Neuropathology of Neurodegenerative Diseases:

Current concepts and the practical diagnostic approach

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Summary of presentation

• Current concepts of neurodegeneration

• Brief update on classification of neurodegenerative diseases (NDDs)

• Diagnostic guideline:
  – Clinical and general autopsy, sampling protocols
  – Essential stainings
  – Evaluation of stainings
  – Comprehensive evaluation of lesions: setting up the diagnosis: Which regions to evaluate? Which criteria to use? When to suspect atypical or genetic forms?
Current concept of neurodegenerative diseases

A common feature of NDDs is the deposition of β-sheet rich protein aggregates

Molecular classification of NDDs is protein-based

The concept of conformational disease underpins the role of protein processing systems
  • Unfolded protein response
  • Ubiquitin-proteasome system
  • Autophagy-lysosome pathway

There are modifications of NDD-related proteins intrinsic to disease
  • Phosphorylated forms
  • Nitrated forms
  • Oligomers
  • Protease-resistant forms
  • Protein cleavage products

Protein deposits in the brain show “maturation“
  • From non-ubiquitinated/non-argyrophilic to ubiquitinated/argyrophilic structures
  • Protein deposits with or without amyloid staining properties (birefringence in Congo)

Appearance of protein deposits in anatomical regions brain show hierarchy
  • Basis for the theory of prion-like cell-to-cell spreading of misfolded proteins
  • Definition of stages or phases of protein deposition
Current concept of neurodegenerative diseases

Neuronal loss is associated with a plethora of pathogenetic pathways

• Apoptosis
• Cell cycle disturbance
• Molecular damage (i.e. lipid peroxidation, DNA oxidation)
• Energetic dysregulation (i.e. oxidative stress and mitochondrial instability)
• Metabolic changes (i.e. alterations of cholesterol metabolism)
• Dysregulation of ion homeostasis
• Dysregulation of adaptation like anti-inflammatory cytokines, microglial activation, anti-apoptotic or antioxidant processes

Neuronal vulnerability shows selectivity in early phases of disease

• Detection of focal atrophy (neuroradiology)
• Basis for early symptomatic therapy

NDDs associate with tissue reactions detectable during the neuropathological evaluation

• Neuronal loss
• Synaptic degeneration, dendritic atrophy, axonal degeneration and transport failure
• Reactive astrogliosis
• Reactive microgliosis
• Neuronal alterations (ballooned cells, inclusion bodies)
• Extracellular plaques
• Lack of relevant inflammatory cell infiltration
• Other (e.g. spongiform change of the neuropil)

There is considerable overlap in the accumulation of different proteins in NDDs
Definition and classification

- Neurodegenerative disorders are characterized by regionally distinct neuronal loss and astrogliosis, and

- Deposition of proteins with altered physicochemical properties
  - Extracellular
    - Cytoplasmic/Cell process or Intranuclear
  - Intracellular
    - Not only neurons but glial cells
Classification of neurodegenerative diseases

- Extracellular
- Intracellular
  - Deposition of misfolded proteins
Classification of neurodegenerative diseases

Deposition of misfolded proteins

Extracellular

- PrP
- CA-Proteins
- Aβ

Parenchymal Deposits: Various morphology

Vascular Deposits: CAA

Intracellular

- Tau
- α-synuclein
- TDP-43
- FUS/FET
- Trinucleotide R
- Other

Predominant cell type with protein-pathology

- GLIA
- NEUR
- GLIA
- NEUR
- GLIA
- NEUR
- GLIA
- NEUR
- NEUR
- GLIA ± NEUR

Predominant subcellular distribution of protein-pathology

- Cyt/CPr
- Cyt/CPr
- Nucl
- Cyt/CPr
- Nucl
- Cyt
- Nucl
- Nucl
- Cyt/CPr
Classification of neurodegenerative diseases

Deposition of misfolded proteins

Extracellular
- PrP
- CA-Proteins
- Aβ

Parenchymal Deposits: Various morphology
Vascular Deposits: CAA

Intracellular
- Tau
- α-synuclein
- TDP-43
- FUS/FET
- Trinucleotide R
- Other

Predominant cell type with protein-pathology
- GLIA
- NEUR
- GLIA
- NEUR
- GLIA
- NEUR
- GLIA
- NEUR
- NEUR
- NEUR + GLIA

