Hierarchical clustering uncovered disease patterns and further untangled complexities in immune complex-mediated idiopathic MPGN and C3 glomerulopathy

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Abstract

Introduction: Membranoproliferative glomerulonephritis (MPGN) is currently stratified into complement C3 glomerulopathy (C3G) and immune complex-mediated MPGN (IC-MPGN). However, classification is subject to continued debate.

Methods: Here, we applied hierarchical clustering to a much larger cohort of patients with C3G/IC-MPGN (295 individuals), extensively characterized for genetic and autoimmune complement abnormalities, with the goal of unraveling specific disease patterns. We also designed a user-friendly web application that with input of data at diagnosis could make cluster classification clinically applicable.

Results: Five clusters with unique phenotypic and complement profiles were identified. Cluster 1 and 2 patients showed systemic complement activation until C5. Consistently, C5 nephritic factor and anti-factor B antibodies were prevalent in these clusters. Cluster 2 was distinguished from cluster 1 for classical pathway activation markers in biopsy. Cluster 3 showed C3-restricted systemic complement activation associated with the prevalence of C3 nephritic factor. Cluster 4 and 5 patients shared a normal complement profile and intense glomerular C3 staining, consistent with solid-phase complement activation, but cluster 5 distinguished for the higher prevalence of genetic abnormalities. Cluster 4 patients had the highest incidence of kidney failure during follow-up, while cluster 1 had the best kidney prognosis. However, clusters 1 and 2 showed a high risk of post-transplant recurrence. Through our web application, we could visually compare the predicted profile of new patients with those of patients included in clustering analysis and assign these patients to different clusters. The cluster-based classification allows etiologic diagnosis of

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C3G/IC-MPGN and had better prognostic value than current approaches.

Conclusions: Our proposed strategy may possibly guide anti-complement treatment.

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KEYWORDS: C3 glomerulopathy; complement inhibitory drugs; complement pathways; hierarchical cluster analysis; membranoproliferative glomerulonephritis; rare kidney diseases

Lay Summary

Membranoproliferative glomerulonephritis (MPGN) is a rare and complex group of kidney diseases with no specific treatment currently available. Its classification remains a topic of ongoing debate. MPGN is typically divided into C3 glomerulopathy and immune complex–mediated MPGN. In this study, we analyzed detailed medical data from 295 patients with MPGN and applied a statistical method called hierarchical clustering to group them into 5 distinct clusters based on shared disease features. These clusters were found to be associated with long-term kidney outcomes and the risk of disease recurrence after kidney transplantation. To support validation of this approach in real world, we developed a user-friendly web application that helps clinicians assign new patients to one of these clusters. This study improves our understanding of MPGN and is *hypothesis-generating*, suggesting that clustering may be a promising approach for predicting outcomes and aiding future research and treatment strategies.

Membranoproliferative glomerulonephritis (MPGN) represents a heterogeneous group of rare kidney disorders[1–3] that are currently classified into alternative pathway complement-mediated C3 glomerulopathy (C3G) and immune complex–mediated MPGN (IC-MPGN). The term C3G is also used to define complement-mediated glomerulopathies that do not have a membranoproliferative pattern.[1] This classification, which is based on only histologic findings,[4–6] does not reflect the pathophysiology and is inefficient in predicting clinical course and response to therapy.[7–10] C3G and idiopathic IC-MPGN share the same prevalence of alternative pathway abnormalities, including the nephritic factors (NeFs), heterogeneous antibodies that stabilize the C3 and C5 convertases,[11–14] and complement gene abnormalities.[15–19] Consequently, the diagnosis and disease management are challenging.

Given the central role of complement dysregulation in the pathogenesis of C3G and IC-MPGN, several drugs targeting different molecules of the complement cascade are on clinical development[1,2] and promising results of clinical trials have been recently published.[20,21] However, clinical trials to test new therapeutics may be heavily influenced by the fact that different patients have abnormal activation at different levels of the complement system.

Through a hierarchical cluster analysis using histologic, genetic, and serum/plasma complement parameters and clinical features at disease onset from 173 patients with C3G/IC-MPGN, our group previously identified 4 clusters, shown to be instrumental to define early and late complement activation either in the fluid phase or in the solid phase.[22,23]

Here, we aimed to advance the hierarchical clustering analysis to a step further, with the ultimate goal of identifying specific disease patterns amenable to targeted treatment. To this purpose, we established the DECODE consortium and studied a much larger cohort of patients more deeply characterized for genetic and autoimmune complement abnormalities. We took NeFs and genetic abnormalities as separate variables, whereas the previous cluster analysis[22] considered both as a single parameter. We describe a fifth cluster that had not been reported previously. The new analysis also resulted in better prognostic power of the clusters for long-term kidney outcomes and identified 2 specific clusters that can predict disease recurrence after transplantation. Fine investigation of the causes underlying complement activation in each cluster provided novel hints to understand pathophysiology and heterogeneity of these complex diseases.

