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Multisite assessment of aging-related tau astrogliopathy (ARTAG)

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Abstract

Neuropathology is important to understand the normal aging-related changes versus disease-related pathologic changes. The recent description of a harmonized evaluation strategy to evaluate aging-related tau astrogliopathy (ARTAG) aimed to facilitate our understanding of its relevance. In the present study we evaluated how consistently neuropathologists recognize i) different astroglial tau immunoreactivities including those of ARTAG and those associated with primary tauopathies (study 1); ii) ARTAG types (study 2A); and iii) the severity of ARTAG (study 2B). A total of twenty-two cases were selected for these studies. For study 1, thirty-five microphotographs of astrocytic tau AT8 stained immunoreactivities were provided. For study 2A+B, AT8 immunostained sections representing different anatomical regions were scanned and made available for download and review. Percentage (%) of agreement and kappa values with 95% confidence interval (CI) were calculated for each evaluation. The overall agreement for study 1 was > 60% with a kappa value of 0.54 (95% CI 0.433, 0.645). Good agreement (> 90%, kappa: 0.48, 95% CI 0.457, 0.900) was reached in study 2A for the identification of ARTAG pathology and also for each ARTAG type (kappa: 0.37-0.71). Less agreement (kappa: 0.39, 95% CI 0.341, 0.445) was reached for the evaluation of ARTAG severity. The overall assessment of ARTAG showed good agreement (kappa: 0.59, 95% CI 0.534,0.653) between raters. Our study supports the application of the current harmonized evaluation strategy of ARTAG with a slight modification of the evaluation of its severity. This allows comparison of worldwide data on ARTAG for research purposes.

Key words: ageing; ARTAG; tau-astrogliopathy; digital pathology; interrater agreement; tau; neuropathology;

Introduction

Neuropathologic assessment of neurodegenerative conditions and the aging brain is witnessing a renaissance. Novel bodily fluids and neuroimaging biomarkers are being developed, which require constant diagnostic certainty established by neuropathological assessment [32]. In parallel, in the last two decades, molecular pathology has entered the diagnostic practice with the application of new disease markers. New disease concepts and diagnostic criteria have emerged. The National Institute on Aging (NIA) in collaboration with the Alzheimer's Association (AA) revised consensus guidelines for the neuropathologic assessment of Alzheimer disease (AD) [28]. The concept of primary age-related tauopathy (PART) was published, which focuses on the evaluation and interpretation of neurofibrillary tangle (NFT) pathology in the medial temporal lobe [12]. Although pathological accumulation of abnormally phosphorylated tau protein in astrocytes has been frequently noted in the aging brain [22, 24, 30], a consensus on how to describe these was not available for the neuropathology community. In addition, clinicians and biomarker researchers were not aware of this type of tau pathology. To stimulate clinicopathological studies and research into the pathobiology of astrocytic tau pathology, an international group of neuropathologists and researchers published a strategy for the harmonized consensus evaluation of aging-related tau astrogliopathy (ARTAG)[21]. This strategy includes four steps: i) identification of subpial, subependymal, perivascular, white and gray matter types of ARTAG; ii) documentation of the regional involvement such as medial temporal lobe, lobar, subcortical, or brainstem; iii) description of subregional involvement; and iv) documentation of the severity [21].

Interlaboratory studies of the BrainNet Europe Consortium to evaluate the reproducibility of the assessments of various neuropathological variables have shown that there are many factors leading to inconsistencies, such as different fixation or staining methods [5], but also differences in the interpretation of immunoreactive morphologies or staging systems [2-4, 6, 7]. Therefore, evaluation of the reproducibility of consensus guidelines is still required. Indeed, AD neuropathologic evaluation yielded data that have high agreement with potential modifications for modest improvements [27].

The spectrum of astroglial tau pathologies expands beyond ARTAG and comprise various morphologies thought to be characteristic for so called primary frontotemporal lobar degeneration (FTLD)-tauopathies [19, 20]. Accordingly, tufted astrocytes are associated with progressive supranuclear palsy (PSP) [13, 17], astrocytic plaques with corticobasal

degeneration (CBD)[13, 15], globular astroglial inclusions with globular glial tauopathies (GGT)[1], and ramified astrocytes with Pick's disease (PiD)[14]. Most of these have been defined using silver staining and later by immunohistochemical studies using different antibodies against different modifications of the tau protein [16]. In ARTAG, two morphologies of astroglial tau immunoreactivities have been defined: the thorn-shaped astrocytes (TSAs) and the granular/fuzzy astrocytes (GFAs)[21]. The bushy astrocytes reported in argyrophilic grain disease (AGD)[9] were included in the morphological spectrum of GFAs. In addition to ARTAG, there is a lack of studies on how consistently can neuropathologists recognize astrocytic tau immunoreactivities of primary tauopathies. Based on these, we set up a study to evaluate how consistently neuropathologists recognize i) different astroglial tau immunoreactivities including those of ARTAG and those associated with primary tauopathies; ii) ARTAG types; and iii) the severity of ARTAG.

