A Reevaluation of Routine Electron Microscopy in the Examination of Native Renal Biopsies

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Abstract. Electron microscopy is routinely utilized in most centers in the evaluation of native renal biopsies. Several studies, primarily from the 1960s and early 1970s, provide justification for its use. Conducted by Siegel et al. (1), the largest study evaluated 213 consecutive renal biopsies and found that electron microscopy was needed for a correct diagnosis in 11%, as well as for confirmation or additional information in another 36%. However, nearly all of these studies were conducted before the use of immunofluorescence in renal biopsy diagnosis became widespread and before several new glomerular diseases and variants were described. In light of this situation and the expense of the procedure, the routine use of electron microscopy in native renal biopsies also examined by immunofluorescence and routine light microscopy was re-evaluated. From January 1996 to June 1996, 288 native renal biopsies were received, and all were evaluated by the same pathologist. Of those, 233 met criteria for inclusion in this study, which were ≥5 glomeruli for light microscopy, ≥2 for immunofluorescence, and ≥1 for electron microscopy, not including globally scarred glomeruli. Light microscopy (hematoxylin and eosin, periodic acid-Schiff stains) and immunofluorescence—for immunoglobulin (Ig) G, IgA, IgM, C3, C1q, fibrinogen; kappa/lambda when needed—were evaluated on each biopsy within 48 h of receipt, and a preliminary diagnosis was recorded if possible. Electron microscopy was then performed, and a final diagnosis was made. In 50 cases (21%), electron microscopy was needed to make the final diagnosis; in two of these cases, the preliminary diagnosis was incorrect, and in 48, a firm preliminary diagnosis could not be made. In the other cases, the preliminary diagnosis was correct, but in 48 (21%), ultrastructural study was felt to provide important confirmatory data, and in eight cases (3%), an additional, unrelated diagnosis was supported by the ultrastructural findings. Diagnoses most frequently requiring electron microscopy included minimal change nephropathy, early diabetic nephropathy, membranous lupus nephritis, membranoproliferative glomerulonephritis, postinfectious glomerulonephritis, thin basement membrane nephropathy (or exclusion of this in cases of otherwise unexplained hematuria), and human immunodeficiency virus-associated nephropathy (or exclusion of it in cases of collapsing glomerulopathy). Common diagnoses usually not requiring electron microscopy included IgA nephropathy, diffuse proliferative lupus nephritis, focal segmental glomerulosclerosis (not collapsing glomerulopathy variant), pauci-immune crescentic glomerulonephritis, acute interstitial nephritis, and amyloid nephropathy. This study confirms that, as was the case 20 to 30 yr ago, electron microscopy provides useful diagnostic information in nearly half of native renal biopsies. If electron microscopy cannot be performed routinely on all such biopsies, it is recommended that tissue for ultrastructural studies be set aside in each case. (J Am Soc Nephrol 8: 70-76, 1997)

In most major medical centers where native renal biopsies are performed and evaluated, electron microscopy is routinely performed as part of the biopsy evaluation, together with routine light microscopy and immunofluorescence studies. Justification for the routine use of electron microscopy in renal biopsy evaluation has come from several studies, the majority of which were performed during the late 1960s and early 1970s (1–5). In the largest of these studies, Siegel et al. (1) evaluated a series of 213 consecutive renal biopsies using light and electron microscopy. They found that in 11% of these cases, electron microscopy indicated a substantially different diagnosis than light microscopy and that in an additional 36% ultrastructural studies provided important information by substantiating or subclassifying the light microscopic diagnosis, and/or in providing information directly relevant to patient management. Similarly, Olsen et al. (2) found that electron microscopy indicated a different diagnosis than light microscopy alone in 11 (13%) of 91 renal biopsies. Muehrcke et al. (3) found that electron microscopy was necessary to establish a correct diagnosis in only 11 (6%) of 179 cases. Neither Olsen et al. (2) or Muehrcke et al. (3) considered confirmatory or subclassifying information. Tighe and Jones (4) found that ultrastructural studies were the most helpful in distinguishing cases of minimal change nephropathy from early membranous nephropathy and other glomerular diseases causing the nephrotic syndrome, a conclusion also reached by Pearson et al. (5) in a more recent study of 88 cases.