Finally, we have developed an innovative clinical support, that is, a computational tool featuring a clean and easy-to-use interface that will allow new patients to be assigned to a cluster using a limited set of genetic, laboratory, and clinical data available to clinicians at the time of diagnosis.

METHODS

Participants and procedures

Clinical data and biopsy reports from all consecutive 561 patients included in the Italian Registry of MPGN (https://www.marionegri.it/eng/mpgn-c3g-registry) from 2006 to 2022 were retrospectively reviewed by two independent clinicians and a pathologist, respectively. According to current guidelines,[6] patients with "dominant C3" glomerular staining on immunofluorescence were diagnosed with C3G and further classified through electron microscopy as having either dense deposit disease (DDD) or C3 glomerulonephritis (C3GN) according to the presence or the absence of highly electron dense deposits in the glomerular basement membrane.[6] Patients with an MPGN pattern and glomerular deposits of both immunoglobulin and complement proteins were diagnosed with IC-MPGN. We excluded patients with alternative diagnoses, those without a native kidney biopsy or lacking immunofluorescenc data, and cases secondary to autoimmune diseases, monoclonal gammopathies, neoplasms, thrombotic microangiopathy preceding or concomitant to MPGN onset, hepatitis B virus, hepatitis C virus, and HIV infections, or typical post-infectious glomerulonephritis (triggered by streptococcus bacteria or other pathogens, with clinical and/or C3 recovery within 8–12 weeks, and no

recurrence).[24] On the basis of the above criteria, we selected 300 patients (Supplementary Figure S1).

Methods used for evaluation of genetic (likely pathogenic variants and copy number variations), biochemical, and autoimmune (NeFs, anti–factor H, and anti–factor B [anti-FB] antibodies) complement abnormalities, and the characterization of NeFs as C3NeF (properdin-independent IgG stabilizing the C3 convertase) and C5NeF (properdin-dependent IgG stabilizing the C5 convertase)[11,13,18,22] are reported in Supplementary Methods. This study was approved by the ethical committee of ASST Papa Giovanni XXIII (Bergamo) and conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from patients or their guardians. Biological samples were stored in the Biobank for Rare Diseases and Kidney Diseases at the Mario Negri Institute.

Clustering

We used 29 clinical, histologic, genetic, and biochemical variables as an input (Table 1). Gender, trigger at onset, family history for nephropathy, mesangial, subepithelial, subendothelial, and intramembranous highly electron-dense deposits and NeFs were regarded as binary variables. Age and clinical parameters at onset (hematuria, proteinuria, and renal function); serum C3, C4 and plasma sC5b-9 levels (first available measure); light microscopy features (sclerotic

Table 1 | Variables included in clustering analysis

Variable	Codification
Gender (male/female)	0: male and 1: female
Age at onset	Years
Hematuria at onset	0: absence of hematuria, 1: microhematuria, and 2: gross hematuria
Proteinuria at onset	0: absence of proteinuria, 1: proteinuria, and 2: nephrotic syndrome[a]
Renal impairment at onset	0: absence of renal impairment, 1: renal impairment, and 2: need of dialysis
Trigger event at onset	0: no and 1: yes
Family history of nephropathy	0: no and 1: yes[b]
% Sclerotic glomeruli on LM	%
% crescents on LM	%
Degree of mesangial proliferation/hypercellularity on LM	0–3+ scale
Degree of endocapillary proliferation on LM	0–3+ scale
Degree of interstitial inflammation on LM	0–3+ scale
Degree of interstitial fibrosis on LM	0–3+ scale
Degree of arteriolar sclerosis on LM	0–3+ scale
C3 on IF	0–3+ scale
IgA on IF	0–3+ scale
IgG on IF	0–3+ scale
IgM on IF	0–3+ scale
C1q on IF	0–3+ scale
Fibrinogen on IF	0–3+ scale
Mesangial deposits on EM	0: no and 1: yes
Subepithelial deposits on EM	0: no and 1: yes
Subendothelial deposits on EM	0: no and 1: yes
Intramembranous highly electron-dense ribbon-like deposits on EM	0: no and 1: yes
Serum C3 first value	0: very low (<50 mg/dl), 1: low (≥50 and <90 mg/dl), and 2: normal (≥90 mg/dl)
Serum C4 first value	0: low (<10 mg/dl), 1: lower normal range (≥10 and <20 mg/dl), 2: medium and higher normal range (≥20 mg/dl)
Plasma sC5b-9 first value	Continuous values (ng/ml)
Number of alleles with LPV or rare CNV	0, 1, and 2[c]
NeF positivity	0: no and 1: yes

