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See related article, "Allogeneic Mesenchymal Stem Cells for Treatment of AKI after Cardiac Surgery," on pages 260–267.

Clusters Not Classifications: Making Sense of Complement-Mediated Kidney Injury

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In 2010, we suggested the name C3 glomerulopathy to encompass a group of glomerular diseases characterized by the presence of glomerular C3 in the absence of substantial Ig and without deposition of the early components of the classic or lectin pathways of complement activation.[1] Within this new disease classification, two morphologic appearances could be recognized—dense deposit disease (DDD), first described in 1963,[2] with typical highly osmiophilic deposits on electron microscopy (EM), and C3GN, more recently recognized as a distinct entity,[3] showing isolated C3 without very dense deposits. The distinction between DDD and C3GN is not always straightforward, and there are patients with overlap cases on which not all pathologists would agree. By light microscopy, about 70% of patients with C3 glomerulopathy showed a membranoproliferative pattern, whereas the others showed mesangial proliferation.[4] Superimposed on these basic patterns were variable degrees of endocapillary hypercellularity, crescent formation, and glomerulosclerosis.

The implication of the finding of isolated glomerular C3 was that there was an inability to regulate activation of complement through the alternative pathway. We considered that identifying the factor(s) that caused this would help us to both understand the mechanism of renal injury and identify patients most likely to benefit from complement-inhibiting therapies.[1] This was our motivation for the classification. Mechanisms of alternative pathway dysregulation in C3 glomerulopathy include genetic and acquired factors. Genetic causes include deficiency of factor

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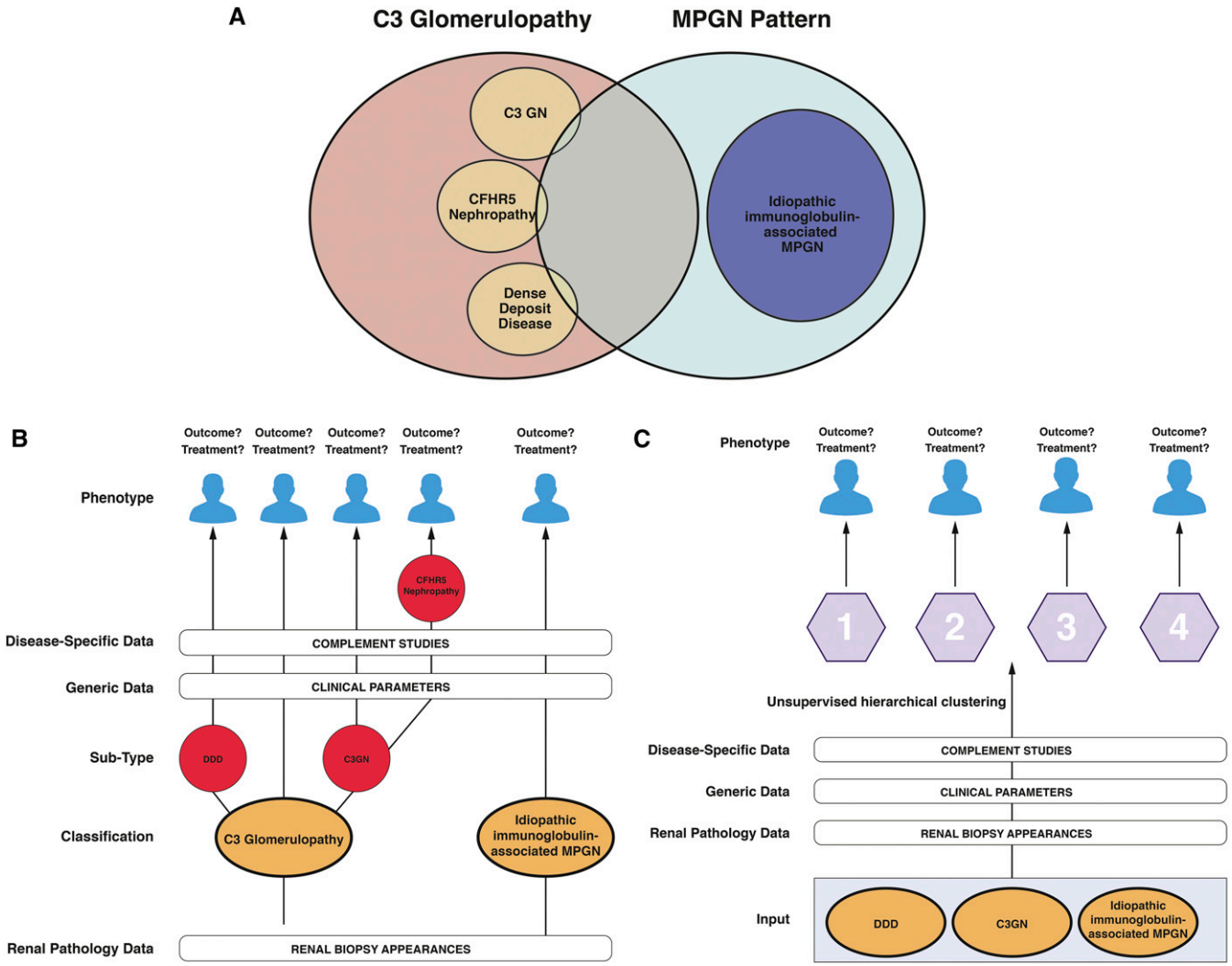

