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Review article

Nephropathology: A Cornerstone for Understanding and Estimation of Recent Advances in Glomerular Diseases

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Abstract

The developments in the field of kidney pathology are major objectives for nephrology worldwide, since the histopathologic diagnosis is a cornerstone for all glomerulopathies (either primary or secondary related to systemic diseases-for tubulointerstitial and vascular lesions as well as renal allograft nephropathy). Moreover, the correct interpretation of kidney tissue samples is a challenge for pathologists too. Consequently, a new subspecialty - nephropathology, was accepted by many medical schools in various universities, while dedicated scientific meetings, journals and websites were also created. In the following few pages, a short overview on the history, classic and novel meanings of the renal pathology for the understanding of glomerular pathophysiology will be discussed.

Keywords: chronic kidney disease, glomerulopathies, kidney histology, nephropathology, proliferative pattern

Introduction

Nephropathology (or renal pathology) represents a branch of pathology aimed to study and diagnose kidney diseases (both in native kidneys and renal allografts) by microscopic examination of biopsy-obtained renal tissue. It provides useful data for everyday clinical practice in nephrology, but it also contributes to the scientific research related to kidney diseases.
Primary and secondary glomerulopathies are prevalent causes of chronic kidney disease (CKD), which could affect around 10% of the general population and thus earning its place among chronic non-communicable diseases. Although not always recognized [1,2] CKD provides a significant burden on any health care system. Kidney biopsy certifies the diagnosis of glomerular diseases, thus allowing stratification of patients into specific disease sub-groups which is important for treatment purposes in order to possibly influence the outcome on an individual basis. In addition, it also provides tissue samples for transcriptomic, genomic and proteomic studies, finally meant to select novel biomarkers for the prevention, early detection and proposing new therapies for CKD.

Historical evolution of nephropathology

Even if the ancient roots of pathology could be tracked back to the Galen's period, the real dawn of this specialty can be traced in the fifteenth century, when the first case histories and autopsies were done [3]. However, only two centuries later, after the invention of the microscope in Holland, the seeds of renal pathology were seen (Figure 1). Marcello Malpighi was the first to study not only the gross anatomy, but also the microanatomy of the kidneys and discover a part of its functional unit (i.e. the nephron), which he called a "gland" attached to arteries [4]. Malpighi assumed that it is the place of urine formation through a process of filtration from blood, according to substances' size and shape [4,5]. Thus, it can be said that both renal pathology and renal physiology were born at that time. Despite this first step, the next three centuries brought rather meagre achievements in the field of kidney histopathology. For example, in the mid-nineteenth century, the proximal (William Bowman) and then the lower parts of nephron (Friedrich Henle) were described, new dyes became available to enrich the staining options, and the technique of paraffin embedding for tissues preservation was created (Edwin Klebs) [5]. Klebs was also the first who introduced the term of "glomerulonephritis".
During the same period, which can be designated as the pre-biopsy era (Figure 1), other important landmarks are the Richard Bright's basis of clinical nephrology (with his studies on kidney-related symptoms and the first attempt to classify kidney diseases), and the integration of clinical and pathologic findings which lead Sir Arthur Ellis to describe two types of nephritis (the first with glomerular hypercellularity and the second with glomerular sclerosis) [3,5]. Another forward move-

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ment took place that time in the field of renal physiology by the work of Carl Ludwig who applied the laws of physics (hydrodynamics) to explain the glomerular filtration process of urine formation [4]. His results were confirmed and further developed by the micropuncture experiments of JT Wearn and AN Richards, which demonstrated a differential reabsorption of glomerular filtered solutes into the tubules, and hence put the basis of the modern renal physiology [4].

At the beginning of the 20th century, the first collaboration between a (renal) pathologist-Theodor Fahr and a clinician (treating internist)-Franz Volhard resulted in a new classification of kidney diseases driven by the combination of pathology findings and clinical signs of the

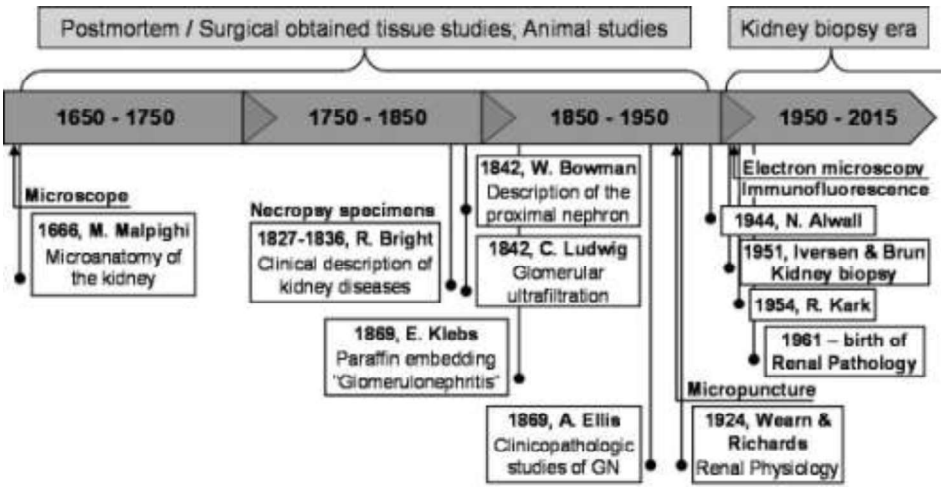

Fig. 1. The timeline of historical evolution of nephropathology as used in clinical routine

