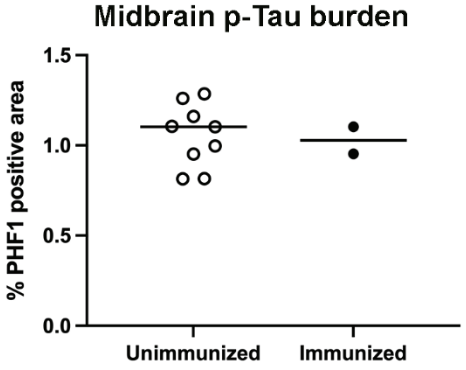
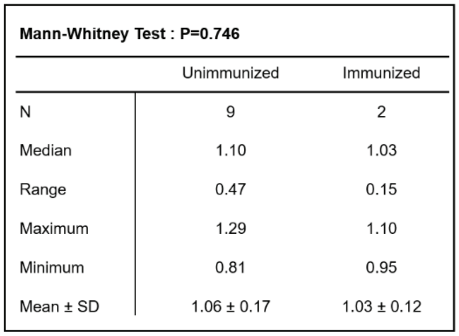




**Mann-Whitney Test : P=0.746**

| | Unimmunized | Immunized |
|---|---|---|
| N | 9 | 2 |
| Median | 1.10 | 1.03 |
| Range | 0.47 | 0.15 |
| Maximum | 1.29 | 1.10 |
| Minimum | 0.81 | 0.95 |
| Mean ± SD | 1.06 ± 0.17 | 1.03 ± 0.12 |

**Supplemental Fig. 1 Quantitative analysis of total tau burden in immunized versus unimmunized PSP cases**

The percent area occupied by PHF1 positive staining was calculated in the midbrain of unimmunized PSP (n=9) and immunized PSP (n=2). Unimmunized cases 5 and 7 were excluded from analysis due to high background which interfered with digital image analysis. Mann-Whitney U test revealed no differences in the median values between two groups. Descriptive results are shown in the summary table.

**a** # Vesicular Astrocytes in the middle frontal cortex

**Perivascular**

**Mann-Whitney Test : P=0.013**

| | Unimmunized | Immunized |
|---|---|---|
| N | 11 | 2 |
| Median | 1 | 27 |
| Range | 4 | 16 |
| Maximum | 4 | 35 |
| Minimum | 0 | 19 |
| Mean ± SD | 1.36 ± 1.43 | 27.00 ± 11.31 |

**Non-perivascular**

**Mann-Whitney Test : P=0.218**

| | Unimmunized | Immunized |
|---|---|---|
| N | 11 | 2 |
| Median | 1 | 1.5 |
| Range | 1 | 1 |
| Maximum | 1 | 2 |
| Minimum | 0 | 1 |
| Mean ± SD | 0.55 ± 0.52 | 1.50 ± 0.71 |

**b** # Tufted Astrocytes in the middle frontal cortex

**Perivascular**

**Mann-Whitney Test : P=0.423**

| | Unimmunized | Immunized |
|---|---|---|
| N | 11 | 2 |
| Median | 3 | 6.5 |
| Range | 22 | 7 |
| Maximum | 22 | 10 |
| Minimum | 0 | 3 |
| Mean ± SD | 4.64 ± 6.27 | 6.50 ± 4.95 |

**Non-perivascular**

**Mann-Whitney Test : P=0.923**

| | Unimmunized | Immunized |
|---|---|---|
| N | 11 | 2 |
| Median | 31 | 25 |
| Range | 68 | 4 |
| Maximum | 5 | 27 |
| Minimum | 63 | 23 |
| Mean ± SD | 33.00 ± 23.42 | 25.00 ± 2.83 |

**c** # Vesicular Astrocytes in the angular cortex

**Perivascular**

**Mann-Whitney Test : P=0.013**

| | Unimmunized | Immunized |
|---|---|---|
| N | 11 | 2 |
| Median | 0 | 10 |
| Range | 3 | 2 |
| Maximum | 3 | 11 |
| Minimum | 0 | 9 |
| Mean ± SD | 0.46 ± 0.94 | 10 ± 1.41 |