CNV, copy number variations; EM, electron microscopy; IF, immunofluorescence; LM, light microscopy; LPV, likely pathogenic variants; NeF, nephritic factors.
[a]0, absence of proteinuria (<0.3 g/24 h; or – or –/+ with dipstick, or ≤20 mg/dl); 1, proteinuria (0.3–3.4 g/24 h; +, ++ with dipstick, or >20 mg/dl and <300 mg/dl); 2, nephrotic-range proteinuria (≥3.5 g/24 h, +++ with dipstick or ≥300 mg/dl).
[b]Family history of nephropathy was defined as the presence of at least one first- or second-degree relative reported to have any form of nephropathy.
[c]1: heterozygous and 2: homozygous or compound heterozygous for the same or for different genes. % crescents: % involvement of sampled glomeruli, includes all ages of crescents (cellular, fibrocellular, and fibrous). The % of sclerotic glomeruli includes both globally sclerotic and segmentally sclerotic glomeruli. The IF grading of 0–3+ was based on intensity of stain only. LM findings were graded as follows: Mesangial proliferation/hypercellularity and endocapillary proliferation: 0 (absent), 1 (mild), 2 (moderate), and 3 (severe) for 0%, 1%–25%, 26%–50%, and >50% involvement of sampled glomeruli, respectively. Interstitial inflammation: 0–3 for % of cortical tubulointerstitial area with inflammation in nonfibrotic cortex, <10%, 10%–25%, 26%–50%, and >50%. Interstitial fibrosis: 0–3 for % of cortical tubulointerstitial area with fibrosis, <10%, 10%–25%, 26%–50%, and >50%. Arteriolar sclerosis: 0–3 for intimal thickening absent, mild, moderate, and severe.

glomeruli, crescents, mesangial proliferation, endocapillary proliferation, interstitial inflammation, interstitial fibrosis, and arteriolar sclerosis); immunofluorescence data (C3, IgA, IgG, IgM, C1q, and fibrinogen staining); and likely pathogenic variants or copy number variations were considered numerical variables. Compared with our previous publication,[22] NeFs and genetic abnormalities were considered as separate variables, whereas the *CFH* (p.V62I and p.Y402H), *CD46* (c.-366A>G), *CFB* (p.Q/W32R), *C3* (p.R102G), and *THBD* (p.A473V) polymorphisms were not included because of their high frequency in the normal population.

Of the 300 selected patients, we excluded 5 with >15% missing values, so the final study cohort included 295 patients, of whom 168 were from the previously published study (Supplementary Figure S1).[22] Imputation was performed by iterative robust model-based imputation implemented in the R package VIM (R: R Foundation for Statistical Computing). Variables were then submitted to a mixed principal component analysis, followed by hierarchical clustering[22,25–28] (see details in Supplementary Methods).

Statistical learning models for patient classification

To assign novel patients to one of the clusters, we verified multiple classification approaches. Best cluster recall in leave-one-out bootstrapping was achieved by support vector machine (89%) and k-nearest neighbors classification (k-NN; 85%–87% depending on k; Supplementary Methods). For k-NN, we selected k = 3 and k = 9 to represent a small and a broad neighborhood, respectively, and to avoid ties between 2 clusters.

The impact of variables on cluster recall was evaluated to judge their relevance for diagnosis. Therefore, support vector machine and k-NN models were trained accordingly with missing variables, and loss of cluster recall in leave-one-out bootstrapping was calculated as difference to the original cluster recall of the models including all variables (Supplementary Methods).

Interactive data browsing and classification tool

We then designed a user-friendly, interactive web application implemented in R-Shiny.[29] We adapted our established self-organizing map analysis and data portraying workflow[30,31] to the specifics of the C3G/IC-MPGN cohort and the 29 parameters to generate detailed data landscapes. Self-organizing map data portraying generates 2-dimensional fingerprint images of the multidimensional variable space of the patients and the clusters, which support diagnosis by visual perception with personalized resolution. The tool combines these feature landscapes with patient landscapes, which were generated using "traditional" t-distributed stochastic neighbor embedding and self-organizing map approaches to identify mixed-class cases and cases of unclear class assignment, and with support vector machine and k-NN classification probabilities. An overview of the tool is given in the Supplementary Material (section "A user-friendly classification platform for MPGN patients").

Statistical analysis

Summary statistics analyses of the clusters' parameters were performed, excluding missing imputed data. Statistical methods are detailed in Supplementary Methods.

RESULTS

Description of the cohort

Among the 295 selected patients, 125 had a diagnosis of C3GN (42%), 35 DDD (12%), and 135 IC-MPGN (46%, Table 2 and Supplementary Table S1). A trigger event was reported at onset in 87 patients (C3GN: 30%, DDD: 36%, and IC-MPGN: 30%), of whom 79 had infections (mostly upper respiratory tract or gastrointestinal) and 6 developed the disease during pregnancy. Complement gene abnormalities (likely pathogenic variants and copy number variations) were identified in 46 patients (16%), and their prevalence did not substantially differ among the histology groups (C3GN: 19%; DDD: 15%; and IC-MPGN: 13%).

NeFs were more frequently found in DDD (77%) than in C3GN (32%) and IC-MPGN (42%) (Table 2). The overall prevalence of genetic and/or acquired complement abnormalities did not differ between C3G (51%) and IC-MPGN (47%), and the same was observed for the fraction of patients with low serum C3 (C3G: 88% and IC-MPGN: 82%) or elevated plasma sC5b-9 (C3G: 50% and IC-MPGN: 48%), confirming the overlapping characteristics between the histology groups.

