Published in final edited form as:

Semin Nephrol. 2015 May ; 35(3): 266–278. doi:10.1016/j.semnephrol.2015.04.006.

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

Cynthia C. Nast, M.D.¹, Kevin V. Lemley, M.D., Ph.D.², Jeffrey B. Hodgins, M.D., Ph.D.³, Serena Bagnasco, M.D.⁴, Carmen Avila-Casado, M.D.⁵, Stephen M Hewitt, M.D., Ph.D.⁶, and Laura Barisoni, M.D.⁷

¹Dept of Pathology, Cedars-Sinai Medical Center, Los Angeles, CA

²Division of Nephrology, Children's Hospital Los Angeles, Los Angeles, CA

³Department of Pathology, University of Michigan, Ann Arbor, MI

⁴Department of Pathology, Johns Hopkins University Medical Center, Baltimore, MD

⁵Department of Pathology, University of Toronto, Toronto, ON, Canada

⁶Experimental Pathology Laboratory, Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, Bethesda MD

⁷Department of Pathology, U of Miami, Miami, FL

Abstract

Conventional light microscopy (CLM) 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 (WSI) for viewing and manipulating on a computer monitor, provides real and potential advantages over CLM. Software tools such as annotation, morphometry and image analysis can be applied to WSIs for studies or educational purposes, and the digital images are globally available 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, WSI, which require additional quality assurance steps, provide efficiencies by reducing slide shipping and consensus conference costs, and allowing anytime anywhere slide viewing. While 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.

Corresponding Authors: Laura Barisoni, M.D., Phone: 305-243-7284, Fax: 305-689-7284, LBarisoni@med.miami.edu, Department of Pathology, U. of Miami Health System, 1611 NW 12th Ave, Miami, FL 33136, Cynthia C. Nast, M.D., 310-423-6658, 310-423-5581, Cynthia.Nast@cshs.org, Department of Pathology, Cedars-Sinai Medical Center, 8700 Beverly Blvd., Los Angeles, CA 90048.

Financial Disclosure and Conflict of Interest Statements: None

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

Digital pathology; Whole slide imaging; Virtual slide; Virtual microscopy; Neptune; Renal pathology; Glomerulus

Introduction

Percutaneous renal biopsy initially was popularized by Iversen and Brun in 1951¹ and further refined by Kark,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 last 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 and segmental glomerulosclerosis.⁹ Rare mutations in more than 20 genes have been found to cause NS.^{10–14} Additionally, common risk alleles with large effects sizes in *APOL1*,¹⁵ *PLA2R1*, & *HLA-DQA1*¹⁶ have been reproducibly associated with glomerular diseases. What remains unclear however, is how genetic profiles correlate with specific morphologic features in general. As 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 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. While pathologic analysis is still 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, and facilitating standardization of protocols across multiple study centers ¹⁷ (Figure 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 whole slide image (WSI), a slide now can be managed using a computer and viewed *virtually*, on demand, in 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 utilized 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 biopsies from patients with a diagnosis of minimal change disease (MCD), focal and segmental glomerulosclerosis (FSGS) and membranous nephropathy (MN) 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 biopsies are centrally scanned, 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. Additionally, the NEPTUNE digital pathology workflow is integrated into the overall operational environment and utilized 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 biopsies. (Figure 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 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 is generally collected at least three 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 into 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 correctly annotated 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 management) is essential. Currently, visual evaluation of the images by a human is necessary to ensure quality. In practical experience, more challenges are encountered due to broken slides, poor coverslipping, wrong stain information and incomplete patient de-identification rather than with image quality.

It should be noted that digital pathology systems currently are not approved by the FDA for primary diagnosis in the United States. Their use is permitted for consultation (second opinion and frozen section review) and research applications including clinical trials.^{18,19,20} The use of digital pathology in the clinical setting outside of the USA is governed by local regulation and varies from country to country. The clinical implementation of digital pathology is not cost-neutral, unlike the conversion to digital radiology, and no doubt has slowed its adoption. Current efforts in the US are focused on validation of digital review for primary diagnosis; however, the FDA's classification of whole slide imaging as Class III, requiring a pre-market approval (PMA), places the onus of validation on the system vendors.²¹

WSI versus Glass Slide Analysis

The clinical use of WSIs requires demonstration in an appropriate study that WSIs are non-inferior to glass slide conventional light microscopy (CLM) with respect to diagnostic accuracy. The College of American Pathologists (CAP) has issued guidelines for validation of WSIs; however, these guidelines do not supersede the FDA's classification of the whole slide imaging process as a class III device.²² In some settings, use of WSIs improves inter- and intra-reader reproducibility, and may be a more sensitive way to identify subtle microscopic features.¹⁸ Inter-observer and inter-modality agreement are relatively high for immunohistochemistry as well, although further validation studies are needed.^{23,24} With regard to renal pathology, Furness found web-based virtual slide examination of European quality assurance cases of lupus nephritis and CLM of the same cases were diagnostically equivalent.²⁵ Jen, et al compared CLM and WSIs of renal transplant biopsies assessed for Banff '07 classification of rejection and other histologic features. They showed good ($\kappa = 0.68$) to excellent ($\kappa = 0.74$) intra-observer and comparable inter-observer non-chance agreement using the kappa coefficient statistical method.^{26,27,28} Another study of transplant biopsy WSIs evaluated 11 pathologic lesions using Banff scoring with similar intra-observer and inter-observer kappa scores indicating comparable agreement.²⁹ This study also

