Renal Diseases With Organized Deposits
An Algorithmic Approach to Classification and Clinicopathologic Diagnosis

Guillermo A. Herrera, MD; Elba A. Turbat-Herrera, MD

Context.—Most renal diseases with organized deposits are relatively uncommon conditions, and proper pathologic characterization determines the specific diagnosis. Different entities with specific clinical correlates have been recognized, and their correct diagnosis has an impact on patient management, treatment options, and determination of prognosis.

Objective.—The diagnosis of these conditions depends on careful evaluation of the findings by light microscopy together with immunofluorescence and electron microscopy. The objective of this manuscript is to delineate an algorithmic approach helpful in the pathologic assessment of these conditions at the light microscopic level. In some diseases, the immunomorphologic parameters short of electron microscopy provide solid information to suggest or make a definitive diagnosis. Nevertheless, electron microscopy plays a crucial role, because the criteria to separate these entities often are heavily influenced by the electron microscopic findings. Accepted diagnostic criteria for each of these conditions are discussed.

Evaluation of renal diseases with organized deposits requires a careful integration of information emanating from a number of sources (Figure 1). Clinical history and pertinent laboratory data may provide useful information, at times suggesting a specific diagnosis. Findings at the light microscopic level enhanced by data obtained from the examination of special stains (periodic acid–Schiff, methenamine silver, and trichrome stains) generally become the first source of information and can guide the diagnostic investigation in the right direction. Immunofluorescence evaluation can provide specific and occasionally virtually diagnostic data in some of these diseases. Electron microscopy can be crucial in resolving diagnostic dilemmas.1,2 Other sophisticated diagnostic techniques, including ultrastructural immunogold labeling, may be of help in a minority of the cases, when information emanating from the other diagnostic modalities is not enough to establish a definitive diagnosis. The role of the renal pathologist is to carefully evaluate all of the data available to generate a final, unequivocal diagnosis and to be able to deal intelligently with circumstances that may arise when the information provided by the various diagnostic techniques is conflicting or not definitive enough to make a diagnosis.

The accurate diagnosis of these diseases can be arrived at by using more than one algorithmic approach. Several have been proposed. Renal diseases with organized deposits are divided by some into 2 categories: Congo red–positive diseases, encompassing all of the amyloidoses, and those that are Congo red negative, which include all of the other disorders mentioned. This approach is sound, but it must consider carefully all of the pitfalls associated with Congo red staining, including the common absence of staining when the amounts of amyloid are small and the situations in which tissue congophilia may be artifactual.3 Another way that these diseases have been conceptualized is by separating them into those in which the deposits are composed of immunoglobulins, including truncated forms, and those in which they are not. With the exception of non–light chain–associated amyloidosis and diseases with fibronectin and abnormal collagen deposition, the other disorders included in this manuscript are immunoglobulin related.

The algorithmic approach proposed in this manuscript takes into account that the first and foremost manifestation of diseases with organized deposits is mesangial expan-
Figure 1. Schematic representation of the use of various diagnostic techniques available to reach a final diagnosis. The accurate diagnosis of renal diseases with organized deposits requires careful evaluation of information obtained through the evaluation of the renal biopsy using a variety of ancillary diagnostic techniques. This information should be fully integrated as the diagnosis is made and carefully correlated with the clinical information and laboratory data. H&E indicates hematoxylin-eosin.

The different diseases will be discussed separately, but in the differential diagnosis section there will be reference to the other conditions within this group of diseases, because the final diagnosis requires separation from the other entities with which they may overlap. Emphasis will be placed on the diagnostic criteria and the clinicopathologic importance of making the correct diagnosis.

Following the algorithm on the left side, this manuscript will initially discuss conditions characterized by an increase in mesangial matrix (increased staining of the mesangium with the silver stain), followed by those on the right side (decreased mesangial argyrophilia).

THE NORMAL AND THE ALTERED MESANGIUM

The normal mesangial matrix is composed of a number of extracellular matrix proteins, collagen IV being the most common. Ultrastructurally, mesangial matrix is composed of fine fibrils, which usually measure 6 to 8 nm in diameter.5–7 Under normal circumstances, the mesangial matrix does not clearly show its finely fibrillar appearance unless it is viewed at very high magnification with an electron microscope (Figure 6). The microfibrillary composition of the mesangial matrix can be better appreciated after tannic acid staining.7 Some of the mesangial microfibrils in the rat mesangium can measure up to 15 nm in diameter. The same is true of the human mesangium, further complicating the differential diagnosis because of the overlap with a number of pathologic processes. In certain conditions, such as those associated with mesangiolysis, the fibrillar nature of the mesangium may become more apparent (Figure 7).8,9 Among the extracellular matrix proteins in the mesangium is fibronectin...
Figure 2. Algorithm for evaluation of renal diseases with organized deposits. Mesangial expansion is the main finding in these diseases, at least in the early stages. Whether the mesangium maintains its silver positivity or reveals decreased staining represents a good way to initially stratify these diseases. Congo red stain is the next logical step for those cases with decreased mesangial argyrophilia. As a viable alternative, both silver methenamine and Congo red stains can be performed concomitantly as the initial step for the work-up of a given case. Information from the ultrastructural evaluation provides solid evidence for classification of these disorders in most cases. Diabetic fibrillosis is highlighted in blue because it portrays a combination of increased matrix—increased silver staining—due to the diabetic nephropathy, and decreased argyrophilia due to the accumulation of fibrils in some mesangial areas. TMA indicates thrombotic microangiopathy; GP, glomerulopathy; LHCDD, light/heavy chain deposition disease; ED, electron dense; FGN, fibrillary glomerulonephritis; AA-amyloid, serum protein A–associated amyloidosis; and AL-amyloid, light chain–associated amyloidosis.

