The Rise of Renal Pathology in Nephrology: Structure Illuminates Function

Vivette D. D’Agati, MD,1 and Michael Mengel, MD2

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We would make a plea that so far as any conclusions are drawn as to the mechanism of human disease, the evidence derived from man should at least be considered.

George W. Pickering and Robert H. Heptinstall1

When considering the origins of medical renal pathology as a discipline and how it has influenced the field of nephrology, it is difficult to set a starting point for discussion. Renal pathology evolved gradually over decades within and alongside the context of nephrology, and the 2 have become so inextricably linked that it is hard to imagine one without the other. Yet there was a time when neither existed in any defined form and the pathology of the kidney was both primitive and confused. The early history of renal pathology dating from 1650 and the first applications of light microscopy to renal tissues are the subject of a recent review.2 If one dates the formal origins of “nephrology” to the coining of the term in 1960 by Jean Hamburger as Chairman of the first International Society of Nephrology (ISN) meeting in Evian, France, it seems appropriate that this discussion should focus on the parallel developments in renal pathology, beginning around the middle of the 20th century.

THE PREBIPSY ERA

Prior to the 1950s, the study of diseased human kidneys was restricted to postmortem examination by gross pathology and histology. Light microscopic findings often were obscured by autolysis, passive congestion, and 5-μm-thick sectioning. Inevitably, most of the medical renal conditions identified at autopsy were so chronic and advanced that they could elucidate little to nothing about the early stages and evolution of disease. Because preterminal kidney function studies and blood chemistry tests were rarely obtained or difficult to interpret, clinical-pathologic correlations were limited. Clinical syndromes such as acute nephritis, nephrosis, asymptomatic hematuria, and chronic kidney failure were understood by clinicians since Richard Bright,3 but how they related to distinct pathologic processes remained obscure. The concepts that a particular clinical syndrome could be caused by more
than one pathologic process and conversely, that one pathologic process could manifest as different clinical syndromes, were not yet appreciated.4

The resulting state of confusion is evident in the few available textbooks from the first half of the 20th century. Bell’s Renal Diseases,5 published in 1946, was widely used, and the textbook by McManus,6 published in 1950, was a major advance by applying periodic acid–Schiff staining to highlight renal basement membranes. Yet to many students of that period, the older 1914 monograph entitled Die Brightsche Nierenkrankheit by Franz Volhard,7 clinician at the University of Frankfurt am Main, and Theodor Fahr, director of the Institute of Pathology at the University of Hamburg, together with the chapter by Fahr in the 1925 edition of Henke and Lubarsch’s Handbuch der Speziellen Pathologischen Anatomie und Histologie,8 remained unsurpassed in precision and detail. The talented pathologist Fahr provided painstakingly accurate histologic drawings (easily mistaken for photographs) of renal diseases, which were accompanied by Volhard’s descriptions of the clinical manifestations. This successful collaboration can be considered the first pathologist-clinician team in nephrology. For example, a drawing entitled “lipoid nephrosis with glomerular degeneration” (Fig 1) illustrates what we now recognize as focal segmental glomerulosclerosis (FSGS), a prescient view considering that their publication preceded any knowledge of podocytopathies by more than 80 years.

Unique among pathologists of that era was Jean Oliver, who used nephron dissection as a primary means of studying renal diseases.9 His meticulous 3-dimensional microdissections of entire nephrons allowed precise localization of the site of a lesion in early disease and demonstration of its multifocal effects on other portions of the nephron and neighboring nephrons in the chronic stages.

The increasing attention to pathology met considerable resistance among many clinicians. In the introduction to their book, The Renal Lesions in Bright’s Disease, Addis and Oliver10 state that “it is more important to know what the kidney does than what it looks like.” The supremacy of function over structure reflected the early bias toward renal physiology. As indicated by the introductory epigraph (which has been used as an unofficial motto by the Renal Pathology Society [RPS]), in the early 1950s human renal pathology often was overlooked in favor of experimental pathology because living animal tissues were more accessible and conditions could be controlled. Obviously renal pathology had to advance and mature before it would be embraced by renal physicians.