demonstrated better inter-observer reproducibility for WSIs than for CLM, indicating that different pathologists assessing the same material make the same observations more often using WSIs.^{27,29} Further validation of WSIs for diagnosis and other analyses, such as more granular descriptions of morphologic abnormalities, still are needed for application in research, education and clinical settings.^{30,31}

Conventional and Historical Pathology Review

Interpretative morphologic classification systems currently are being used for patients with lupus nephritis,³² IgA nephropathy,³³ membranoproliferative glomerulonephritis,⁶ focal segmental glomerulosclerosis,³⁴ diabetic nephropathy³⁵ and ANCA vasculitis.³⁶ Such classifications provide a framework for assessing clinical diagnostic features, selecting patients for clinical trials, elucidation of disease pathophysiology, allowing communication among healthcare providers and, most importantly, determining diagnosis, prognosis and therapeutic options. To some extent, the interpretative classifications currently in use in renal pathology have been successfully used for conducting clinical trials and to guide therapy for specific disease entities. However, they represent a “qualitative” interpretation of pathologic features without providing granular quantitative assessment of components of parenchymal injury. With the advancement of the molecular characterization of renal diseases and substantial gains in knowledge of the etiologies and mechanisms of kidney dysregulation and injury, there is a need to redefine the association of etiology with morphologic phenotype. The current qualitative pathologic approaches may not provide adequate detail to identify lesions correlating with newly described mechanisms of disease. The goal is to refine the capacity to subtype an injury pattern, improving diagnostic specificity, prognostic value, and approach to therapy. To support this goal, the evaluation of morphologic findings must expand from qualitative to detailed quantitative assessment of the renal biopsy.

There are several factors contributing to the limitations inherent in conventional morphologic classifications. These are demonstrated by inconsistencies in validation studies, the lack of useful additional information provided by morphology in some clinical settings, and the frequent revisions in classifications. As an example, there have been at least seven iterations of classification schemes for lupus nephritis over the past four decades. Most classification systems are based on correlations of morphologic features with clinical phenotypes and represent a consensus opinion but are not evidence based,³⁷ with the exception of the Oxford IgA classification.³³ This general lack of rigorous application of an unbiased approach, while allowing development of classifications that have clinical-pathologic correlations, may reinforce pre-conceived ideas and obfuscate pathogenetic links. Lastly, the use of CLM and glass slide review carries intrinsic limitations, such as lack of consistency in examining the same renal structures, contributing to the poor reproducibility of current morphologic classification systems. Although many of the classification schemes are not supported by reproducibility studies, when reproducibility and validity studies have been performed, they included consensus meetings and/or multiple mailings of glass slides for pathologists to review at their local sites (Figure 1A). These methods are cumbersome, expensive, may result in damage or loss of part of the pathology materials, do not ensure that pathologists are examining the same structures in the slides, and lack transparency.

Applications of Digital Technology in Nephropathology; the NEPTUNE Experience

The introduction of the WSI technology has provided new opportunities in the use of pathologic materials for studying renal disease, and is particularly useful in the setting of multicenter translational research and clinical trials.^{17,19} (Figure 1B) Specifically, the use of WSIs offers the capacity to reduce the number of variables in reproducibility studies by removing potential inconsistencies of the object visualized by CLM and replacing it with an image that is functionally identical to all readers. Furthermore, the ability to annotate specific objects for evaluation ensures that the same features are evaluated in generating summary interpretations. Such technology is also a very powerful training tool, facilitating cost and time-effective webinar-based consensus meetings before, during and at the conclusion of the analysis.³⁸ Lastly, the application of digital pathology provides a permanent record and full transparency of the data collected.

The NEPTUNE digital pathology protocol has exploited the advantages offered by digital pathology technology, such as viewing of multiple images simultaneously, overlapping and aligning of images to examine specific components of the renal parenchyma through multiple slide levels, annotating glomeruli, and enumeration and measurement of morphologic features. After development and population of the DPR (described above), the NEPTUNE pathology committee developed a standardized and reproducible morphologic analysis approach that would be complementary to the molecular, genetic and clinical profile data generated for each study patient. Creating the elements of and process for this analysis represented the most challenging component of the pathology study protocol. It resulted in development of the NEPTUNE Pathology Scoring System to obtain detailed morphologic data for comprehensive granular profiling of all study renal biopsies, free from interpretative bias for use in an evidence-based systems biology approach. (Table 1, Table 2) With the application of this approach, the advantages of WSIs over CLM became clear. Glomerular annotation allows profiling of each glomerulus in all levels in which it appears, for a more precise evaluation of the extent of damage. It also has served to increase precision of glomerular count, ultimately contributing to a more accurate assessment of the extent of renal injury.³⁹ The use of multilevel glomerular annotation with the collection of observational data (descriptors) applied to all renal parenchymal compartments provides both detailed quantitative pathologic analysis and conventional qualitative assessment.^{17,40} (Figure 2) To collect data efficiently, an electronic scoring sheet (descriptor scoring matrix) was generated and used for scoring the digital renal biopsies for comprehensive morphologic profiling. (Figure 3)