The high levels of fibronectin are related to the need for firm connections between the different structures within the mesangium.10 The matrix can influence cell shape, cell survival, and cell proliferation,11,12 and its composition may provide clues to various pathologic processes. In some instances, it may indicate a specific disease process (ie, collagenofibrotic glomerulopathy, or collagen III glomerulopathy).

Maintenance of mesangial homeostasis is a very well-regulated process.13 Excessive production of matrix proteins or excessive catabolism results in alterations of the normal mesangium, and these are the 2 fundamental processes seen in pathologic conditions. Distortion of the delicate balance between matrix synthesis and turnover also may result in altered composition of the mesangial matrix. The altered matrix can then have positive or negative effects on the progression of the disease process.11-13 Understanding the pathogenesis of increased matrix production and/or decreased proteolysis may help in designing new therapeutic strategies.12
DISEASES AND GENERIC PATHOLOGIC PROCESSES CHARACTERIZED BY AN INCREASE IN EXTRACELLULAR MATRIX

The most common condition characterized by an increase in matrix either in a focal, segmental, or global fashion is glomerulosclerosis. In this particular situation, the increase of extracellular matrix proteins may vary. The matrix proteins that accumulate include collagen IV, fibronectin, laminin, and a variety of other glycoproteins. One such protein that has been emphasized lately is tenascin, which appears to deposit in large quantities rather late in the process of glomerulosclerosis and is difficult to digest, often becoming the main component of irreversible glomerulosclerotic processes. Fibrillary collagen, which is never present in the normal glomerulus, is at times identified in the sclerotic matrix. The process of glomerulosclerosis may be associated with mesangial nodule formation, as is typically seen in diabetic nephropathy (Figure 3), or with collapse of the glomerular architecture and replacement of its normal structures, resulting in segmental areas replaced with sclerotic matrix, as is noted in focal, segmental glomerulosclerosis.

Collagenofibrotic Glomerulopathy

Collagenofibrotic glomerulopathy is a very rare condition that has a genetic component. In most cases, the inheritance pattern has been autosomal recessive, which is consistent with the usual onset of symptoms in early childhood. Fewer than 50 cases have been described in the English-language literature under several names, including primary glomerular fibrosis, collagen III glomerulopathy, and collagenofibrotic glomerulopathy. The patients have ranged in age from 6 to 72 years, with no sex predilection. Manifestations of renal dysfunction may occur in childhood or later in life. The most common clinical presentation is proteinuria with or without associated nephrotic syndrome, with minor alterations in renal function. These patients also can exhibit varying degrees of hematuria and hypertension. Many of the cases have been reported in Japan, suggesting racial and geographic predilection. At least 2 cases have been associated with factor H deficiency. This condition is a progressive disease in at least a subset of the patients. Renal failure has been described within 3 years of diagnosis in 1 patient. In other patients, symptomatology has progressed steadily to an increase in clinical manifestations, renal insufficiency and, eventually, failure. The disease appears to be primarily a renal process, but liver involvement has been reported in 1 case. Hemolytic anemia, hemolytic uremic syndrome, and unexplained respiratory symptoms also have been reported. The severity of the disease at presentation is highly variable, and its pace of progression is unpredictable.

Diagnosis and Pathogenesis.—Because of its rarity, many renal pathologists will never encounter this disorder.

Figure 3. Diabetic nephropathy. Mesangial nodularity with increased silver staining in the mesangium in a case of diabetic nephropathy with nodular glomerulosclerosis (silver methenamine, original magnification ×750).

Figure 4. Diabetic nephropathy. Transmission electron microscopy. The mesangium is expanded with an increase in matrix, which explains the increased argyrophilia (uranyl acetate and lead citrate, original magnification ×22 500).

Figure 5. Amyloidosis. Expanded mesangium with decreased argyrophilia, indicative of replacement of normal mesangial matrix (in this case due to amyloid deposition; silver methenamine, original magnification ×500).
in their career. The light microscopic findings of this condition are very nonspecific, with an increase in mesangial matrix, which results in enhanced argyrophilia (Figures 8 and 9) and occasional thickening of peripheral capillary walls. Because of expansion of subendothelial spaces, some cases may be confused with thrombotic microangiopathy. However, the pathologic findings are rather peculiar and should be recognized with certainty when identified in the routine ultrastructural evaluation of a renal biopsy specimen. The collagen fibrils deposited predominantly in the mesangium reveal unusual features ultrastructurally (Figures 10, A and B). The fibrils appear curved, frayed, and worm- and comma-shaped when sectioned transversely, and they show a distinct periodicity from 43 to 65 nm. The fibrils typically arrange in irregular bundles when cut longitudinally. The diagnosis requires confirmation with a stain for collagen III using immunohistochemistry techniques (Figure 10, C). Reliable antibodies to collagen III are commercially available. Normal human glomeruli do not have collagen III, but collagen III may be found in the interstitium, especially when fibrosis is present.

The pathogenesis of this disorder remains unclear. Mesangial cells appear to be the most reasonable candidates for the overproduction of collagen III. Serum pro–collagen III is usually elevated significantly in these patients and could be considered a marker for this disease when interpreted in the proper clinicopathologic context.

**Differential Diagnosis.**—Making a diagnosis of collagenofibrotic glomerulopathy on the basis of light microscopic findings is virtually impossible. Mesangial expansion, either focal, segmental, or diffuse and generalized, is the most characteristic finding. The expanded mesangium displays increased argyrophilia. Demonstration of collagen III in the expanded mesangial areas is imperative to confirm the diagnosis. Collagen III immunohistochemistry shows either focal, segmental, or diffuse and generalized mesangial staining, primarily depending on the stage of...
Figure 10. Collagenotibrotic glomerulopathy. The collagen III fibers deposited in the mesangium display a disorganized arrangement and a peculiar periodicity distinct from other fibrillary collagen. Collagen III stain in C shows strong mesangial positivity. A and B, Transmission electron microscopy (uranyl acetate and lead citrate, original magnifications ×7500 [A] and ×18 500 [B]). C, Immunohistochemical stain for collagen III (diaminobenzidine, original magnification ×500).