Predominant subcellular distribution of protein-pathology
- Cyt/CPr
- Nucl

- Morphology
- Distribution
- Isolorms

CJD
GSS
FFi
Molecular subtypes
- PrP isotypes
- PRNP gene
- Etiology
- Genetic background
- ABri, Adan CA
- ACys CAA
- Aβel CAA
- ATTR FOLMA

AD
3R+4R
3R
4R

NFT-D FTDP-17T
PJD FTDP-17T
PSP CBD AGD FTDP-17T
GGT

ALS/MND
BIBD
NIFD
ALS/MND
FTLD-ALS

FTLD-UPS/NOS

Amyloid-β

Protein deposition

Extracellular

A-beta

PrP

Deposition in Vessels

Amyloid

Non-amyloid

Plaques

AD

CAA

CJD

sPr

Codon 129 in PRNP
Western blotting of PrP-Res

Molecular subtyping

Prion protein
Ph-TDP43     

phospho-Tau

a-synuclein

Protein deposition

Extracellular

Intracellular
Important for the diagnostic practice

1. “Maturation“ of protein deposits

2. Hierarchical spreading → Stages and Phases

3. Concomitant pathologies are more the rule than the exception
Braak & Braak staging

Neurofibrillary changes

Fig. 4. Distribution pattern of neurofibrillary (NF) changes [neurofibrillary tangles (NFT) and neuropil threads (NT)]. Six stages (I–VI) can be distinguished. Stages I–II show alterations which are virtually confined to a single layer of the transentorhinal region (transentorhinal I–II). The key characteristic of stages III–IV is the severe involvement of the entorhinal and transentorhinal layer Pre-α (limbic III–IV). Stages V–VI are marked by isocortical destruction (isocortical V–VI). Increasing density of shading indicates increasing severity of NF changes.
**Phase 1**: frontal, parietal, **temporal**, or **occipital** neocortex. These deposits appear focally in small groups of diffuse plaques in layers II, III, IV, and V.

**Phase 2**: Abeta appears in the **entorhinal region, CA1**, and in the **insular cortex**. In 33–50% of the cases, single Abeta deposits occur in the amygdala, the cingulate gyrus, the presubicular region, the molecular layer of the fascia dentata.

**Phase 3**: **subcortical regions**: caudate nucleus, putamen, claustrum, basal forebrain nuclei, substantia innominata, thalamus, hypothalamus, molecular layer of the fascia dentata. **Variable**: central gray in the midbrain, the colliculi superiores and inferiores, CA4, red nucleus, subthalamic nucleus.

**Phase 4**: **further brainstem regions**: inferior olivary nucleus, the reticular formation of the medulla oblongata, and the substantia nigra.

**Phase 5**: **further brainstem regions and cerebellum**: the reticular formation of the pons, the pontine nuclei, the central and dorsal raphe nuclei, the locus coeruleus, the parabrachial nuclei, the dorsal tegmental nucleus (Gudden), the reticulotegmental nucleus of the pons (Bechterew).
What does it mean: spreading?
Example: alpha-Synuclein

According to the theory of Heiko Braak the dorsal vagus nucleus is the first site in the central nervous system.
Stages of alpha-Synuclein deposition
Stages of alpha-Synuclein deposition
Stages I-II: Premotor phase

Stage III
Substantia nigra

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Affected brain region (Braak St.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disorder of olfaction</td>
<td>Bulbus und Tractus olfactorius (I)</td>
</tr>
<tr>
<td>Obstipation</td>
<td>Dorsal Vagus nucleus (I)</td>
</tr>
<tr>
<td>REM-sleep disorder</td>
<td>Raphe Nuclei (II)</td>
</tr>
<tr>
<td>Depression</td>
<td>Locus coeruleus, Raphe Nuklei (II)</td>
</tr>
<tr>
<td>Parkinsonism</td>
<td>Substantia nigra (III)</td>
</tr>
<tr>
<td>Cognitive decline</td>
<td>Amygdala, Cortex (IV-VI)</td>
</tr>
</tbody>
</table>
Parkinson's disease: a systemic disorder?

- Sympathetic Ganglion cells
- Gastrointestinal system
- Cardial sympathetic nerves
- Adrenal medulla
- Subcutan
Wide spectrum of alterations in the brain

The possible number of combinations is very high.
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• Diagnostic guideline:
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  – Evaluation of stainings
  – Comprehensive evaluation of lesions: setting up the diagnosis: Which regions to evaluate? Which criteria to use? When to suspect atypical or genetic forms?
Before starting....