Clusters characteristics

On the basis of the results from the NbClust package and our objective of achieving a balanced patient distribution (Supplementary Methods), we identified 5 clusters (Figure 1), which shared intense glomerular C3 staining, but differed by phenotype and complement profile. The first branching separated patients with low levels of C3 (block A) from those with basically normal blood complement profile (block B). Block A split into 3 clusters (clusters 1–3), whereas in block B, we identified 2 clusters (clusters 4 and 5) (Table 3 and Supplementary Table S2, Figure 1).

The average *z* scores of the variables used in the analysis (Figure 2) showed separation of clusters based on the age at onset, presence of NeFs, renal impairment at onset, serum C3 and plasma sC5b-9 levels, the presence of intramembranous highly electron-dense deposits, crescents, interstitial fibrosis and inflammation, sclerosis and degree of C1q, fibrinogen, and immunoglobulin staining (Figure 2).

Cluster 1 included 70 patients and was characterized by low serum C3, high plasma sC5b-9 levels, and by high prevalence of mesangial and subepithelial deposits in biopsy (Table 3, Figure 2). The mean age at onset was 16.5 years, and a trigger event was reported in about half of the patients (Table 3). The majority (69%) had a diagnosis of C3GN (Supplementary Figure S2).

Patients in cluster 2 (n = 67) showed low serum C3 and very high plasma sC5b-9 levels. However, compared with the other clusters, cluster 2 distinguished for strong glomerular

Table 2 | Clinical features at onset, histologic parameters, and complement abnormalities in patients classified according to histologic classification

Parameter	C3GN (n = 125)	DDD (n = 35)	IC-MPGN (n = 135)	*P* value
Gender (female)	0.4 (50 of 125)	0.34 (12 of 35)	0.44 (59 of 135)	NS
Data at onset				
Age, yr	13.322 (9–21; n = 125)	12.339 (9.611–19.597; n = 35)	16 (10–30.489; n = 135)	NS
Trigger	0.31 (36 of 118)	0.36 (12 of 33)	0.3 (40 of 134)	NS
Family history of nephropathy	0.16 (20 of 125)	0.17 (6 of 35)	0.11 (15 of 134)	NS
Hematuria (total)	0.86 (107 of 125)	0.83 (29 of 35)	0.79 (106 of 134)	NS
Gross hematuria	0.3 (37 of 125)	0.34 (12 of 35)	0.27 (36 of 134)	NS
Proteinuria (total)	0.93 (116 of 125)	0.94 (33 of 35)	0.9 (122 of 135)	NS
Urine protein, g/24 h	2.34 (0.885–4.452; n = 74)	2.7 (0.97–5.3; n = 25)	4 (1.69–7.615; n = 75)	NS
Nephrotic syndrome	0.3 (37 of 125)	0.34 (12 of 35)	0.46 (62 of 135)	NS
Renal impairment (total)	0.26 (32 of 125)	0.14 (5 of 35)	0.32 (43 of 135)	NS
Serum creatinine, mg/dl	0.8 (0.64–1.24; n = 69)	0.67 (0.547–0.78; n = 26)	0.925 (0.562–1.3; n = 76)	NS
Need of dialysis	0.04 (5 of 125)	0 (0 of 35)	0.01 (2 of 135)	NS
Histology				
Time onset to biopsy, yr	0.589 (0.164–2.453; n = 125)	0.559 (0.14–2.857; n = 35)	0.474 (0.094–2.294; n = 135)	NS
Subendothelial deposits	0.7 (81 of 116)	0.23 (8 of 35)[a]	0.8 (99 of 124)	*P*
Intramembranous DDD deposits	0.02 (2 of 125)	1 (35 of 35)[a]	0.03 (4 of 134)	*P*
Mesangial deposits	0.79 (95 of 121)	0.6 (21 of 35)	0.61 (78 of 128)	NS
Subepithelial deposits	0.57 (66 of 116)[b]	0.12 (4 of 34)[a]	0.38 (47 of 123)	*P*
Crescents	0 (0–0; n = 125)	0 (0–0; n = 33)	0 (0–0.033; n = 133)	NS
Mesangial proliferation	2 (1–3; n = 125)	2 (1.25–2.75; n = 35)	2 (2–3; n = 134)	NS
Endocapil proliferation	1 (0–2; n = 124)	0.25 (0–2; n = 34)	1.5 (0–2; n = 134)	NS
Interstitial fibrosis	0 (0–1; n = 125)	0 (0–0; n = 35)	0 (0–1; n = 132)	NS
Interstitial inflammation	0 (0–1; n = 125)	0 (0–1.25; n = 35)	1 (0–1; n = 134)	NS
Sclerotic glomeruli	0 (0–0.1; n = 125)	0 (0–0; n = 35)	0.024 (0–0.11; n = 133)	NS
Arteriolar sclerosis	0 (0–0; n = 124)	0 (0–0; n = 35)	0 (0–0; n = 132)	NS
C3	3 (2.5–3; n = 125)	3 (2.5–3; n = 34)	3 (2–3; n = 134)	NS
Fibrinogen	0 (0–0; n = 125)	0 (0–0; n = 32)	0 (0–0.5; n = 131)	NS
C1q staining	0 (0–0; n = 124)	0 (0–0.5; n = 34)	1 (0–2; n = 132)[a]	*P*
IgA	0 (0–0; n = 125)	0 (0–0; n = 31)	0 (0–1; n = 134)[a]	*P*
IgG	0 (0–0; n = 125)	0 (0–0.5; n = 31)	2 (0.5–2.5; n = 134)[a]	*P*
IgM	0 (0–1; n = 125)[b]	0.5 (0–1; n = 31)[a]	1 (0.5–2; n = 134)	*P*
Complement				
Plasma sC5b-9	561 (209–1576; n = 115)	304 (200–566; n = 34)	364 (252–1436; n = 128)	NS
Serum C3	38 (17–76; n = 121)	20 (12–47; n = 33)	45 (15–80; n = 130)	NS
Serum C4	21 (16–26; n = 116)	22 (15–29; n = 34)	19 (14–27; n = 132)	NS
LPV/CNV carriers	0.19 (23 of 124)	0.15 (5 of 34)	0.13 (18 of 134)	NS
NeF positive	0.32 (35 of 111)	0.77 (27 of 35)[a]	0.42 (54 of 129)	*P*
Treatment[c]				
RAAS inhibition	0.7 (86 of 122)	0.71 (25 of 35)	0.76 (99 of 130)	NS
Immunosuppression	0.66 (82 of 124)	0.59 (20 of 34)	0.81 (108 of 133)	NS
Corticosteroids (no MMF)	0.45 (56 of 124)	0.57 (20 of 35)	0.63 (84 of 133)	NS
MMF ± corticosteroids	0.21 (26 of 124)	0 (0 of 35)	0.18 (24 of 133)	NS
Supportive only	0.11 (14 of 124)	0.17 (6 of 35)	0.03 (4 of 133)	NS