THE EARLY KIDNEY BIOPSY ERA

It was not until the introduction of kidney biopsy as a study of living tissue that renal pathology entered the modern era. Although kidney biopsy had been used as early as the 1930s for renal tumors,11 the earliest reports of its use in the diagnosis of medical kidney disease were in 1951 by Iversen and Brun12 in Denmark, in 1952 by Alwall13 in Sweden, and in 1953 by Pardo and colleagues14 in Cuba. In Denmark, kidney biopsy was first used as an aspiration biopsy for the practical purpose of triaging patients for dialysis. In 1953, the technique entered the United States, as per-
formed by Parrish and Howe.\textsuperscript{15} It was soon modified at the University of Illinois by Robert Kark and Robert Muehrcke,\textsuperscript{16} who introduced the Vim-Silverman cutting needle and adopted a prone, rather than sitting, position to improve safety and biopsy yield.

Thus began one of the most fortuitous pairings of pathologists and clinicians in the history of renal medicine. Much to his initial dismay, Conrad Pirani (Fig 2), a pathologist at the University of Illinois, was entrusted by his chairman Granville Bennett with the dubious task of interpreting these minute specimens. Bennett actually apologized to Pirani for burdening him with this responsibility because he had so little confidence in the future of the enterprise.\textsuperscript{18} The Chicago team of Pirani, Kark, and Muehrcke would soon prove him wrong. Over the ensuing decade, a stream of groundbreaking clinical-pathologic studies would emerge from the study of kidney biopsies,\textsuperscript{19} sometimes performed sequentially to demonstrate the evolution and resolution of disease.\textsuperscript{20} Although ethical issues later curtailed the use of repeated biopsy for investigative purposes, the frequent practice of repeated kidney biopsies in these early years provided invaluable information about the natural history of disease. Pirani’s application of systematic semiquantitative evaluation of active and chronic lesions in all renal compartments is one of his greatest contributions.\textsuperscript{21} As kidney biopsy became widely adopted and interpreted by other pioneers in renal pathology, including Jacob Churg at Mount Sinai School of Medicine (Fig 3), Robert Jennings at Northwestern University, Benjamin Spargo at University of Chicago, Robert McCluskey at New York University, Robert Heptinstall at Johns Hopkins Hospital, Jay Bernstein at Albert Einstein College of Medicine, and Renée Habib at Institut National de la Santé et de la Recherche Médicale, Paris (Fig 4), among others, the stage was set for renal pathology to emerge as its own subspecialty of pathology.

However, several important things would have to happen first. The advantages of thin sections (2-3 μm) cut serially to standardize assessment of glomerular cellularity and detect focal lesions were emphasized by Churg and Grishman.\textsuperscript{22} Masson trichrome stain was adopted as a useful means to quantitate interstitial fibrosis and differentiate fibrin, hyaline, and immune deposits as red against the blue-stained glomerular basement membrane (GBM) and mesangial matrix. David Jones at The State University of New York at Buffalo developed the Jones methenamine silver stain, which remains the most helpful GBM stain for demonstrating such textural changes as spikes, gaps, vacuolizations, and lamellations.\textsuperscript{23} Periodic acid–Schiff had the advantage of vividly delineating all renal basement membranes and highlighting the brush border of intact proximal tubular cells.

Importantly, acquisition of living tissues allowed application of emerging modalities that require maximal tissue preservation: immunofluorescence and electron microscopy. In the 1950s, Coons de-
veloped the method of coupling fluorescent probes to antibody (at that time gamma globulin) to detect immunoglobulin in frozen sections. In parallel with the experimental models of serum sickness by Dixon et al and Germuth et al, these advances opened the way for identification of human immune complex–mediated glomerulonephritides. Robert Mccluskey and Gloria Gallo at New York University were pioneers in the application of immunofluorescence to human kidney biopsies. Similarly, development of the experimental Masugi (nephrotoxic serum) nephritis model together with the identification of circulating antibodies with anti-GBM activity producing linear, as opposed to granular, staining in human crescentic glomerulonephritis introduced major immunologic disease paradigms. With the coupling of fluorescein to other antibodies, it became possible to identify immunoglobulin A (IgA) deposits in IgA nephropathy, as well as complement deposits in postinfectious glomerulonephritis and membranoproliferative glomerulonephritis (MPGN).