- **Critical evaluation of your aims and possibilities**
  - Performed in neuropathology diagnostic service with or without special focus on neurodegeneration,
  - It may be performed in the frame of brain banking primarily for research purposes.
  - Also general pathologists may be required to indicate their first opinion on a neuropathological condition and know further when consultation of specialised neuropathological service is essential.

  All approaches should reach a common level of diagnostic quality!
Clinical information and general autopsy

- Sex and age of the patient; duration of symptoms; presence of dementia, movement disorder or both; consumption of drugs/alcohol; evidence for concomitant and systemic disease,
- Clinico-neuropathological correlation
- Neuroradiology reports and images are also helpful (are vascular lesions compatible with the clinical course?; distribution of brain atrophy?; hydrocephalus? etc.).
- Importantly, **a suspicion of prion disease** should be considered before start of the neuropathological work-up (result of CSF examination; 14-3-3 performed or not; EEG report: periodic sharp wave complex, triphasic waves mentioned or not; rapidly progressive disease course of several months might raise the suspicion).

- **Information from the general autopsy**: Cause of death; state of the cardiovascular system; neoplastic, inflammatory or infectious disease, etc.
Sampling
## Sampling protocols


<table>
<thead>
<tr>
<th>No.</th>
<th>Region in block</th>
<th>Recommended</th>
<th>Diagnostic</th>
<th>Orientation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Frontal Cx</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Anterior Cingular Cx</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Inferior Parietal Cx</td>
<td></td>
<td></td>
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<tr>
<td>4</td>
<td>Temporal Cx (Gyrus Sup. and Med.)</td>
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<tr>
<td>5</td>
<td>Occipital Cx (Area striata &amp; peristriata)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>6</td>
<td>Precentral cortex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Post. Hippocampus (with entorhinal Cx)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Ant. BGG (Caud.; Put., Accumbens)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Post. BGG (Put., GP, Basal nucleus)</td>
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<tr>
<td>10</td>
<td>Thalamus (and subthalamic nucleus)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>11</td>
<td>Amygdala (and gyrri ambiens &amp; parahipp.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Mesencephalon (Level of IIIrd nerve)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Pons (Level of Locus coeruleus)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Medulla oblongata (Inferior olives; DVN)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Anterior vermis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Chbl. Hemisphere (with dentate nucleus)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Ant. hippocampus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Ant. hippocampus other side</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19-29</td>
<td>1-11 other side</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-32</td>
<td>12-14 further levels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>16 other side</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34-</td>
<td>Spinal cord if available (several levels)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1) **Hematoxylin and Eosin** (HE) and **luxol fast blue counterstained with nuclear fast red** (LFB/NFR) on all blocks;

2) As silver stain, generally we use **Bielschowsky**, or in selected cases **Gallyas**.

3) For amyloid, **Congo red** or alternatively Thioflavine may be used;

4) For the detection of protein markers, we use antibodies against the six most important proteins related to NDDs: **anti-Aβ** (detecting both 40 and 42), **anti-prion protein** (PrP), **anti-α-synuclein**, **anti-phospho-Tau**, **anti-phospho-TDP-43** (if not available, then **anti-TDP-43** can be used as well), and **anti-FUS**.

5) Further antibodies to be eventually used are **anti-ubiquitin**, **anti-p62**, microglial/macrophage markers (**HLA-DR, CD68**; e.g. to detect tract degeneration), **glial fibrillar acidic protein** (**GFAP**; e.g. to detect reactive astrogliosis, or where there is a discrepancy between protein deposits and tissue pathology), or **myelin basic protein and axonal markers** (e.g. **SMI-31**).