However, all but one (5) of the above studies were conducted without the aid of immunofluorescence microscopy. The original description of IgA nephropathy by Berger and Hinglais (6), who utilized immunofluorescence to detect and localize the immune complex deposits in glomeruli, was published in 1968, and it was not until 5 to 10 yr later that
immunofluorescence studies for immunoglobulin (Ig) and complement deposits became a routine part of renal biopsy evaluation in most centers. In addition, over the past 25 yr, several new glomerular diseases and variants have been described, including human immunodeficiency virus (HIV)-associated nephropathy, fibrillary glomerulonephritis, and C1q nephropathy, in which ultrastructural findings are useful in establishing the diagnosis (7–9). Ultrastructural correlations have also been made with other glomerular diseases (e.g., Alport’s syndrome, thin basement membrane nephropathy/benign familial hematuria, membranous lupus nephritis) that aid in diagnosis on renal biopsy (10–12). In light of these developments and in consideration of the fact that electron microscopy remains a costly procedure, this study was undertaken to reevaluate the routine use of electron microscopy for native renal biopsies also examined by immunofluorescence and light microscopy.

**Methods**

The Renal Pathology Laboratory of The University of Chicago Medical Center received 288 native renal biopsies during the first 6 months of 1996. A single pathologist (M.H.) evaluated all of the biopsies, which were included in this study if tissue was received for light microscopy, immunofluorescence, and electron microscopy and contained ≥5 glomeruli for light microscopic examination, ≥2 glomeruli for immunofluorescence, and at least one glomerulus for ultrastructural studies, not counting globally sclerotic glomeruli. Of the 233 biopsies that met these inclusion criteria, 28 were performed in-hospital, and 205 were received from over 30 outside hospitals, most in the midwestern United States. Our laboratory also processed renal transplant biopsies during this period; tissue for electron microscopy was received with fewer than 10% of these biopsies, and in these cases, ultrastructural studies were performed at the discretion of the pathologist. No renal transplant biopsies were included in this study.

Light microscopy (eight consecutive slides, with two to three sections per slide and slides alternately stained hematoxylin and eosin and periodic acid-Schiff stain) and immunofluorescence (for IgG, IgA, IgM, C3, C1q, and fibrinogen) were evaluated on each biopsy within 48 h of receipt. In some cases, additional studies (Congo Red stain, immunofluorescence for kappa and lambda light chains, immunohistochemical stain for amyloid AA) were performed based on the light microscopic and/or immunofluorescence findings and the clinical history; these were also evaluated before ultrastructural studies were done on the biopsy. Based on the clinical data provided, light microscopy, immunofluorescence, and (where applicable) special studies, a preliminary diagnosis was recorded if possible. In 185 of the 233 cases, the pathologist thought that a firm preliminary diagnosis could be made and communicated to the appropriate clinician. In the remaining 48 cases, no better than a differential diagnosis of two or more diseases and/or variants could be made and communicated; in some of these cases, one diagnosis was clearly favored by the pathologist but the other(s) could not be sufficiently excluded to warrant a firm preliminary diagnosis.

After the preliminary diagnosis was recorded, ultrastructural studies were performed on one or two glomeruli from the biopsy, and a primary or major final diagnosis was recorded. The biopsy was then assigned to one of the following three categories on the basis of how the ultrastructural findings contributed to this primary diagnosis.

**Not Required.** Electron microscopy resulted in no change in the preliminary diagnosis, was not needed to confirm this diagnosis, and did not supply other clinically pertinent information related to the primary final diagnosis.

**Crucial for Diagnosis.** Electron microscopy was needed to make the primary final diagnosis, either changing the preliminary diagnosis or resolving a differential diagnosis in cases where a firm preliminary diagnosis could not be made. Examples include cases of postinfectious glomerulonephritis beyond the acute exudative stage, identified by large subepithelial deposits (“humps”) ultrastructurally; cases of minimal change nephropathy, where immunofluorescence showed modest (1+, on a scale from zero to 4+), predominantly linear IgG in the glomerular capillaries and electron microscopy was needed to exclude an early membranous lesion; and a case of membranous nephropathy with normocellular glomeruli in a patient with a weakly positive ANA but without overt systemic lupus erythematosus (SLE), in whom immunofluorescence showed only weak glomerular capillary staining for IgA and C1q, but electron microscopy showed both mesangial deposits and tubulovascular bodies (tubuloreticular inclusions) typical of membranous lupus nephritis, World Health Organization (WHO) Class V (12).