**Non-perivascular**

**Mann-Whitney Test : P=0.346**

| | Unimmunized | Immunized |
|---|---|---|
| N | 11 | 2 |
| Median | 1 | 2 |
| Range | 5 | 0 |
| Maximum | 5 | 2 |
| Minimum | 0 | 2 |
| Mean ± SD | 1.27 ± 1.62 | 2 ± 0 |

**d** # Tufted Astrocytes in the angular cortex

**Perivascular**

**Mann-Whitney Test : P=0.205**

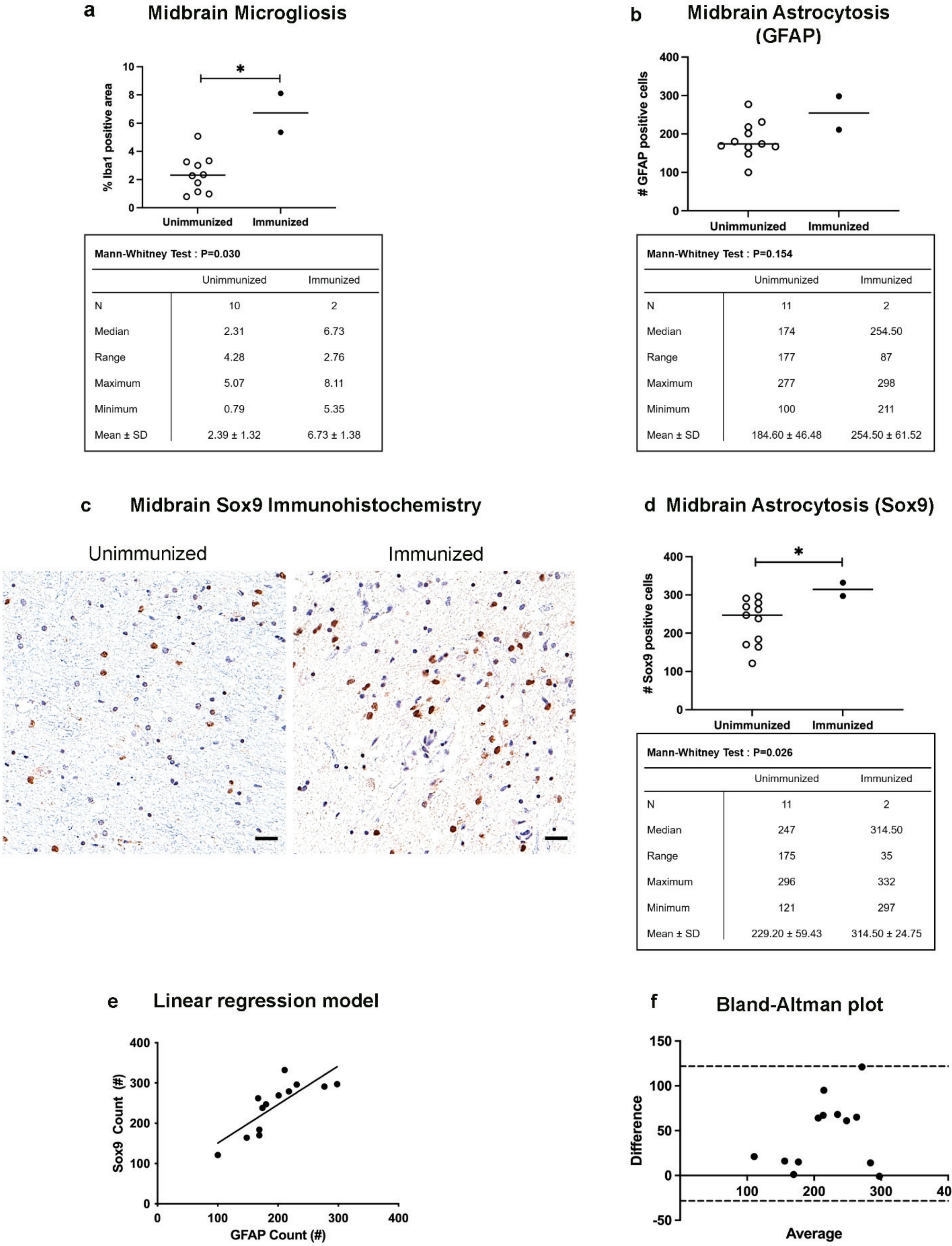
| | Unimmunized | Immunized |
|---|---|---|
| N | 11 | 2 |
| Median | 3 | 6 |
| Range | 12 | 4 |
| Maximum | 12 | 8 |
| Minimum | 0 | 4 |
| Mean ± SD | 3.09 ± 3.33 | 6.00 ± 2.83 |