6) Rarely we use **anti-huntingtin** immunostain, and other disorders with intranuclear protein deposits (e.g. polyglutamine repeat disorders) may require additional antibodies.
### Evaluation of sections-1

<table>
<thead>
<tr>
<th>Alteration/Feature</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclei, nucleoli, and cell bodies including Nissl substance</td>
<td>Normal condition</td>
</tr>
<tr>
<td>Eosinophilic neurons</td>
<td>Acute change; indicates ischaemic/hypoxic damage</td>
</tr>
<tr>
<td>Mineralisation (incrustation) of neurons</td>
<td>Indicates chronic ischaemic/hypoxic damage</td>
</tr>
<tr>
<td>Atrophic neurons with shrunken cell bodies and corkscrew-like dendrites</td>
<td>Frequently seen in NDDs</td>
</tr>
<tr>
<td>Chromatolysis, achromasia, ballooned neuron</td>
<td>Various aetiology indicating axonal/metabolic damage</td>
</tr>
<tr>
<td>Accumulation of lipofuscin</td>
<td>Can be seen in the aging brain and NDDs</td>
</tr>
<tr>
<td>Extracellular neurormelanin pigment</td>
<td>Suggestive of a parkinsonism-related disorder</td>
</tr>
<tr>
<td>Distension of cytoplasm</td>
<td>Indicates neurometabolic (storage) disease</td>
</tr>
<tr>
<td>Granulovascular degeneration</td>
<td>In dentate nucleus: consider PSP</td>
</tr>
<tr>
<td>Eosinophilic crystalloid deposits</td>
<td>Mostly seen in hippocampus; suggestive of the presence of AD or senile change</td>
</tr>
<tr>
<td>Viral inclusion</td>
<td>Rabies: intraeytoplasmic; others (e.g. HSV, VZV, CMV) are intranuclear</td>
</tr>
<tr>
<td>Cytoplasmic neuronal eosinophilic / basophilic inclusion</td>
<td>Consider Lewy body, basophilic inclusion body, neurofilament-inclusion body, Pick body, Collins body (in Neuroserpinopathy), Lai fora bodies, iron/ferritin bodies</td>
</tr>
<tr>
<td>Cytoplasmic fibrillar inclusion</td>
<td>Consider neurofibrillary degeneration</td>
</tr>
<tr>
<td>Intranuclear neuronal eosinophilic inclusion</td>
<td>Consider Marinesce body if only in the substantia nigra. Intranuclear inclusion body disease if widespread.</td>
</tr>
<tr>
<td>Hirano body</td>
<td>Mostly seen in the hippocampus; suggestive of AD or senile change</td>
</tr>
<tr>
<td>Spongiform vacuolation of the neuropil in several anatomical regions</td>
<td>Highly suggestive of prion disease; distinguish from hypoxic/ischaemic damage</td>
</tr>
<tr>
<td>Spongiform vacuolation in upper layers of frontal and temporal cortices</td>
<td>Suggestive of FTLD or other long standing NDD but may be noted in elderly individuals</td>
</tr>
<tr>
<td>Amyloid plaques</td>
<td>Can be seen in different amyloidoses</td>
</tr>
<tr>
<td>Dystrophic neurites</td>
<td>Suggestive of amyloid plaques</td>
</tr>
<tr>
<td>Axonal bulbs</td>
<td>Indicative of axonal damage</td>
</tr>
<tr>
<td>Axonal torpedo</td>
<td>Seen in the cerebellum - Purkinje cells</td>
</tr>
<tr>
<td>Prominent mineral deposition</td>
<td>Evaluate anatomical location and distribution; consider NBIA, Fahr disease or DNTC, Ferritinopathy</td>
</tr>
</tbody>
</table>
# Structures visible in H&E staining

<table>
<thead>
<tr>
<th>Pick bodies</th>
<th>Cortical Lewy bodies</th>
<th>Marinesco-body Lewy body</th>
<th>Intranuclear inclusion body</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td>Neurofilament inclusions</td>
<td>Neurofibrillary tangles</td>
<td>Neurofibrillary tangles</td>
<td>Granulovacuolar degeneration</td>
</tr>
<tr>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
</tr>
<tr>
<td>Amyloid plaque-1</td>
<td>Amyloid plaque and neurites</td>
<td>Dystrophic neurites</td>
<td>Amyloid plaque-2 + Torpedo</td>
</tr>
</tbody>
</table>
These structures are argyrophilic

- Neurofibrillary tangles
- Granulovacuolar degeneration
- Dystrophic neurites
- Plaques and neurites
- Diffuse plaques
- Pick bodies
- Astrocytic plaque
- Tufted astrocyte
- Coiled bodies
- Oligodendroglial deposits-1
- Oligodendroglial deposits-2
- Grains
### Evaluation of sections-3
Immunohistochemistry: Extracellular deposits