Although the first transmission electron microscopes had been developed by Siemens and Halske in the 1930s, electron microscopy was not used to examine human kidney biopsy specimens until the late 1950s. Ultrastructural images settled the long-standing dispute over the existence of an intralobular cell by identifying the mesangial cell as a distinct cell type and elucidating its relationship to the mesangial matrix and glomerular endothelium. For the first time, it was possible to resolve the fine structure of podocyte foot processes, as well as glomerular endothelial fenestrations and filtration slit diaphragms, which were both originally thought by some to be fixation artifacts. As the technical quality of electron microscopic images improved, particularly in the expert hands of Churg and Spargo, a host of new diseases were discovered. For example, GBM thinning was identified in families with benign hematuria, whereas widespread lamellation of GBM was a diagnostic lesion in Alport syndrome. Fusion or effacement of foot processes was the major pathologic finding in minimal change disease. Fibrillar deposits were detectable in fibrillary glomerulonephritis, and microtubules in immunotactoid glomerulonephritis. Inclusion bodies were found in lipid storage diseases such as Fabry disease. As discussed by Heptinstall, the use of electron microscopy even informed light microscopic interpretations by fostering better understanding of the normal anatomic relations of cells to matrix. To this day, renal pathology is one of the few subspecialties of pathology in which electron microscopy remains an essential part of the diagnostic armamentarium.

At first there was skepticism among clinicians that such minute needle biopsy samples could provide an accurate representation of disease. A famous case in point was the submission of a paper to The Lancet by Pirani and colleagues in 1957. It described sequential kidney biopsies in a young...
man with nephrotic syndrome. The first biopsy specimen showed no obvious glomerular changes visible upon light microscopy, but complete effacement of foot processes by electron microscopy in each of the 7 glomeruli examined ultrastructurally, leading to a diagnosis of lipoid nephrosis. The patient received a course of corticosteroids, which resulted in complete remission of nephrotic syndrome. A second biopsy was performed at this time and showed normal-appearing glomeruli by light microscopy and complete restoration of foot processes in the 5 glomeruli examined ultrastructurally. This was the first description of the resolution of foot-process effacement after glucocorticoid therapy for minimal change disease and identified the visceral epithelial cell as the major target of injury. The reviewers rejected the paper outright with the critique that the number of glomeruli studied by electron microscopy was insufficient for definitive conclusions. It would take time for clinicians and pathologists to develop confidence in the adequacy of minute renal samples studied under expert hands. Fortunately for the Chicago group, the Annals of Internal Medicine had a more enlightened view.32

CIBA SYMPOSIUM (1961)

A milestone in the investiture of renal pathology as a specialty was the 1961 Ciba Symposium in London. Twenty-nine clinicians and pathologists convened to discuss the benefits, risks, and future of kidney biopsy in medical renal disease. As described by Pirani:

This meeting, attended by physicians with much experience in the study of renal diseases, was a most significant event and a turning point in the history of renal biopsy. Until then this procedure had been carried out in relatively few centers around the world. Thereafter renal biopsy became an integral part of every major renal center and played a vital role in the birth of nephrology as a major medical specialty.33

In attendance were Arnold Rich, who chaired the symposium, and the following pathologists: A. Bergstrand (Sweden), R. Habib (France), R.H. Heptinstall (Great Britain), R.B. Jennings (United States), H.Z. Movat (Canada), and C.L. Pirani (United States), representing a combined experience of approximately 5,000 biopsies. What impressed Pirani most was the clinicians’ intense interest and enthusiasm for renal pathology. He added that the stimulating effects of a midmorning glass of sherry and a leisurely afternoon cup of tea fostered a lively exchange of ideas. The symposium was a huge success and a crucible for the melding of renal pathology with clinical nephrology into a synergistic whole.