**Non-perivascular**

**Mann-Whitney Test : P=0.539**

| | Unimmunized | Immunized |
|---|---|---|
| N | 11 | 2 |
| Median | 19 | 30 |
| Range | 80 | 8 |
| Maximum | 85 | 34 |
| Minimum | 5 | 26 |
| Mean ± SD | 27.00 ± 24.06 | 30.00 ± 5.66 |

**Supplemental Fig. 2 Descriptive statistics for the number of astrocytic tau inclusions in immunized versus unimmunized PSP cases.** The sample size (N), median, range, maximum, minimum, mean ± standard deviation (SD), and p value as determined by Mann-Whitney test are presented for (a) perivascular and non-perivascular vesicular astrocytes in the middle frontal cortex, (b) perivascular and non-perivascular tufted astrocytes in the middle frontal cortex, (c) perivascular and non-perivascular vesicular astrocytes in the angular cortex, and (d) perivascular and non-perivascular tufted astrocytes in the angular cortex.



a Midbrain Microgliosis

Mann-Whitney Test : P=0.030

| | Unimmunized | Immunized |
|---|---|---|
| N | 10 | 2 |
| Median | 2.31 | 6.73 |
| Range | 4.28 | 2.76 |
| Maximum | 5.07 | 8.11 |
| Minimum | 0.79 | 5.35 |
| Mean ± SD | 2.39 ± 1.32 | 6.73 ± 1.38 |

b Midbrain Astrocytosis (GFAP)

Mann-Whitney Test : P=0.154

| | Unimmunized | Immunized |
|---|---|---|
| N | 11 | 2 |
| Median | 174 | 254.50 |
| Range | 177 | 87 |
| Maximum | 277 | 298 |
| Minimum | 100 | 211 |
| Mean ± SD | 184.60 ± 46.48 | 254.50 ± 61.52 |

c Midbrain Sox9 Immunohistochemistry

d Midbrain Astrocytosis (Sox9)

Mann-Whitney Test : P=0.026

| | Unimmunized | Immunized |
|---|---|---|
| N | 11 | 2 |
| Median | 247 | 314.50 |
| Range | 175 | 35 |
| Maximum | 296 | 332 |
| Minimum | 121 | 297 |
| Mean ± SD | 229.20 ± 59.43 | 314.50 ± 24.75 |

e Linear regression model

f Bland-Altman plot

**Supplemental Fig. 3 Quantitative analysis of gliosis in immunized versus unimmunized PSP cases**

The percent area occupied by positive thresholds for (a) Iba1 (n=10, immunized PSP, n=2, unimmunized PSP) was calculated. Case 5 exhibited high background which interfered with digital image analysis was excluded from the percent of Iba1 positive area measurements. (b) The number of GFAP positive cells in the midbrain of unimmunized PSP (n=11) and immunized PSP (n=2) is shown. Representative images of (c) Sox9 immunohistochemistry and (d) quantification of Sox9 positive cells show increased number of astrocytes in the immunized PSP cases compared to unimmunized PSP cases. Median values within each group of cases are indicated and *p<0.05 was determined by Mann-Whitney U test. Descriptive results are presented in summary tables. (e) Linear regression model demonstrates a positive correlation between GFAP positive and Sox9 positive cell counts. (f) Bland-Altman plot shows 95% confidence limits of the bias for two counting measurements within two dotted lines, demonstrating that Sox9 was more sensitive in terms of identifying astrocytes.