<table>
<thead>
<tr>
<th>Protein</th>
<th>Morphology of deposit</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aβ</strong></td>
<td>Focal deposits: Cored plaque (classic neuritic plaques)</td>
</tr>
<tr>
<td></td>
<td>Primitive (without dense core) or compact (only a dense core)</td>
</tr>
<tr>
<td></td>
<td>Diffuse deposits (including fleecy, lake-like and subpial)</td>
</tr>
<tr>
<td></td>
<td>IR in vessel walls (with or without amyloid features): check vessel type</td>
</tr>
<tr>
<td><strong>Prion protein</strong></td>
<td></td>
</tr>
<tr>
<td>Fine IR</td>
<td>Diffuse/synaptic</td>
</tr>
<tr>
<td></td>
<td>Perineuronal</td>
</tr>
<tr>
<td></td>
<td>Axonal</td>
</tr>
<tr>
<td>Coarse IR</td>
<td>Patchy in neuropil</td>
</tr>
<tr>
<td></td>
<td>Patchy perivacuolar</td>
</tr>
<tr>
<td></td>
<td>Coarse perineuronal</td>
</tr>
<tr>
<td></td>
<td>Plaque-like PrP deposit without amyloid characteristic</td>
</tr>
<tr>
<td></td>
<td>Plaque with amyloid characteristic (uni- or multicentric, “kuru plaque”)</td>
</tr>
<tr>
<td>Other IR</td>
<td>Rarely: IR in vessel walls (with or without amyloid features)</td>
</tr>
</tbody>
</table>
Capillary deposition of A-beta
Diffuse/synaptic
Patchy/perivacuolar

Patchy/perivacuolar
Plaque-like PrP IR

Perineuronal-I

Kuru type
amyloid plaque

Perineuronal-II

Multicentric
amyloid plaque
Evaluation of sections-3

Immunohistochemistry: Intracellular deposits

<table>
<thead>
<tr>
<th>Protein</th>
<th>Cellular location</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tau</td>
<td>Neuron-cytoplasm</td>
<td>Neurofibrillary tangle</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diffuse cytoplasmic granular IR (&quot;pretangle&quot;)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spherical inclusion (compare size to nucleus)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ring-like IR hugging the nucleus</td>
</tr>
<tr>
<td></td>
<td>Neuron-process</td>
<td>Other</td>
</tr>
<tr>
<td></td>
<td>Threads (axons)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neurites (periplaque)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grains (dendrites)</td>
<td></td>
</tr>
<tr>
<td>Astrocyte</td>
<td>Tufted</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Astrocytic plaque</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other (ramified, thorny, bush-like, dots in processes etc)</td>
<td></td>
</tr>
<tr>
<td>Oligodendroglia</td>
<td>Coiled body</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Small globular inclusion (≤ nucleus) (e.g. in FTLD-Pick)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Globular /Conical/Crescent shaped inclusion (&gt; nucleus)</td>
<td></td>
</tr>
<tr>
<td>α-synuclein</td>
<td>Neuron-cytoplasm</td>
<td>Spherical inclusion (Lewy body) or pale body</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Granular cytoplasmic IR (diffuse or as small aggregates)</td>
</tr>
<tr>
<td></td>
<td>Neuron-nucleus</td>
<td>Immunoreactive deposit without well-defined shape</td>
</tr>
<tr>
<td></td>
<td>Neuron-process</td>
<td>Thin and thick (Lewy) neurites</td>
</tr>
<tr>
<td></td>
<td>Astrocyte</td>
<td>Star or crescent shaped (e.g. in LBD)</td>
</tr>
<tr>
<td>Oligodendroglia</td>
<td>Globular /Conical/Crescent shaped inclusion (&gt; nucleus; MSA)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Thin coiled-body-like or circular (e.g. in PD or LBD)</td>
<td></td>
</tr>
<tr>
<td>TDP-43</td>
<td>Neuron-cytoplasm</td>
<td>Periauricular granules</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Compact round or skein-like inclusions</td>
</tr>
<tr>
<td></td>
<td>Neuron-nucleus</td>
<td>Round, rod, or lentiform (&quot;cat-eye&quot;) inclusions</td>
</tr>
<tr>
<td></td>
<td>Neuron-process</td>
<td>Thin and thick neurites</td>
</tr>
<tr>
<td></td>
<td>Oligodendroglia</td>
<td>Thin coiled-body-like, small flame-shaped or round</td>
</tr>
</tbody>
</table>
I.a. Pretangle (more correct: diffuse cytoplasmic neuronal IR)
Diffuse or dispersed fine granular staining of neuronal cytoplasm and, to a variable extent, the processes. Perinuclear enhancement may be noted.