**Important Contribution.** The ultrastructural findings did not alter the preliminary diagnosis and were not essential to making the primary final diagnosis. However, the ultrastructural findings did provide important information confirming/strengthening this primary diagnosis and/or provided clinically relevant insight into the patient’s historical data, light microscopic findings, and/or immunofluorescence findings related to the primary diagnosis. Examples include a case of membranous lupus nephritis similar to that noted above but with prominent (2+ or more) C1q staining by immunofluorescence strongly suggesting this diagnosis; a case of a child with nephrotic syndrome and a renal biopsy showing unremarkable light microscopy and negative immunofluorescence, with electron microscopy confirming the strongly suspected diagnosis of minimal change nephropathy; and a biopsy from a diabetic patient with proteinuria showing diffuse mesangial matrix expansion by light microscopy without nodular glomerulosclerosis or specific immunofluorescence findings, in whom the diagnosis of diabetic nephropathy is confirmed by the ultrastructural finding of thickened glomerular basement membranes (13). Another case in this category would be a focal or diffuse proliferative glomerulonephritis in a patient with a long history of SLE, in whom immunofluorescence showed only weak staining for immunoglobulins and complement components and in whom electron microscopy showed extensive resorption of the immune complex deposits present, explaining the immunofluorescence findings. Finally, cases where ultrastructural findings suggested a possible disease etiology were categorized as “important contribution,” including three cases of membranous nephropathy, unrelated to SLE, in which the presence of mesangial deposits ultrastructurally indicated a possible secondary membranous lesion.

The above categories refer only to determination of the primary final diagnosis. In eight cases, ultrastructural findings provided information supporting a distinct, additional diagnosis. In one of these cases, electron microscopy was also crucial in making the primary diagnosis, whereas in the other seven, it was not required to make the primary diagnosis. These eight cases are given special consideration in the Results section.

Assignment of biopsies to the above categories was based not only on histological, immunofluorescence, and ultrastructural findings, but also on clinical data. For example, a biopsy from a 7-yr-old child with normal renal function and nephrotic syndrome that showed only mild mesangial matrix expansion histologically, trace linear IgG in glomerular capillaries by immunofluorescence, and typical ultrastructural findings of minimal change nephropathy would be categorized as “important contribution.” By contrast, a biopsy showing similar findings but from a 50-yr-old, mildly hypertensive patient with nephrotic syndrome and a creatinine clearance rate of 90 mL/min would be...
categorized as "crucial for diagnosis" because of the greater possibility that ultrastructural findings might yield a different diagnosis (e.g., early membranous nephropathy or thickened glomerular basement membranes, suggesting early diabetic nephropathy). In most cases, the clinical data used was provided with the biopsy in the form of a cover letter, history sheet, and/or summary of laboratory data. In cases where the clinical data was considered inadequate for biopsy interpretation, the appropriate clinician was called before ultrastructural studies were performed.

Results

Of the 233 biopsies meeting the study criteria, electron microscopy was thought necessary for making the primary final diagnosis (Crucial for Diagnosis category) in 50 (21%), including the 48 for which a firm preliminary diagnosis could not be reached based on the light microscopic and immunofluorescence findings. In two cases, electron microscopy resulted in a change from the preliminary diagnosis. One of these involved a woman with SLE and the nephrotic syndrome. By light microscopy and immunofluorescence, the lesion in question appeared to be membranous lupus nephritis with focal proliferative glomerulonephritis (GN), WHO Class Vc. However, ultrastructural studies performed on two glomeruli showed subendothelial and mesangial deposits only, with no subepithelial deposits. The final diagnosis was focal proliferative lupus nephritis, WHO Class III. The second case involved a nondiabetic, normotensive, young adult male with recent onset of the nephrotic syndrome; light microscopy showed 10 normocellular glomeruli with segmental sclerosis in two. Immunofluorescence showed fairly strong (2+) interrupted linear staining for IgG in the glomerular capillaries, with trace IgM, C3, and fibrinogen in a similar pattern. A preliminary diagnosis of membranous nephropathy was made, yet electron microscopy performed on two glomeruli showed no electron-dense deposits or significant irregularities in the glomerular basement membranes. The final diagnosis was focal segmental glomerulosclerosis (FSGS).