From a practical standpoint, the WSI web-based process allows for anytime, anywhere slide review, thus maximizing efficiency. Glomerular annotation by the reviewing pathologist provides a framework for the scoring pathologist to ensure complete review of the glomeruli. Using multiple webinar-based consensus meetings during which WSIs and digital images are reviewed simultaneously by multiple investigators, all study pathologists involved in the analysis had the opportunity to participate in defining the language applied to each descriptor, which was recapitulated in the descriptor reference manual. Additionally,

webinar-based consensus meetings were used for cross-training to increase agreement and reproducibility of the descriptor-based scoring system, both within the NEPTUNE community in North America and also in Europe, facilitating sharing of protocols and application of similar morphologic analysis with other international consortia. Using a protocol including webinar-based consensus meetings with alternating independent scoring, reproducibility studies have shown promising data for intra- and, most importantly, inter-reader reproducibility.³⁸

Changing the Qualitative Assessment of Renal Biopsies

Conventional classification systems are based on interpretation of renal biopsy findings summarized into a diagnostic entity or category (qualitative assessment). The application of a descriptor-based scoring system allows a “new look at old diseases” and an opportunity to organize diagnostic categories according to different criteria. Thus, whereas conventional classification systems would divide certain diseases causing NS into MCD and FSGS, the descriptor-based quantitative assessment, utilizing specific descriptors and algorithms, provides the opportunity to derive and test different and unexplored categories in addition to conventional ones. As an example, cases can be divided into two major groups, those with glomerular lesions by histology and those without (minimal lesions) (Figure 4). In parallel, the presence or absence of segmental glomerular obliterative lesions can be used to further divide cases in the MCD/FSGS cohort into two additional groups. Therefore, biopsies with segmental obliteration can be termed FSGS and FSGS-like. In this algorithm, FSGS encompasses lesions mimicking conventional classification approaches, while FSGS-like indicates a group of lesions (descriptors) currently not included in any classification systems and that, when detected in the absence of segmental solidification or collapse, remain unclassified or labeled as “of uncertain significance”. Using this approach, biopsies lacking segmental obliterative lesions also can be stratified further into sub-categories, for example using the presence of global sclerosis or deflation and the extent of foot process effacement. According to this system, the presence of extensive effacement with the absence of any global or segmental sclerotic lesions defines a category mimicking typical MCD. Those biopsies without segmental obliteration but with only partial foot process effacement and/or global sclerosis do not fulfill the strict criteria for MCD and are termed MCD-like (for lack of a better term). This latter category includes: 1) MCD-like without global sclerosis and with partial effacement. 2) MCD-like with focal global sclerosis and with partial effacement. 3) MCD-like with focal global sclerosis and with extensive effacement. This algorithmic approach allows stratification of cases into groups sharing similar features, and also serves as a framework for hypothesis generation. For example, molecular studies may elucidate whether MCD-like with partial effacement but no global sclerosis represents MCD partially responsive to therapy or under-sampled FSGS, and whether MCD-like with partial effacement and with focal global sclerosis is under-sampled FSGS or an entirely new category of diseases. Alternatively, there may be no discovered clinical or molecular differences between MCD-like with focal global sclerosis and extensive effacement, MCD, and/or under-sampled FSGS.

Preliminary studies indicate that there are clinical and demographic associations with some of these new categories with respect to age, hypertension, and pre-biopsy steroid use.⁴¹

Therefore, specific glomerular descriptors may identify distinct clinical cohorts within a disease category such as MCD, and demonstrate the potential for this approach in developing new or more refined classifications of renal disease.