Comment: By definition these are ubiquitin immunonegative and are not argyrophilic.

I.b. Neurofibrillary tangle
Densely stained fibrillar, usually circumscribed, intracellular cytoplasmic structures showing flame or globose shape.

Comment: These are ubiquitin immunopositive and are argyrophilic.
I.c. Pick body
Well demarcated usually single, rarely multiple cytoplasmic fibrillar spherical structures usually as big or bigger than the neuronal nucleus.

Comment: The full definition requires argyrophilia with Bielshowsky but not with Gallyas method, furthermore ubiquitin/p62 and 3R tau immunoreactivity.

I.d. Spherical inclusion
This term is used in some scientific reports to indicate usually round cytoplasmic structures that are smaller than the neuronal nucleus, when the anatomical distribution or staining patterns are not fitting with current definitions of Pick body.
**Perinuclear ring**

Strong perinuclear ring-formed tau immunoreactivity usually with a lack of tau immunopositivity of other parts of the neuronal perikaryon.
Tau immunoreactivity

Dystrophic neurites  Neuropil threads  Grains
Tufted astrocyte  Fine granular IR in astrocytic process  Astrocytic plaque
Coiled bodies

Globular oligodendroglial inclusions
Brainstem and cortical Lewy bodies

Granular cytoplasmic IR Thick and thin neurites

A-Synuclein
"Maturation" of Lewy bodies
Astrocytic and oligodendroglial coiled body-like inclusions in Parkinson’s disease and Lewy body dementia
Neuronal cytoplasmic and nuclear and oligodendroglial cytoplasmic inclusions in multiple system atrophy
<table>
<thead>
<tr>
<th>Skein-like NCI</th>
<th>Skein-like NCI</th>
<th>Compact and granular NCI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compact round NCI</td>
<td>Granular NCI</td>
<td>Compact NCI</td>
</tr>
</tbody>
</table>

**phTDP-43: NEURONAL**

**TDP-43**
### Use stageing and diagnostic criteria

<table>
<thead>
<tr>
<th>Disease</th>
<th>Protein</th>
<th>Stage</th>
<th>Grade/Phase</th>
<th>Subtypes</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD</td>
<td>α-syn</td>
<td>0-VI</td>
<td>-</td>
<td>-</td>
<td>[51]</td>
</tr>
<tr>
<td>DLB</td>
<td>α-syn</td>
<td>-</td>
<td>-</td>
<td>Brainstem; Limbic; Neocortical</td>
<td>[52]</td>
</tr>
<tr>
<td>MSA*</td>
<td>α-syn</td>
<td>-</td>
<td>-</td>
<td>MSA-P; MSA-C</td>
<td>[53, 54]</td>
</tr>
<tr>
<td>AD</td>
<td>Aβ</td>
<td>-</td>
<td>0-5</td>
<td>-</td>
<td>[55]</td>
</tr>
<tr>
<td>CAA</td>
<td>Aβ</td>
<td>-</td>
<td>-</td>
<td>Capillary/Non-capillary type</td>
<td>[56]</td>
</tr>
<tr>
<td>Prion dis.**</td>
<td>PrP</td>
<td>-</td>
<td>-</td>
<td>sCJD, iCJD, vCJD; gCJD, GSS, F/sFI</td>
<td>[50]</td>
</tr>
<tr>
<td>AD</td>
<td>Tau</td>
<td>0-VI</td>
<td>-</td>
<td>-</td>
<td>[13, 57]</td>
</tr>
<tr>
<td>AGD</td>
<td>Tau</td>
<td>0-III</td>
<td>-</td>
<td>-</td>
<td>[58]</td>
</tr>
<tr>
<td>PSP***</td>
<td>Tau</td>
<td>-</td>
<td>0-12 score</td>
<td>PSP-P; Richardson’s Sy.; PSP-CST</td>
<td>[26, 27]</td>
</tr>
<tr>
<td>FTLD-Pick</td>
<td>Tau</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CBD</td>
<td>Tau</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NFTD</td>
<td>Tau</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FTLD-TDP</td>
<td>TDP-43</td>
<td>-</td>
<td>-</td>
<td>4 subtypes</td>
<td>[17]</td>
</tr>
</tbody>
</table>
**Step-8**

