Congophilic Fibrillary Glomerulonephritis: A Case Series

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Rationale & Objective: Congo Red positivity with birefringence under polarized light has traditionally permitted classification of organized glomerular deposits as from amyloid or nonamyloid diseases. The absence of congophilia has been used to differentiate fibrillary glomerulonephritis (GN) from amyloidosis. We describe a series of fibrillary GN cases in which the deposits are Congo Red–positive (congophilic fibrillary GN) and discuss the role of DNAJB9 in distinguishing congophilic fibrillary GN from amyloidosis.

Study Design: Case series.

Setting & Participants: Analysis of the clinicopathologic characteristics of 18 cases of congophilic fibrillary GN. Mass spectrometry was performed and compared with 24 cases of Congo Red–negative fibrillary GN, 145 cases of amyloidosis, and 12 apparently healthy individuals. DNAJB9 immunohistochemistry was obtained for a subset of cases.

Results: The proteomic signature of amyloid was not detected using mass spectrometry among cases of congophilic fibrillary GN. DNAJB9, a recently discovered proteomic marker for fibrillary GN, was detected using mass spectrometry in all cases of fibrillary GN regardless of congophilia and was absent in cases of amyloidosis and in healthy individuals. DNAJB9 immunohistochemistry confirmed the mass spectrometry findings. The congophilic fibrillary GN cases included 11 men and 7 women with a mean age at diagnosis of 65 years. Concomitant monoclonal gammopathy, hepatitis C virus infection, malignancy, or autoimmune disease was present in 35%, 22%, 17%, and 11% of patients, respectively. No patient had evidence of extrarenal amyloidosis. Patients presented with proteinuria (100%), nephrotic syndrome (47%), hematuria (78%), and chronic kidney disease (83%). After a mean follow-up of 23 months, 31% of patients progressed to end-stage kidney disease and the remaining 69% had persistently reduced kidney function.

Limitations: Retrospective nature. Blinded pathology evaluations were not performed.

Conclusions: The congophilic properties of organized fibrillary deposits should not be solely relied on in differentiating fibrillary GN from renal amyloidosis. Mass spectrometry and DNAJB9 immunohistochemistry can be useful in making this distinction.

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Fibrillary glomerulonephritis (GN) is characterized by amyloid-like deposits in glomeruli that lack the histochemical staining characteristics of amyloid.[1] Its defining features include Congo Red–negative randomly oriented fibrils that lack a hollow center, measure <30 nm in thickness, and typically stain with antisera to immunoglobulins using routine immunofluorescence.[2,3] The importance of distinguishing fibrillary GN (Congo Red–negative fibrillary deposits) from amyloidosis (Congo Red–positive fibrillary deposits) lies in the different cause, treatment, and prognosis of these 2 entities. Most cases of fibrillary GN are idiopathic and renal-limited. Although renal survival in fibrillary GN is poor (nearly 50% of patients reach end-stage kidney disease within 4 years), patient survival is good (80% at 4 years in one study).[4] Mortality is typically due to comorbid conditions.[4] Aside from kidney transplantation, which can be associated with disease recurrence,[4,5] there is currently no effective therapy for fibrillary GN. In contrast, immunoglobulin-derived (most commonly immunoglobulin light chain [AL]) amyloidosis, the most common form of renal amyloidosis, is due to a plasma cell dyscrasia and is a systemic disease in most patients. Treatment includes cytotoxic chemotherapy, autologous stem cell transplantation, and novel amyloid deposit–targeted therapy.[6] Despite the improved prognosis for AL amyloidosis during the past 2 decades, early mortality, largely due to cardiac involvement, remains high, with 2-year overall survival of only 60%.[7]

Mass spectrometry has become the gold-standard analytical technique for the classification of amyloidosis. During the past few years, we have observed several kidney biopsy specimens containing Congo Red–positive glomerular deposits sent for amyloid typing that after comprehensive analysis were judged to be unusual examples of fibrillary GN. In this work, we detail the clinicopathologic and proteomic characteristics of 18 patients with congophilic fibrillary GN. The goals of this study are to: (1) increase awareness among nephropathologists of this diagnostic pitfall in the evaluation of fibrillary GN to avoid misdiagnosing congophilic fibrillary GN as AL amyloidosis, and (2) show that DNAJB9, a specific biomarker for fibrillary GN, is a more specific marker than congophilia for differentiating fibrillary GN from amyloidosis.