In addition to the 50 cases in the Crucial for Diagnosis category, ultrastructural studies were thought to provide important confirmatory and/or clinically relevant information related to the primary diagnosis (Important Contribution category) in another 48 (21%). The final line of Table 1 (All Cases) summarizes these results. Table 1 also examines the value of electron microscopy in the diagnosis of individual renal diseases, as assessed by the categories defined in the Methods section (also see the Discussion section). In eight (3%) of the total cases, ultrastructural studies revealed an unexpected finding indicative or strongly suggestive of an additional diagnosis unrelated to the primary final diagnosis. Table 2 lists both primary and additional diagnoses for each of these cases, together with the ultrastructural finding supporting the additional diagnosis.

Discussion

Three major changes in the practice of renal pathology and of medicine in general have provided impetus for us to reevaluate the routine use of electron microscopy in native renal biopsy diagnosis. First, the economic climate of medical practice has changed dramatically even in the past decade, with increasing pressure to reduce the number of high-cost diagnostic tests. Diagnostic electron microscopy remains a costly procedure; at our institution, the charges for electron microscopy are approximately equivalent to those for light microscopy plus immunofluorescence. Second, immunofluorescence was not in widespread use for renal biopsy diagnosis during the late 1960s and early 1970s when the issue of routine use of electron microscopy was considered in greatest detail; by contrast, immunofluorescence is now performed routinely on native renal biopsies in most centers to detect and localize immune complex deposits. Third, several new glomerular diseases and variants have been described in the past 25 yr. Electron microscopy is needed, or at least useful, in properly identifying many of these, and newer ultrastructural correlations have also been made with several long-recognized glomerular diseases, aiding in their diagnosis on renal biopsy.

Several studies published between 1969 and 1983 (1-4), and most notably that of Siegel et al. (1), provided important justification for the routine use of electron microscopy in evaluation of native renal biopsies and, most likely, played an important role in dictating this practice, which remains in effect today in most major medical centers. In their 1973 article, Siegel et al. (1) reported that ultrastructural findings on renal biopsy contributed to diagnosis and/or patient management in 48% of cases also studied by light microscopy but not by immunofluorescence.

Perhaps somewhat surprisingly, the results of our study are remarkably similar to those of Siegel et al. (1), with ultrastructural studies providing required diagnostic information, important confirmatory data, and/or findings that support an additional diagnosis on 105 (45%) of 233 native renal biopsies evaluated during the first six months of 1996. What makes this result surprising is that the addition of immunofluorescence to our diagnostic procedure has essentially negated the need for electron microscopy in the diagnosis of several common glomerular diseases, as detailed below. However, the influence of immunofluorescence on diagnosis appears to have been offset by those glomerular diseases and variants described since 1973 that require electron microscopy for definitive diagnosis, as well as by newer ultrastructural correlations that aid in the diagnosis of other long-recognized glomerular diseases. As summarized in Tables 1 and 2 (see the Results section), we found that ultrastructural studies were most helpful in the diagnosis of the following diseases or classes of diseases.