Figure 11. Fibrillary collagen in mesangium in a case of focal glomerulosclerosis. In contrast to Figure 10, mature fibers from other types of fibrillary collagens (not collagen III) are generally disposed in a rather organized parallel arrangement and display a characteristic periodicity (65 nm) noticeable in B. Transmission electron microscopy (uranyl acetate and lead citrate, original magnifications ×7500 [A] and ×13 500 [B]).

the disease process. It is also common to find collagen III in the interstitium, but this is not of diagnostic significance, because it may also be seen in interstitial fibrosis unassociated with this condition.

Differential diagnosis includes separation of collagen III deposition in the mesangium from other fibrillary collagens at the ultrastructural level. Regular fibrillary collagen generally appears composed of straight fibrils with periodicity at 65 nm when sectioned longitudinally, and circular when cut transversely. These collagen fibrils typically dispose themselves in an organized parallel arrangement, and in rare cases they represent the main extracellular material in the mesangium (Figure 11). Collagen III reveals a variety of ultrastructural appearances.

Differentiation from nail-patella syndrome also is important in some instances. The clinical manifestations of the latter disorder are rather specific in most cases, but skeletal manifestations may be absent in sporadic cases. Ultrastructurally, the collagen fibrils are of the classic type with the usual periodicity, and they are found along the glomerular basement membranes rather than in the mesangium.

Glomerulosclerosis

Glomerulosclerosis is the final common pathway for irreversible glomerular injury. It occurs in association with all renal disorders that have progressed to the point of irreversibility. Mesangial expansion with nodularity represents a type of glomerulosclerosis that is shared by a number of the pathologic conditions. It is important to differentiate nonspecific glomerulosclerosis from a specific process.

Diagnosis and Pathogenesis.—Congo red–negative fibrils may be found in the sclerotic mesangium, creating diagnostic difficulties. The diameter of these fibrils is variable (5–25 nm), and they frequently, but not always, stain intensely black with the silver methenamine stain. Perhaps the best example of lack of staining is the entity known as diabetic fibrillosis. In this condition, silver
methenamine stain is most commonly negative, which suggests that the composition of the fibrils determines the degree of argyrophilia.

The incidence of fibrils in sclerosing glomerular lesions has been stated to be 3% in one study, emphasizing the importance of adequate characterization of such fibrils. Precollagen fibrils can create significant confusion, because they may mimic other types of fibrils. Fortunately, in most occasions, precollagen coexists with typical collagen, and this may facilitate the pathologic assessment. Collagen fibrils are most often arranged in bundles and aligned in a parallel fashion. However, this is not a constant finding, and there are instances in which the fibrils are randomly disposed. These disorganized fibrils likely represent collagen in various stages of development that lack the typical interstitial collagen periodicity (65 nm) present in mature collagen fibers. Such fibrils also can be seen in combination with other diagnostic entities with glomerulosclerosis; for example, in association with nodular glomerulosclerosis in patients with multiple myeloma. In these cases, irregularly arranged precollagen and collagen fibers have been described, predominantly in the marginal sections of the mesangial nodules. Some of these fibers have been shown to exhibit a periodicity of about 77 ± 15.5 nm, and they may even have the appearance of long-spacing collagen.

Immunofluorescence evaluation in the cases with glomerulosclerosis shows the findings associated with the original renal disease. The areas with glomerulosclerosis also can trap immunoglobulin M (IgM) and C3. The mechanisms involved in the formation of interstitial (fibrillary) types of collagen in the mesangium have been debated for quite some time. It is possible that several mechanisms are involved, depending on the situation. The mesangial cells can be programmed to secrete these types of collagen in some pathologic conditions. In vitro studies have demonstrated that mesangial cells in culture can produce these types of collagen (collagens I and III, predominantly). The activation of genes responsible for this unusual secretory activity by mesangial cells can occur and be modulated by a variety of conditions. In diseases such as necrotizing, crescentic glomerulonephritis, where there is disruption of Bowman capsule, fibroblasts can migrate from the interstitium into glomeruli and produce interstitial collagens. A third possibility is that smooth muscle cells from the vasculature can produce interstitial collagens at the vascular pole that would eventually infiltrate the glomerulus.

Differential Diagnosis.—One of the most difficult diagnostic challenges is to differentiate some of these fibrils from amyloid and from those seen in fibrillary glomerulopathy, because their ultrastructural features overlap. Obviously, those fibers with periodicity can be identified readily as mature fibrillar collagen; the confusion arises when precollagen and altered collagen fibers. Amyloid is Congo red positive, Thioflavin T positive, and Thioflavin S positive, and fibrillary glomerulopathy has a typical glomerular fluorescence pattern; these 2 characteristics are of crucial diagnostic value when these differential diagnoses are under consideration.

Accentuation of Mesangial Matrix Appearance

The normal fibrillary mesangial matrix may become more apparent and be confused with other fibrils, such as amyloid. The mesangial fibrils have a serpentine, intertwined appearance. An accentuated fibrillary matrix appearance can occur in association with a number of diseases, but it is most prominent in cases where mesangiolysis has occurred, such as in thrombotic microangiopathy. Because most of the normal mesangial fibrils are in the range of 6 to 10 nm in diameter, there may be overlap with amyloid fibrils (in the 8- to 12-nm diameter range). Some of the fibrils in the altered mesangium may be thicker (10–25 nm in diameter), overlapping with those present in fibrillary glomerulonephritis. Congo red and Thioflavin T or S stains again are important to address the differential diagnosis, because the fibrils composing the mesangial matrix in these situations would be negative. Some of the fibrils identified in the mesangium, particularly when the normal matrix has been pathologically altered, may be thicker (10–25 nm in diameter), overlapping with those seen in fibrillary glomerulonephritis. Negative immunofluorescence is helpful in these instances to exclude a diagnosis of fibrillary glomerulonephritis.