Material and methods

Case selection and evaluation

For this study twenty-two cases were selected from the Brain Bank of the Institute of Neurology, Medical University of Vienna. Cases with PSP, CBD, PiD, GGT and combined age-related pathologies (e.g. ARTAG, PART, AD, AGD) were included. The latter cases were selected from the ongoing longitudinal VITA (Vienna Transdanubian Ageing) study [22]. Neuropathological data of the cases included in the study are summarized in **Table 1**. The same cases were used for studies 1 and 2 A and B (see below).

For study 1 in sum thirty-five microphotographs (x400; 15x 11.3 cm 300 dpi) of astrocytic tau (AT8) immunoreactivities were provided from seventeen cases (**Table 1**). The evaluators had to choose from six different tau-morphologies (i.e. tufted astrocyte, astrocytic plaque, globular astroglial inclusion, ramified astrocyte, GFA, and TSA) or as a seventh option "none of these". The first and, if felt equivocal, the second most likely morphology had to be selected.

In addition a set of AT8-immunostained sections were scanned (study 2 A, B) and made available for download (courtesy of Histotech3D, Hungary) for each neuopathologist participating in the study. The images and sections were evaluated alone or in small (2-3) groups of neuropathologists in different Departments and Institutions. In sum twenty-five

AT8-immunostained sections were scanned from twenty cases (**Table 1**). The Digital Slide Viewer Application was suitable for Windows and MacOs systems. The sections represented different ARTAG types showing different severity but also sections from primary tauopathy cases have been included. For each case a separate excel sheet was provided (see **online supplemental file 1**). For gray and white matter, ARTAG specific anatomical regions were asked. The evaluators had i) to decide whether ARTAG is present (yes/no); ii) to indicate which type of ARTAG is present (yes/no question for each type); iii) to indicate for each ARTAG type visible on the section whether the severity/extent is occasional or numerous; iv) to indicate for each ARTAG type visible on the section if numerous is focally accentuated or widespread; and v) to indicate whether other non-ARTAG type of astroglial tau immunoreactivity is seen in the section or not (yes/no).

Neuropathologists at different sites were blinded to the overall neuropathological diagnosis for each case. Examples of the different forms of ARTAG and astrocytic tau immunoreactivities and also a table summarizing the key features of each type of pathology were provided as published recently [21]. A reference group (GGK, JQT, EBL, DJI, JLR, VJ, JBT, DS) evaluated all images and scanned sections to reach a consensus. This meeting was held in the Department of Pathology and Laboratory Medicine, Center for Neurodegenerative Disease Research, Institute on Aging, University of Pennsylvania School of Medicine, Philadelphia, PA, USA.

Statistical analysis

Percentage (%) of agreement with 95% confidence interval (CI) was calculated for each evaluation and also for the mean % of agreements. In addition, a weighted kappa value [11] was calculated to assess agreement between each rater's response and the reference opinion, resulting in 45 kappa values for each study. Then the overall Kappa value was generated by averaging the 45 kappa values and the 95% confidence interval was generated using the bootstrap procedure. The bootstrap resampling method was performed by resampling cases 1000 times. The above process was done for ten different study questions (**Table 2**). When a kappa value can't be generated for a particular rater due to no variation of his/her responses for all sub-questions in a given study, Maxwell's random error coefficient of agreement [25] was applied as an alternative. Kappa value or Maxw ell's statistic above 0.75 was considered "excellent" agreement, 0.4–0.75 as "good" agreement, and below 0.4 as "poor" agreement.

Both kappa value and Maxwell‘s statistic correct for random chance agreement, and thus they are in general lower than the percentage agreement.

Results

Evaluation of astrocytic tau immunoreactivities

For 35 images (**Fig. 1**) the reference group defined the astrocytic morphology (**Table 3**): 11 GFA, 10 TSA, 4 of each astrocytic plaques and tufted astrocytes and 3 of each globular astroglial inclusions and ramified astrocytes. For six images (i.e., 6, 10, 15, 16, 19, and 20) the reference group provided a second option (**Table 3**) since the image was not equivocal. For 24 images the agreement was above 60%, including 9 out of 10 (9/10) images of TSAs, 7/11 of GFAs, 4/4 of astrocytic plaques, 2/4 of tufted astrocytes, 2/3 of globular astroglial inclusions and 1/3 images of ramified astrocytes. Less agreement was reached for 1 out of 10 TSA, 4/11 GFAs, 2/4 tufted astrocytes, 1/3 globular astrocytic inlcusions and for 2/3 ramified astrocytes.