Glomerular Basement Membrane Abnormalities, Including Thin Basement Membrane Nephropathy, Alport's Syndrome, and Diabetic Nephropathy Without Nodular Glomerulosclerosis

As noted in the note to Table 1, two biopsies performed on patients with unexplained hematuria were histologically unremarkable and negative by immunofluorescence; in these cases, ultrastructural studies were needed to exclude thin basement membrane nephropathy. Notably, seven of the eight cases where ultrastructural findings supported an additional diagnosis distinct from the primary diagnosis (Table 2) showed either increased glomerular basement membrane (GBM) thickness
Table 1. Value of electron microscopy in renal biopsy diagnosis of individual diseases

<table>
<thead>
<tr>
<th>Primary final diagnosis</th>
<th>No. of cases</th>
<th>No. (%) of cases in each category</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Not required</td>
</tr>
<tr>
<td>Focal Segmental Glomerulosclerosis (includes collapsing glomerulopathy)</td>
<td>34</td>
<td>25 (74)</td>
</tr>
<tr>
<td>IgA Nephropathy and Henoch-Schönlein Nephritis</td>
<td>28</td>
<td>25 (89)</td>
</tr>
<tr>
<td>Diabetic Nephropathy</td>
<td>20</td>
<td>10 (50)</td>
</tr>
<tr>
<td>Pauci-Immune Crescentic GN (with or without vasculitis)</td>
<td>20</td>
<td>18 (90)</td>
</tr>
<tr>
<td>Membranous Nephropathy</td>
<td>18</td>
<td>11 (61)</td>
</tr>
<tr>
<td>Lupus Nephritis, WHO Class V (includes V&lt;sub&gt;c&lt;/sub&gt; and V&lt;sub&gt;d&lt;/sub&gt;)</td>
<td>16</td>
<td>4 (25)</td>
</tr>
<tr>
<td>Lupus Nephritis, WHO Class IV</td>
<td>16</td>
<td>12 (75)</td>
</tr>
<tr>
<td>Lupus Nephritis, WHO Class III</td>
<td>7</td>
<td>2 (29)</td>
</tr>
<tr>
<td>Lupus Nephritis, WHO Class II</td>
<td>5</td>
<td>1 (20)</td>
</tr>
<tr>
<td>Minimal Change Nephropathy</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Hypertensive Nephrosclerosis</td>
<td>10</td>
<td>5 (50)</td>
</tr>
<tr>
<td>Acute Interstitial Nephritis and Acute Pyelonephritis</td>
<td>9</td>
<td>9 (100)</td>
</tr>
<tr>
<td>Membranoproliferative GN (includes types I, II, and III)</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Amyloid Nephropathy (types AA and AL)</td>
<td>5</td>
<td>5 (100)</td>
</tr>
<tr>
<td>Post-Infectious GN</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Clq Nephropathy</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Thin Basement Membrane Nephropathy</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Acute Tubular Necrosis</td>
<td>3</td>
<td>3 (100)</td>
</tr>
<tr>
<td>Anti-GBM Nephritis</td>
<td>2</td>
<td>2 (100)</td>
</tr>
<tr>
<td>HIV-Associated Nephropathy</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Light Chain Cast Nephropathy</td>
<td>2</td>
<td>1 (50)</td>
</tr>
<tr>
<td>No Pathologic Diagnosis</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>All Other Diagnoses (see Notes)</td>
<td>6</td>
<td>2 (33)</td>
</tr>
<tr>
<td>All Cases</td>
<td>233</td>
<td>135 (58)</td>
</tr>
</tbody>
</table>

*This table considers only the role of ultrastructural findings in making the primary final diagnosis; Table 2 considers cases where the findings also supported an additional, unrelated diagnosis. The two cases listed as having no pathologic diagnosis both involved patients with unexplained hematuria and normal light microscopic and negative immunofluorescence findings; in these cases, electron microscopy excluded a diagnosis of thin basement membrane nephropathy. "Other" diagnoses included (N=1 for each case): sarcoid nephropathy (not required), findings consistent with Bartter's syndrome (not required), Alport's syndrome (crucial for diagnosis), chronic immune-complex GN (crucial for diagnosis), light chain deposition disease (important contribution), and hemolytic-uremic nephropathy (important contribution). GN, glomerulonephritis; GBM, glomerular basement membrane; HIV, human immunodeficiency virus; WHO, World Health Organization.

suggestive of probable diabetic nephropathy (13) or concurrent thin basement membrane nephropathy.

**Minimal Change Nephropathy**

As noted in previous studies (1,4,5,14), electron microscopy is useful in the diagnosis or confirmation of these lesions, and distinguishing them from possible early membrandous lesions that do not show capillary loop thickening by light microscopy and may lack convincing immunofluorescence findings.