DISEASES CHARACTERIZED BY A DECREASE IN EXTRACELLULAR MATRIX

In these diseases, there is extraneous material replacing the normal mesangial matrix. As a result, the silver methenamine stain is negative in the areas where normal mesangial matrix has disappeared. Because the replacement of the mesangium is a progressive process, alternating silver-positive and silver-negative areas are typically seen until the final stages of the disease process, when virtually all of the mesangium has been replaced and there is virtually no remaining silver staining. A corollary of the above is that in the early stages, it may be difficult to appreciate a decrease in mesangial argyrophilia because of the focal and mild matrix replacement that may be present.

Amyloidoses

Most of the patients with amyloidosis are older than 50 years. Amyloidosis is rarely seen before 40 years of age. The presenting signs and symptoms are generally non-specific, including fatigue, weight loss, anemia, bleeding, and peripheral neuropathy. Hepatomegaly, splenomegaly, and macroGLOSSIA may be present. Another common presentation is characterized by renal manifestations, which typically include proteinuria with or without nephrotic syndrome and varying degrees of renal insufficiency. A renal biopsy often is the diagnostic procedure that makes the diagnosis of amyloidosis. Light chain–associated amyloidosis with a male predominance is the most common type in the Western hemisphere, and most of these cases are associated with lambda light chains. Lambda VI has renal tropism. Serum protein A–associated amyloidosis is more prevalent and the most common in underdeveloped countries. Hereditary types of amyloidosis are a source of recent interest and are being diagnosed more frequently. Because of the consequences associated with the diagnosis, a diagnosis of amyloidosis requires confirmation beyond any doubt. There are new therapeutic interventions that require speciation of the type of amyloid because they are specific for the various types. Therefore, making an unequivocal diagnosis of amyloidosis is required, but typing the amyloid is also extremely important. Amyloidosis is a systemic disorder, and deposits may be found anywhere in the body. Recurrence in trans-
the diagnosis of amyloidosis. Birefringence, generally but not always apple green, needs to be demonstrated in association with Congo red–positive material for a diagnosis of amyloidosis (Figure 14, A).

Currently, typing of amyloid is the standard of care and is performed by detecting the precursor protein associated with it. Careful evaluation of the routine battery of immunofluorescence stains employed in the evaluation of renal biopsies provides useful and sometimes unequivocal diagnostic information. Immunochemistry may provide information regarding the type of amyloid deposited by demonstrating light or heavy chain monotypically (Figure 14, B), supporting a diagnosis of light chain–associated or heavy chain–associated amyloidosis (Figure 15). However, because of limitations associated with the commercially available antibodies, a negative stain for mononclonal light chains does not rule out light chain–associated amyloidosis. Fibrinogen stain may be positive, supporting a diagnosis of α-fibrinogen–associated amyloidosis. To confirm other types of amyloid, immunohistochemistry may be used if commercially available antibodies are available, as is the case for serum amyloid A protein, transthyretin, calcitonin, lysozyme, lactoferrin, and β2-microglobulin–associated amyloidoses. In other instances, there may be antibodies in research laboratories, primarily to establish the diagnosis of hereditary types of amyloidosis. Undoubtedly, the most accurate technique for amyloid typing is by microextracting the material from the tissue sample, followed by amino acid sequencing. Although heavily supported by some, routine use of this technique is currently impractical in the diagnostic arena for most samples. The amount of material that can be recovered from small biopsies and the difficulties associated with the extraction and sequencing procedures limit the diagnostic usefulness of this methodology to analyze amyloid deposits.

Electron microscopy can confirm a generic diagnosis of amyloidosis by showing the classic, randomly oriented, 8–12-nm diameter, nonbranching fibrils in various renal compartments (Figure 16). Different types of amyloid share light microscopic, tinctorial, and ultrastructural features and cannot be differentiated by morphologic means.

The situation can be complicated in cases in which the amount of amyloid present in the renal biopsy is small (Figure 17, arrow). Congo red may be negative in these cases, even when the sections are cut at 9-nm thickness, as recommended to detect small quantities of amyloid. Thioflavin T and S stains may be challenging to interpret, because the fluorescence may be quite focal and punctuate, thus raising an issue regarding specificity. Sampling can be an issue in some of these cases, because the presence/absence and amounts of amyloid deposition may vary from one area of the kidney to another, and can even vary within renal compartments. However, in our experience, Thioflavin T and S stains appear to be more sensitive in detecting small amounts of amyloid than the traditional Congo red stain. This opinion is not shared by all individuals, with some maintaining that Congo red is the stain of choice.

Challenges and pitfalls associated with amyloid detection are carefully reviewed in Iskandar and Herrera, Howell et al, and Linke. In some of these cases, electron microscopy can be useful if the specimen available for ultrastructural evaluation samples the proper arteries and interstitial areas containing amyloid have lost their normal argyrophilia. In some, a spicular arrangement of mesangial matrix remains, a characteristic, although not specific, finding in amyloidosis (hematoxylin-eosin, original magnification ×500).

Diagnosis and Pathogenesis.—The diagnosis of amyloidosis is rather easy in most cases. Light microscopy may suffice by showing the presence of Congo red–positive, eosinophilic, amorphous, weakly periodic acid–Schiff–positive material on the hematoxylin-eosin stain in glomerular, interstitial, and/or vascular locations (Figure 12). Amyloid deposition first occurs in the mesangium in most cases, although exceptions may occur, and cases with initial vascular and even intratubular amyloidosis have been described. Amyloid in the peripheral capillary walls in glomeruli appears to occur after extensive mesangial deposition. A peculiar appearance is sometimes noticeable along peripheral capillary walls and in mesangial areas, with spikes of silver–positive material appreciable on the silver methenamine stain (Figure 13). This is such a characteristic but not pathognomonic (because it may also be seen in some cases of fibrillary glomerulonephritis)
Figure 14. Light chain–associated amyloidosis. A, The typical apple green birefringence of amyloid when the Congo red–stained specimen is polarized is shown (original magnification ×500). B, Mesangial staining for λ light chains with negative staining for κ light chains established a diagnosis of light chain–associated amyloidosis, λ light chain related. Direct immunofluorescence for λ light chains (fluorescein, original magnification ×500).