Astrocytic plaques were interpreted only by a few evaluators as GFA, globular astroglial inclusion, or as unclassifiable astrocytic morphology. One image (Nr. 12) of a globular astroglial inclusion was interpreted as astrocytic plaque by 37.78% of the evaluators. This image was taken from the amygdala of an elderly individual showing no further neuropathological features of either GGT or CBD. The reference group felt that the distinct globular structures in the astrocytic processes had to be distinguished from a GFA morphology, which has fine granular deposits. Since the tau immunoreactivity was seen in the proximal segments of the astrocyte, the possibility of this image representing an astrocytic plaque was not raised by the reference group. GFAs were mostly interpreted as astrocytic plaques or rarely as TSAs or tufted astroctyes, while TSAs were interpreted with the widest range of possible astrocytic morphologies (**Table 3**). Ramified astrocytes photographed from Pick´s disease cases were interpreted as TSA or tufted astrocyte by some of the evaluators. Finally tufted astrocytes photographed from cases showing the neuropathological features of PSP, were interpreted as ramified astrocytes, GFA or TSA by a few evaluators. In summary the overall % agreement for study I was above 60% with a kappa value of 0.54 (95% CI 0.433, 0.645) (**Table 2**).

Recognition of ARTAG and other astrogliopathies

Evaluation of 25 scanned tau-immunostained sections revealed high agreement whether ARTAG pathology is present or not (**Table 4**). In this series three cases with neuropathological features of either PSP or CBD were included. Indeed, in these three cases was the least agreement. Scan Nr. 9 represented the basal ganglia from a case showing ARTAG and PSP. While the presence of ARTAG was recognized by 68.29% of the evaluators, only 26.83% recognized tufted astrocytes in the section. Precise evaluation of the section revealed astrocytes compatible with GFAs (**Fig. 2a**) and also tufted astrocytes (**Fig. 2b**). Scan 16 represented temporal cortex from a case with CBD (**Figs. 2c, d**): here more than 50% of evaluators thought ARTAG was also present. Regarding scan 19, the section of the temporal cortex was evaluated from a case with PSP; while ARTAG was recognized, only 52.5% agreed that tufted astrocytes could be seen as well. Finally, for scan 21, approximately 30% of the evaluators felt that the scan showed astrocytic plaques; the reference opinion was that only occasional GFAs were noted in the scanned temporal cortex. In spite the high % of agreement regarding the recognition of ARTAG and other astrogliopathies, kappa values were lower for these (0.48, 95% CI 0.457, 0.900; and 0.24, 95% CI 2.89E-16, 0.374; respectively)(**Table 2**).

Recognition of ARTAG types

Next we examined the agreement of the recognition of different ARTAG types. For each subpial, subependymal, gray and white matter, and perivascular ARTAG high agreement was reached (approximately 80%), with kappa values ranging from 0.37-0.71 (**Table 2**). Only a few examples can be listed where considerable disagreement was observed (**Table 5**). Regarding ARTAG in scans 3, 10, and 25 the reference group thought that the tau immunoreactive dots in subpial (**Fig. 3a**), subependymal (**Fig. 3c**), or perivascular (**Fig. 3e**) locations, respectively, were not sufficient to call ARTAG. On the other hand, in scan 13 occasional subpial TSAs (**Fig. 3b**), in scan 24 subependymal (**Fig. 3d**) and in scan 7 perivascular (**Fig. 3f**) TSAs and thick astrocytic processes were recognized and interpreted as ARTAG. In scan 17 the reference group did not interpret the tau immunoreactivity in the white matter as ARTAG, but rather as oligodendroglial coiled bodies (**Fig. 4a, b**). On the other hand, in scan 20, the reference group interpreted the tau immunoreactivity in the white matter in the vicinity of the inferior ventricle as white matter ARTAG (**Fig. 4c, d**). Regarding scan 10, the reference group did not interpret the single astrocytic-like tau immunoreactivity (**Fig. 4e**) in the dentate gyrus as ARTAG, while in the CA4 region similar immunoreactivities

were interpreted as such, leading to disagreement (**Table 5**). On the other hand, in scan 13, TSAs in the dentate gyrus were interpreted as ARTAG (**Fig. 4f**) with a high level of agreement (80.49%). In scan 12 several neuritic plaque-related tau profiles were observed in the inferior temporal gyrus (**Fig. 4g**), and due to the unequivocal characteristics of GFAs or TSA, were not interpreted as ARTAG. In the temporal cortex as seen in scan 15, occasional GFAs (**Fig. 4h**) were interpreted as ARTAG with high agreement (72.5 %; **Table 5**). Finally, in scan 12, ARTAG was seen in both the hippocampal dentate gyrus (**Fig. 4i**) and the CA4 subregion (**Fig. 4j**), however with different agreement among raters (87.8% versus 70.73%).

Evaluation of the severity and extent of ARTAG

In all scans where ARTAG was observed, severity was scored in a total of 90 locations by the reference group. Agreement for these 90 locations ranged from 12.5 to 87.5% (mean 50.49%) with a kappa value of 0.39±0.049 (**Table 2**). Next we evaluated the agreement to decide whether the severity is occasional or numerous, without further stratification of numerous for focally accentuated or widespread. For this the agreement was better (ranged from 22.5-100%) with a mean agreement of 65.9% (**Table 2**).

Finally, the overall assessment of ARTAG pathologies (all aforementioned aspects calculated) revealed 82.3% percentage of agreement and a kappa value of 0.59 (95% CI 0.534, 0.653) (**Table 2**).

