

Developments in Neuropathology in the Past Decade



Nicki R Cohen,
DPhil, MRCP, FRCPath

Nicki is a trainee in Neuropathology at Southampton and is applying for her CCT having recently gained FRCPath. She has a background in neuroscience and has also developed an interest in medical education. She was the series editor for the last series of Neuropathology articles in ACNR.



Roy O Weller,
BSc, PhD, MD, FRCPath

is Emeritus Professor of Neuropathology in the University of Southampton. He trained in Neuropathology in London and New York. In addition to the Clinical Diagnostic Neuropathology Service in the Wessex Region, he has focused his research on peripheral nerve and muscle disease, tumour biology, experimental neuroimmunology and the pathogenesis of neurodegenerative diseases. He is the author of several books, chapters and many research papers.

Correspondence to:
Dr Nicki Cohen
Department of Cellular Pathology
Southampton University Hospitals
NHS Trust,
Mailpoint 002,
Level E South Block
Southampton General Hospital,
SO16 6YD, UK.
Email: nicki@ookypooky.com

Neuropathology is a discipline that has broad applications across clinical and research fields involving the central and peripheral nervous systems and skeletal muscle. As a clinical service, neuropathology defines diseases of the nervous system by their structural features through the macroscopic, microscopic and, occasionally, ultrastructural examination of biopsy and autopsy material. Much of the research in neuropathology is centred on the study of human disease and observations that generate hypotheses to be tested by experiments in animal models or in tissue culture. Conversely, results from experimental studies aid the interpretation of pathological features of human disease.

Scientific and clinical advances in the last ten years affecting Neuropathology have been driven by the introduction of new techniques and concepts and by the need to communicate more effectively with those involved in the direct clinical care of patients and with patients and relatives themselves. A spirit of communication and collaboration has strengthened over the last decade through regular Multidisciplinary Team Meetings and collaborative research projects.

Many developments have occurred in the last ten years across the whole spectrum of adult, paediatric

and forensic neuropathology. However, in this article, we focus upon some of the changes that have evolved in tumour pathology, an area of primary responsibility for most practicing neuropathologists, and in muscle pathology. We go on to describe progress in the post-mortem diagnosis and in the understanding of neurodegenerative diseases.

Advances in tumour pathology

Neuropathology is often at the centre of the clinical decision making process. The pathological diagnosis of tumours in patients is crucial for their management and treatment. Major changes in clinical practice in relation to tumours of the nervous system have come with the introduction of molecular diagnostics. Molecular evaluation of tumours is now routine (Table 1), but diagnosis remains dependent primarily on the microscopic appearances of a tumour in routine sections stained with haematoxylin and eosin.

Scientific discoveries that hold most promise for a change in clinical practice in relation to tumours of the nervous system have been due to the application of array-based and deep sequencing technologies and by the use of mouse models as a foundation for in vivo therapeutic testing. During the last decade, application of immunocytochemistry

Table 1: Some of the more commonly investigated molecular characteristics of primary CNS tumours. Citation of all of this work is not possible, for a review see reference 1.

Tumour	Test	Implication
Glioblastoma	EGFR amplification Pten /p53 mutations	Distinguish from anaplastic astrocytoma
	MGMT methylation	Associated with improved response to chemotherapy and improved survival
Oligodendroglioma	IDH-1 or 2	May be associated with progression from a lower grade glial tumour and hence possibly improved prognosis
	1p/19q Loss (qPCR or FISH)	Deletion on both loci associated with superior survival and treatment response. Linked to histopathological features
Medulloblastoma	NMYC / CMYC PCR	Gains associated with poor prognosis
	Wnt pathway mutations	Associated with improved prognosis
Atypical Teratoid Rhabdoid tumour	INI-1 or BAF47 immunohistochemistry	Mutation in this gene is linked closely with development of such tumours sporadically or genetically

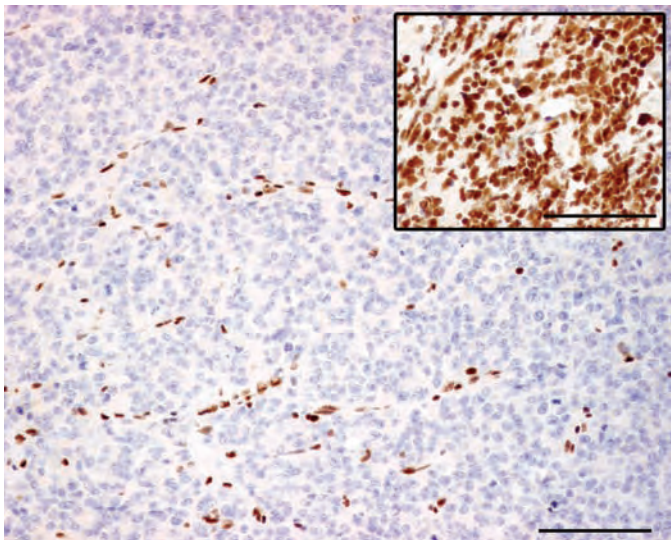


Figure 1: INI-1 immunohistochemistry of an atypical teratoid/rhabdoid tumour (main image, no staining within tumour cells but vascular endothelial cells show nuclear brown staining). By comparison, a primitive neuroectodermal tumour (PNET), inset, shows strong nuclear staining of tumour cells. Scale bar = 100 μ m.