Terminology that is used for classification, without implying etiology or pathogenesis, is a crucial feature of a descriptor-based scoring system. The use of parameters not currently incorporated in the qualitative assessments used in current classification systems may contribute to a better understanding of pathophysiology of specific types of injury, and potentially identify new diagnostic entities. For example, retraction and wrinkling of the glomerular tuft in the absence of epithelial cell (podocyte) hypertrophy and hyperplasia historically has been described as a lesion resulting from ischemic injury. For this pattern of glomerular injury, the NEPTUNE descriptor language, along with others, has replaced the term “ischemic” retraction with the term segmental or global “deflation”, thus avoiding any assumed etiologic factors. Glomerular deflation and frequently associated features, such as glomerular basement membrane wrinkling and periglomerular fibrosis, were observed in a recent morphometric study of 89 normal kidneys. This study revealed that these features were common in patients older than 60 years of age, but without evidence of significant arteriosclerosis, arguing against ischemic injury as the main driver of glomerulosclerosis through this intermediate morphologic stage in this patient cohort. Deflation of the glomerular tuft in the absence of epithelial cell hyperplasia correlated with increased global glomerulosclerosis and was associated with very low overall podocyte density. In addition, the deflated glomeruli demonstrated detached cells and proteinaceous material in Bowman’s space, representing glomerular capillary collapse associated with podocyte detachment, indicating a leaky glomerular filtration barrier.⁴² (Figure 5A) While ischemic injury is known to be associated with glomerular capillary wall wrinkling, this study demonstrates that the general application of the moniker “ischemic glomerulopathy” to all glomeruli with such features may be misleading. (Figure 5B). Additionally, this study serves as proof of concept that conventional interpretative diagnoses are not fully adequate to characterize the nature of morphologic injury, and that a descriptor-based approach may ultimately improve and change current classification systems. It is anticipated more such examples will come to light, as descriptor-based scoring systems continue to be employed and analyzed.

Implementation of Quantitative Pathology

The importance of quantitative pathology, including morphologic and morphometric analysis, is widely recognized, and the application of digital pathology has allowed standardization of the quantitative approach. Although WSIs are not yet in wide use among pathologists or an economically viable replacement for CLM, the benefits of WSIs in quantitative evaluation are changing our understanding of and capacity to accurately classify disease. The application of the NEPTUNE protocol allows collection not only of qualitative pathology assessments derived from the quantitative data, but also of granular data including the exact number of glomeruli demonstrating one or more descriptors, percent of tubulointerstitium affected by acute or chronic damage, degree of vascular disease, and other features identified on ultrastructural analysis. Individual glomerular and parenchymal descriptors, and the frequency with which they are detected in a given biopsy, can be analyzed individually or grouped according to certain common characteristics, and

correlated with other critical parameters. Such a detailed approach allows detection of features that have been overlooked and for which the significance has not yet been explored. Descriptors such as epithelial cell (podocyte) hypertrophy, synechia, hyalinosis or foam cells, in the absence of segmental solidification of the tuft, may represent early lesions preceding definitive evolution into FSGS. Therefore, the quantification of granular lesions currently not used in diagnosis or classification schema may provide important information with regard to therapeutic approaches, pathogenesis or genetic underpinnings of disease, and prognosis.

The NEPTUNE DPR provides a wealth of material for research examining the use of pathologic and morphometric features for predicting outcomes. Preliminary work by Lemley, et al correlated changes in estimated glomerular filtration rate (eGFR) over 24 months with morphometric assessments of average glomerular tuft cross-sectional area, cortical glomerular density and fractional interstitial area measured on digital images. It was demonstrated that these morphometric measurements are strong predictors of changes in eGFR⁴³ and may significantly enhance the value of the renal biopsy in determining prognosis. There is much interest in nephrology in developing predictive biomarkers, including histologic evaluation of biopsies, to discern disease diagnosis, therapeutic response and prognosis. The use of descriptors may identify morphologic biomarkers of disease that are either broadly applicable to many glomerulonephritides or more disease-specific than the current pathologic classification systems provide for abnormalities such as FSGS and IgA nephropathy. There are several biomarkers thought to predict prognosis or specific disease entities, among them the soluble urokinase-type plasminogen activator receptor (suPAR)⁴⁴ which has been suggested as a specific marker for FSGS. Using the NEPTUNE cohorts, Spinale, et al determined in multivariable linear regression that plasma suPAR concentrations did not correlate with diagnostic groups (FSGS, MCD, MN, IgA nephropathy) when adjusted for proteinuria or eGFR. However, higher baseline and larger increases in plasma suPAR concentrations were independently associated with reduced eGFR levels.⁴⁵

An area of great interest is the role of APOL1 risk alleles in the progression of renal disease. These risk variants have been shown to be associated with worse outcomes regardless of the cause of kidney disease. The use of descriptors allows a wealth of morphologic correlates to explore the response of renal compartments to this milieu in the hopes of identifying specific lesions associated with this genetically based risk. The same principle is applicable to other genetic variants yet to be identified.¹⁴

Summary and Conclusions

The use of WSIs and digital pathology provide new opportunities in all aspects of pathology by offering a platform for quantitative and qualitative morphologic evaluation that is challenging to implement using CLM.⁴⁶ Potential clinical benefits of using a digital pathology-based descriptor approach include the ability to evaluate and improve diagnostic criteria including refining the language of renal pathology, carefully examining diagnostic algorithms, honing classification schema, improving prognostic information, and providing anywhere, anytime remote access for cross-training, consultation and consensus review of

renal biopsies. The NEPTUNE digital pathology approach has served as a model for other international consortia and other diseases (Figure 6). In the kidney research arena, providing unified protocols for DPR of study materials allows its use by many investigators over decades. It abrogates the need for shipping slides with the associated costs and the potential for material loss or damage, and for slide review meetings. It increases efficiencies with pathology materials available at all locations at all times, and allows examination and measurement of the same identified structures, achievable through the application of software tools such as annotation, morphometry and image analysis. This approach holds the promise of rapid progress in our ability to better understand and use morphology in the context of genomics, proteomics and other advances in nephrology.