HEPTINSTALL’S PATHOLOGY OF THE KIDNEY

The many technical advances set the stage for the codification of kidney disease in the first modern textbook of renal pathology, Pathology of the Kidney, by Robert Heptinstall34 (Fig 5). Heptinstall is both a diagnostic and experimental pathologist who made significant contributions to the pathophysiology of hypertensive nephrosclerosis and pyelonephritis. This scientific background gave him a probing critical perspective that few morphologists of his day could match. The first edition, published in 1966, was written over a 3-year period after Heptinstall returned from St Mary’s Hospital, London, to his permanent position at Johns Hopkins Hospital.

The impact of this book on generations of pathologists and nephrologists cannot be underestimated. It was the first modern systematic approach to the classification of kidney disease and provided a synopsis of all the relevant literature to date in a carefully organized well-illustrated text, seasoned by personal observations. Heptinstall speaks to his reader in the first person, often giving insightful anecdotes from his own experience and providing a much needed critique of earlier publications and classifications. His writing is clear and direct, yet nuanced, with sprinklings of wry humor. The many personal vignettes make the book as intimate and enjoyable as a picaresque novel, despite the reams of scientific data. For example, when describing glomeruli in lipoid nephrosis, Heptinstall remarks:

The tufts have a somewhat fixed and rigid appearance in spite of there being no thickening of the wall and have been fittingly described as “almost too handsome” by Habib et al. [see 35]. These proud-looking glomeruli, however, are not always seen, and in some cases there may be nothing peculiar about them.33(p377)

Descriptions of each entity were divided neatly into sections on gross pathology and the morphologic findings within each renal compartment, followed by sections on clinical and experimental correlations and prognosis. Subsequent editions incorporated increasingly more electron microscopy and immunofluorescence.

THE MODERN ERA OF RENAL PATHOLOGY

Before renal pathology could rise within nephrology it had to be recognized as a viable subspecialty by pathologists. Although this may seem obvious, patholo-
gists have their own esteemed societies, even older and arguably more venerable than those of nephrologists. In the early 1960s, both the United States and Canadian Academy of Pathology and the International Academy of Pathology held subspecialty conferences, “short courses” and “long (full-day) courses” devoted to the emerging specialty of medical renal pathology. In 1977, Conrad Pirani founded the Renal Pathology Club, which began by invitation to the following members: Peter Burkholder, Jacob Churg, Ramzi Cotran, Francis Cuppage, Robert Heptinstall, David Jones, Michael Kashgarian, Richard Kempsion, John Kissane, Robert McCluskey, Kash Mostofi, Benjamin Spargo, and Gary Striker. It would meet at the International Academy of Pathology and American Society of Nephrology (ASN) meetings and was open to any interested attendees. In 1993, the club metamorphosed into the RPS (www.renalpathsoc.org), which now boasts more than 400 international members and promotes a wealth of educational and investigative activities.

Jean Hamburger’s comments at the Sixth Congress of the ISN held in Florence in 1975 are a testament to the enormous advances attributable to renal pathology: “The history of nephrology of the last 25 years could be entitled ‘The Decline and Fall of Bright’s Disease and the Birth of Individual Renal Diseases from its Ashes.’” She attributed the rise of renal pathology as a force within the nephrology community to the respect and inclusion accorded renal pathologists by nephrology societies. Renal pathologists are regular members of the ASN Program Committee and Postgraduate Education Committee, and certain sessions of the annual meetings are held jointly with the RPS. Renal pathologists deservedly serve on the editorial boards of the major renal journals. A Renal Pathology precourse is given annually at the ASN since 1998 under the direction of Agnes Fogo. The RPS has cosponsored many reclassifications of disease, such as the new ISN/RPS classification of lupus nephritis, new approaches to antineutrophil cytoplasmic antibody–associated glomerulonephritis, and diabetic nephropathy. Renal pathologists were recipients of the John Peters Award of the ASN: Conrad Pirani and Jacob Churg in 1987, Robert Heptinstall and Priscilla Kincaid-Smith in 1993, and Ramzi Cotran in 1999. As ultimate achievements, pathologists Robert Heptinstall and Ramzi Cotran served as presidents of the ASN in 1972 and 1995, respectively, and pathologist Jan Weening was ISN president in 2003.