**Suggest or characterize**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Anatomical region</th>
<th>Staining</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frontal Cx</td>
<td>Tau</td>
</tr>
<tr>
<td></td>
<td>Anterior cingular Cx</td>
<td>Tau</td>
</tr>
<tr>
<td></td>
<td>Parietal Cx</td>
<td>Tau</td>
</tr>
<tr>
<td></td>
<td>Temporal Cx</td>
<td>Tau</td>
</tr>
<tr>
<td></td>
<td>Motor Cx</td>
<td>Tau</td>
</tr>
<tr>
<td></td>
<td>Occipital Cx</td>
<td>Tau</td>
</tr>
<tr>
<td></td>
<td>Striatum</td>
<td>Tau</td>
</tr>
<tr>
<td></td>
<td>Globus Pallidus</td>
<td>Tau</td>
</tr>
<tr>
<td></td>
<td>Thalamus/Subthalamus</td>
<td>Tau</td>
</tr>
<tr>
<td></td>
<td>Hippocampus anterior</td>
<td>Tau</td>
</tr>
<tr>
<td></td>
<td>Hippocampus posterior</td>
<td>Tau</td>
</tr>
<tr>
<td></td>
<td>Amygdala (+ gyrus ambiens)</td>
<td>Tau</td>
</tr>
<tr>
<td></td>
<td>Mesencephalor/substancia nigra</td>
<td>Tau</td>
</tr>
<tr>
<td></td>
<td>Pons</td>
<td>Tau</td>
</tr>
<tr>
<td></td>
<td>Medulla oblongata (DVN)</td>
<td>Tau</td>
</tr>
<tr>
<td></td>
<td>Cerebellum</td>
<td>Tau, silver</td>
</tr>
<tr>
<td></td>
<td>Dentine nucleus</td>
<td>Tau</td>
</tr>
</tbody>
</table>

- **Suggest** (indicated with **black** boxes)
- **characterize** (indicated with **gray** boxes)
Evaluating unusual forms or variants of sporadic disorders, and suspicion of genetic NDD

Atypical patterns and variants reported in the literature

or

Further proteins…
If none of the above steps was helpful, neuroserpinopathy and ferritinopathy might be considered.
It is also important to keep polyglutamine repeat diseases in mind; here anti-ubiquitin immunostaining and careful screening for intranuclear inclusions may help.
In FTLD cases without TDP-43 and tau pathology, anti-FUS or further immunostaining for ubiquitin and p62 may be useful
And when you think you are finished....
Alpha-synuclein

TDP-43

AD

AD

AD

AD

AD

AD

Amygdala

Concomitant other tauopathies

Brainstem (+ other)

Limbic

Limbic + Subcortical

None

Alzheimer's disease is a multi-proteinopathy
Evaluate concomitant pathology

Screening with p62 can be sufficient in the regions indicated by a red box

- **Subcortical tauopathies**
  - Atypical TDP-43 proteinopathy
  - A-beta in PD with dementia
- **Sufficient to suspect tauopathy**
  - Sufficient to suspect TDP-43 proteinopathy
- **Sufficient to suspect tauopathy, including early AGD; early limbic TDP-43 proteinopathy; Amygdala Lewy-body variant form**
- **Sufficient to detect early stage Lewy-body pathology and atypical forms restricted to the substantia nigra**
- **Sufficient to detect early phase cortical A-beta deposits**

**Step-10**

- **Tau (AT8)**
  - phTDP-43
  - A-beta
- **Tau (AT8)**
  - phTDP-43
- **Tau (AT8)**
  - phTDP-43
  - a-synuclein
- **a-synuclein**
  - Medulla oblongata
- **A-beta**
  - Temporal cortex
  - Frontal cortex
  - Parietal cortex
Conclusions

• Widespread sampling and staining is needed to evaluate the spectrum of pathological alterations in the neurodegenerating brain

• “Prion-like“ spreading: research should be followed whether it has any implications for public health

• Involvement of peripheral tissues: a tool for in vivo diagnostic biopsies? First when relevant therapy can be offered....
Thank you!