

## The Diagnostic Utility of Transmission Electron Microscopy in Contemporary Human Medicine: Experience at a University Hospital

Karen M. Weidenheim<sup>1</sup> and Gloria W. Stephney<sup>2</sup>.

<sup>1</sup> Departments of Pathology, Neurology and Neurosurgery, Montefiore Medical Center and Albert Einstein College of Medicine, Bronx, NY USA

<sup>2</sup> Electron Microscopy Laboratory, Department of Pathology, Montefiore Medical Center and Albert Einstein College of Medicine, Bronx, NY USA.

Transmission electron microscopy (TEM) became widely available for diagnosis of human disease in the last quarter of the twentieth century. Examination of subcellular organelles has determined the directions of investigations that have produced major breakthroughs in the understanding of several categories of disease. For example, ultrastructural study of neoplasia sparked biochemical study of characteristic subcellular structures found in the various tumor types. After their molecular composition was known, antibodies against the tumor-associated epitopes were made and these antibodies now represent “state-of-the-art” in tumor diagnosis. The study of abnormal cytoplasmic filaments has elucidated the pathophysiology of degenerative brain disorders such as Alzheimer’s disease and prion disorders. The study of lysosomes and peroxisomes provided the direction for biochemical identification of the abnormal degradative enzymes that are defective in metabolic storage disorders such as Tay-Sachs disease and adrenoleukodystrophy. Identification of the structure of abnormal structures in of skeletal muscle provoked biochemical investigations of their origin. After biochemical identification of the abnormal substance, testing for a given molecule and/or identification of the genetic mutation(s) has provided clinical testing for several disorders prior to birth, enabling prevention of cases. These successes have led to the impression that ultrastructural study is no longer necessary for the diagnosis of human disease, except for the study of kidney biopsies. However, there are numerous situations in human medicine where ultrastructural study is still a valuable diagnostic tool, and indeed, there are occasions where a given disease process can only be further investigated with the use of transmission electron microscopy. The purpose of this paper is to illustrate these situations.

Thorough study of skeletal muscle biopsies requires electron microscopy to elucidate the status of mitochondria, glycogen, lysosomes, and various abnormalities of fibrillary nature. Skeletal muscle is the most accessible tissue for biopsy for identification of abnormal mitochondria, which will enable the clinician, by means of biochemical analysis, to place the patient in the appropriate category of mitochondrial encephalomyopathies. Glycogen storage disorders are often unsuspected by the clinician, and a muscle biopsy in a child or young adult should always be examined for lysosomal glycogen even if the light microscopy is unremarkable. The myofibrillar myopathies are an emerging group of disorders in which ultrastructural study is useful to confirm the nature of the often confusing light microscopic findings, again permitting the clinician to order appropriate genetic testing to determining the specific genetic abnormality present in a given family.

Many muscle biopsies are performed on clinical suspicion of “myositis.” Inclusion body myositis (IBM) is a well-established entity that must not be missed, and at our institution, we use electron microscopy to confirm the presence of intrasarcoplasmic amyloid and/or 15-21 nm tubulofilaments in the setting of a red-rimmed vacuole myopathy, with or without inflammation. While some workers use immunohistochemistry for this purpose, we have found overlap in protein expression between IBM and

other entities with immunohistochemical methods, and so prefer the gold standard of ultrastructural study when making the very serious diagnosis of definite IBM.

Tumors of the central nervous system (CNS) occasionally require ultrastructural evaluation. We have found that protein marker studies using immunohistochemical methods, may show equivocal results in our most difficult cases. For example, neoplastic ependymal cells often express glial fibrillary acidic protein, generally considered a marker for astrocytes. In this situation, ultrastructural study will reveal intra- and extra-cellular microlumina with microvilli, and complex intercellular junctions if the tumor is an ependymoma. Glio-neuronal tumors represent a relatively new category of CNS lesions, and their light microscopic appearance overlaps with the histology of the more common astrocytomas and oligodendrogliomas. Ultrastructural study may reveal the presence of synapses or dense-core vesicles, which enables the pathologist to correctly classify these tumors. Correct classification is important because glio-neuronal tumors often have a better prognosis, and different treatment, than conventional gliomas.

A high clinical prevalence of asthma and related infections has produced interest in the ultrastructural evaluation of cilia, to rule out ciliary dyskinesia, a rare cause of repeated broncho-pulmonary infections. We prefer to examine ciliated mucosa obtained by brush biopsy, a relatively non-invasive technique, and immediately transferred to fixative solution, because this method produces better preserved microtubules and associated protein structures such as radial spokes, nexin links and dynein arms. We advise our clinicians that brush biopsy produces a greater quantity of better-preserved cilia with a much smaller chance of clinical hemorrhage, than traditional biopsies.

Skin biopsies are sometimes performed to elucidate complex neurological disorders, generally in cases where all other investigations have failed to produce a diagnosis. A three millimeter punch biopsy of axillary skin including the dermis, which has a greater content of adnexal structures than other regions, is the preferred sample. While observation of squamous epithelium is not the point of the biopsy, its presence permits proper orientation of the specimen. Careful ultrastructural study of the many tissue types available in the dermis, including apocrine and eccrine glands, myoepithelial cells, fibroblasts, endothelial cells of capillaries with pericytes, smooth muscle cells from the arector pili muscles, and axons and Schwann cells from included nerve twigs, are examined for unusual inclusions that might suggest a genetic-metabolic disorder such as neuronal ceroid lipofuscinosis.

The rare peripheral nerve biopsy always requires ultrastructural study to define the relationships of myelin and axons, as well as to look for amyloid and inclusions of genetic-metabolic disorders.

In summary, electron microscopy is a critical diagnostic tool for a range of interesting disease entities including but not limited to disorders of the kidney. The well-prepared electron microscopist will find that study of the biology, physiology and disorders of skeletal muscle, CNS tumours, cilia, peripheral nerve and skin are invaluable in helping physicians better understand their most difficult and most ill patients. Ultrastructural analysis guides physicians in selecting the most appropriate and cost-effective additional genetic and metabolic testing for a given patient. Electron microscopy should remain available for those patients and families whose unfortunate illnesses are not diagnosable by any other means.