Discussion

The goal of this study was to evaluate the variation in the neuropathologic assessment of ARTAG. While several studies have been conducted to determine interrater variability of AD-related neuropathological changes or Lewy body pathologies [3, 27], there was a paucity of data how astrocytic tau immunoreactivities are described. In spite the definition of tufted astrocytes and astrocytic plaques as hallmark lesions of PSP and CBD [13], respectively, the spectrum of astrocytic tau immunoreactive morphologies expand beyond these two, even within PSP and CBD. Some of these morphologies are thought to represent early forms of tufted astrocytes [29], analogous to the concept of pretangles preceding neurofibrillary tangles [8]. Further astrocytic morphologies have been described in primary FTLD-tauopathies, such as the ramified astrocytes in PiD [14] and globular astrocytic inclusions in GGT [1]. The recent consensus statement on the evaluation of ARTAG aimed to harmonize the description of astrocytic tau morphologies and added GFAs and TSAs to this spectrum of tau

immunoreactivities [21]. Importantly, our study revealed that the overall assessment of ARTAG shows a good agreement (kappa: 0.59, 95% CI 0.534, 0.653) between raters across multiple international centres.

To be able to include researchers and neuropathologists from all over the world we decided to use the cost-effective method of digital pathology, which is already widely applied for some diagnostic aspects, including post mortem neuropathology evaluations. Our first study focused on images of single astrocytic tau immunoreactivities, and the second on the evaluation of scanned slides. We are aware that evaluating single images and scans of circumscribed anatomical regions might have led to a certain proportion of disagreement. In the recent study on the multisite assessment of NIA-AA criteria of AD-related pathologies, whole-slide images decreased the performance for the evaluation of severity and scores of amyloid-β plaques [27]. On the other hand with this approach in the current study we were able to eliminate bias during the evaluation of cases. This means, for example, that if the evaluator looks across many anatomical regions and decides that the diagnosis is PSP or CBD, then the spectrum of astrocytic morphologies may be overlooked or not described in detail, with most classified as tufted astrocytes or astrocytic plaques, respectively. Our study, however, was able to show that GFA type morphologies in the gray matter occur in both PSP and CBD, suggesting that these have a pathogenic relation to the tau lesions in primary FTLD-tauopathies. Importantly, several cases included in this study were above 85 years of age where some evaluators suspected primary FTLD tauopathy-related astrocytic tau pathologies. Therefore, evaluating only a small number of anatomical regions or brain biopsies for the diagnosis of neurodegenerative diseases could potentially lead to misinterpretations in the classification of astrocytic pathologies. We noted variability in the evaluation of ramified astrocytes, which were either under-recognized, or in some cases, TSAs and tufted astrocytes were misinterpreted for these ramified morphologies. This might be due to the fact that PiD is a rare disorder, which shows variability in the presence and severity of astrocytic tau immunoreactivity [14, 18, 23]. Indeed, ramified astrocytes are less studied astrocytic morphologies. Although we cannot exclude that ramified astrocytes are present in the ageing brain without the classical neuronal tau pathology characteristic of PiD, these astrocytes frequently show 3R tau isoform immunoreactivity in PiD [16, 23], which might be useful tool to reconcile these discrepancies. Some degree of difficulty was also observed for the recognition of individual TSAs in microphotographs. This was not a problem for scanned sections when, depending on the location (i.e., subpial, subependymal,

perivascular), even without the classical thorny appearance, astrocytes were interpreted as ARTAG. Accordingly, when single astrocytic tau morphologies are evaluated in these locations, the TSA-like appearance may be more ambiguous and might not be recognized in all cases.

The least agreement was observed for the evaluation of severity and extent of ARTAG. While the distinction between occasional and numerous was better, there are several aspects, which need to be addressed. For the original recommendation for the description of ARTAG severity, instead of the widely used three-tiered semiquantitative strategy (mild, moderate, severe) we aimed to concede that ARTAG astrocytes may be focally accentuated (e.g. subpial TSAs in cortical areas in the depths of sulci) or may appear throughout (i.e., widespread) the gray matter as GFAs, or in the white matter as TSAs. Many evaluators expressed difficulty in describing the situation when single gray matter GFAs or subpial TSAs are seen following the cortical ribbon with 500-2000 μm distance between them: whether this is to be interpreted as widespread even if the amount of astrocytes on a birds-eye view is not numerous. The way one has to manipulate the digital slides, such as zooming in and out, may have also contributed to the discordance in determining the amount of ARTAG. It is a challenge to incorporate the different distribution patterns of ARTAG in a simple scoring system, especially that morphometry methods are difficult to develop that distinguish between neuronal and astrocytic tau immunoreactivities.

