3R tau
Pick bodies & BN in Ctx & hippocampus
Pick's disease (r/o FTDP-17)
No tau & no synuclein lesions
4R tau
Tufted astrocytes & NFT in BG, Thal, STN & BS
PSP (r/o FTDP-17)
Ubiquitin immunostain
4R tau
Threads, astrocytic plaques, BN & preNFT in Ctx & BG
CBD (r/o FTDP-17)
FTLD-U
4R tau
Grains, BN & pre-NFT in limbic lobe
AGD
MND & CST degeneration
(myelin & macrophage stains)
Intranuclear inclusions
Trinucleotide repeat (Huntington, INHID etc.)
Axonal spheroids
Neuroaxonal dystrophies
FTLD-MND
NF immunostain
No ubiquitin lesions
DLDH
NFID

◄

Fig. 2 An algorithm for diagnosis of major neurodegenerative diseases, highlighting the role of histologic methods (*underlined* and *italics*) in their neuropathologic evaluation. The disorders are grouped by major category: amyloidoses, synucleinopathies, tauopathies, motor neuron and frontal degenerations, and rare disorders with diverse ubiquitin-immunoreactive inclusions

cially in the pontine base) [1], but there is little difficulty in differentiating MSA from LBD in the overwhelming majority of cases.

Lewy body pathology is currently classified by the density and distribution of inclusions [51] into cases with predominant pathology in brainstem nuclei (so-called brainstem LBD), involvement of brainstem nuclei as well as limbic cortices (so-called transitional LBD) or involvement of brainstem, limbic and higher order neocortical areas of especially frontal and temporal lobes (so-called diffuse LBD). At present it is difficult to predict the clinical presentation based upon the pathologic findings, but there is a wide range of clinical presentations of LBD, including PD, dementia in the setting of long-standing PD [so-called Parkinson's disease dementia (PDD)], as well as dementia with Lewy bodies (DLB). Clinical correlation is essential to arrive at a final clinicopathologic diagnosis [54].

Tau pathology is essential to the diagnosis of AD, but when it is found in glia as well as neurons, and the process is distributed to basal ganglia, thalamus, brainstem and cerebellum, it is not AD that is producing this change, it is most likely a degenerative tauopathy. The diagnostic considerations that must go into the differential diagnosis of the degenerative tauopathies is beyond the scope of the present discussion, but differential diagnosis is discussed in several recent reviews and must take into account not only the morphology of the glial and neuronal lesions, but also their anatomic distribution [18].

In cases with neither tau nor synuclein pathology, it is essential to study brain sections with ubiquitin immunohistochemistry to exclude the possibility of frontotemporal lobar degeneration (FTLD). The majority of cases of FTLD will have ubiquitin-immunoreactive neuronal inclusions in the cortex, hippocampus and striatum [40, 50] (Fig. 1D–G). This has been referred to as FTLD-U. Many, but not all such cases may have degeneration of motor neurons, and it is important to determine the status of the motor neurons and the corticospinal tract in such cases. If neuronal loss is detected in brainstem motor neurons (or spinal cord anterior horns if the spinal cord is available for study) or degeneration is detected in the cortical spinal tract, a diagnosis of FTLD-motor neuron disease (MND) is warranted. Almost all cases of FTLD-MND will also have ubiquitin-immunoreactive neuronal inclusions [50].

Ubiquitin immunostaining is a good marker for intranuclear inclusions that are found in many trinucleotide-repeat disorders, such as Huntington's disease and several of the spinocerebellar ataxias [22]. The specific distribution of the pathology will assist in the proper diagnosis. For example, in Huntington's disease the brunt of the pathology will be in corpus striatum and frontal lobe. In intranuclear hyaline inclusion disease, brainstem and cerebellar pathology may be striking. Intranuclear inclusions that are not associated with heredodegenerative disorders are much less common. Finally, intranuclear inclusions that are positive for ubiquitin can also be detected in FTLD-U and FTLD-MND, especially in the striatum of familial cases [83]. In MSA intranuclear inclusions can sometimes be detected in neurons in the pontine base or the inferior olive, but these inclusions can be distinguished from those in trinucleotide-repeat disorders by their immunoreactivity with synuclein, which labels nuclear inclusions in MSA [49]. In FTLD-U and FTLD-MND the substrate protein (or proteins) that make up the inclusions is unknown, whereas specific genetic defects in the trinucleotide-repeat disorders offers the prospect of specific immunohistochemical differentiation of these disorders.

A minority of FTLD cases will have no inclusions with tau, synuclein or ubiquitin immunostaining. These cases currently are referred to a dementia lacking distinctive histopathology (DLDH) [42] or simply as FTLD. The term DLDH is misleading because most of these cases have quite remarkable and quite distinctive histopathology. These cases are not to be confused with cases of dementia with no significant histopathology. When the latter is encountered, it is essential to obtain clinical information to explore the possibility of a pseudo-dementia, often due to late-life depression. Some prion disorders may also present only subtle histopathology.

In a small subset of cases of FTLD one is able to detect hyaline inclusions in cortical neurons with routine histologic methods, such as HE stains. The ubiquitin-immunoreactive inclusions in FTLD-U and FTLD-MND are inconspicuous or invisible with HE stains. When hyaline inclusions are detected, it is important to determine if the proteinaceous material in the cytoplasmic inclusions is composed of neurofilament or the developmentally regulated neurofilament, α-internexin, which is a sensitive and specific marker for these lesions [11, 39]. Presence of these lesions warrants a diagnosis of NFID. The inclusions in NFID are also positive for ubiquitin, but neuronal inclusions in FTLD-U or FTLD-MND are not visible with routine histologic methods studies, while those in NFID are.

Final comments

The minimal methods that are needed to evaluate and diagnose the majority of neurodegenerative disorders can be summarized as follows: (1) routine histologic methods and gross morphology to document the presence and distribution of cerebrovascular pathology, (2) a method to detect Alzheimer-type pathology (e.g., thioflavin-S or Bielschowsky stains), (3) synuclein immuno-

histochemistry to detect Lewy body pathology (and MSA in rare cases of parkinsonism without Lewy bodies), (4) a sensitive method to detect non-Alzheimer tau pathology (either immunohistochemistry for phospho-tau or Gallyas silver stains), and (5) ubiquitin immunohistochemistry for disorders in which neither tau nor synuclein pathology is detected. The latter has value in a number of disorders ranging from FTLD to trinucleotide-repeat disorders to neuroaxonal dystrophies.

Immunohistochemical methods to show reactive gliosis (e.g., immunohistochemistry for glial fibrillary acidic protein) are ancillary and provide little differential diagnostic value. The same can be said for immunohistochemical methods for microglia, although the latter is particularly sensitive for demonstrating tract degeneration in FTLD and MND. Neurofilament immunohistochemistry is another ancillary tool for detection of tract degeneration. α-Internexin immunohistochemistry is useful to detect inclusion bodies in NFID, while prion immunostaining is an ancillary method to evaluate cases with atypical clinical or pathologic features, but neither is likely to provide be a high yield since NFID and prion disorders are rare. Other rare disorders that are not readily detected in this simplified scheme are disorders associated with inclusions composed of specific proteins that are not within this minimal battery, such as neuroserpin [13] or neuroferritin [80], or there are also disorders characterized by mineral or pigmentary deposits or accumulation of glucose polymers (i.e., polyglucosan disease) [3].

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