Supplemental Table 1. Antigen and antibody list

| Antigen | Antibody | Host | Concentration | Source |
|---|---|---|---|---|
| Phospho-tau (pS396 and pS404) | PHF1 | mouse monoclonal | 1:2000 for IHC<br>1:600 for IF | Gift from Peter Davies |
| 4-repeat tau | Anti 4R-tau | mouse monoclonal | 1:5000 for IHC | Cosmo Bio |
| 3-repeat tau | RD3 | mouse monoclonal | 1:1000 for IHC | Millipore |
| AD-specific conformation tau | GT38 | mouse monoclonal | 1:1000 for IHC | Gift from CNDR |
| Iba1 | Anti-Iba1 | rabbit polyclonal | 1:2000 for IHC | Wako |
| Sox9 | Anti-Sox9 | rabbit monoclonal | 1:2000 for IHC | Abcam |
| GFAP | 2.2B10 | rat monoclonal | 15000 for IHC | Gift from CNDR |
| EEA1 | PA1-063A | rabbit polyclonal | 1:100 for IF | ThermoFisher Scientific |
| LC3B | D11 | rabbit polyclonal | 1:1000 for IF | Cell Signaling Technology |
| LAMP1 | Anti-LAMP1 | rabbit polyclonal | 1:450 for IF | Abcam |
| Human $IgG_4$ | MA5-32169 | rabbit polyclonal | 1:1000 for IF | Invitrogen |

*IHC* Immunohistochemistry, *IF* Immunofluorescence, *AD* Alzheimer's disease, *CNDR* Center for Neurodegenerative Disease Research, *GFAP* Glial fibrillary acidic protein, *EEA1* Early Endosome Antigen 1, *LC3B* Microtubule-associated proteins 1A/1B light chain 3B, *LAMP1* Lysosomal-associated membrane protein 1