Considering all aspects we recommend the following strategy with modest changes as compared to the original ARTAG consensus harmonization paper [21]:

1) After the recognition of the morphology of astrocytic tau immunoreactivity using high magnification (x200-x400), the extent of involvement in a selected anatomical area should be evaluated using low magnification (x50-x100);

2) If the occasional tau immunoreactive astrocytes appear in a circumscribed area of the specific anatomical region then it should designated as “occasional” (score 1, corresponding to mild in a semiquantitative evaluation strategy);

3) If the occasional tau immunoreactive astrocytes are scattered throughout the anatomical region then it should be designated as “widespread” with the note that the degree is mild/moderate (score 2, corresponding to moderate in a semiquantitative evaluation strategy);

4) If numerous tau immunoreactive astrocytes appear in a circumscribed area of the specific anatomical region then it should designated as “numerous, focally accentuated” (score 2, corresponding also to moderate in a semiquantitative evaluation strategy);
5) If numerous tau immunoreactive astrocytes appear throughout the anatomical region then it should be designated as “numerous widespread” (score 3, corresponding to severe in a semiquantitative evaluation strategy).

For several evaluations we observed considerable discrepancy between the % of agreement and the calculated kappa values. This is because kappa value corrects for random chance agreement, while percentage of agreement does not. Thus kappa value is a more conservative summary measure than percentage agreement.

In summary, we find that application of a harmonized consensus evaluation strategy for the description of ARTAG [21] yields a good inter-rater agreement and good comparison across research and neuropathology sites. Improvement is needed regarding comparable evaluations of the severity and extent of ARTAG types. Our study suggests that the spectrum of co-existing astrocytic tau immunoreactivities might be wider than generally assumed in primary FTLD-tauopathies if more care is taken to describe the morphologies. This concept does not weaken the diagnostic importance of tufted astrocytes, astrocytic plaques, ramified astrocytes and globular astrocytic inclusions as specific morphologies associated with certain primary FTLD-tauopathies. On the other hand this notion might help to better understand the pathogenic relevance of ARTAG and its relation to primary FTLD-tauopathies or other disease conditions with astrocytic tau pathologies such as chronic traumatic encephalopathy [26]. Overall, our study supports the application of the current harmonized consensus evaluation strategy of ARTAG [21] with a slight modification of the evaluation of its severity and extent. This allows collection and comparison of worldwide data on ARTAG for research purposes.

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Legend to Tables

Table 1. Clinicopathological data of the cases included in study 1 (evaluation of photos) and study 2 A and B (evaluation of scanned sections). Abbreviations: m: male; f: female; CERAD: Consortium to Establish a Registry for Alzheimer Disease; Cx: cortex. Braak stage refers to neurofibrillary degeneration [10] and Thal phase to Aβ deposition [31]. *indicates that presence of subcortical neurofibrillary tangles were suggestive of early form of PSP; **indicates that ARTAG was represented by single GFAs.

Table 2. Summary of kappa values (± 95 confidence interval, CI) and mean % of agreement (± 95 CI) for different aspects of study I and II.

Table 3. Reference opinion and interrater agreement (% of agreement ± 95 confidence interval, CI) for the microphotographs (1-35) representing astroglial tau immunoreactivities. GAI:globular astrocytic inclusion; AP: astrocytic plaque; TA: tufted astrocyte; RA: ramified astrocyte.

Table 4. Interrater agreement (% of agreement ± 95 confidence interval, CI) for the recognition of ARTAG and other astroglial tau pathologies. Note that for case 14, only 14 evaluations were received due to technical reasons.

Table 5. Interrater agreements (AGR; % of agreement ± 95 confidence interval, CI) for different ARTAG types (SP: subpial; SE: subependymal; PV: perivascular; WM: white matter; GM: gray matter) in different anatomical regions (TEM: temporal; FRO: frontal; IC: internal capsule; FRB: frontobasal; MES: mesencephalon; MED-MID: medulla oblongata midline; PYR: pyramid; AMY: amygdala; CING: anterior cingulate; CAUD: caudate; ACC: accumbens; HYPOG: hypoglossal nucleus; CA1-4: cornu ammonus 1-4 subregion; GYAMB: gyrus ambiens; DG: dentate gyrus.

Legend to figures

Figure 1. Microphotographs used in study 1. The numbering corresponds to that listed in Table 2 with the reference opinion. Arrows indicate the astroglial tau immunoreactivities that were specifically asked to be evaluated.

Figure 2. Representative images of tau immunoreactive astrocytes in cases where the interrater agreement was less than in others when the presence of ARTAG had to be decided.

GFA (A) and TA (B) in the striatum in case 9. Astrocytic plaques (C, D) in a case 16 (corticobasal degeneration). Astrocytic tau immunoreactivities (E) in a case 19 with early form of PSP with an example of a tufted astrocyte (left upper) and granular/fuzzy astrocyte (right lower) in image F. Occasional GFAs in the temporal cortex in case 21 (G, H).

Figure 3. Representative images of different ARTAG types where discrepancy was observed between the reference opinion and the evaluations (see also Table 4). In scans 3, 10 and 25 the reference group did not interpret the fine dots (arrows) as subpial (A), subependymal (C), or perivascular (E) ARTAG, respectively. On the other hand, in cases 13, 24 and 7 thorny astrocytes and thick astrocytic processes (arrows) were interpreted as subpial (B), subependymal (D) and perivascular (F) ARTAG, respectively.