By 1990, the specialty of renal pathology was well established and major centers were training nephropathology fellows. Kidney biopsy had become such a successful diagnostic tool that its rise paralleled the decline in urine microscopy as performed by the nephrologist. The automated gun technique has made it easier and quicker to biopsy, especially if performed by a radiologist. As the gauge of the biopsy needle has risen from 14 with the old Vim-Silverman needles to as high as 18 or even 20 with many of the automated guns, the pathologist’s skills and resourcefulness at salvaging tissue from one modality, such as light microscopy, for another, such as immunofluorescence or electron microscopy, have had to compensate for the increasingly marginal adequacy of many specimens.
By 2000, our ability to diagnose kidney disease had far outstripped our knowledge of pathogenesis. It is astounding to consider that even at the time the sixth edition of Heptinstall’s Pathology of the Kidney was published in 2007, the etiology of most forms of glomerular disease remained unknown. For example, the specific causes of the 3 main forms of idiopathic nephrotic syndrome, minimal change disease, primary FSGS, and membranous glomerulopathy, were obscure. Likewise, the pathogenic antigens in acute postinfectious glomerulonephritis and IgA nephropathy were enigmatic. A notable exception was the elucidation by the pathologist-clinician team of J. Charles Jennette and Ronald Falk of antineutrophil cytoplasmic antibody as mediator of most pauci-immune crescentic and necrotizing glomerulonephritis, thereby closing the gap with anti-GBM nephritis, for which etiology had been known for decades.

**RENAL PATHOLOGY IN THE MOLECULAR AGE**

Many of the diagnostic criteria used by pathologists are empirically derived from morphology, immunopathology, and clinical correlates, but lacking an etiologic basis. In this emerging era of personalized medicine, precise mechanism-based diagnoses are essential to refine the taxonomy of disease and deliver targeted therapy to the individual patient. In addition, diagnostic precision of the kidney biopsy is a prerequisite to validate new noninvasive biomarkers, such as urine proteomics and metabolomics, against the biopsy as gold standard.

Recent studies, mostly in the oncology field, suggest that including molecular information as part of a classification fosters more precise diagnoses and predictions for response to treatment. A compelling illustration in the area of nephropathology is the classification of MPGN. Since the 1970s, MPGN had been classified into types I, II, and III based on the location and ultrastructural appearance of deposits, rather than their immune composition. It now is understood that subsets of type I and type III MPGN that contain C3 only, without immunoglobulin, are mediated by inherited or acquired dysregulation of the alternative complement pathway. Accordingly, this subset of types I and III are now called C3 glomerulonephritis, and together with type II (dense deposit disease), constitute the umbrella of C3 glomerulopathies. A diagnosis of C3 glomerulopathy has opened the way to improved diagnostic and treatment algorithms, such as investigations of genetic defects in or autoantibodies to complement factor H, genetic deficiencies in other complement regulatory proteins, and the presence of a C3 nephritic factor.

Similarly, primary membranous glomerulopathy now is understood as pathogenetically distinct subsets largely mediated by antibody to phospholipase A2 receptor, and some unusual antigens such as cationic bovine serum albumin and neutral endopeptidase in infantile forms. In some cases, the target antigen can be detected in glomerular deposits by immunohistochemistry even when anti-phospholipase A2 receptor antibody is undetectable in serum. The application of stains for IgG subtypes has identified new monoclonal forms of proliferative glomerulonephritis and IgG4-associated autoimmune interstitial nephritis.

Mass spectrometry after laser-capture microdissection has emerged as a valuable proteomic tool for more precise subtyping of renal amyloidosis. For the first time, rare forms of amyloidosis caused by deposition of leukocyte cell-derived chemotaxin 2, fibrinogen α chain, apolipoprotein A-I and A-IV, transthyretin, gelsofin, and β2-microglobulin can be diagnosed from human kidney biopsy specimens, providing a diagnostic granularity beyond what could be accomplished by standard immunohistochemical staining approaches for detection of more common precursor proteins, such as immunoglobulin light and heavy chains or serum amyloid A. Such precise typing of renal amyloidosis should guide genetic counseling and disease-specific treatments.

