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Diagnosis of Glomerular Disease With Podocyte Infolding, Microspherical, and Microtubular Glomerular Basement Membrane Inclusions

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In 1992, Sato *et al.*[1] described novel pathologic findings in 3 patients with autoimmune disease who presented with stable kidney function and subnephrotic range proteinuria. Their kidney biopsies showed thickened glomerular basement membrane (GBM) on light microscopy and were distinguished from membranous nephropathy by the following: (i) the presence of only faint or negative glomerular capillary wall staining by immunofluorescence; (ii) lack of usual forms of subepithelial electron dense deposits and GBM "spikes" on ultrastructural examination; and (iii) the presence of fine and minute intramembranous deposits (40–80 nm in diameter) and "membrane degradation products," likely representing invagination of podocyte foot processes into the GBMs (i.e., "podocyte infolding").[1]

In 2008, the term podocytic infolding glomerulopathy (PIG) was proposed as a new entity by Joh *et al.*[2] in a case series of 25 patients, including the cases first described by Sato *et al.*[1] This series defined PIG based largely on ultrastructural features of podocytic infolding into the GBM, with or without "microspherical" or "microtubular" substructures. This report significantly expanded the clinical and pathologic spectrum of PIG described by Sato *et al.*[1] by inclusion of cases with nephrotic range proteinuria; those with varying light microscopic features, such as mesangial hypercellularity or membranoproliferative changes; those with significant immunofluorescence staining and corresponding usual electron dense deposits; and those with known etiology, including hepatitis B virus associated membranous nephropathy. Joh *et al.*[2] defined 3 subtypes of PIG based on ultrastructural characteristics: (i) podocytic infolding only (akin to the cases described by Sato); (ii) podocytic infolding with intramembranous microspherical/microtubular inclusions; and (iii) intramembranous clusters of microspherical/microtubular inclusions, without podocytic infolding (but presumably derived from entrapped podocyte cytoplasm). However, all 25 cases showed podocytic infolding and no data were presented to suggest these subtypes had any clinical differences. The intramembranous substructures are similar to the "spherical microparticles" previously described by Burkholder *et al.*[3] and Kowalewska *et al.*,[4] and membranous nephropathy with circulating antineutral endopeptidase antibody,[5] but distinguished from these entities by the presence of diffuse podocytic infolding. In addition, intramembranous substructures in most cases of PIG are not confined within the electron dense deposits. A recent review of 44 published cases of PIG confirms a strong association with East Asian origin and systemic lupus erythematosus (SLE); however, approximately 20% of cases were idiopathic.[6] A single case of PLA2R-associated membranous nephropathy with PIG features has been described,[7] though some may not consider this case to be PIG, and rare genetic associations have been reported (e.g., with *SMARCAL1* and *INF2*).[6] However, the pathogenesis of PIG remains unknown.

Although PIG is a highly memorable acronym, the nature of this rare entity is poorly understood. Indeed, it is unclear whether PIG constitutes a single disease entity or is simply a peculiar morphologic variant of other forms of glomerular disease, particularly membranous nephropathy. In this issue, Hong *et al.* (*in press*) present the largest single-center series of PIG to-date

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and shed more light on its epidemiology and clinical features.[8] One hundred sixteen cases of PIG were identified among 126,086 biopsied patients (biopsy prevalence 0.09%). PIG was defined by diffuse (>75%) microsphere or microtubular structure correlated with podocytic cytoplasm infolding into the GBM on electron microscopy, and diffuse GBM thickening with craters resembling membranous nephropathy on light microscopy. The podocytic origin of these structures was confirmed by alpha actinin 4 and collagen IV alpha 3 immunostaining. Most (59.5%) patients carried a diagnosis of SLE and most (76.7%) demonstrated significant immune complex deposition by immunofluorescence and immune-type electron dense deposits on electron microscopy. A minority (23.3%) lacked immune complex deposition ("isolated PIG"); however, half of these cases had other causes of glomerular disease (e.g., autoimmune disease, diabetic nephropathy, and focal segmental glomerulosclerosis).