disease: nephrosis-i.e. the degenerative diseases, nephritides-i.e. inflammatory diseases, and chronic nephrosclerosis [5]. The successive progress regarding the techniques of tissues fixation, embedding, cutting, staining, microscopy, and image processing constantly yielded more accurate diagnostic tools [3], but the true turning point in the evolution of nephropathology is the introduction in practice of the percutaneous kidney biopsy around the mid-20th century (firstly used but not reported by Nils Alwall, then by Paul Iversen and Claus Brun, and finally refined by Robert Kark). Along with the almost simultaneous discover of immunofluorescence (Albert Coons and Melvin Kaplan, 1950) and the use of electron microscopy (firstly invented by Ernst Ruska and Max Knoll, 1931) in the medical field, this marked the entrance in the biopsy era [6]. One decade later, the nephrology was recognized as an independent specialty and the inaugural symposium on nephropathology was held, a fact that contributed to connect clinical nephrology and renal pathology as a synergistic entity [6]. From then onward, the rhythm of discoveries and new insights into the understanding of kidney disease pathogenesis (especially glomerular diseases) gathered speed in an unprecedented manner, leading to today's knowledge.

Nephropathology and established advances in glomerular pathology

Once introduced in the daily nephrological practice, kidney biopsy not only became the foundation of the great improvements in the diagnosis accuracy, but it also contributed to the deep understanding of disease mechanisms and provided important clues for the classification of kidney diseases, which opened the way to more efficient therapies. Light microscopy, immunofluorescence and electron microscopy are complementtary techniques and all concur to the precise description of the histopathologic changes in the kidney [7].

The understanding of glomerular pathology was the main benefit of the obtained knowledge from nephropathology. The diagnosis, pathogenetic insights, prediction of prognosis and therapeutic decisions of various nephropathies are stronger than in any other diseases relying on the microscopic assessment of the biopsy-extracted kidney tissue. Firstly, new histological patterns were observed and gradually characterized. Furthermore, during the late '60s and early '70s, the time-related classification of glomerulopathies (acute, subacute and chronic) was replaced by the morphologic classification, which was considered a great step forward because it assured the reproducibility of diagnosis and opened the pathway to a pathogenetic classification [8].

A good example is immunoglobulin A nephropathy (IgAN)-the most common primary glomerulonephritis worldwide, accounting for almost one third of cases [9]. It was initially described by Jean Berger and Nicole Hinglais in 1968 with the help of immunofluorescence which showed dominant mesangial deposits of IgA (with/without IgG) associated with moderate mesangial hypercellularity [10]. Since this first report, successive attempts of histological classifications and grading of

IgAN were made in order to obtain indices for predicting the outcome. Lee *et al*. (1982) and Haas *et al.* (1997) both proposed five-grade systems based on the spread of glomerular lesions (focal and segmental versus diffuse mesangial proliferation), the presence and spread of crescents, glomerular sclerosis and of the tubulointerstitial fibrosis [9]. Alamartine *et al*. (1990) developed a quantitative scoring system (the global optical score) ranging from 0 to 20, based on the sum of the glomerular, vascular, tubular and interstitial indices of injury [9]. However, none of these efforts were widely accepted, and the lack of consensus triggered the work of an international expert group to conceive a reliable and clinically significant, evidence-based new classification. The Oxford classification of IgAN is built on the MEST score [11]:

- mesangial hypercellularity-in ≤50% glomeruli=M0 and in >50%=M1;
- endocapillary proliferation-absent=E0 and present=E1;
- segmental glomerulosclerosis or adhesion-absent=S0 and present=S1;
- tubular atrophy and/or interstitial fibrosis-in <25% glomeruli=T0, in 26-50%=T1, and in >50%=T2.

The independent prognostic value of these four histological indices for IgAN patients was subsequently confirmed by numerous validation studies [9]. The only probable drawback of the Oxford classification is the lack of crescents from the scoring system (due to their low prevalence in the original investigated cohort [11]), since several studies found the extracapillary proliferation as an independent risk factor for the disease progression [12,13].