Supplementary Table 2. Regional scoring of cellular tau pathologies in PSP

| | | | Cortex-Grey matter | | | | | Medial Temporal Lobe | | | | Subcortical Nuclei | | | Brain Stem | | | | | Cerebellum |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Case | Group | Tau/Cell | FR | TE | AN | VI | CI | AM | HC DG | HC CA | HC EC | PU | GP | TH | SN | MB | LC | PB | MO | DN |
| 1 | PSP with treatment effect | Neuron | 0.5 | 0.5 | 1 | 0.5 | 1 | 2 | 0.5 | 0.5 | 0.5 | 0.5 | 1 | 2 | 3 | 2 | 2 | 2 | 2 | 2 |
| | | Astro | 0.5 | 0.5 | 1 | 0.5 | 0.5 | 2 | - | 0 | 0 | 3 | 2 | 2 | 0 | 2 | 0 | 0.5 | 0.5 | 0 |
| | | Astro-PVA | 2 | 0.5 | 2 | 0.5 | 1 | 0.5 | - | 0 | 0.5 | 1 | 0.5 | 1 | 0 | 0.5 | 0 | 0.5 | 0.5 | 0 |
| | | Oligo | 0.5 | 0 | 0.5 | 0.5 | 0.5 | 1 | - | 0 | 0 | 1 | 2 | 2 | 0 | 3 | 0 | 0.5 | 1 | 0.5 |
| 2 | PSP with treatment effect | Neuron | 0.5 | 1 | 0.5 | 0 | 1 | 2 | 1 | 2 | 2 | 1 | 1 | 2 | 3 | 2 | 2 | 2 | 2 | 1 |
| | | Astro | 0.5 | 0.5 | 0.5 | 0 | 1 | 2 | - | 0 | 0 | 3 | 2 | 2 | 0.5 | 1 | 0.5 | 0.5 | 0.5 | 0.5 |
| | | Astro-PVA | 2 | 0.5 | 2 | 0.5 | 1 | 0.5 | - | 0.5 | 1 | 1 | 1 | 0.5 | 0.5 | 0.5 | 0 | 0 | 0.5 | 0 |
| | | Oligo | 0.5 | 0.5 | 1 | 0.5 | 0.5 | 0.5 | - | 0 | 0 | 0.5 | 1 | 2 | 0.5 | 2 | 0.5 | 1 | 0.5 | 0.5 |
| 4 | Sibling of case 2-PSP | Neuron | 0.5 | 0.5 | 0.5 | 0 | 2 | 2 | 1 | 0.5 | 0.5 | 1 | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 2 |
| | | Astro | 0.5 | 0 | 0.5 | 0.5 | 0.5 | 1 | - | 0 | 0 | 3 | 3 | 1 | 1 | 2 | 0.5 | 1 | 0.5 | 0.5 |
| | | Oligo | 0.5 | 0 | 0.5 | 0 | 1 | 0.5 | - | 0 | 0 | 0.5 | 2 | 2 | 0.5 | 2 | 0.5 | 1 | 0.5 | 1 |
| 5 | PSP | Neuron | 1 | 1 | 2 | 0 | 2 | 0.5 | 0.5 | 1 | 1 | 0.5 | 1 | 2 | 2 | 1 | N/A | 3 | 0 | 2 |
| | | Astro | 2 | 1 | 3 | 0 | 1 | 0.5 | - | 1 | 1 | 2 | 1 | 1 | 0 | 1 | N/A | 0.5 | 0 | 1 |
| | | Oligo | 2 | 0.5 | 1 | 0 | 1 | 0.5 | - | 0.5 | 0.5 | 0 | 1 | 2 | 1 | 1 | N/A | 0.5 | 0 | 1 |
| 6 | PSP | Neuron | 1 | 2 | 1 | 0.5 | 1 | 2 | 1 | 2 | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 1 | 1 | 0 |
| | | Astro | 2 | 2 | 2 | 0.5 | 1 | 2 | - | 2 | 2 | 2 | 1 | 1 | 0.5 | 1 | 0.5 | 0 | 0 | 0 |
| | | Oligo | 0.5 | 1 | 1 | 0 | 0.5 | 2 | - | 3 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| 7 | PSP | Neuron | 1 | 0.5 | 1 | 0.5 | 2 | 1 | 0.5 | 2 | 2 | 2 | 2 | 3 | 2 | 2 | 1 | 2 | 3 | 2 |
| | | Astro | 0.5 | 0.5 | 1 | 0.5 | 0.5 | 0 | - | 0 | 0 | 3 | 2 | 1 | 0 | 0.5 | 0 | 0 | 0.5 | 0 |
| | | Oligo | 0.5 | 0 | 0.5 | 0 | 0.5 | 0.5 | - | 0 | 0 | 1 | 2 | 3 | 0 | 2 | 0.5 | 1 | 2 | 1 |
| 8 | PSP | Neuron | 1 | 1 | 1 | 0.5 | 1 | 2 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 2 | 2 | 2 | 3 | 2 |
| | | Astro | 3 | 1 | 1 | 0 | 3 | 3 | - | 2 | 2 | 2 | 3 | 3 | 0 | 1 | 0.5 | 0.5 | 2 | 0 |
| | | Oligo | 1 | 3 | 2 | 0.5 | 1 | 1 | - | 0.5 | 1 | 1 | 2 | 3 | 0.5 | 3 | 0.5 | 1 | 3 | 1 |
| 9 | PSP | Neuron | 1 | 1 | 1 | 0.5 | 0.5 | 2 | 1 | 2 | 1 | 2 | 1 | 3 | 2 | 2 | 2 | 2 | 2 | 2 |
| | | Astro | 1 | 0 | 0.5 | 0 | 0 | 1 | - | 0 | 0 | 1 | 2 | 2 | 0 | 0.5 | 0 | 0.5 | 0 | 1 |
| | | Oligo | 0.5 | 0 | 0.5 | 0 | 0 | 0.5 | - | 0.5 | 0.5 | 1 | 2 | 3 | 0.5 | 3 | 0 | 1 | 2 | 2 |
| 10 | PSP | Neuron | 0 | 0.5 | 0.5 | 0 | 0 | 1 | 0.5 | 1 | 1 | 0.5 | 0.5 | 2 | 3 | 2 | 2 | 1 | 1 | 1 |
| | | Astro | 2 | 1 | 2 | 1 | 0.5 | 2 | - | 0.5 | 1 | 2 | 2 | 3 | 2 | 2 | 0 | 0.5 | 0.5 | 0.5 |