Among 61 patients with available clinical information, those with immune complex PIG were significantly more likely to carry a diagnosis of SLE or display other features of autoimmunity, such as antinuclear and antidouble stranded DNA antibody seropositivity and hypocomplementemia, confirming the association with autoimmunity in previous reports.[2] Isolated PIG cases typically had subnephrotic proteinuria and were associated with autoimmune diseases in approximately a third of cases, including SLE (without lupus nephritis), primary Sjogren syndrome, and unclassified connective tissue disease. Despite the diffuse (>75%) podocyte foot process effacement in all patients, the absence of nephrotic syndrome in most cases of isolated PIG argues against PIG being a primary diffuse podocytopathy, such as minimal change disease. Therapeutic response is difficult to assess, due to the retrospective design, lack of follow-up information in half of the patients, and variability in the treatment regimen. However, most patients with PIG appear to achieve remission (95.1%), including 45.9% and 49.2% complete and partial remission, respectively, after some immunosuppressive therapy. Hong *et al*.[8] graded the severity of PIG based on the thickness of GBM involvement, and higher grades correlated with heavier proteinuria (in isolated PIG cases only) and reduced remission rates. However, there were no significant differences in proteinuria and rate of nephrotic syndrome among the PIG subtypes A, B, and C. Interestingly, 3 patients (2 with membranous nephropathy and 1 with class IV + V lupus nephritis) were diagnosed with PIG on second biopsies performed after therapy, suggesting that PIG may be a sequela of membranous nephropathy or lupus nephritis.

The pathogenesis of PIG is unknown; however, the available evidence suggests that this is closely related to membranous nephropathy, particularly class V lupus nephritis. Mass spectrometry has uncovered novel target antigens in membranous nephropathy, notably PLA2R in primary disease and exostosin 1/2 in class V lupus nephritis.[9] Hong *et al*.[8] could not identify consistently enriched antigens in PIG cases by mass spectrometry of laser captured and microdissected glomeruli, although a decrease in alpha actinin 4 and dysregulation of the actin cytoskeleton was seen compared to normal donor kidneys. Whether further analysis using other more relevant disease controls, such as membranous nephropathy and lupus nephritis, might yield a culprit antigen remains to be seen. Novel imaging techniques, such as serial blockface scanning electron microscopy and focused ion beam-scanning electron microscopy, may provide a better picture of the altered 3-dimensional architecture in PIG, and suggest insights into pathogenesis.[S1] Although the predominance of reported cases from Japan and China may reflect publication bias, there could also be population-based genetic or epigenetic differences that await further elucidation.

In summary, Hong *et al*.[8] provide a wealth of data on the full clinicopathologic spectrum of patients with PIG, but many important questions remain. Do cases of lupus nephritis with PIG features differ clinically and pathogenetically from lupus nephritis without these features? How should PIG be treated? In particular, should cases of isolated PIG and immune complex PIG be treated differently, particularly when the underlying cause (e.g., lupus nephritis) is known?

Should PIG even be considered a single disease entity? Based on the above evidence, we believe the answer is no. Although many forms of glomerulonephritis (GN) are named for pathologic features, (including focal segmental glomerulosclerosis, IgA nephropathy, C3GN, minimal change disease, and fibrillary GN), this morphology-based nosology reflects the fact that the etiology and pathogenesis in most cases remains unknown. If a secondary cause is identified, such as collagen-vascular disease or infection, this should be included in the diagnosis (e.g., lupus nephritis, HCV-related cryoglobulinemic GN, HIV-associated collapsing glomerulopathy), with the goal of targeting therapy to the underlying cause.[S2] Following this rationale (Figure 1),