Acknowledgments

This research was supported in part by the Intramural Research Program, of the NIH, National Cancer Institute, Center for Cancer Research. It was also supported by the Nephrotic Syndrome Study Network Consortium (NEPTUNE; U54-DK-083912), which is a part of the National Institutes of Health (NIH) Rare Disease Clinical Research Network (RDCRN), supported through a collaboration between the Office of Rare Diseases Research (ORDR), NCATS, and the National Institute of Diabetes and Digestive and Kidney Diseases

References

1. Iversen P, Brun C. Aspiration biopsy of the kidney. *Am J Med.* 1951; 11(3):324–330. [PubMed: 14877837]
2. Kark R, Muehrcke R. Biopsy of kidney in prone position. *The Lancet.* 1954; 263(6821):1047–1049.
3. Cameron JS, Hicks J. The introduction of renal biopsy into nephrology from 1901 to 1961: a paradigm of the forming of nephrology by technology. *Am J Nephrol.* 1997; 17(3–4):347–358. [PubMed: 9189255]
4. Jie-Liu, Li XY, Zhao YQ, et al. Genotype-phenotype correlation in Chinese patients with pulmonary mixed type adenocarcinoma: Relationship between histologic subtypes, TTF-1/SP-A expressions and EGFR mutations. *Pathol Res Pract.* 2014; 210(3):176–181. [PubMed: 24370340]
5. Luinetti O, Fiocca R, Villani L, et al. Genetic pattern, histological structure, and cellular phenotype in early and advanced gastric cancers: evidence for structure-related genetic subsets and for loss of glandular structure during progression of some tumors. *Hum Pathol.* 1998; 29(7):702–709. [PubMed: 9670827]
6. Sethi S, Fervenza FC. Membranoproliferative glomerulonephritis: pathogenetic heterogeneity and proposal for a new classification. *Semin Nephrol.* 2011; 31(4):341–348. [PubMed: 21839367]
7. Patrakka J, Kestilä M, Wartiovaara J, et al. Congenital nephrotic syndrome (NPHS1): features resulting from different mutations in Finnish patients. *Kidney Int.* 2000; 58(3):972–980. [PubMed: 10972661]
8. Lenkkeri U, Männikkö M, McCready P, et al. Structure of the gene for congenital nephrotic syndrome of the finnish type (NPHS1) and characterization of mutations. *Am J Hum Genet.* 1999; 64(1):51–61. [PubMed: 9915943]
9. Koziell A, Grech V, Hussain S, et al. Genotype/phenotype correlations of NPHS1 and NPHS2 mutations in nephrotic syndrome advocate a functional inter-relationship in glomerular filtration. *Hum Mol Genet.* 2002; 11(4):379–388. [PubMed: 11854170]
10. Trautmann A, Bodria M, Ozaltin F, et al. Spectrum of steroid-resistant and congenital nephrotic syndrome in children: the PodoNet registry cohort. *Clin J Am Soc Nephrol.* 2015
11. Bullich G, Trujillano D, Santín S, et al. Targeted next-generation sequencing in steroid-resistant nephrotic syndrome: mutations in multiple glomerular genes may influence disease severity. *Eur J Hum Genet.* 2014
12. Lovric S, Fang H, Vega-Warner V, et al. Rapid detection of monogenic causes of childhood-onset steroid-resistant nephrotic syndrome. *Clin J Am Soc Nephrol.* 2014; 9(6):1109–1116. [PubMed: 24742477]