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Oligo | 0.5 | 0 | 0.5 | 1 | 0 | 0 | - | 0 | 0 | 0.5 | 2 | 2 | 2 | 2 | 0.5 | 0.5 | 0.5 | 0.5 |
| 11 | PSP | Neuron | 2 | 1 | 1 | 0 | 1 | 1 | 1 | 2 | 1 | 0 | 0.5 | 2 | 1 | 1 | N/A | 1 | 2 | 1 |
| | | Astro | 3 | 2 | 3 | 1 | 3 | 1 | - | 1 | 0.5 | 2 | 1 | 3 | 2 | 2 | N/A | 0 | 0.5 | 1 |
| | | Oligo | 1 | 1 | 1 | 1 | 1 | 1 | - | 0.5 | 0 | 1 | 2 | 3 | 1 | 2 | N/A | 1 | 0.5 | 1 |
| 12 | PSP | Neuron | 1 | 0.5 | 0.5 | 0.5 | 1 | 1 | 0.5 | 1 | 0.5 | 0.5 | 1 | 3 | 2 | 1 | 2 | 2 | 2 | 2 |
| | | Astro | 1 | 0.5 | 0.5 | 0.5 | 1 | 0.5 | - | 1 | 0.5 | 3 | 3 | 3 | 2 | 3 | 2 | 0.5 | 1 | 1 |
| | | Oligo | 1 | 0 | 0 | 1 | 1 | 0 | - | 0.5 | 0 | 1 | 2 | 3 | 2 | 1 | 1 | 1 | 3 | 2 |
| 13 | PSP | Neuron | 0.5 | 0.5 | 0 | 1 | 0.5 | 0.5 | 2 | 2 | 2 | 0.5 | 0.5 | 2 | 2 | 2 | N/A | 2 | 2 | 2 |
| | | Astro | 2 | 1 | 0.5 | 2 | 2 | 0.5 | - | 2 | 2 | 3 | 2 | 2 | 2 | 2 | N/A | 1 | 1 | 2 |
| | | Oligo | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | - | 1 | 1 | 1 | 1 | 1 | 1 | 1 | N/A | 1 | 2 | 2 |
| 14 | PSP | Neuron | 0.5 | 0.5 | 1 | 0 | 2 | 0.5 | 0 | 1 | 0.5 | 1 | 0.5 | 2 | 3 | 2 | 3 | 2 | 2 | 1 |
| | | Astro | 0.5 | 0.5 | 2 | 0 | 2 | 0 | - | 0 | 0 | 2 | 1 | 2 | 2 | 3 | 1 | 1 | 1 | 1 |
| | | Oligo | 1 | 0.5 | 3 | 0 | 1 | 0 | - | 0 | 0 | 0.5 | 2 | 2 | 1 | 3 | 0.5 | 0.5 | 1 | 3 |

*FR* frontal, *TE* temporal, *AN* angular, *VI* visual, *CI* anterior cingulate, *AM* amygdala, *HC* hippocampus, *DG* dentate gyrus, *CA* cornu ammonis, *EC* entorhinal cortex, *PU* putamen, *GP* globus pallidus, *TH* thalamus, *SN* substantia nigra, *MB* midbrain, *LC* locus coeruleus, *PB* pontine base, *MO* medulla oblongata, *DN* dentate nucleus, *Astro* astroglia, *Oligo* oligodendroglia. Semi-quantitative scoring system: 0 (none), 0.5 (rare), 1 (mild), 2 (moderate), 3 (severe).