Tissues, Pathology, and Diagnostic Microscopy

LS.2.031
Impact of electron microscopy in clinical context.

J. Schröder[1], H. Siegmund[1], F. Hofstädter[1]

[1]Central EM-Lab, University Hospital, Pathology, Regensburg, Germany

josef.schroeder@klinik.uni-regensburg.de

Transmission electron microscopy (TEM), with the potential of 1000x higher resolving power compared with light microscopy (LM), is still used as an ancillary tool, quality control method or gold standard to complement, support or confirm the result of specific histopathological diagnoses. TEM was a very efficient approach in pathological diagnosis in the 1960s till early 1990s, it importance declined with the emerging use of immunohistochemistry (IHC) and molecular methods as well as due to some intrinsic EM limitations like scanty sample processing automation and long turnaround time. Today a resurgence of TEM as a complementary diagnostic modality can be observed [1].

Due to the relative small size (approx. 1-2mm^3) of the examined tissue samples, the right sample collection and processing is a crucial step to avoid sampling errors and to secure adequate quality of tissue preservation for the TEM examination. The optimal standard approach is to immerse the biopsy specimen immediately in a buffered fixative (Karnovsky formulation), subsequent osmium tetroxide postfixation for lipid stabilization and structure contrast enhancement, dehydration in graded ethanol, and embedding in epoxy resin for heat polymerisation into hard blocks. In a number of cases formalin-fixed and wax-embedded tissue for light pathology can be reprocessed for EM as well as frozen samples to obtain useful diagnostic information by TEM examination.

Routine sample processing is performed in our lab by a computer-controlled tissue processor (LYNX) saving reagents, time, and labour (standardized batch processing overnight); the total sample turnaround time (TAT) is 3-5 workdays. For urgent clinical cases, the use of microwave-assisted tissue processing (AMW, Leica/Austria; REM, Milestone/Italy) reduce the TAT to less than 6 hours, facilitating the “same-day diagnosis” [2]. After ultramicrotomy the resin sections are examined in a TEM (LEO912AB, ZEISS) equipped with a customized side-entry digital camera image acquisition system (iTEM, OSIS/Münster). Interactive remote TEM operation via Internet allows instant and live “second opinion” consultation of difficult cases worldwide (“ultrastructural telepathology”) [3].

Based on the experience of our centralized EM unit, which is integrated in the diagnostic service of the pathology department of the medical centre, we confirm the continuing value of TEM diagnosis in a broad spectrum of diseases.

This includes the classical rapid virus detection in herpetiform skin lesions by the negative-staining procedure of particle suspensions which is a very efficient and low-cost method (e.g. herpesvirus: yes/no in 30 minutes TAT). The intrinsic “open view” of the method can be of great help in identification and morphological classification of infectious agents also in emerging situations (e.g. SARS, bird flu, Schmalenberg virus, parapox and many others). Another example of routine virus diagnosis, polyoma viruses detected in urine of a patient suffering complications after kidney transplantation, is shown in Figure 1.

Ultrastructural study of tissue sections improves significantly diagnostic interpretation in a number of neoplastic and especially non-neoplastic conditions of the kidney, muscle, nervous system, skin, cilia defects, storage diseases, liver biopsies, respiratory diseases, toxic lesions, male infertility (centriolopathy), microsporidia and opportunistic infections, as referred in detail elsewhere [4]. Distinct diagnostic immunophenotype is lacking in many tumours, aberrant immunostaining or small amounts of antigens are not detectable by LM, these and other pitfalls of affinity labelling systems makes TEM findings indispensable for exact diagnosis of poorly differentiated e.g. carcinomas, sarcomas, melanomas, mesotheliomas, and neuroendocrine neoplasms.

We display a number of examples of non-neoplastic disorders, in which a diagnosis cannot be rendered without TEM studies like numerous renal glomerular lesions, mitochondriopathy or sarcomere structure lesions in skeletal muscle and in ptosis condition (Figure 2), amyloid deposition in hearth and skin, structural abnormalities in immotile cilia of the respiratory tract causing insufficient airways clearing; skin biopsies from patients suffering of granulomatous, CADASIL (=inherited vascular disorder), and NSF (=Nephrogenic Systemic Fibrosis) condition with spectroscopic imaging (EELS=Electron Energy Loss Spectroscopy) of the causative Gadolinium deposits, and more.

In conclusion, we demonstrate the impact of TEM studies as a complementary tool in modern pathological diagnostic approach with obvious implications for prognosis and selection of therapy. This becomes very important with the paradigm shift in pathology and future aspects of personalized patient pathological predictive diagnosis and medical care management [5].

1. R. Erlandson in “Modern Surgical Pathology”, ed. W. Cote, (Saunders, Philadelphia) (2003), p. 81.
2. J. Schroeder et al., Micron 37,6 (2006), p. 577.
3. J. Schroeder in “Telepathology”, ed. S. Kumar, (Springer, Berlin) (2009), p. 179.
4. J. Stirling, A. Curry and B. Eyden, ed. “Diagnostic Electron Microscopy”, (Wiley, Chichester) (2013), p. 1.
5. M. Dietel and R. Schafer, Virchows Archiv 453,4 (2008), p. 309.