13. Hildebrandt F. Genetic kidney diseases. *Lancet*. 2010; 375(9722):1287–1295. [PubMed: 20382325]
14. Sampson MG, Hodgin JB, Kretzler M. Defining nephrotic syndrome from an integrative genomics perspective. *Pediatr Nephrol*. 2015; 30(1):51–63. quiz 59. [PubMed: 24890338]
15. Freedman BI, Murea M. Target organ damage in African American hypertension: role of APOL1. *Curr Hypertens Rep*. 2012; 14(1):21–28. [PubMed: 22068337]
16. Lv J, Hou W, Zhou X, et al. Interaction between PLA2R1 and HLA-DQA1 variants associates with anti-PLA2R antibodies and membranous nephropathy. *J Am Soc Nephrol*. 2013; 24(8):1323–1329. [PubMed: 23813219]
17. Barisoni L, Nast CC, Jennette JC, et al. Digital pathology evaluation in the multicenter Nephrotic Syndrome Study Network (NEPTUNE). *Clin J Am Soc Nephrol*. 2013; 8(8):1449–1459. [PubMed: 23393107]
18. Barisoni L, Jennette JC, Colvin R, et al. Novel quantitative method to evaluate globotriaosylceramide inclusions in renal peritubular capillaries by virtual microscopy in patients with fabry disease. *Arch Pathol Lab Med*. 2012; 136(7):816–824. [PubMed: 22742555]
19. Mroz P, Parwani AV, Kulesza P. Central pathology review for phase III clinical trials: the enabling effect of virtual microscopy. *Arch Pathol Lab Med*. 2013; 137(4):492–495. [PubMed: 23544938]
20. Brachtel E, Yagi Y. Digital imaging in pathology--current applications and challenges. *J Biophotonics*. 2012; 5(4):327–335. [PubMed: 22213680]
21. Treanor D, Gallas B, Gavrielides A, Hewitt A. Evaluating whole slide imaging: A working group opportunity. *J Pathol Info*. 2015
22. Pantanowitz L, Sinard JH, Henricks WH, et al. Validating whole slide imaging for diagnostic purposes in pathology: guideline from the College of American Pathologists Pathology and Laboratory Quality Center. *Arch Pathol Lab Med*. 2013; 137(12):1710–1722. [PubMed: 23634907]
23. Gavrielides MA, Conway C, O'Flaherty N, Gallas BD, Hewitt SM. Observer performance in the use of digital and optical microscopy for the interpretation of tissue-based biomarkers. *Analytical Cellular Pathology*. 2014 ID 157308.
24. Ordi J, Castillo P, Saco A, et al. Validation of whole slide imaging in the primary diagnosis of gynaecological pathology in a University Hospital. *J Clin Pathol*. 2015; 68(1):33–39. [PubMed: 25355520]
25. Furness P. A randomized controlled trial of the diagnostic accuracy of internet-based telepathology compared with conventional microscopy. *Histopathology*. 2007; 50(2):266–273. [PubMed: 17222256]
26. Viera AJ, Garrett JM. Understanding interobserver agreement: the kappa statistic. *Fam Med*. 2005; 37(5):360–363. [PubMed: 15883903]
27. Bartlett JW, Frost C. Reliability, repeatability and reproducibility: analysis of measurement errors in continuous variables. *Ultrasound Obstet Gynecol*. 2008; 31(4):466–475. [PubMed: 18306169]
28. Jen KY, Olson JL, Brodsky S, et al. Reliability of whole slide images as a diagnostic modality for renal allograft biopsies. *Hum Pathol*. 2013; 44(5):888–894. [PubMed: 23199528]
29. Ozluk Y, Blanco PL, Mengel M, et al. Superiority of virtual microscopy versus light microscopy in transplantation pathology. *Clin Transplant*. 2012; 26(2):336–344. [PubMed: 21955102]
30. Nelson D, Ziv A, Bandali KS. Going glass to digital: virtual microscopy as a simulation-based revolution in pathology and laboratory science. *J Clin Pathol*. 2012; 65(10):877–881. [PubMed: 22791768]
31. Weinstein RS, Graham AR, Richter LC, et al. Overview of telepathology, virtual microscopy, and whole slide imaging: prospects for the future. *Hum Pathol*. 2009; 40(8):1057–1069. [PubMed: 19552937]
32. Weening JJ, D'Agati VD, Schwartz MM, et al. The classification of glomerulonephritis in systemic lupus erythematosus revisited. *Kidney Int*. 2004; 65(2):521–530. [PubMed: 14717922]
33. Roberts IS, Cook HT, Troyanov S, et al. The Oxford classification of IgA nephropathy: pathology definitions, correlations, and reproducibility. *Kidney Int*. 2009; 76(5):546–556. [PubMed: 19571790]