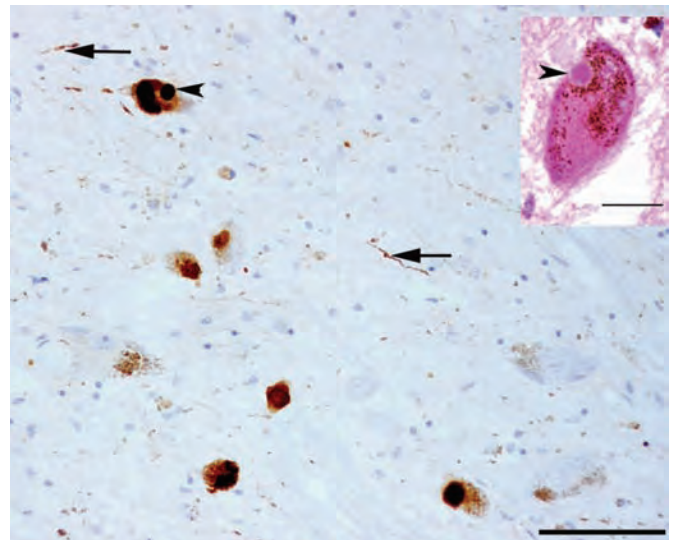


Figure 2: Lewy neurites and Lewy bodies in the substantia nigra of an 82 year-old male with Dementia with Lewy Bodies. Main image – α -synuclein immunohistochemistry showing fine wire-like Lewy neurites (arrows to two) and Lewy bodies (arrow-head to one), scale bar = 100 μ m. Inset: H&E appearance of a single nigral neuron with some remaining melanin pigment, but also a single Lewy body with a dense core and paler periphery (arrowhead). Scale bar = 20 μ m.

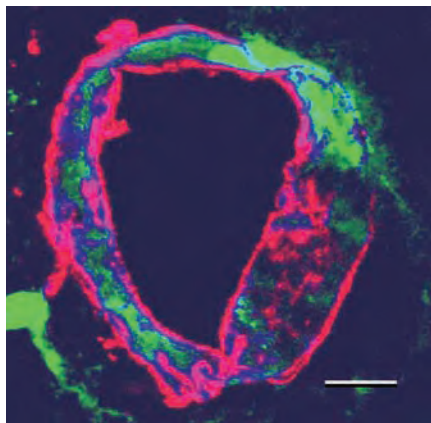


Figure 3: A confocal image of a mouse cerebral artery in transverse section showing how tracers such as dextran (green) can be co-localised with laminin (red) in the artery wall. Co-localisation of dextran and laminin is seen as dark blue. By such use of confocal microscopy it was shown that interstitial fluid and solutes drain from the brain along perivascular basement membranes. Scale bar = 10 μ m. Reproduced with permission from reference 9.

(Figure 1) has greatly improved the classification of brain tumours. In addition, neuropathology plays a key role in establishing the primary diagnosis for tumour tissue used in array-based techniques and fluorescence in situ hybridization (FISH) for the molecular classification of glioblastoma,¹ astrocytoma,² oligodendroglioma³ and medulloblastoma.⁴ Such work has had a direct effect on the management and treatment of brain tumours.

Advances in muscle pathology

The characterisation of new forms of inherited muscle disease has grown exponentially over the last ten years. New proteins whose mutations cause certain phenotypes are described several times a year. Basic histological and histochemical evaluation of frozen muscle specimens remains as an important investigation for categorising muscle pathology.⁵ More in-depth analysis of biopsies, including genetic testing, is

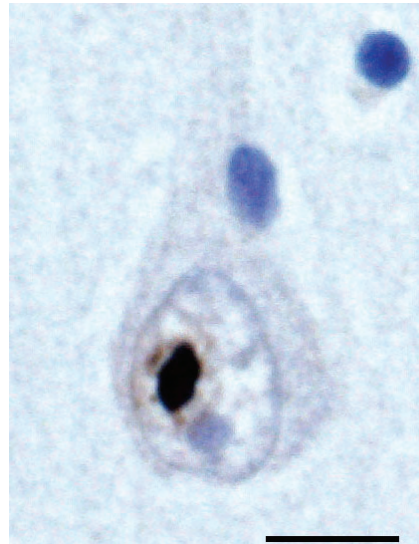


Figure 4: P62 immunohistochemistry labels a neuronal intranuclear inclusion within a cortical neuron in an uncommon neurodegenerative disease associated with dementia. Scale bar = 10 μ m.

often carried out at NCG (National Commissioning Group) centres. This illustrates one way in which super-specialist high-cost services and professional working practices can be developed and flourish. Data on muscle pathology, biochemistry and genetics are produced at such a rapid rate that published papers and books have difficulty in keeping pace, so many websites (e.g. www.neuromuscular.wustl.edu/) are now the main sources of up-to-date information.