**Membranous Lupus Nephritis**

The presence of mesangial deposits and tubulovesicular bodies (tubuloreticular inclusions) ultrastructurally remains a primary means of distinguishing membranous lupus nephritis (WHO Class V) from idiopathic membranous nephropathy (12). This means of distinction is of particular value when there is no strong clinical history of SLE (e.g., latent membranous lupus nephritis) and/or when immunofluorescence for Clq is weak.

**Membranoproliferative and Postinfectious GN**

Membranoproliferative GN (MPGN) and postinfectious GN may be difficult to distinguish from each other by light microscopy and immunofluorescence, particularly beyond the acute phase. Three of the four postinfectious lesions diagnosed were at least several weeks old by clinical history and showed some evidence of chronicity by light microscopy, and the demonstration of subepithelial "humps" ultrastructurally was needed for the diagnosis. Electron microscopy permits proper subtyping of membranoproliferative lesions, even when the diagnosis of MPGN is apparent from light microscopy, immunofluorescence, and clinical data.

**HIV-Associated Nephropathy**

Ultrastructural demonstration of tubulovesicular bodies in endothelial cell cytoplasm is important for this diagnosis (e.g., References 7 and 15). It is also important to demonstrate the absence of these structures in cases of the collapsing glomeru-
Tubulointerstitial Diseases

These diseases include acute interstitial nephritis, acute pyelonephritis, and acute tubular necrosis.

IgA Nephropathy and Glomerulonephritis of Henoch-Schönlein Purpura

In three cases in which IgA staining by immunofluorescence was very weak, demonstration of mesangial electron-dense deposits was considered helpful diagnostically.

Pauci-Immune Crescentic Glomerulonephritis and Anti-GBM Nephritis

Clinical and serologic data, light microscopy, and immunofluorescence were typically sufficient to make the former diagnosis, with or without vasculitis present on the biopsy, and to distinguish these cases from anti-GBM nephritis. In 16 of the 20 patients diagnosed with pauci-immune crescentic GN, anti-neutrophil cytoplasmic autoantibodies (ANCA) were reported to be positive at the time of biopsy or in discussions with the clinician before ultrastructural studies were performed; ANCA were reported negative in one case and pending in three. Notably, four of the 20 cases (all ANCA-positive and categorized as Not Required) showed a small number of electron-dense deposits ultrastructurally, in each case limited to mesangial areas. Such deposits have been noted previously—e.g., in 19% of cases reported by Jennette and Falk (17)—and their significance is unclear; the electron-dense deposits do not appear to correspond to immune complexes identifiable by immunofluorescence. Of the four cases of pauci-immune crescentic GN with deposits ultrastructurally, two showed focal, 1+ glomerular staining for IgM and/or C3, without staining for IgG, IgA, or C1q. However, immunofluorescence showing ≥1+ IgM and/or C3 was also noted in four cases where deposits were not seen ultrastructurally; all of these cases were likewise ANCA-positive. Two of the latter cases showed 1–2+ IgM and C3 (mainly mesangial) in all or most glomeruli, without IgG, IgA, or C1q. In these two cases, the absence of deposits ultrastructurally was considered helpful in confirming the preliminary diagnosis of pauci-immune crescentic GN, and both were categorized as Important Contribution.

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Table 2. Cases in which electron microscopic findings supported an additional diagnosis*