Figure 4. Representative images of different ARTAG types where discrepancy was observed between the reference opinion and the evaluations (see also Table 4). In scan 17 the reference group did not interpret the tau immunoreactivity in the white matter as ARTAG but rather as oligodendroglial coiled bodies (A; enlarged in B). In scan 20 the reference group interpreted the tau immunoreactivity in the white matter in the vicinity of the inferior ventricle as white matter ARTAG (C, D). In scan 10 the reference group did not interpret the single astrocytic-like tau immunoreactivity (E; arrow) as ARTAG leading to disagreement. In scan 13 thorny astrocytes in the dentate gyrus were interpreted as ARTAG (F). Neuritic plaque tau profiles in the temporal cortex (G) were not interpreted as ARTAG in case 12. In the temporal cortex of case 15, occasional granular/fuzzy astrocytes (H) were interpreted as ARTAG. In scan 12 ARTAG was seen in both the hippocampal dentate gyrus (I) and the CA4 subregion (J) but with different levels of agreement.

Online supplemental file 1. Evaluation sheets used for this study.

Table 1.

			Neuropathology										Photo number and region					Scan number and region							
Case Nr.	**Age**	**Sex**	AD/PART	Braak stage	Thal phase	CERAD score	AGD	ARTAG	PSP	CBD	GGT	Pick's disease	Frontal Cx	Temporal Cx	Hippocampus	Amygdala	Basal ganglia	Frontal Cx	Temporal Cx	Ant. cingulate	Hippocampus	Amygdala	Basal ganglia	Mesencephalon	Medulla obl.
Case-1	80	f	+	3	1	1	+	+	-	-	-	-		1					1						
Case-2	85	f	+	2	1	1	-	+	-	-	-	-		2-5			6-10	3	2				4	5	6
Case-3	87	f	+	3	1	1	-	+	-	-	-	-				11,12						7			
Case-4	89	f	+	3	2	1	+	+	-	-	-	-				13,14						8			
Case-5	83	f	+	6	5	3	+	+	+*	-	-	-					15,16						9		
Case-6	83	f	+	3	2	2	+	+	-	-	-	-		17-19							10				
Case-7	85	m	+	2	1	1	+	+	-	-	-	-				20						11			
Case-8	77	m	+	3	2	2	+	+	-	-	-	-			21				12					25	
Case-9	82	m	+	5	3	2	+	+	+	-	-	-			22						13				
Case-10	86	m	+	5	3	3	+	+	-	-	-	-			23						14				
Case-11	82	m	+	3	1	1	+	+	-	-	-	-	24					17							
Case-12	79	m	+	4	3	2	-	-	-	+	-	-		25, 28, 30					16						
Case-13	76	f	+	2	0	0	-	-	+	-	-	-		26,27,29					19						
Case-14	88	f	+	4	3	3	+	+	-	-	-	-	31					22							
Case-15	63	f	-	-	-	-	-	-	-	-	+	-	32,34												
Case-16	55	m	-	-	-	-	-	-	-	-	-	+		33											
Case-17	65	f	-	-	-	-	-	-	-	-	-	+		35											
Case-18	82	f	+	2	1	1	-	+	-	-	-	-									15				
Case-19	87	f	+	3	0	0	+	+	+*	-	-	-								18			23		
Case-20	85	m	+	1	0	0	-	+	-	-	-	-									20				
Case-21	81	m	+	1	0	0	-	+**	-	-	-	-							21						
Case-22	83	m	+	2	1	1	+	+	-	-	-	-											24		

Table 2.

Study and question	Kappa value	95% CI	Mean % of agreement	95% CI
1: Recognition of astrocytes	0.54	0.433, 0.645	63.8	±7.5
2A: Recognition of presence of ARTAG	0.48	0.457, 0.900	91.1	±5.1
2A: Recognition of other astrogliopathy	0.24	2.89E-16, 0.374	73.1	±6.3
2A: Recognition of SP ARTAG	0.60	0.468, 0.739	81.8	±7.02
2A: Recognition of SE ARTAG	0.71	0.584, 0.828	87.2	±5.9
2A: Recognition of GM ARTAG	0.37	0.288, 0.536	83.1	±5.6
2A: Recognition of WM ARTAG	0.44	0.323, 0.551	79.5	±6.05
2A: Recognition of PV ARTAG	0.57	0.442, 0.672	78.1	±6.4
2B: Semiquantitative scoring	0.39	0.341, 0.445	50.4 (65.9)	±3.8 (4.6)
2A+B: Overall assessment of ARTAG	0.59	0.534,0.65	82.3	±2.4

Table 3.