Figure 15. Heavy chain–associated amyloidosis. Replacement of mesangial areas by eosinophilic, amorphous material consistent with amyloid and characterized as such (positive for Congo red and Thioflavin T). The expanded mesangial areas were strongly positive for IgM (μ) heavy chains and negative for κ and λ light chains, resulting in a diagnosis of heavy chain–associated amyloidosis (hematoxylin-eosin [A]; direct immunofluorescence stain for μ heavy chains, fluorescein [B], original magnifications ×500).

Much information has been elucidated regarding renal amyloidogenesis, primarily in light chain–associated amyloidosis. Amyloid formation first begins in the mesangium. The normal smooth muscle phenotype of mesangial cells changes into a macrophage phenotype on recognition of precursor light chains in the immediate envi-

nique appears to be more sensitive than routine fluorescence and eliminates the background that is so common in immunohistochemistry procedures. Immunogold labeling also can unequivocally identify small amyloid deposits when other diagnostic modalities may have difficulties doing so, or when morphologic manifestations are unusual.

Much information has been elucidated regarding renal amyloidogenesis, primarily in light chain–associated amyloidosis. Amyloid formation first begins in the mesangium. The normal smooth muscle phenotype of mesangial cells changes into a macrophage phenotype on recognition of precursor light chains in the immediate envi-

Figure 16. Serum amyloid A–associated amyloidosis. Amyloid fibrils replacing normal mesangium. Typical amyloid fibrils are randomly oriented, nonbranching, and measure 8 to 12 nm in diameter, as clearly seen in B. The different types of amyloid cannot be distinguished ultrastructurally. Transmission electron microscopy (uranyl acetate and lead citrate, original magnifications ×8500 [A] and ×22 500 [B]).

Figure 17. Early light chain–associated amyloidosis. Focal, segmental expansion of mesangial areas with deposition of eosinophilic and amorphous material. This one expanded mesangial area (arrow) was the only finding in this biopsy with 7 glomeruli (hematoxylin-eosin, ×500).

The amyloidogenic light chains are internalized in mesangial cells and delivered to the mature lysosomal compartment, where amyloid fibrils are formed. This process is regulated by a number of crucial steps. Once amyloid is formed and deposited in the extracellular matrix, metalloproteinases are activated, and the normal mesangial matrix is destroyed and is eventually totally replaced by the amyloid fibrils. Amyloid also inhibits transforming growth factor β activity, impairing the rebuilding and repair of the mesangial matrix.44 The end result is that the normal mesangium is replaced by amyloid, explaining the absence of mesangial argyrophilia in the advanced stages of glomerular amyloidosis.4,44 Another effect of amyloid deposition is enhancement of apoptosis, which explains why in the advanced stages of the disease there is significant mesangial cell deletion, to the point where these cells essentially disappear and only amyloid remains in the mesangium.

Differential Diagnosis.—Following the algorithmic approach, mesangial expansion can be noted in the initial evaluation of the hematoxylin-eosin–stained sections, and the silver stain typically shows decreased or absent staining in mesangial areas because of the replacement of the normal mesangial matrix with a non–extracellular matrix.
protein. The only entity that is Congo red positive is amyloidosis, clinching the diagnosis. Amyloid P component colocalizes with amyloid deposits but can also be seen associated with fibrils in fibrillary glomerulopathy.45

Amyloid should be differentiated from the normal fibrillary mesangial matrix, which can become more apparent when certain disease processes are present (see discussion above). Also, fibrils seen in fibrillary glomerulonephritis are arranged similarly and do not branch. The difference is in their diameter, because fibrils in fibrillary glomerulonephritis measure 15 to 25 nm, and are therefore almost twice as thick as amyloid fibrils.1,2

**Fibrillary Glomerulonephritis**

The average age for patients with fibrillary glomerulonephritis is about 50 years. The clinical presentation is generally proteinuria with or without associated nephrotic syndrome, sometimes accompanied by hematuria, hypertension, and some degree of renal functional impairment. In some cases, the clinical presentation is that of a rapidly progressive renal disease.46–50 Fibrillary glomerulonephritis was first recognized as an entity in 1977, when Rosenmann et al49 published a case report of a patient with nephrotic syndrome with Congo red–negative, amyloid-like glomerular deposits. The absence of congophilia and lack of staining with Thioflavin T or S represent crucial reproducible features of this disease that are very useful for diagnostic purposes, especially when the immunofluorescence profile, which is quite characteristic, is present.50 The disease occurs in older adults and is almost invariably localized to the kidneys, with no systemic deposits.50–52 Fibrillary deposits have been reported rarely in the lungs, heart, and liver53–56 in association with pathologic manifestations in these organs.

A few cases (less than 5% of all cases of fibrillary glomerulonephritis) have been shown to be associated with an underlying neoplastic, lymphoplasmacytic disorder.57,58 Fibrillary glomerulonephritis has been reported in human immunodeficiency virus–positive patients.59 A diagnosis of fibrillary glomerulopathy portends a poor prognosis, and most cases progress to renal failure rather quickly.48,50,52 Some controversy still exists as to whether this condition and immunotactoid glomerulopathy are part of the spectrum of one disease process.46,47,51,52 The latter view is only held by a few individuals.51

It has been documented that fibrillary glomerulonephritis can recur in renal transplants.47 This fact emphasizes that the fibrils result from the deposited immunoglobulins and/or host peculiarities, and not the microenvironment of the affected kidney.