Figure 1. Concepts of phenotyping in complement-mediated disease have evolved over the last decade. (A) The complex relationship between the pathologic features of MPGN and C3 glomerulopathy. Subgroups of C3 glomerulopathy can be defined by pathologic features (DDD and C3GN) or the presence of a specific genetic defect (CFHR5 nephropathy and a familial C3GN). (B) Classification on the basis of the renal biopsy. Patients are classified on the basis of renal biopsy features. Clinical data (generic and specific; *e.g.*, complement assays) are then interpreted and analyzed within these categories together with outcome and treatment responses. (C) Unsupervised hierarchical clustering is used to discover unknown subgroups within the dataset. Existing biopsy classifications are used to determine which patients to include in the dataset.

H, the key negative regulator of the alternative pathway (very rare); gain of function changes in C3 (very rare); and an autosomal dominant disease that is endemic in Cyprus, termed Complement Factor H Related 5 (CFHR5) nephropathy. In CFHR5 nephropathy, there is an internal duplication in the gene that codes for factor H–related protein 5.[5] This results in a mutant FHR5 protein that reduces the ability of factor H to control C3 activation within the kidney. Notably, what prompted us to look carefully for complement gene mutations in CFHR5 nephropathy, where circulating complement levels are typically normal, was the presence of dominant C3 staining within the biopsies. There are now multiple reports of other genetic changes within the factor H–related genes in familial C3GN. Acquired factors include C3 nephritic factors (common), and autoantibodies to factor H, factor B, and C3 (all rare). However, in many patients, no clear pathogenic mechanism is identifiable. Moreover, the link between C3 nephritic factors and disease pathogenesis remains controversial (are these factors the cause of the alternative pathway dysregulation or a consequence of it?), and the relevance of complement gene variants in sporadic disease in many patients is unclear.

The recognition of C3 glomerulopathy led to a major revision of our understanding of the entity of membranoproliferative GN (MPGN). Thus, it seemed that the previous categorization of MPGN types 1–3 on the basis of light microscopy and EM could be replaced by a classification on the basis of immunofluorescence findings into immune complex–associated MPGN and