<table>
<thead>
<tr>
<th>Primary diagnosis</th>
<th>Additional diagnosis</th>
<th>Ultrastructural findings supporting additional diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Focal Segmental Glomerulosclerosis</td>
<td>Thin basement membrane nephropathy</td>
<td>Diffusely thin glomerular basement membranes with attenuated but intact lamina densa</td>
</tr>
<tr>
<td>(two cases)</td>
<td>Probable early diabetic nephropathy</td>
<td>Thickened glomerular basement membranes without deposits</td>
</tr>
<tr>
<td>Focal Segmental Glomerulosclerosis</td>
<td>Membranous nephropathy</td>
<td>Subepithelial electron-dense deposits</td>
</tr>
<tr>
<td>Diabetic Nephropathy</td>
<td>Probable early diabetic nephropathy</td>
<td>Thickened glomerular basement membranes without deposits</td>
</tr>
<tr>
<td>Pauci-Immune Crescentic GN</td>
<td>Thin basement membrane nephropathy</td>
<td>Diffusely thin glomerular basement membranes with attenuated but intact lamina densa; the subepithelial deposits present barely extended into the glomerular basement membranes</td>
</tr>
<tr>
<td>Lupus Nephritis, WHO Class V</td>
<td>Diffusely thin glomerular basement membranes with attenuated but intact lamina densa</td>
<td></td>
</tr>
<tr>
<td>Minimal Change Nephropathy</td>
<td>Thickened glomerular basement membranes without deposits</td>
<td></td>
</tr>
<tr>
<td>Acute Interstitial Nephritis</td>
<td>Probable early diabetic nephropathy</td>
<td>Thin basement membrane nephropathy</td>
</tr>
</tbody>
</table>

*The ultrastructural findings listed are only those supporting the additional diagnosis. In seven of the eight cases listed, the ultrastructural findings did not contribute to the primary diagnosis (not required), whereas in one (minimal change nephropathy), electron microscopy was needed to establish the primary diagnosis (crucial for diagnosis). Each of the four patients diagnosed with thin basement membrane nephropathy had microscopic hematuria on a urinalysis before the renal biopsy. None of the three patients with probable early diabetic nephropathy was noted diabetic in the clinical history provided with the renal biopsy, although subsequent discussions with the appropriate clinicians revealed that two of these patients had known non-insulin-dependent diabetes of three and five years' duration, respectively. The patient with diabetic nephropathy (with nodular glomerulosclerosis) and membranous nephropathy had proteinuria of 3.5 g/24 h; immunofluorescence for IgO showed relatively weak (1+) and linear staining in glomerular capillaries and in tubular basement membranes, considered consistent with diabetic nephropathy.
Diabetic Nephropathy with Nodular Glomerulosclerosis

Congo Red stain, immunofluorescence, and immunohistochemistry could be used to exclude amyloid and light chain deposition disease where appropriate.

Amyloid Nephropathy

Congo Red stain, immunofluorescence, and immunohistochemistry were sufficient to make this diagnosis and to distinguish AA and AL amyloid in all five cases received.

Diffuse Proliferative Lupus Nephritis, WHO Class IV

In most cases, light microscopy, an appropriate clinical history and a “full house” pattern of Ig and complement deposits by immunofluorescence were sufficient to make this diagnosis. Electron microscopy tended to be more useful in cases of lupus nephritis where the proliferative glomerular lesion was mild and focal (WHO Classes II and III), or very chronic; in such cases, the immunofluorescence findings were often less convincing. In WHO Class II lesions with more prominent mesangial cellularity, exclusion of subendothelial deposits was considered helpful in confirming that the lesion was, in fact, properly classified as WHO Class II. Three cases of C1q nephropathy were also diagnosed; in all three, the patients were children or young adult females with no serologic or clinical evidence of SLE. Light microscopy showed a mesangial proliferative GN with mesangial C1q deposits by immunofluorescence; demonstration of mesangial electron-dense deposits (and absence of tubulovesicular bodies) ultrastructurally was considered helpful in confirming the diagnosis of this relatively uncommon and recently described disease (9,18).

Focal Segmental Glomerulosclerosis (FSGS), Not of the Collapsing Glomerulopathy Variant

FSGS was our most frequent diagnosis, accounting for nearly 15% of our native renal biopsy diagnoses. D’Agati (16) has noted a similar experience in her active renal biopsy practice based in New York City. Histologic lesions resembling primary FSGS may be seen in other glomerular diseases, including membranous nephropathy and IgA nephropathy (19). In addition, certain ultrastructural findings, such as separation of glomerular epithelial cells from the underlying basement membrane, are reported to occur more frequently in primary FSGS than in other glomerular diseases (although they are not present in every case of FSGS) and may reflect disease severity and pathogenesis (20–22). Still, in cases with appropriate clinical and serologic findings, the characteristic light microscopic lesions of FSGS, together with negative or nonspecific immunofluorescence findings, are sufficient for diagnosis of this entity.