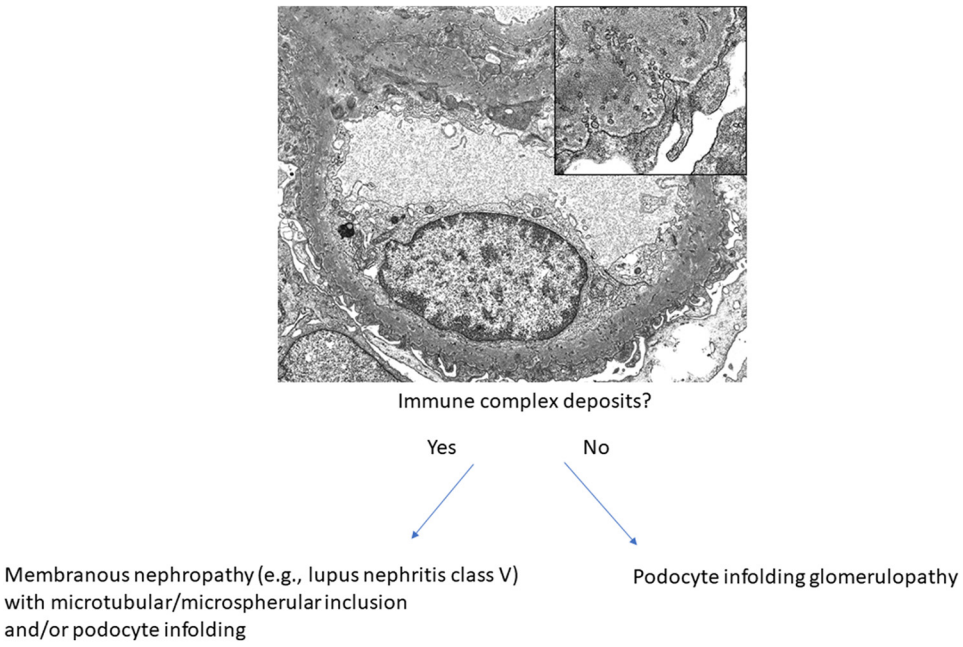

Figure 1. Diagnostic algorithm for glomerular disease with podocyte infolding, microspherical, and/or microtubular glomerular basement membrane inclusions.

most cases of PIG are probably best classified as lupus nephritis with podocyte infolding and/or microspherical/microtubular inclusions because SLE is the main pathogenetic driver. However, further studies are needed to determine whether PIG features affect the course of lupus nephritis and how best to classify PIG in patients with SLE, given that PIG is not currently included in the International Society of Nephrology/Renal Pathology Society classification of lupus nephritis. Similarly, cases with immune complex deposition and only rare podocyte infolding or microspherical inclusions, especially where such inclusions are confined to usual or resorbing electron dense deposits, likely do not warrant designation as PIG and, instead, should be diagnosed as membranous nephropathy, particularly when associated with a known pathogenic antibody. In addition, cases of membranous nephropathy with microspherical deposits should be distinguished from PIG by the larger size of the microspheres and the lack of podocyte infolding.[S3]

Lastly, it is important not to confuse PIG with other inclusions that are occasionally encountered in the GBM, such as cellular debris and fragments of mesangial or endothelial cell cytoplasm.

Diagnosis of PIG without immune complex deposits (isolated PIG) is more challenging. Some of these cases may represent the advanced stage of immune-complex GN; however, this can only be inferred if there was a prior biopsy. In cases with coexisting glomerular disease (e.g., focal segmental glomerulosclerosis or diabetic glomerulosclerosis), the clinical significance of podocyte infolding and whether to diagnose PIG separately or together with the coexisting glomerular disease, requires case-by-case consideration. Ultimately, this leaves a vanishingly small number of cases that might reasonably be classified as primary, nonimmune complex associated PIG suitable for clinicopathologic studies (Figure 1). Given the rarity of primary PIG, further insights will require multicenter collaboration. Whatever is causing PIG, there is a clear need for careful use of this term, because isolated PIG and the more common immune complex GN with podocyte infolding are likely not a single disease.

DISCLOSURE

All the authors declared no competing interests.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplemental References.

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