34. D'Agati VD, Fogo AB, Bruijn JA, Jennette JC. Pathologic classification of focal segmental glomerulosclerosis: a working proposal. *Am J Kidney Dis.* 2004; 43(2):368–382. [PubMed: 14750104]
35. Tervaert TW, Mooyaart AL, Amann K, et al. Pathologic classification of diabetic nephropathy. *J Am Soc Nephrol.* 2010; 21(4):556–563. [PubMed: 20167701]
36. Berden AE, Ferrario F, Hagen EC, et al. Histopathologic classification of ANCA-associated glomerulonephritis. *J Am Soc Nephrol.* 2010; 21(10):1628–1636. [PubMed: 20616173]
37. Haas M, Rastaldi MP, Fervenza FC. Histologic classification of glomerular diseases: clinicopathologic correlations, limitations exposed by validation studies, and suggestions for modification. *Kidney Int.* 2014; 85(4):779–793. [PubMed: 24088958]
38. Barisoni L, Troost J, Bagnasco S, et al. Reproducibility of the NEPTUNE digital pathology morphologic profiling of NS. *J Am Soc Nephrol.* 2014; 25:744A.
39. Rosenberg A, Palmer M, Merlino L, et al. The NEPTUNE digital pathology protocol increases accuracy in glomerular number assessment. *J Am Soc Nephrol.* 2014; 2014:955A.
40. Gadegbeku CA, Gipson DS, Holzman LB, et al. Design of the Nephrotic Syndrome Study Network (NEPTUNE) to evaluate primary glomerular nephropathy by a multidisciplinary approach. *Kidney Int.* 2013; 83(4):749–756. [PubMed: 23325076]
41. Nast C, Barisoni L, Troost J, et al. Glomerular descriptors enhance standard renal biopsy diagnoses. *J Am Soc Nephrol.* 2013; 24:824A.
42. Hodgin J, Bitzer M, Wickman L, et al. Glomerular aging. A podometric perspective. *J Am Soc Nephrol.* 2015 In press.
43. Lemley K, Bagnasco S, Nast C, et al. Morphometry on baseline biopsies predicts early GFR changes in nephrotic syndrome. *J Am Soc Nephrol.* 2014; 25:955A.
44. Reiser J, Nast CC, Alachkar N. Permeability factors in focal and segmental glomerulosclerosis. *Adv Chronic Kidney Dis.* 2014; 21(5):417–421. [PubMed: 25168830]
45. Spinale J, Mariani L, Kapoor S, et al. A reassessment of soluble urokinase-type plasminogen activator receptor in glomerular disease.
46. Pantanowitz L. Digital images and the future of digital pathology. *J Pathol Inform.* 2010;1. [PubMed: 20805964]

Figure 1. Conventional versus digital pathology review for multicenter consortia

A) Representation of conventional glass slide review, wherein pathology material is mailed to multiple study pathologists who are located in different institutions/cities. Pathologists collect data via glass slide review, and meet for reconciliation or consensus in a selected location, requiring time and financial expenditures. **B)** Representation of the NEPTUNE digital pathology creation and review process. Glass slides and other pathology materials, including immunofluorescence and electron microscopy digital images, and de-identified pathology report pdfs, are collected and scanned locally or mailed to the Image-managing coordinating center for scanning and uploading into the cloud. Investigators remotely access the NEPTUNE digital materials anywhere and anytime, annotate renal structures, and collect data from the annotated digital slides. Annotation allows reviewing pathologists to score exactly the same structures for more precise, targeted and efficient analysis.

Figure 2. Annotation and multilevel representation of glomeruli for accurate glomerular counts and analysis

Up to four sections from the same biopsy can be visualized simultaneously for annotation or scoring. Individual glomeruli can be followed along the sectioning levels and annotated with a single unique number on all levels. This process is facilitated by the application of specific digital functions such as overlapping of 2 different whole slide images, allowing for precise mapping of glomeruli within the biopsy. The figure shows three glomeruli disappearing; one (black circle) disappearing from level (Lev) 1 to Lev 5 and two (green and orange circles) from Lev 5 to Lev 8. On Lev 8 there are two new glomeruli appearing (red and yellow circles) that were not present on the previous levels. The overlap of Lev 1 and Lev 8 demonstrates all the glomeruli.

Figure 3. The NEPTUNE Digital Pathology Repository (DPR) with annotated Whole Slide Images (WSI) and electronic scoring sheet (descriptor matrix)

A) The left computer monitor shows WSI from a selected case in the DPR with visualization of four slide levels after glomerular annotation. The right monitor shows the electronic scoring spreadsheet into which the collected data are entered. **B)** The electronic scoring spreadsheet has individual numbered glomeruli across the top x axis, and descriptors along the y axis. Descriptor data boxes are pre-populated by “0” = “absent” as indicated in the blue cells. When a descriptor is selected using a drop down menu to indicate the appropriate response (yes, no, %, etc.) the cell changes color to red, making it easier to identify the structures with lesions. Glomeruli are profiled by the selection of all descriptors applicable to that glomerulus on all levels, as illustrated by the glomerular lesions on the right.

Example of descriptor-based categories

Figure 4. Example of descriptor-derived categories for the focal and segmental glomerulosclerosis (FSGS)/minimal change disease (MCD) cohort

Cases with a clinical-pathologic diagnosis of FSGS or MCD can be stratified into two major categories of “glomerular lesions” or “minimal lesions” based on the presence or absence (respectively) of specific descriptors of any form of sclerosis detectable on WSI. The presence or absence of segmental obliterative lesions defines two additional categories. Within the segmental obliteration groups, are conventionally named FSGS cases and a novel category termed FSGS-like, which can be distinguished using descriptors of segmental non-sclerosing obliteration, which typically are not incorporated into conventional classification systems. The significance of these latter lesions, in the absence of defined segmental sclerosis or collapse, is uncertain. Algorithms can be applied to cases with no segmental obliteration dividing them into “minimal lesions” or “glomerular lesions” categories depending on the presence or absence of global sclerosis/deflation. Further subclassification based on global sclerosis/deflation and degree of foot process effacement allows the identification of well-known categories, such as MCD with extensive foot process effacement, as well as not yet explored categories such as MCD-like, which is characterized by partial effacement and/or global sclerosis or deflation. Such MCD-like cases with global sclerosis or deflation are grouped with “glomerular lesions” and, based on the degree of podocyte foot process effacement, can be divided into MCD-like with global sclerosis/deflation and extensive effacement, or MCD-like with global sclerosis/deflation and partial effacement. The degree of effacement further separates cases with “minimal lesions” into those considered true “MCD” group (>75% effacement) and those with partial effacement that not fulfill criteria for MCD, considered MCD-like. The dotted lines indicate hypotheses, see text.

