Morphology in the Digital Age: Integrating High-Resolution Description of Structural Alterations With Phenotypes and Genotypes

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Summary: Conventional light microscopy has been used to characterize and classify renal diseases, evaluate histopathology in studies and trials, and educate renal pathologists and nephrologists. The advent of digital pathology, in which a glass slide can be scanned to create whole slide images (WSIs) for viewing and manipulating on a computer monitor, provides real and potential advantages compared with conventional light microscopy. Software tools such as annotation, morphometry, and image analysis can be applied to WSIs for studies or educational purposes, and the digital images are available globally to clinicians, pathologists, and investigators. New ways of assessing renal pathology with observational data collection may allow better morphologic correlations and integration with molecular and genetic signatures, refinements of classification schema, and understanding of disease pathogenesis. In multicenter studies, WSIs, which require additional quality assurance steps, provide efficiency by reducing slide shipping and consensus conference costs, and they allow slide viewing anytime and anywhere. Although validation studies for the routine diagnostic use of digital pathology still are needed, this is a powerful tool currently available for translational research, clinical trials, and education in renal pathology.

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Percutaneous renal biopsy initially was popularized by Iversen and Brun in 1951[1] and further refined by Kark and Muehrcke,[2] leading to the acceptance of histopathologic evaluation of small samples of kidney tissue to define diseases affecting the kidney. The interpretation of renal biopsies constituted a major advance in the field of nephrology, with the advent of immunofluorescence (IF) and electron microscopy (EM) further adding to the diagnostic and investigative use of the renal biopsy.[3] In oncology, the application of immunophenotyping and genotyping in disease classification and prognostication has become the standard of care in some cancers.[4,5] In contrast, over the past 25 years, little has been added to this method of renal biopsy evaluation to better characterize renal diseases, with diagnoses remaining largely dependent on conventional morphologic characteristics. Only recently have refinements to renal biopsy evaluation been introduced, correlating morphologic findings with molecular and genetic signatures. For example, the classification of membranoproliferative glomerulonephritis, previously based on the morphology of light and electron microscopy, was revised to reflect correlation of IF findings with the underlying immunologic and molecular pathogenetic mechanisms.[6]

Similar approaches are being explored in the area of nephrotic syndrome (NS). There are now numerous examples of genetic alterations correlating with the risk of developing specific glomerular disorders. *NPHS1* mutations have been found to be a cause of congenital nephrotic syndrome of the Finnish type,[7,8] and the unique di-genic inheritance of *NPHS1* and *NPHS2* mutations results in a "tri-allelic" hit and manifests as congenital focal segmental glomerulosclerosis (FSGS).[9] Rare mutations in more than 20 genes have been found to cause NS.[10–14] In addition, common risk alleles with large effects sizes in *APOL1*,[15] *PLA2R1*, and *HLA-DQA1*[16] have been associated reproducibly

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with glomerular diseases. What remains unclear, however, is how genetic profiles correlate with specific morphologic features in general. Because the recent advances in genomic science have contributed to a better understanding of the pathophysiology of different glomerular diseases, these new observations challenge the utility of conventional pathologic classifications and emphasize the need for morphologic analysis that is more suitable for integration with molecular nephrology in the era of systems biology. The growing awareness of the complexity of clinical, morphologic, and genotype profiles of individuals affected by these disorders also has stimulated the establishment of large consortia to develop a better understanding of the pathogenesis, classification, and, ultimately, treatment of glomerular diseases. Although pathologic analysis still is essential to classify and study these diseases, the current approach to morphologic classification is inadequate to support the current molecular nephrology trials.

The Nephrotic Syndrome Study Network (NEPTUNE) was the first consortium for translational research to deploy digital pathology for evaluation and consensus review. The digital pathology consensus review platform has provided a mechanism for overcoming the limitations of traditional pathology review methods, enabling novel approaches to morphologic analysis by using observational data on annotated whole slide images (WSIs), and facilitating standardization of protocols across multiple study centers[17] (Fig 1).

THE NEPTUNE DIGITAL PATHOLOGY REPOSITORY

Digital pathology encompasses the capacity to generate a digital image of a microscope slide at the optical resolution of a light microscope. With the generation of a WSI, a slide now can be managed using a computer and viewed virtually, on demand, at multiple locations. Adding other capacities of computers and information systems, the WSI can be annotated by reviewers and subjected to morphologic assessment that can be recorded in a database. All related data can be linked not only to the WSI, but tags and annotation can be applied to individual lesions for evaluation by multiple reviewers, or used for computerized image analysis with the appropriate software.[17] A digital pathology repository (DPR) also can host other electronic documents and images in addition to WSIs, including static images of IF, immunohistochemistry or EM, and scanned reports.

The NEPTUNE investigators have exploited 21st century digital technology by systematically collecting and storing digital renal biopsy specimens from patients with a diagnosis of minimal change disease (MCD), FSGS, and membranous nephropathy from the more than 30 NEPTUNE recruitment sites. NEPTUNE renal biopsy WSIs, as well as EM and IF digital images, and clinical reports are deposited in a central online DPR. The majority of the renal biopsy specimens are scanned centrally, and EM and IF images along with de-identified original pathology reports are uploaded to the servers. The DPR is backed up, access is limited to authenticated users, and all material is rigorously de-identified for patient protection. In addition, the NEPTUNE digital pathology workflow is integrated into the overall operational environment and used for information sharing. The NEPTUNE pathologists are cross-trained to address the specific type of analysis implemented by the NEPTUNE digital pathology protocol for morphologic profiling of renal biopsy specimens (Fig. 1B).

THE NEPTUNE DPR: NEW TOOLS REQUIRE NEW RULES

The application of whole slide imaging as a research tool requires a consistently high level of technical specification, quality assurance, and standardization. Implementation of this technology is far more complex than having a scanner available and a server with a database in which to upload the images. The process begins with the development of a slide and report retrieval protocol by study coordinators, who are responsible for the de-identification of the material, as well as the timely and complete submission of the pathology material for inclusion in the DPR. Pathology material generally is collected at least 3 months after the biopsy is performed to ensure no disruption in clinical care. The glass slides are shipped to the central scanning facility where they are imaged at 40× resolution, or slides are scanned locally following the same protocol. The central scanning process lasts approximately 2 weeks, after which the pathology material is returned to the originating center. A rapid-return procedure is available if clinical care requires pathology re-review of the glass slides (needed in approximately $<0.5\%$ of centrally scanned cases). WSIs, IF and EM digital images, and the clinical report are uploaded to the server and stored in the NEPTUNE DPR. Replicates of the data are made on a local storage site before the images are uploaded to the DPR, which resides on a secure cloud server. Quality assurance occurs at this point and includes review of all WSIs by the technical staff to ensure the images are complete, in focus, and annotated correctly as to stain and level. Complete de-identification of the pathology material is confirmed in all components of the digital case.

Obtaining a high level of quality is critical and requires dedicated well-trained staff, routinely engaged in WSI capture. Maintaining both the hardware (imagers and servers) and software (including access