Membranous Nephropathy

The majority of membranous lesions could be diagnosed by light microscopy and immunofluorescence; with negative SLE serologies, histology of normocellular glomeruli, and absence of IgA and (particularly) C1q deposits by immunofluorescence, membranous lupus nephritis could be excluded with confidence. In membranous lesions showing weak C1q deposits, absence of mesangial deposits and tubulovesicular bodies ultrastructurally was considered helpful in confirming that the lesion was not related to SLE. The presence of mesangial deposits (with negative C1q by immunofluorescence and negative SLE serologies) suggested a possible hepatitis B-related membranous lesion on one biopsy from a patient with positive hepatitis B serologies. Similar findings on two biopsies from patients with unknown serologies (except negative ANA) led to the suggestion of a possible secondary (but not SLE-related) membranous lesion. Each of the latter three membranous lesions was categorized as Important Contribution.

Our study included only native renal biopsies. Renal transplant biopsies were not included because tissue for immunofluorescence and electron microscopy was received with less than 10% of these biopsies, and for these latter cases, ultrastructural studies were performed at the discretion of the pathologist. By contrast, the study of Siegel et al. (1) included an unspecified number of renal allograft biopsies performed to exclude acute rejection. As the value of electron microscopy in this regard remains questionable, the fraction of native renal biopsies to which ultrastructural studies contributed important information may have actually been higher than the 48% indicated by that study.

Our study found that electron microscopy was crucial to making the primary final diagnosis on 21% of renal biopsies. This finding is in good agreement with that of Pearson et al. (5), who examined the diagnostic role of electron microscopy on 88 consecutive renal biopsies that were also studied by light microscopy and immunofluorescence, and found that electron microscopy was necessary for diagnosis in 22 (25%). Pearson et al. (5) also found that ultrastructural studies were helpful in another 53% of their cases, which exceeds the 24% (adding the Important Contribution category plus those cases in which ultrastructural findings supported an additional diagnosis) determined by our study. Most of this difference, however, can be accounted for by two diagnoses: IgA nephropathy and pauci-immune crescentic GN. Pearson et al. (5) considered electron microscopy helpful but not necessary in the diagnosis of all nine of their IgA nephropathy cases and in eight of their 10 cases of “crescentic GN” (not otherwise specified; presumably idiopathic or pauci-immune). By contrast, we thought that electron microscopy was not needed for diagnosis in 25 of 28 cases of IgA nephropathy and in 18 of 20 cases of pauci-immune crescentic GN. If the 17 cases of IgA nephropathy and “crescentic GN” from the study of Pearson et al. (5) are shifted from the Helpful to the Not Needed category, their revised results (electron microscopy helpful but not necessary in 30 of 88, or 34% of cases) become fairly similar to those of this study.

Our findings suggest that routine use of electron microscopy to evaluate native renal biopsies is not wasteful or frivolous, even in the current medical economic climate. We feel the same conclusion applies to immunofluorescence—although this was not tested systematically—which is required for diagnosis of certain renal diseases (e.g., IgA nephropathy/Henoch-Schönlein nephritis, anti-GBM nephritis, C1q nephropathy) and is extremely helpful in the diagnosis and/or classification of others (e.g., lupus nephritis, light chain-related diseases). Receiving unfixed tissue for immunofluorescence studies also
affords the pathologist the opportunity to reprocess any remaining tissue for additional light or electron microscopy (although with some reduction in histologic or ultrastructural detail) should the latter specimens prove inadequate. An obvious limitation of this study is that it represents the judgment of a single pathologist, albeit one who has read >2000 native renal biopsies over the past 10 yr. We hope that this study encourages a more extensive, multicenter investigation that may ultimately be needed to justify such costly diagnostic procedures as electron microscopy and immunofluorescence if economic restraints on health care continue to increase. Based on the findings of our study, for cost-conscious centers we recommend that if ultrastructural studies cannot be routinely performed on all native renal biopsies, a small portion of renal cortical tissue should be saved in an appropriate fixative for electron microscopy, which could then be performed at the discretion of the pathologist.

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References