C3GN, C3 glomerulonephritis; DDD, dense deposit disease; IC-MPGN, immune complex–mediated membranoproliferative glomerulonephritis; IF, immunofluorescence; MMF, mycophenolate mofetil; NeF, nephritic factors; NS, not significant; RAAS, renin angiotensin aldosterone system.
P: significant after Bonferroni correction for multiple comparisons ($P < 5 \times 10^{-4}$).
[a]$P < 0.05$ vs. all.
[b]$P < 0.05$ vs. IC-MPGN.
[c]During the first year after diagnosis.
Numerical variables are expressed as proportion (frequency/N) or median (interquartile range). N = number of patients with raw data available.
Serum C3: reference 90–180 mg/dl, serum C4: reference 10–40 mg/dl, and plasma sC5b-9: reference ≤400 ng/ml.
Degrees of arteriolar sclerosis, mesangial proliferation, endocapillary proliferation, interstitial fibrosis, and interstitial inflammation, as well as IF stainings were scored with a scale 0–3.

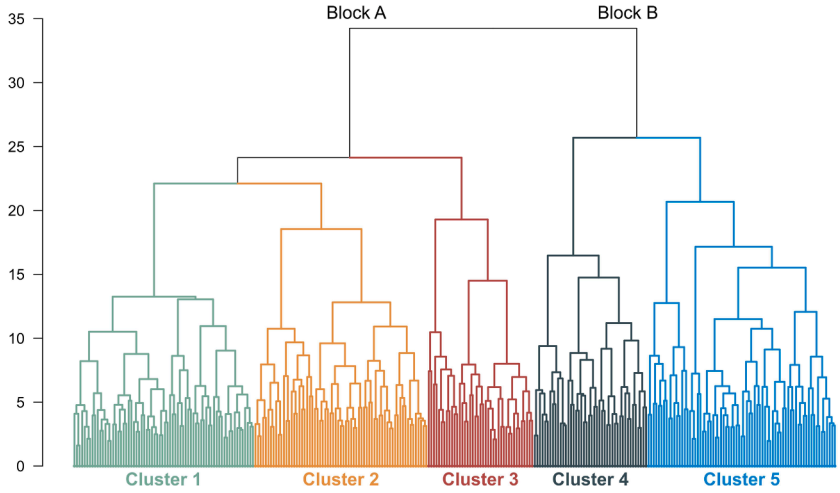


Figure 1 | Dendrogram showing the identification of 5 clusters in the 295 patients. The length of the vertical lines represents the degree of dissimilarities between groups and subgroups of patients.

staining of C1q, IgG, IgM, and fibrinogen (Table 3 and Supplementary Table S2, Figures 2 and 3). In addition, this cluster showed a high frequency of genetic abnormalities (21% of carriers) and NeFs (64%), and a high prevalence of nephrotic syndrome (52%) at onset (mean age: 15.5 years). Histology showed IC-MPGN in 75% of patients (Supplementary Figure S2).