**Diagnosis and Pathogenesis.**—The light microscopic findings in this condition are quite variable. Most cases reveal mesangial expansion and some mesangial cell proliferation (Figure 20, A), whereas in other cases, mesangial deposition of eosinophilic material, difficult to differenti-
ate from that seen in amyloidosis, represents the most noticeable finding. In most cases, there is also thickening of peripheral capillary walls, which may create confusion with a membranous nephropathy (Figure 20, B). The expanded mesangial areas are silver negative (Figure 21, A), and a spicular arrangement similar to that described in amyloidosis may be seen. In most instances, the material replacing the mesangium is much more periodic acid–Schiff positive than amyloid. Approximately 15% to 20% of cases with fibrillary glomerulonephritis have crescents.

Using immunofluorescence, this disease reveals a classic pattern, with smudgy, ribbonlike to granular glomerular staining for IgG, C3, and κ and λ light chains (sometimes with light chain restriction) along peripheral capillary walls and in mesangium. If IgGotyping is performed, IgG4 is dominant in most fibrillary glomerulonephritis cases. The overall fluorescence pattern is so characteristic that it should suggest the diagnosis. Fibrillary glomerulonephritis can be diagnosed with relative certainty when the light microscopic and the immunofluorescence findings are characteristic, as described above (Figure 21, B).

Ultrastructural examination identifies randomly disposed, 15- to 25-nm diameter fibrils in the mesangium, and frequently also along peripheral capillary walls (Fig-
Figure 22. Fibrillary glomerulonephritis. A, Replacement of mesangial matrix by fibrillary material. Note similar material along peripheral capillary walls. B, Details of the fibrillary material. Transmission electron microscopy (uranyl acetate and lead citrate, original magnifications ×7500 [A] and ×12,500 [B]).

Figure 23. Fibrillary glomerulonephritis. Randomly disposed, nonbranching, 15- to 25-nm fibrils typical of this disease along the peripheral capillary wall, replacing the normal basement membrane. Transmission electron microscopy (uranyl acetate and lead citrate, original magnification ×22,500).

ures 22 and 23). Rarely, fibrils also have been found in the interstitium, usually adjacent to tubular basement membranes. Infrequently, the typical fibrils described above coexist with microtubules that are of a diameter much smaller than those seen in most cases of immunotactoid glomerulopathy.

The pathogenesis of this disorder has been a source of controversy over the years. It is believed that the fibrils occur because of polymerization of immune complexes, and possibly monoclonal light chains in some cases. The role that amyloid P component plays is still not clear. The fact that a particular IgG (IgG4) is dominant in most (possibly, in all, although IgG1 has been reported dominant in a few cases) of the cases probably represents an important pathogenetic consideration, which may imply that this particular immunoglobulin in the proper setting can polymerize into fibrils.

Differential Diagnosis.—The most important differential diagnosis is amyloidosis. As previously stated, stains for Congo red and Thioflavins T and S are very helpful. Fibrillary glomerulonephritis may be confused with membranous glomerulopathy when a granular pattern of fluorescence predominates, because the positive immunoreactants and the distribution of the staining pattern are similar to what is typically seen in membranous nephropathy. However, most fibrillary glomerulonephritis cases exhibit a highly characteristic quality of the immunofluorescence signal, which should alert the pathologist to the diagnosis.

At the ultrastructural level, the fibrils are randomly arranged and nonbranching, similar to the features of amyloid fibrils. The difference is in the diameter of the fibrils. Fibrils in this condition are thicker (15–25 nm in diameter). They are mostly present in mesangial areas but can extend into peripheral capillary walls. In some instances, segments of the glomerular basement membrane become entirely replaced by the fibrils.

An important variant of fibrillary glomerulonephritis is represented by electron-dense deposits regularly disposed epimembranously in a pattern most suggestive of membranous nephropathy. When some or most of these electron-dense deposits are examined at high magnification, they are found to contain randomly arranged 15- to 25-nm diameter fibrils similar to those seen in classic fibrillary glomerulopathy. These deposits are usually kappa-restricted. Some of these patients have an underlying lymphoproliferative disorder.

Another important differential diagnosis is that of diabetic fibrillosis, which is discussed in the next section. In this condition, there may be fibrils measuring 15 to 25 nm in diameter in the mesangium. The difference is that the typical fluorescence staining pattern seen in fibrillary glomerulopathy is not present, and the other immunomorphologic features are those of diabetic nodular glo-
merosclerosis. Linear staining along peripheral capillary walls for IgG and albumin is typically noted. The lamina densa of the glomerular basement membranes is distinctly thickened, and mesangial nodules with increased extracellular matrix material are invariably present.

**Diabetic Fibrillosis**

This condition was first described in 1970 by Sohar et al, when they observed peculiar fibrils in the expanded mesangium of diabetic patients with nodular glomerulosclerosis. Recognition of this entity is important, because the fibrils may be confused with those present in amyloidosis or fibrillary glomerulonephritis. There were no clinical peculiarities in the patients in whom this finding became apparent, and the overall immunomorphologic features of diabetic nodular glomerulosclerosis were not unique in any way other than the presence of the peculiar fibrils. Furthermore, the presence of diabetic fibrillosis does not appear to have any specific clinical connotations, because patients with this disorder behave similarly clinically to those without the fibrils.

**Diagnosis and Pathogenesis.**—By light microscopy, the only finding that may be apparent is that the typical intense mesangial argyrophilia noted in diabetic nodular glomerulosclerosis may be somewhat decreased, because the areas with the fibrillary material are commonly silver negative. The degree of decreased argyrophilia depends on the extent of fibril deposition. The fibrils are only identifiable at the ultrastructural level and can measure from 10 to 25 nm in diameter (Figure 24). The fibrils are negative for Congo red and Thioflavins T and S, and the immunofluorescence pattern in this disorder is that seen in classic nodular glomerulosclerosis associated with diabetes (linear IgG and albumin, as well as focal, segmental mesangial IgM and C3), the diagnosis can be made with certainty.