In the last 5 years, there have been exciting methodological advances in molecular biology. The fundamental breakthrough after completion of the human genome project was to shift the focus of biologic research from investigating single genes, transcripts (messenger RNA [mRNA]), or proteins to assaying essentially all molecules of a given class in parallel with array technologies. Since its advent in the early 1990s, the field has battled skepticism concerning platform comparability and biostatistical analysis. The expectation is that such “omics” approaches, serving as a “molecular microscope,” ultimately will elucidate disease mechanisms and identify novel biomarkers that inform diagnosis, prognosis, and therapy.

The largest experience has been in the study of kidney transplant biopsy specimens with Affymetrix microarrays for quantitative genome-wide mRNA expression analysis. It is a general observa-
tion that groups of genes (sometimes several hundred per group) change their expression in a coordinated fashion reflecting major biological processes, such as inflammation and repair. Microarray results thus can be scored as the geometric mean of fold changes across selected pathogenesis-based transcript sets. In this way, large-scale cumbersome microarray data can be collapsed into a small number of transcript set scores representing molecular measurements of distinct pathogenetically relevant biological processes. Currently, microarray processing and interpretation require highly qualified personnel, have a turnaround time of about 3-4 days, and cost approximately $2,000-$3,000 per biopsy, making them prohibitive for widespread routine application. With economy of scale and the advent of refined hardware and software, costs should decrease such that comprehensive transcriptome analysis will soon become feasible for routine diagnostics.

A limitation is that broad elements of the molecular phenotype are not disease specific. Many progressive diseases operating in transplanted kidney have similar molecular phenotypes. Parallel observations have been made in native kidneys, for which microarray mRNA analysis from biopsy specimens of patients with diverse chronic kidney diseases has revealed unexpectedly high frequencies of shared gene networks. Glomerular genes identified by laser-capture microdissection and genome-wide expression analysis in cases with primary FSGS compared to minimal change disease revealed dysregulated slit diaphragm and podocyte transcripts, as well as differences in the biological processes of development, differentiation, cell motility, and signal transduction. Thus, in addition to disease-specific transcripts, many differentially expressed molecules represent a stereotypic injury response for which the extent correlates with disease activity.

An example of how the addition of molecular assessments can improve diagnostic precision is in antibody-mediated rejection. Although the identification of complement component C4d deposition in peritubular capillaries has revolutionized our ability to diagnose antibody-mediated rejection, it soon was realized that its sensitivity as a biomarker is limited. Interaction of anti-HLA antibodies with their targets on endothelial cells causes endothelial stress with upregulation of endothelium-specific molecules such as factor VIII, cadherin 13, and CD31 and promotes inflammation by binding to Fc receptors on natural killer cells. Molecules specific for endothelial cell activation and natural killer cell infiltration have been demonstrated to associate with antibody-mediated rejection even in the absence of C4d staining.

A futuristic approach to interpreting high-dimensional data derived from biopsies uses sophisticated biostatistics to predict the diagnosis of a new sample solely from the “omic” assessment. Mathematical algorithms assign probabilities that the new sample belongs to a specific class/diagnosis based on various input data, such as the result of a microarray experiment. Probability algorithms are built using machine-based supervised learning after training on a set of known classes of correctly diagnosed cases. Recent investigations are applying this technique to native and transplant kidney biopsy specimens and to the classification of renal neoplasms.

Despite these methodologic advances, with the exception of diseases with a strong genotype-phenotype association, such as hereditary diseases, it is unlikely that even comprehensive molecular assessments of kidney biopsy specimens will provide absolute diagnostic precision. In some areas, the molecules are superior, as in the identification of early injury responses, which may be invisible to morphology. Histopathology will always carry greater specificity and sensitivity in other areas, such as focal glomerular diseases. An integrated approach factoring morphologic, immunopathologic, serologic, clinical, genetic, and molecular information likely will provide the greatest diagnostic precision. Thus, in the end, it is only through the continued close partnering and interdisciplinary efforts of pathologists and nephrologists that we can meet the future challenge of implementing more refined pathogenesis-based diagnosis into clinical management.

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REFERENCES

4. Pirani CL. Renal biopsy: an historical perspective. In: Silva FG, D’Agati VD,