Figure 5. Deflating glomerulopathy

A) Global deflation of the glomerular tuft (historically considered “ischemic collapse”). In contrast to other glomerulopathies characterized by wrinkling and folding the glomerular basement membranes (such as collapsing glomerulopathy), there is no epithelial cell (podocyte) hypertrophy or hyperplasia. (PAS 40×) **B)** Segmental deflation with a perihilar scar and periglomerular fibrosis, the significance of which are uncertain. (HE 40×)

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 6. Morphologic analysis in the 21st century

The NEPTUNE digital pathology protocol is a model for the evolution of pathology material review in the setting of multicenter studies and for other applications. Conventional light microscopic review and interpretative histologic assessment are replaced by digital image review and quantitative descriptor-based morphologic profiling for implementation of precision medicine.

Glomerular Descriptors Used in The NEPTUNE Pathology Scoring System*

Table 1

Whole Slide Image Observations	Whole Slide Image Observations	Electron Microscopy Image Observations
Global glomerular obliteration	Pod Injury	Podocyte injury
Global sclerosis/obsolescence	Halo (detachment, neocollagen)	Foot process effacement
Global sclerosis & hyalinosis	Hyaline (protein) droplets	0 (0–10%)
Global sclerosis, no hyalinosis	Pod HT	1 (11–25%)
Obsolescence	Segmental	2 (26–50%)
Global collapse (with Pod HT/HP)	Global	3 (51–75%)
Global deflation (no Pod HP)	Pod HP	4 (> 75%)
Segmental glomerular obliteration	Segmental	Condensation of actin-based cytoskeleton
Segmental solidification/sclerosis (SS)	Global	0 (0–10%)
Perihilar	Abnormal basement membranes (GBM)	1 (11%–50%)
At the tip (tubular pole)	Subepithelial spikes	2 (greater than 50%)
Mid-glomerular	Segmental	Microvillus transformation
Away from hilum and tip	Global	0 (0–10%)
Undetermined location	GBM Duplication	1 (11%–50%)
Cellular (not at tip)	Segmental	2 (> 50%)
Segmental collapse (with Pod HT/HP)	Global	Loss of primary processes
Segmental deflation (without Pod HP)		Podocyte detachment (halo)
Hyalinosis (H)	Other Lesions	Electron dense deposits
At the hilum	Periglomerular fibrosis	Mesangial deposits
At the tip	Endocapillary hypercellularity	Subepithelial deposits
Away from hilum and tip	Segmental	Stage I
Undetermined location	Global	Stage II
Foam cells	Intracapillary neutrophils	Stage III
Adhesions	Karyorrhexis	Stage IV
	Necrosis	Transmembranous deposits
Mesangiopathic changes	Cellular crescents	Nuclear pore configuration
Matrix expansion only	25% glomerulus involved	Subendothelial deposits
Segmental	>25% glomerulus involved	

<u>Whole Slide Image Observations</u>		<u>Whole Slide Image Observations</u>	<u>Electron Microscopy Image Observations</u>
Mesangial hypercellularity	Global	Fibrocellular crescents	Other Lesions
	Segmental	25% glomerulus involved	Tubulo-reticular inclusions
		>25% glomerulus involved	GBM thickness
	Global	Fibrous crescents	Normal
		25% glomerulus involved	Thick
		>25% glomerulus involved	Thin
			Mixed thick and thin

Choices are Yes/No unless otherwise indicated

Pod = podocytes and parietal cells (glomerular epithelium)

HT = hypertrophy

HP = hyperplasia

GBM = glomerular capillary basement membrane

Tubulointerstitial and Vascular Descriptors Used in The NEPTUNE Pathology Scoring System * Whole Slide Image Observations

Table 2

Tubulointerstitial Lesions	
Acute/Active	
Tubular cell injury	
None	
Mild	
Moderate	
Severe	
Interstitial edema	
Interstitial inflammation (% involvement)	
Neutrophils (if > 10%)	
Eosinophils (if > 10%)	
Chronic	
Tubular atrophy (% involvement)	
Interstitial fibrosis (% involvement)	
Other	
Tubular microcysts	
Vascular Lesions	
Arteriosclerosis	
None	
Mild	
Moderate	
Severe	
Arteriolar hyalinosis	
None	
Mild	
Moderate	
Severe	

* Choices are Yes/No unless otherwise indicated