**Immunotactoid Glomerulopathy**

*Immunotactoid glomerulopathy* was a term introduced by Schwartz et al in 1980. They described the disease as a “glomerular disease characterized by highly organized crystalline structure of immune deposits in the absence of systemic diseases such as amyloidosis, cryoglobulinemia, paraproteinemia, and systemic lupus erythematosus.” The average age at presentation is 62 years, and the typical manifestations include proteinuria, often with associated nephrotic syndrome and hematuria. One clinical association that is very important is that this disorder is associated with underlying lymphoproliferative diseases in most, if not all, instances. Immunotactoid glomerulopathy has been reported in at least 1 human immunodeficiency virus–positive patient. Thus, it is mandatory that when a diagnosis of immunotactoid glomerulopathy is rendered, the clinician must carefully rule out the presence of a concomitant lymphoproliferative process. Interestingly, there is not a tendency toward progression to end-stage renal disease in most cases. Recurrence in transplanted kidneys has been reported.

**Diagnosis and Pathogenesis.**—The light microscopic findings in this condition are nonspecific. Glomeruli may exhibit varying degrees of mesangial expansion (Figure 25), with negative silver staining and variable thickening.
of peripheral capillary walls. They also can have mesangial proliferative activity, which may create an accentuated lobular appearance. Fluorescence varies from case to case, but these cases generally show staining for IgG and C3 in mesangium and along peripheral capillary walls, with a granular to occasionally pseudolinear pattern (Figure 26, A). Staining for IgA, IgM, and C1q is more variable and may be negative. Light chain restriction, more often κ than λ, is seen in some cases. The diagnosis is not made until hollow microtubular or cylindrical structures measuring 10 to 90 nm (most commonly more than 30 nm) in diameter without periodicity or substructure are documented ultrastructurally to be present, predominantly in the mesangium but also possibly subendothelially and in other glomerular locations (Figure 26, B). These deposits may organize in parallel arrays or in rather complex and intricate formations. Microtubules may be disposed on a background of granular to amorphous electron-dense material or can be embedded in the mesangial matrix. Extraglomerular deposits have not been described.

The pathogenesis of this disease is currently not understood. It is possible that certain immunoglobulin products produced by the lymphoproliferative processes associated with this disease polymerize, forming the microtubules that characterize this process.

**Differential Diagnosis.**—In patients with lupus nephritis, the immune complexes may polymerize and form microtubular structures that may mimic those seen in immunotactoid glomerulopathy. However, in these cases there are also classic immune complexes in various renal locations. Electron-dense deposits in lupus nephritis may exhibit characteristic fingerprints. In immunotactoid glomerulopathy there are no electron-dense deposits of the immune complex type.

Cryoglobulins also can polymerize and form unusual microtubular structures, but the other clinicopathologic features of the disease should be present, permitting separation from this disorder (see “Cryoglobulinemic Nephropathy”).

Differentiation from amyloidosis and fibrillary glomerulopathy should not be a significant problem, because in these 2 diseases there are fibrils rather than microtubules. Strict adherence to morphologic criteria is needed.

**Cryoglobulinemic Nephropathy**

Cryoglobulinemic nephropathy occurs in approximately 24% of patients with cryoglobulinemia and is characterized by remissions and exacerbations. Among the manifestations are nephrotic syndrome, isolated proteinuria, hematuria, purpura, arthralgias, and other symptoms and signs of systemic vasculitis. There are a number of systemic conditions associated with cryoglobulinemia, including hepatitis C, lymphoproliferative disorders, infections, systemic lupus erythematosus and other collagen vascular diseases, and chronic liver disease of various et-
ologies. In some cases, no associated systemic conditions can be discovered, and the term essential cryoglobulinemia is used to categorize these cases. Presently, most cases are associated with hepatitis C. Cryoglobulins have been classified into 3 categories by Brouet et al: type I, monoclonal, isolated, and often essential; type II, monoclonal, generally IgM with anti–polyclonal immunoglobulin activity (mostly IgG); and type III, polyclonal with more than one isotype. Type III is the most common and is often associated with collagen vascular diseases, such as systemic lupus erythematosus. Only a small percentage of patients with cryoglobulinemic nephropathy progress to end-stage renal disease.

**Diagnosis and Pathogenesis.**—The main light microscopic manifestations include glomerular cellular proliferation, segmental necrotizing lesions, and hyaline thrombi in capillaries. In some cases, distinct accentuation of glomerular lobularity creating an appearance reminiscent of membranoproliferative glomerulonephritis is present, whereas in others, proliferative activity is not pronounced (Figure 27, A). Silver stain frequently demonstrates segmental duplication of peripheral capillary walls resulting from mesangial cell cytoplasm interposition, and it also highlights expanded mesangial areas, which appear argyrophilic (Figure 28). In a minority of cases, a true vasculitis is identified predominantly in arterioles and small-sized vessels, but it may also involve larger arteries in the kidneys and extrarenal locations. Hyaline thrombi also can be seen infrequently in the extraglomerular vasculature. Immunofluorescence may show distinct staining of capillary thrombi that are sometimes monoclonal for light chains (Figure 27, B), and this finding should suggest type I or II cryoglobulinemia, the latter being more common in renal biopsies.

Cryoglobulins may exhibit variable ultrastructural appearances. Perhaps the most characteristic cryoglobulins are the ones with paired, curved, microtubular (cylindrical), and/or circular (annular) structures measuring 20 to 30 nm in diameter, but this is only seen in a subset of these cases (Figure 29). Other appearances include fibrillary or amorphous deposits, as well as some with fingerprints. There is no definitive diagnostic substructure associated with all cryoglobulin deposits. In our experience, when intraluminal thrombi are present, they usually exhibit the most distinctive ultrastructural features of the cryoglobulins (Figure 29, B). Cryoglobulin deposits can be identified in virtually all glomerular locations (epimembranous, subendothelial, and mesangial). Osmiophilic spheroid bodies and crystalline inclusions of variable shapes may be detected in the cytoplasm of endothelial and mesangial cells. Using immunofluorescence, there may be staining for various immunoglobulins, and capillary thrombi may be easily identifiable. Light chain restriction is seen in cases
where type I or II cryoglobulins are involved. Although cryoglobulinemic nephropathy may exhibit classic features as described above, a significant number of the cases show findings that are much less specific and require careful pathologic assessment and clinicopathologic correlation for an accurate diagnosis to be reached.