Post-mortem diagnosis and understanding neurodegenerative diseases

Developments in neuropathology in the last decade have played a significant role in the continuing search for the effective diagnosis and treatment of neurodegenerative diseases. An appreciation of neuropathological changes and

mechanisms of disease are essential for the interpretation of neuroimaging and of biomarkers in the blood and CSF used in the diagnosis of patients with dementia.⁶ Relating the changes in the human brain to those in transgenic mouse models has allowed significant progress in our understanding of disease processes during the last 10 years.

Recognition of genetic abnormalities in familial diseases has had a profound effect on neuropathology and vice-versa. For example, the known genetic causes of Parkinson's disease include mutations in the α -synuclein gene, reported in the late 1990s.⁷ The presence of this synapse-related protein, as insoluble accumulations in Lewy bodies and in neurites (Figure 2) in sporadic Parkinson's disease, has stimulated physiological experiments to determine the effects of excessive synuclein on synaptic function. Using transgenic mice that over-express α -synuclein, whole cell patch clamp recordings have shown that high levels of expression of α -synuclein alter synaptic function and suggest that synaptic dysfunction may occur early in Parkinson's.⁸

Technological advances have also increased our understanding of CNS disease. Confocal microscopy, together with continuing advances in immunocytochemistry, have allowed proteins, tracers and ligands to be co-localised with a degree of accuracy that was previously unknown⁹ (Figure 3). This has greatly enhanced our capacity to investigate the functional aspects of tissues and cells in the normal and diseased nervous system.

Alzheimer's disease

Alzheimer's disease remains the most common form of dementia world-wide. With an ageing population, it is estimated that there are 700,000 people with dementia in the UK, most of whom will suffer from Alzheimer's disease. Extracellular plaques of insoluble amyloid- β ($A\beta$) and intracellular, intraneuronal, neurofibrillary tangles

containing tau protein have long been recognised pathological features of Alzheimer's disease. During the last 10 years, there have been very significant advances in our understanding of the disease; too many to mention here. As an example, however, attention has focussed on the failure of elimination of A β from the elderly brain and how such a failure may be responsible for dementia. Age brings loss of enzymes that degrade A β in brain tissue¹⁰, reduced absorption of A β into the blood¹¹ and impaired elimination of A β along perivascular drainage pathways.¹² As the level of soluble A β in the brain appears to correlate with cognitive decline in Alzheimer's disease,¹³ toxicity of soluble A β has also become a focus of research.¹⁴

Immunotherapy whereby plaques of insoluble A β are removed from the brains of patients with Alzheimer's disease has been introduced.¹⁵ Neuropathological studies of post-mortem brains from treated patients have revealed mechanisms by which A β is removed from plaques by microglia and how such removal reduces the accumulation of tau in neurites.¹⁶ These observations supply key data for the future development of immunotherapy.

Other studies, using transgenic mice, have shown that tau spreads from neuron to neuron¹⁷ whereas A β diffuses through the extracellular spaces and drains along perivascular pathways

by which fluid and solutes drain from the brain to regional lymph nodes.^{9,18}

Other neurodegenerative diseases

Animal models of prion diseases have been used to distinguish between the toxic and infective roles of prion proteins and this could lead to the development of therapies for Creutzfeldt-Jacob disease (CJD).¹⁹ Just before the present decade began, the UK was in a public health dilemma with the advent of variant CJD contracted by ingestion of meat from cows infected with bovine spongiform encephalopathy (BSE). In the last ten years there has been intense surveillance by neuropathologists to identify any new cases of vCJD as they occur. Biochemical analyses have shown that the prion protein in variant Creutzfeldt-Jacob disease is remarkably stereotyped, in contrast to the considerable heterogeneity that exists in sporadic CJD.²⁰

The last decade has seen significant advances in our understanding of frontotemporal lobar degeneration (FTLD)^{21, 22} associated with dementia in a slightly younger age group than Alzheimer's disease. Several new types of FTLD have been identified through a combination of gene studies and the neuropathological characterisation of intracellular protein inclusions. One of the more common forms of this disease was previously recognised as a form of

motor neuron disease (FTLD-MnD). Particularly significant has been the identification of new pathological proteins in most tau-negative forms of FTLD. P62 is a protein within the ubiquitin functional pathway and immunohistochemistry for p62 has superseded ubiquitin as a diagnostic tool for intracellular inclusions (Figure 4) in FTLD.²¹

The role of neuropathology in Brain Tissue Banks

Although there was much public concern in the UK at the beginning of the decade about retention of brains at post mortem, there have been significant developments in brain and tissue banking during the past ten years. The formation of the Human Tissue Authority and associated Act is an example of this. Brain-banking entails cooperation of patients, carers and relatives, together with the organisational skills and funding from charities and the Medical Research Council (e.g. www.brainsfordementiaresearch.org.uk). Neuropathologists play a key role in brain and tissue banks, not only in the acquisition and storage of the material but also in its accurate diagnosis and documentation.²³ This is an essential service that ensures that researchers who use material from brain banks are supplied with firm and accurate diagnostic neuropathological data. ♦

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