The 41 patients belonging to cluster 3 had low serum C3 levels, but at variance with clusters 1 and 2, plasma sC5b-9 was normal or mildly elevated. This cluster had the highest prevalence of NeFs (87.8%), and intramembranous highly electron-dense deposits (70.7%), and the highest percentages of glomeruli with crescents (Figure 2, Table 3). Notably, 25 of 35 patients with DDD were in this cluster.

Clusters 4 and 5 included 44 and 73 patients, respectively. They shared a rather normal complement profile (Table 3) and a lower degree of endocapillary proliferation than clusters 1–3 (Figure 2, Table 3).

Among all clusters, cluster 4 revealed the highest percentage of sclerotic glomeruli and the highest degrees of arterial sclerosis and interstitial fibrosis and inflammation (Figure 2, Table 3). In addition, disease onset occurred later (35.6 ± 17.9 years) than in the other clusters, and 11.4% of patients required dialysis at onset (Table 3).

Compared with cluster 4, cluster 5 had a higher prevalence of genetic abnormalities (carriers: 26%), less glomerular and arteriolar sclerosis, and an earlier disease onset (19.1 ± 15.1 years) (Table 3 and Figure 2).

Although cluster 5 was not identified in our previous study,[22] it is noteworthy that among the 168 patients included in both analyses (Supplementary Table S3), the majority of patients originally assigned to cluster 4 in the previous model are now divided between clusters 4 and 5. This reclassification aligns with the subsequent branching step in the earlier cluster analysis, which would have led to the division of cluster 4.

Both clusters 4 and 5 included equal proportions of patients with C3GN and IC-MPGN (Supplementary Figure S2).

The main characteristics of the 5 clusters are summarized in Table 3 and in Figures 2 and 3.

There were no significant differences between clusters in the use of renin-angiotensin-aldosterone system inhibitors, immunosuppressive agents, or the proportion of patients receiving supportive therapy alone during the first year after diagnosis (Table 3). Long-term kidney outcomes did not differ among the various treatment subgroups within each cluster (Supplementary Figure S3).

In-depth characterization of autoimmune and genetic complement abnormalities among clusters

The large majority of NeFs in clusters 1 and 2 showed C5NeF activity[11,13] (83% and 78% among NeF^+ patients, respectively, Figures 3 and 4a), consistently with the evidence of *in vivo* fluid phase terminal complement pathway activation (Figure 3). Conversely, cluster 3 patients showed the highest prevalence of C3NeF (72%, Figures 3 and 4a), according to *in vivo* fluid phase complement activation mainly restricted at the C3 level.

The prevalence of anti–factor H antibodies was low in all clusters except for cluster 3 (11.8%, Figures 3 and 4a). Anti-FB antibodies were most prevalent in clusters 1 and 2 (Figures 3 and 4a); 27 of 38 anti-FB^+ patients (71%) fell in these 2 clusters, and 17 of 27 anti-FB^+ patients (63%) had elevated sC5b-9 levels.

Finally, we found rare functional genetic abnormalities in 46 patients (Supplementary Table S4). Patients in cluster 5 showed mostly isolated genetic variants, whereas in the other clusters, genetic variants were commonly associated with NeFs or other antibodies (Figure 4b). Complement factor H (CFH) and complement factor H-related (CFHR) genetic abnormalities were more frequent in cluster 5, whereas C3 variants were found in all clusters apart from cluster 4 (Supplementary Figure S4).

Prognostic value of clusters

Longitudinal clinical data were available for 293 patients. For the 48 patients who received complement inhibitors

Table 3 | Clinical features at onset, histologic parameters, and complement abnormalities in patients classified according to clusters