The pathogenesis of this condition is directly related to the presence of circulating cryoglobulins, which are delivered to the kidneys, where they can be deposited in various locations or participate in the formation of capillary thrombi. Because the amount of circulating cryoglobulins can vary significantly from patient to patient and at different stages of the disease process in a given patient, renal manifestations are cyclical, with remissions and exacerbations.

Differential Diagnosis.—Because the light microscopic pattern is often similar to that of membranoproliferative glomerulonephritis, type I, cryoglobulinemic nephropathy must be differentiated from other types of membranoproliferative glomerulonephritis. If diagnostic cryoglobulin deposits are present, a definitive diagnosis can be established. If that is not the case, the findings may have to be considered as consistent with cryoglobulinemic nephropathy. Silver stain is commonly intensely positive in the expanded mesangial areas.

When the light microscopic features are less conspicuous (ie, mild glomerular proliferative activity, absence of necrosis, no hyaline microthrombi), the differential diagnosis broadens considerably, and there is a high risk of missing the correct diagnosis. In some cases, the immunofluorescence findings are quite suggestive of cryoglobulinemic nephropathy, but these are in the minority and usually represent those cases that show more typical light microscopic and ultrastructural findings.

Hereditary Glomerulopathy With Fibronectin Deposits

This is a very rare hereditary condition with an autosomal-dominant pattern of inheritance. The first family with this condition was reported on in 1980 by Burgin et al., and the rubric of familial glomerulopathy with giant fibrillary deposits was attached to these cases. Most of the cases reported were collected from a retrospective multi-institutional study. The common denominator of these familial glomerulopathies was strong glomerular immunoreactivity with a monoclonal antibody to serum fibronectin. The clinical presentation was proteinuria, often in the nephrotic range, microhematuria, and hypertension. Progression of the renal disease has been variable in the various kindred reported, but some patients progress generally slowly to end-stage renal disease. Disease recurrence in an allograft has been documented.

Diagnosis and Pathogenesis.—The light microscopic appearance is usually that of mesangial expansion without associated hypercellularity. The glomeruli often appear enlarged and sometimes exhibit an accentuated lob-
Figure 31. Fibronectin glomerulopathy. Expanded mesangial area is filled with markedly electron-dense material (A), which when viewed at high magnification (B) has a fine and somewhat subtle fibrillary appearance. Transmission electron microscopy (uranyl acetate and lead citrate, original magnifications ×12,500 [A] and ×18,500 [B]).

The diagnosis of renal diseases with organized deposits can be challenging. Non–disease-specific deposits can be a significant source of confusion. Some of these, such as immature/aberrant matrix material and fibrillary collagen, have been alluded to in this manuscript. Others, including fibrin tactoids, substructure within immune complex deposits, and true artifacts, are not addressed, but the reader is referred to Howell et al² for a detailed description of these. Definition of specific diagnostic features is essential to properly characterize these disorders and establish solid clinicopathologic correlations. Careful eval-
Diagnostic Features of Renal Diseases With Organized Deposits

<table>
<thead>
<tr>
<th>GN or GP</th>
<th>LM/Special Stains</th>
<th>IF/IH</th>
<th>EM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amyloidosis</td>
<td>Congo red, Thioflavin T or S positive</td>
<td>Precursor protein detection</td>
<td>Nonbranching, 7- to 12-nm fibrils, randomly arranged</td>
</tr>
<tr>
<td>Fibrillary</td>
<td>Congo red, Thioflavin T or S negative</td>
<td>IgG, C3, λ, κ; ribbonlike, smudgy staining</td>
<td>Nonbranching, 15- to 30-nm fibrils, randomly arranged; S/T associated with immune complexes</td>
</tr>
<tr>
<td>Diabetic fibrosis</td>
<td>Nodular GN; Congo red, Thioflavin T or S negative</td>
<td>Linear IgG and albumin</td>
<td>Fibrils 10–25 nm in nodular mesangial areas</td>
</tr>
<tr>
<td>Immunotactoid</td>
<td>Congo red, Thioflavin T or S negative</td>
<td>Variable</td>
<td>Microtubules 10–90 nm</td>
</tr>
<tr>
<td>Cryoglobulinemic</td>
<td>Hyalin thrombi, necrosis, exudative GN, MPGN pattern</td>
<td>Monoclonal λ or κ in thrombi</td>
<td>Microtubules 20–30 nm, often curved and in pairs</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>Hyalin thrombi, necrosis, exudative GN, MPGN pattern</td>
<td>Fibronectin positive</td>
<td>Electron dense, granular to fibrillar material</td>
</tr>
<tr>
<td>Collagenofibrotic</td>
<td>MPGN pattern</td>
<td>Collagen III positive</td>
<td>Fibrils with periodicity; S/T; curved, frayed, worm, and comma shaped appearance</td>
</tr>
<tr>
<td>Various advanced renal diseases</td>
<td>Features associated with the various renal disorders/segmental sclerosis</td>
<td>Those of the corresponding diseases</td>
<td>Banded collagen and precollagen/5- to 25-nm fibrils</td>
</tr>
</tbody>
</table>

Abbreviations: EM, electron microscopy; GN, glomerulonephritis; GP, glomerulopathy; IF, immunofluorescence; IgG, immunoglobulin G; IH, immunohistochemistry; LM, light microscopy; MPGN, membranoproliferative glomerulonephritis.