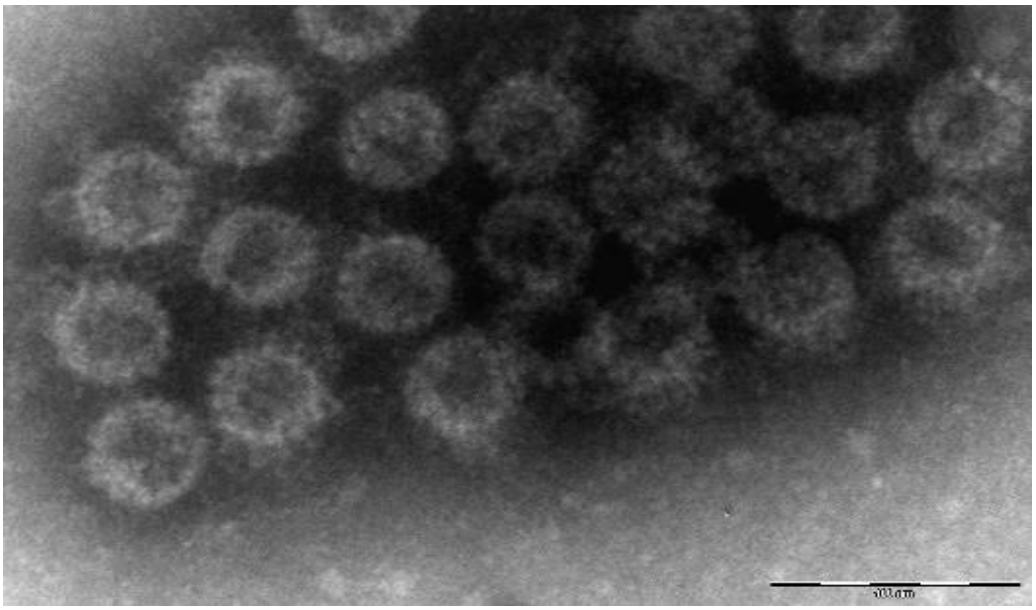

Figure 1. Polyoma viruses from urine of a nephritis patient after kidney transplantation (DD: acute rejection). Rapid negative-staining method (including ultracentrifugation of urine sample), diagnosis rendered in 60 minutes!

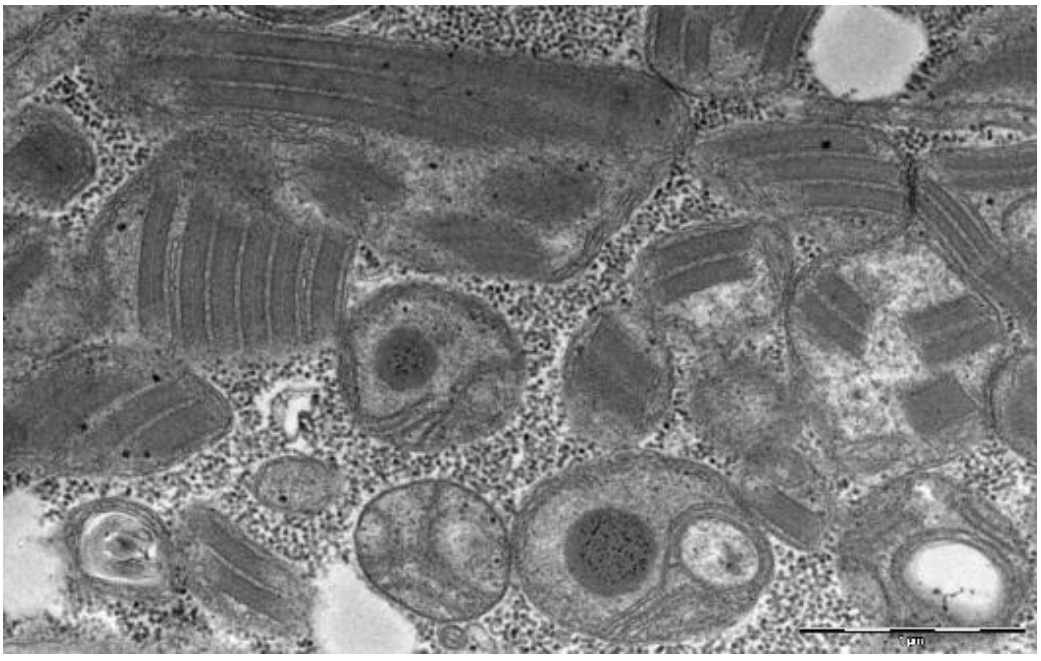

Figure 2. Mitochondriopathy in skeletal muscle biopsy of a patient suffering of myopathy of unknown ethiology. Note numerous paracrystalline and dark inclusions in abnormal sized and shaped mitochondria.