C3 glomerulopathy with a membranoproliferative pattern.[6] In many patients with cases of immune complex–associated MPGN, an underlying cause can be identified, such as autoimmunity, chronic infection, or deposition of a monoclonal Ig, and this led to the suggestion that primary MPGN might now be considered a vanishingly rare condition.[7] Although the division of MPGN into immune complex–associated forms and C3 glomerulopathy with a membranoproliferative pattern seemed to provide a pathogenesis-based classification, there were practical problems with its application. First, patients with cases of C3 glomerulopathy with a membranoproliferative pattern who have only glomerular C3 with absolutely no Ig are relatively uncommon: most patients will have dominant C3 staining with some Ig. In a study of over 300 patients with primary MPGN, the group at Columbia showed that a criterion defined as the presence of dominant C3 staining with the intensity of C3 staining at least two orders of intensity stronger than any combination of IgG, IgM, IgA, and C1q identified 31% of the patients with MPGN type 1, 88% of the patients with MPGN type 2 (DDD), and 39% of the patients with MPGN type 3.[8] Second, there are patients with MPGN who present with a biopsy that has glomerular Ig and complement deposition not fulfilling criteria for C3 dominance, whereas a biopsy later during the disease shows isolated C3. Although not formally proven, it seems likely that this may relate to the role of immune complexes in either initiating or exacerbating the disease in a patient who has an underlying abnormality of complement alternative pathway control. Third, features of complement dysregulation, such as hypocomplementaemia and C3 nephritic factors, are often as frequent in idiopathic Ig–associated MPGN as they are in C3 glomerulopathy.[4,9] We think, therefore, that the idea that we had a clear understanding of MPGN by classification into Ig-associated and C3 dominant forms was too simplistic. Patients with cases of Ig-associated MPGN need to be investigated for an underlying cause, such as autoimmunity or infection, but after secondary causes are excluded, it seems appropriate to consider screening for complement dysregulation.

Among patients with renal biopsies showing C3 glomerulopathy, be it DDD or C3GN, and those with idiopathic MPGN, what both patients and physicians really want to know are what the likely effect of the disease over time is and what can be done to prevent it. Armed with multiple clinical parameters, complement protein measurements, complement genetics, autoantibody results, and a renal biopsy, how does the nephrologist begin to address these questions? In this issue of the *Journal of the American Society of Nephrology*, Iatropoulos *et al.*[10] turn to mathematics to find clinically meaningful patterns in such a dataset. In a well characterized population of patients with renal biopsies reported as DDD, C3GN, or idiopathic MPGN, they adopted a statistical approach termed unsupervised hierarchical clustering, a technique widely used in gene expression analysis. However, it can be used to explore whether individuals can be classified into groups in such a way that differences within a group of patients are small, whereas the differences between groups are large. It has previously been applied in this context in asthma and chronic obstructive airways disease. They studied 178 patients: 68 patients with cases of C3GN, 25 patients with cases of DDD, and 80 patients with cases of idiopathic MPGN. They performed cluster analysis using 34 variables, of which seven were clinical features at onset; 17 were pathologic findings, including light microscopy, immunofluorescence, and EM; and the others were complement measurements and genetic variants. Using these variables, they detected four distinct clusters. Each cluster included patients with C3GN and MPGN. However, patients with DDD fell predominantly into cluster 3. Clusters 1 and 2 had C3 and C5 activation in the circulation with high levels of circulating C5b-9 and were distinguished by more Ig deposition in cluster 2. In cluster 3, C3 convertase activity seemed to predominate over C5 convertase activity, and many patients had very dense deposits on EM. Cluster 4 represented a group that had activation of C3 in glomeruli but did not have activation of C3 in the circulation and did not have either genetic variants or C3 nephritic factor.

The key test for this approach is its validation in a distinct cohort (*i.e.*, do these clusters exist in other cohorts and can they be examined prospectively?). In addition, this approach is clearly dependent on the variables that are selected for the cluster analysis. Some specific scenarios do not seem to require either complex analysis or big data: for example, women with Cypriot ancestry and CFHR5 nephropathy have an excellent long-term prognosis.[11] Nevertheless, their study reinforces the overlap between C3 glomerulopathy and idiopathic Ig–associated MPGN (Figure 1). It also relates some long-standing complement observations (that dysregulated complement activation may occur within the circulation and/or within glomeruli and may deplete C3 or both C3 and C5) to distinct groups. Perhaps most importantly, their cluster analysis did better at predicting renal survival than the division into MPGN, DDD, and C3GN. Therefore, mathematics seems to trump pathology! The ultimate test of the utility of this approach in the clinic will be if it can also identify groups of patients who will benefit from distinct complement-modulating therapies.

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DISCLOSURES

None.

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See related article, "Cluster Analysis Identifies Distinct Pathogenetic Patterns in C3 Glomerulopathies/Immune Complex–Mediated Membranoproliferative GN," on pages 283–294.