Parameter	Cluster 1 (n = 70)	Cluster 2 (n = 67)	Cluster 3 (n = 41)	Cluster 4 (n = 44)	Cluster 5 (n = 73)	*P* value
Gender (female)	0.41 (29 of 70)	0.51 (34 of 67)	0.44 (18 of 41)	0.2 (9 of 44)	0.42 (31 of 73)	NS
Data at onset						
Age (yr)	12 (8–17.97; n = 70)	12 (10–17.29; n = 67)	11.41 (9.5–15; n = 41)	34.94 (20.28–53; n = 44)[a]	15.05 (6–26.94; n = 73)	*P*
Trigger	0.55 (36 of 65)[b]	0.17 (11 of 66)	0.33 (13 of 39)	0.16 (7 of 43)	0.29 (21 of 72)	*P*
Family history of nephropathy	0.04 (3 of 70)[c]	0 (0 of 67)[c]	0.29 (12 of 41)	0.11 (5 of 44)	0.29 (21 of 72)	*P*
Hematuria (total)	0.9 (63 of 70)	0.82 (55 of 67)	0.92 (37 of 40)	0.68 (30 of 44)	0.78 (57 of 73)	NS
Gross hematuria	0.36 (25 of 70)	0.18 (12 of 67)	0.48 (19 of 40)	0.14 (6 of 44)	0.32 (23 of 73)	NS
Proteinuria (total)	0.91 (64 of 70)	0.97 (65 of 67)	0.9 (37 of 41)	0.89 (39 of 44)	0.9 (66 of 73)	NS
Urine protein (g/24 h)	3.16 (1.05–6.05; n = 47)	3.16 (1.11–7.28; n = 44)	3.48 (0.99–4.95; n = 27)	4 (2.56–5.50; n = 23)	1.67 (0.6–5.12; n = 33)	NS
Nephrotic syndrome	0.39 (27 of 70)	0.52 (35 of 67)	0.41 (17 of 41)	0.36 (16 of 44)	0.22 (16 of 73)	NS
Renal impairment (total)	0.19 (13 of 70)	0.25 (17 of 67)	0.2 (8 of 41)	0.5 (22 of 44)	0.27 (20 of 73)	NS
Serum creatinine (mg/dl)	0.75 (0.55–1; n = 43)	0.7 (0.52–0.96; n = 41)	0.65 (0.53–0.77; n = 26)	1.4 (1.07–2.82; n = 30)[a]	0.9 (0.69–1.11; n = 31)	*P*
Need of dialysis	0 (0 of 70)	0.01 (1 of 67)	0 (0 of 41)	0.11 (5 of 44)	0.01 (1 of 73)	NS
Histology						*P*
Time onset to biopsy, yr	0.34 (0.17–1.11; n = 70)	0.43 (0.08–1.59; n = 67)	0.39 (0.12–1.14; n = 41)	0.96 (0.08–3.57; n = 44)	1.30 (0.18–5.44; n = 73)	NS
Subendothelial deposits	0.86 (56 of 65)[d]	0.8 (51 of 64)	0.3 (12 of 40)[a]	0.7 (28 of 40)	0.62 (41 of 66)	*P*
Intramembranous DDD deposits	0.06 (4 of 70)	0.01 (1 of 67)	0.71 (29 of 41)[a]	0.12 (5 of 43)	0.03 (2 of 73)	*P*
Mesangial deposits	0.88 (60 of 68)[e]	0.55 (36 of 66)	0.56 (22 of 39)	0.79 (33 of 42)	0.62 (43 of 69)	*P*
Subepithelial deposits	0.69 (45 of 65)[a]	0.37 (24 of 65)	0.21 (8 of 38)	0.32 (13 of 40)	0.42 (27 of 65)	*P*
Crescents	0 (0–0; n = 69)	0 (0–0; n = 67)[f]	0 (0–0.408; n = 40)[g]	0 (0–0.11; n = 44)	0 (0–0; n = 71)	*P*
Mesangial proliferation	2 (2–3; n = 70)	2.5 (1–3; n = 67)	2 (1.5–3; n = 41)	2 (1.875–3; n = 44)	2 (1–2.5; n = 72)	NS
Endocapil proliferation	2 (1–2.5; n = 69)[f]	2 (1–2.25; n = 67)[f]	1.25 (0–2.63; n = 40)	0 (0–2; n = 44)	0 (0–1; n = 72)[h]	*P*
Interstitial fibrosis	0 (0–1; n = 70)	0 (0–0.25; n = 67)	0 (0–0; n = 41)	2 (1–2; n = 44)[a]	0 (0–1; n = 70)	*P*
Interstitial inflammation	0 (0–1; n = 70)	0 (0–1; n = 67)	0 (0–1; n = 41)	2 (1–2; n = 44)[a]	0.5 (0–1; n = 72)	*P*
Sclerotic glomeruli	0 (0–0; n = 70)[d]	0 (0–0.05; n = 66)	0 (0–0.05; n = 41)	0.28 (0.09–0.45; n = 44)[a]	0 (0–0.14; n = 72)	*P*
Arteriolar sclerosis	0 (0–0; n = 70)	0 (0–0; n = 66)	0 (0–0; n = 41)	1 (0.375–2; n = 44)[a]	0 (0–0; n = 70)	*P*
C3	3 (3–3; n = 70)[i]	3 (3–3; n = 67)[j]	3 (2.5–3; n = 40)	3 (2–3; n = 44)	2.5 (2–3; n = 72)	*P*
Fibrinogen	0 (0–0; n = 70)	0 (0–2; n = 64)[a]	0 (0–0; n = 38)	0 (0–0; n = 44)	0 (0–0; n = 72)	*P*
C1q staining	0 (0–0; n = 69)[f]	1 (0.125–2; n = 66)[a]	0 (0–0.75; n = 39)	0 (0–1; n = 43)	0 (0–1; n = 73)	*P*
IgA	0 (0–0; n = 69)	0 (0–0.5; n = 67)	0 (0–0; n = 38)	0 (0–0; n = 44)	0 (0–1; n = 72)	NS
IgG	0 (0–1; n = 69)	2 (0–2.5; n = 67)[a]	0 (0–1; n = 38)	0 (0–2; n = 44)	0 (0–1.25; n = 72)	*P*
IgM	0 (0–1; n = 69)[k]	1 (0.5–2; n = 67)[l]	1 (0.5–1; n = 38)	0.5 (0–1; n = 44)	0.5 (0–1; n = 72)	*P*
Complement						*P*
Plasma sC5b-9	975 (296–1908; n = 66)[i]	1329 (364–2474; n = 65)[j]	337 (217–774; n = 38)	297 (206–444; n = 40)	267 (202–401; n = 68)	*P*
Serum C3	20 (10–39; n = 68)	20 (12–42; n = 67)	18 (9–47; n = 40)	80 (54–93; n = 41)[h]	88 (55–111; n = 68)[h]	*P*
Serum C4	19 (13–24; n = 68)	17 (12–26; n = 66)	18 (14–27; n = 40)	23 (19–29; n = 41)	24 (16–29; n = 67)	NS
LPV/CNV Carriers	0.07 (5 of 69)	0.21 (14 of 67)	0.1 (4 of 40)	0.09 (4 of 43)	0.26 (19 of 73)	NS
NeF positive	0.33 (20 of 60)[m]	0.64 (42 of 66)	0.9 (35 of 39)[a]	0.36 (15 of 42)	0.06 (4 of 68)[n]	*P*