Methods

Clinical Characteristics of Patients With Fibrillary GN

Eighteen patients with congophilic fibrillary GN received the diagnosis in the Department of Laboratory Medicine and Pathology of Mayo Clinic during 2010 to 2017. This accounted for 4% of the cases of fibrillary GN in which Congo Red staining was performed. Thirteen (72%) of these cases had originally been referred for proteomic analysis using mass spectrometry because the referring clinicians wanted to confirm the diagnosis of amyloidosis (on account of the congophilia of glomerular deposits) and determine its type (8 cases) or distinguish fibrillary GN from amyloidosis (5 cases) before initiating treatment. Four of the 5 remaining cases were sent in as consult cases by outside renal pathologists who were concerned by the congophilia of otherwise typical fibrillary GN. The final case was an in-house case. Subsequently, the kidney biopsy material for all 18 cases was reviewed in the Renal Biopsy Laboratory of Mayo Clinic.

All 18 patients with congophilic fibrillary GN and 56 control patients with Congo Red–negative fibrillary GN included in this study fulfilled the following diagnostic criteria for fibrillary GN: glomerular deposition of fibrils that (1) were randomly oriented, (2) lacked hollow centers at magnification <30,000, and (3) stained with antisera to immunoglobulins using immunofluorescence, as previously defined.[3,4]

Clinical data were obtained from patients' medical records. Clinical definitions used include: (1) nephrotic-range proteinuria (protein excretion ≥ 3.0 g/d), (2) hypoalbuminemia (serum albumin ≤ 3.5 g/dL), (3) nephrotic syndrome (nephrotic-range proteinuria with hypoalbuminemia and peripheral edema), (4) decreased kidney function (serum creatinine > 1.2 mg/dL), and (5) hypertension (systolic blood pressure ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg, or ongoing treatment with antihypertensive medications). Quantification of proteinuria was evaluated using 24-hour collection or spot urine protein-creatinine ratio when 24-hour urine collection was not performed.

Data were analyzed using SPSS Statistics, version 17.0, and StatXact/LogXact, version 9 (Cytel Software Corp). Continuous variables are reported as mean ± standard deviation. Analysis was performed using nonparametric exact statistical methods, including Fisher-Freeman-Halton, Kruskal-Wallis H, Wilcoxon-Mann-Whitney U, Kolmogorov-Smirnov Z, and Jonckheere-Terpstra, as appropriate for variable type. Statistical significance was assumed at $P < 0.05$. The Mayo Clinic Institutional Review Board approved this study, and it was conducted in accordance with the Declaration of Helsinki. The need for informed consent was waived due to exclusive use of deidentified information.

Kidney Biopsy Evaluation

All 18 biopsies included detailed light microscopic, immunofluorescence, and electron microscopy (EM) studies. For light microscopy, all kidney biopsy specimens were stained with hematoxylin and eosin, periodic acid–Schiff, Masson trichrome, Jones methenamine silver, and Congo Red (using the Puchtler alkaline Congo Red method[8]). In 5 cases in which tissue was still available in the paraffin block after mass spectrometry, Congo Red staining was repeated and reviewed by an external renal pathologist who confirmed the congophilia of the glomerular deposits. Thus, in all 18 cases, the Congo Red stain had been reviewed independently by at least 3 pathologists who confirmed the Congo Red positivity. For immunofluorescence, 3- to 4-μm cryostat sections were stained with polyclonal fluorescein isothiocyanate [FITC]-conjugated antibodies to immunoglobulin G (IgG), IgM, IgA, C3, C1q, and κ and λ light chains. Pronase immunofluorescence[9] was performed in 5 biopsies. Tubular atrophy and interstitial fibrosis were graded on a semiquantitative scale based on an estimate of the percentage of renal cortex affected and recorded as 0 (none), 1% to 25% (mild), 26% to 50% (moderate), or >50% (marked).

Laser Microdissection–Assisted Shotgun Proteomics

Biopsies from 18 congophilic fibrillary GN cases, 24 noncongophilic (congophobic) fibrillary GN cases, 145 renal amyloidosis cases, and 12 unaffected individuals were analyzed using a previously established proteomics method.[10] In brief, renal glomeruli present in formalin-fixed paraffin-embedded tissue sections were laser microdissected. For Congo Red–positive cases, tissues were stained with Congo Red and visualized under florescence for microdissection. For Congo Red–negative tissues, sections were stained with hematoxylin and eosin. Multiple independent dissections were performed for each case and proteins were analyzed using liquid chromatography–assisted tandem mass spectrometry (LC-MS/MS).

Bioinformatics of Protein Identification

A previously published bioinformatics pipeline was used for processing the LC-MS/MS data to generate protein identification profiles.[11] Protein identifications with at least 2 confident (identification probability > 0.9) and unique peptide matches were considered to establish the presence of the protein in the sample. A pathologist reviewed proteome profiles of all samples. For amyloid samples, the profile was examined for an amyloid-confirming universal molecular signature (apolipoprotein A-IV [APOA4], serum amyloid P component [SAP], and apolipoprotein E [APOE]),[12] and the type was assigned based on the most abundant amyloidogenic protein[13] (using spectral counts) that was consistently detected in all replicate dissections.[10-12]