Photo Nr.	Ref. Opinion Nr. 1	Interrater agreement (%)	95% CI	Ref. Opinion Nr. 2	Interrater agreement (%): Ref. 1 +2	95% CI	2nd most frequent opinion of the evaluators	% of second most frequent opinion
1	GFA	97.78	±4.31	-	-	-	TA	2.22
2	GFA	73.33	±12.9	-	-	-	TA	11.1
3	GFA	62.22	±14.1	-	-	-	TSA	26.67
4	TSA	84.44	±10.5	-	-	-	GFA	4.44
5	TSA	91.11	±8.32	-	-	-	TA, RA, GFA	2.22
6	GFA	68.89	±13.5	TSA	86.67	±11.2	TSA	17.78
7	GFA	64.44	±13.9	-	-	-	RA	22.22
8	GFA	37.78	±14.1	-	-	-	TSA	37.78
9	TSA	75.56	±12.5	-	-	-	RA, GFA	6.67
10	TSA	64.44	±13.9	GFA	64.44	±13.9	RA	28.89
11	TSA	77.78	±12.1	-	-	-	GAI	11.11
12	GAI	42.22	±14.4	-	-	-	AP	37.78
13	TSA	80.00	±11.6	-	-	-	TA, GFA	11.11
14	GFA	60.00	±14.3	-	-	-	AP	26.67
15	RA	44.44	±14.5	TA	57.78	±14.4	TSA	24.44
16	TA	24.44	±12.5	RA	35.56	±13.9	GFA, TSA	24.44
17	AP	73.33	±12.9	-	-	-	GFA	13.33
18	GFA	68.89	±13.5	-	-	-	AP	15.56
19	GFA	6.67	±7.28	RA	28.89	±13.2	TSA	31.11
20	GFA	31.82	±13.6	AP	86.36	±10.0	AP	54.55
21	TSA	68.89	±13.5	-	-	-	GFA	15.56
22	TSA	73.33	±12.9	-	-	-	RA, GAI, GFA	6.67
23	TSA	57.78	±14.4	-	-	-	GAI	24.44
24	TSA	71.11	±13.2	-	-	-	GFA	15.56
25	AP	88.89	±9.19	-	-	-	GAI	6.67
26	TA	44.44	±14.5	-	-	-	RA	26.67
27	TA	62.22	±13.9	-	-	-	RA	24.44
28	AP	80.00	±11.1	-	-	-	Uncl	6.67
29	TA	68.89	±13.5	-	-	-	RA, Uncl	8.89
30	AP	88.89	±9.19	-	-	-	Uncl	4.44
31	GFA	40.00	±14.3	-	-	-	AP	40.00
32	GAI	91.11	±8.32	-	-	-	GFA	6.67
33	RA	8.89	±8.31	-	-	-	TSA	68.89
34	GAI	91.11	±8.32	-	-	-	Uncl, TSA, GFA, TA	2.22
35	RA	68.18	±13.6	-	-	-	TA	25.00

Table 4.

Scan	ARTAG	Agreement	95%CI	Other AG	Agreement	95%CI	Nr of evaluations
1	yes	95.12	±6.59	no	78.05	±12.67	41
2	yes	100.00	0	no	75.00	±13.42	40
3	yes	100.00	0	no	80.00	±12.4	40
4	yes	100.00	0	no	87.50	±10.25	40
5	yes	100.00	0	no	82.50	±11.78	40
6	yes	100.00	0	no	85.00	±11.07	40
7	yes	80.00	±12.4	no	62.50	±15	40
8	yes	100.00	0	no	75.00	±13.42	40
9	yes	68.29	±14.24	TA	26.83	±13.56	41
10	yes	92.68	±7.97	no	63.41	±14.74	41
11	yes	100.00	0	no	80.49	±12.13	41
12	yes	92.68	±7.97	no	87.80	±10.02	41
13	yes	95.12	±8.45	no	80.49	±12.13	41
14	yes	100.00	0	no	100.00	0	14
15	yes	100.00	0	no	77.50	±12.94	40
16	no	48.78	±15.3	AP	63.41	±14.74	41
17	yes	77.50	±12.94	no	60.00	±15.18	40
18	yes	92.50	±8.16	no	77.50	±12.94	40
19	yes	80.00	±12.4	TA	52.50	±15.48	40
20	yes	100.00	0	no	95.00	±6.75	40
21	yes	70.00	±14.2	no	67.50	±14.51	40
22	yes	92.50	±8.16	no	47.50	±15.48	40
23	yes	100.00	0	no	67.50	±14.51	40
24	yes	97.50	±4.84	no	65.00	±14.78	40
25	yes	95.00	±6.75	no	90.00	±9.3	40

Table 5.