The contribution of electron microscopy should also be emphasized, as the histological hallmark of minimal change disease (one of the four major primary glomerulopathies which are clinically identified by the nephrotic syndrome), the diffuse foot process effacement, is visible only on the ultrastructural evaluation of kidney tissue. Moreover, this lesion was observed in other glomerular diseases associated with nephrotic syndrome too, thus leading to a higher awareness about the involvement of podocytes in glomerulopathies. The final result was the outlining of an array of glomerular diseases induced by altered podocyte functions, namely the podocytopathies: minimal-change disease nephropathy, focal and segmental glomerulosclerosis, diffuse mesangial sclerosis, and collapsing glomerulopathy [14,15].

Membranoproliferative glomerulonephritis (MPGN) is another example of glomerular disease, which understanding about classification and mechanisms closely followed the historical evolution of knowledge in the field of nephropathology. Firstly, it was identified as a descriptive pattern of glomerular injury observed on light microscopy and was included in the histopathologic classification of primary glomerulopathies published by Renee Habib in 1975 [16]. Further studies using electron microscopy were able to divide MPGN in three subtypes according to the location of electron-dense immune deposits inside the glomerular capillary wall [17]. More recently, the extensive evaluation by immunofluorescence found significant differences in the composition of immune deposits, strongly related to the etiopathogenesis of the disease, thus laying the foundation of the current classification into two major groups: immunoglobulin-mediated and complement-mediated MPGN [18]. This new proposed taxonomy has the advantages of being more pathophysiologically-oriented and providing important clues for the subsequent etiological diagnosis. Also, it allowed the surfacing of the C3 glomerulopathy (MPGN with deposition of C3 alone), a new disease entity due to inherited or acquired dysregulation of the alternative complement pathway, with potential implications for the therapeutic decisions [6,17].

However, since the glomeruli have limited possibilities of reaction to various injuries, the glomerular histopathologic changes usually overlap among different glomerulopathies irrespective of their etiology and even pathogenesis. Therefore, it is conceivable that a future step will be to incorporate serologic, genetic and molecular information to the clinical and histological findings, in a broad interdisciplinary effort to enhance the diagnosis and prognosis accuracy in the field of glomerulopathies [6].

Nephropathology and future advances in glomerular pathology

The most recent innovation in kidney biopsy assessment is the introduction of a computer-aided tool, by scanned slides and the setting up of a digital archive, which is easier to access and represents valuable stored information. The method seems also to enhance the performance of histological diagnosis, since in a recent study on whole slide images of 277 biopsies from the Nephrotic Syndrome Study Network (NEPTUNE) digital pathology repository, the digital technique was able to increase the accuracy of glomeruli counting, especially in cases with high number of glomeruli or high proportion of glomerulosclerosis (> 40%) [19].

Further ahead, the cooperation between classic nephropathology and the newest methodological advances in molecular biology (proteomics, metabolomics and transcriptomics) would be most probably the future in the field of glomerular pathology and kidney transplantation [6,20]. Laser capture microdissection and mass spectrometry-based proteomic analysis in biopsy specimens has already emerged as a valuable proteomic tool for the identification and subtyping of renal amyloidosis, with 100% reported specificity and sensitivity [21]. Also, the *in-situ* proteomics technology known as matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS) applied in cases of minimal change disease and membranous nephropathy showed molecular changes expressed as different signals in control ver-

sus patients with the two glomerulopathies, suggesting the possibility to detect the proteomic "signature" of the disease [22]. MALDI-IMS, which can analyze either fresh frozen tissue or formalin-fixed paraffin-embedded samples, opens opportunities to discover new biomarkers with both diagnostic and prognostic value [23].

On the other hand, transcriptomics was used in conjunction with renal pathology as well. Thus, the quantitative genome-wide mRNA expression analysis from kidney biopsy specimens by microarrays technique (next generation sequencing) uncovered abnormalities in slit diaphragm and podocyte transcripts, which are different in primary focal and segmental glomerulosclerosis (FSGS) from minimal change disease [6]. The method also identified complex genetic mutations in the spectrum of FSGS and it can be expected to provide similar new insights for other hereditary glomerular diseases with various genes aberrations but common histological and clinical characteristics, like Alport syndrome [24]. The same is true for the kidney graft pathology, as a recent multicentre study reported a set of 13 genes which was an independent predictor of the risk to develop fibrosis at one year in kidney allograft recipients, with high predictive capacity (area under the curve 0.967), hence it was proposed to use this set of genes as an early predictor of the risk for progressive loss of graft function [20].

However, until now, the high costs of the novel methods of molecular biology and, more important, the high number and heterogeneity of proposed biomarkers hinder the routine clinical utility of proteomics, transcriptomics and genomics. Presently, the correlation of detailed clinical, laboratory, immunological and histopathological data remains the most reliable diagnostic tool, and the place of nephropathology in the management algorithm of patients with glomerulopathies is well established. For this reason, aiming to strengthen this subspecialty in our country, a series of courses in nephropathology for the trainees and young nephrologists have recently been initiated, the first of which just has ended few weeks ago and was highly appreciated by the attendees.

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