Scan Nr SP-SE-PV	SP	AGR	95% CI	SE	AGR	95% CI	PV	AGR	95% CI	Scan Nr WM	Region	WM	AGR	95% CI	Scan Nr GM	Region	GM	AGR	95% CI
1	no	92.68	±7.97	no	100.00	±0	no	95.12	±6.59	1	TEM	no	53.66	±15.2	1	TEM	yes	92.68	±7.97
2	yes	95.00	±6.75	no	100.00	0	yes	80.00	±12.4	2	TEM	yes	97.50	±4.84	2	TEM	yes	97.50	±4.84
3	no	27.50	±13.8	no	75.00	±13.4	yes	60.00	±15.1	3	FRO	yes	92.50	±8.1	3	FRO	yes	100.00	0
4	yes	90.00	±9.3	yes	70.00	±14.2	yes	92.50	±8.16	4	IC	yes	77.50	±12.9	4	CAUD	yes	100.00	0
5	yes	97.50	±4.84	yes	100.00	0	yes	97.50	±4.84	4	FRB	yes	100.00	0	4	ACC	yes	100.00	0
6	yes	100.00	0	yes	95.00	±6.75	yes	97.50	±4.84	5	MES	yes	92.50	±8.1	4	FRB	yes	100.00	0
7	yes	72.50	±13.8	no	72.50	±13.8	yes	37.50	±15.0	6	MED-MID	yes	97.50	±4.8	5	MES	yes	97.50	±4.84
8	yes	95.00	±6.75	yes	100.00	0	yes	92.50	±8.16	6	PYR	yes	100.00	0	6	HYPOG	yes	100.00	0
9	no	95.12	±6.59	no	97.56	±4.72	no	78.05	±12.6	7	AMY	yes	72.50	±13.8	7	AMY	yes	75.00	±13.4
10	yes	73.17	±13.5	no	51.22	±15.3	no	75.61	±13.1	8	AMY	yes	95.00	±6.75	8	AMY	yes	100.00	0
11	yes	92.68	7±.97	no	78.05	±12.7	yes	58.54	±15.0	9	IC	no	73.17	±13.5	9	CAUD	yes	65.85	±14.5
12	yes	82.93	±11.2	yes	65.85	±14.5	yes	56.10	±15.1	10	TEM	no	78.05	±12.6	10	CA1-4	yes	58.54	±15.0
13	yes	70.73	±13.9	yes	90.24	±9.08	yes	56.10	±15.1	11	AMY	yes	68.29	±14.2	10	DG	no	51.22	±15.3
14	no	85.71	±10.8	no	100.00	0	yes	64.29	±14.8	12	TEM	yes	92.68	±7.97	10	TEM	yes	82.93	±11.5
15	yes	100.00	0	no	57.50	±15.3	yes	82.50	±11.7	13	TEM	yes	92.68	±7.97	11	AMY	yes	85.37	±10.8
16	no	58.54	±15.0	no	95.12	6.59	no	85.37	±10.8	14	TEM	yes	100.00	0	11	GYAMB	yes	80.49	±12.1
17	no	90.00	±9.3	no	100.00	0	no	90.00	±9.3	15	TEM	yes	97.50	±4.84	12	CA1-4	yes	70.73	±13.9
18	no	60.00	±15.1	no	92.50	±8.16	no	75.00	±13.4	16	TEM	no	63.41	±14.7	12	DG	yes	87.80	±10.0
19	no	70.00	±14.2	no	100.00	0	no	95.00	±6.75	17	FRO	no	42.50	±15.3	12	TEM	no	34.15	±14.5
20	yes	100.00	0	yes	95.00	±6.75	yes	65.00	±14.7	18	CING	yes	87.50	±10.2	13	CA1-4	no	43.90	±15.1
21	no	77.50	±12.9	no	100.00	0	no	97.50	±4.84	19	TEM	yes	65.00	±14.7	13	DG	yes	80.49	±12.1
22	no	92.50	±8.16	no	100.00	0	no	97.50	±4.84	20	TEM	no	55.00	±15.4	13	TEM	yes	78.05	±12.6
23	no	56.41	±15.5	no	84.62	±11.3	no	76.92	±13.2	20	HIPP	yes	87.50	±10.2	14	CA1-4	no	85.71	±10.8
24	yes	97.50	±4.84	yes	70.00	±14.2	yes	75.00	±13.4	21	TEM	no	82.50	±11.7	14	DG	no	92.86	±7.98
25	yes	72.50	±13.8	no	90.00	±9.3	no	72.50	±13.8	22	FRO	yes	62.50	±15.0	14	TEM	no	64.29	±14.8
										23	IC	no	58.97	±15.4	15	CA1-4	yes	87.50	±10.2
										23	FRB	yes	92.31	±8.36	15	DG	yes	92.50	±8.16
										24	IC	no	72.50	±13.8	15	TEM	yes	72.50	±13.8
										24	FRB	yes	92.50	±8.16	16	TEM	no	63.41	±14.7
										25	MES	no	42.50	±15.3	17	FRO	yes	77.50	±12.9
															18	CING	yes	90.00	±9.3
															19	TEM	yes	80.00	±12.4
															20	CA1-4	yes	85.00	±11.0
															20	DG	yes	100.00	0
															20	TEM	yes	92.50	±8.16
															21	TEM	yes	67.50	±14.5
															22	FRO	yes	92.50	±8.16
															23	CAUD	yes	94.87	±6.92

23	ACC	yes	89.74	±9.52
23	FRB	yes	87.18	±10.4
24	CAUD	yes	92.50	±8.16
24	ACC	yes	85.00	±11.0
24	FRB	yes	90.00	±9.3
25	MES	yes	95.00	±6.75