Treatment[o]						*P*
RAAS inhibition	0.69 (47 of 68)	0.74 (49 of 66)	0.76 (31 of 41)	0.72 (31 of 43)	0.75 (52 of 69)	NS
Immunosuppression	0.7 (49 of 70)	0.82 (54 of 66)	0.72 (29 of 40)	0.66 (29 of 44)	0.69 (49 of 71)	NS
Corticosteroids (no MMF)	0.53 (37 of 70)	0.56 (37 of 66)	0.61 (25 of 41)	0.52 (23 of 44)	0.54 (38 of 71)	NS
MMF ± corticosteroids	0.17 (12 of 70)	0.26 (17 of 66)	0.1 (4 of 41)	0.14 (6 of 44)	0.15 (11 of 71)	NS
Supportive only	0.09 (6 of 70)	0.03 (2 of 66)	0.1 (4 of 41)	0.11 (5 of 44)	0.1 (7 of 71)	NS

CNV, copy number variations; DDD, dense deposit disease; IF, immunofluorescence; LPV, likely pathogenic variants; MMF, mycophenolate mofetil; NeF, nephritic factors; NS, not significant; RAAS, renin angiotensin aldosterone system.
P: significant after Bonferroni correction for multiple comparisons ($P < 1.5 \times 10^{-4}$).
[a]$P < 0.05$ vs. all.
[b]$P < 0.05$ vs. 2, 4, 5.
[c]$P < 0.05$ vs. 3, 5.
[d]$P < 0.05$ vs. 5.
[e]$P < 0.05$ vs. 2, 3, 5.
[f]$P < 0.05$ vs. 4.
[g]$P < 0.05$ vs. 1, 2, 5.
[h]$P < 0.05$ vs. 1, 2, 3.
[i]$P < 0.05$ vs. 4, 5.
[j]$P < 0.05$ vs. 3, 4, 5.
[k]$P < 0.05$ vs. 3.
[l]$P < 0.05$ vs. 1, 4, 5.
[m]$P < 0.05$ vs. 2.
[n]$P < 0.05$ vs. 1, 2, 4.
[o]During first year after diagnosis.
Numerical variables are expressed as proportion (frequency/N) or median (interquartile range). N = number of patients with raw data available.
Serum C3: reference 90–180 mg/dl, C4: reference 10–40 mg/dl, and plasma sC5b-9: reference ≤400 ng/ml.
Degrees of arteriolar sclerosis, mesangial proliferation, endocapillary proliferation, interstitial fibrosis, and interstitial inflammation, as well as IF stainings were scored with a scale 0–3.

Figure 2 | Mean vectors of variables influencing cluster assignment. C3NeF, C3 nephritic factor; CNV, gene copy number variations; DDD, dense deposit disease; Deps, deposits; LPV, likely pathogenic variants; prolif, proliferation.

		Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
Glomerular C3	*Score*	2.8	2.9	2.7	2.4	2.4
Rare genetic abnormalities	%	7	21	10	9	26
NeFs	%	33	64	90	36	6
C3NeF	*Fraction*	0.17	0.22	0.72	0.3	0
C5NeF	*Fraction*	0.83	0.78	0.28	0.7	1
Anti-CFB	%	23	20	3.3	4.8	11
Anti-CFH	%	4.8	3.1	11.8	4.8	1.6
Serum C3	*mg/dl*	↓↓	↓↓	↓↓	N	N
Plasma sC5b-9	*ng/ml*	↑↑	↑↑	N↑	N	N
Glomerular IgG	*Score*	0.5	1.5	0.5	0.7	0.8
Glomerular C1q	*Score*	0.2	1.3	0.3	0.7	0.4
Highly electron-dense deps	%	6	2	71	11	3

Figure 3 | Clusters differentiate for complement abnormalities. Summary representation of complement abnormalities in each cluster. Anti-CFB, anti–complement factor B antibodies; Anti-CFH, anti–complement factor H antibodies; deps, deposits, N, normal; NeF, nephritic factor.

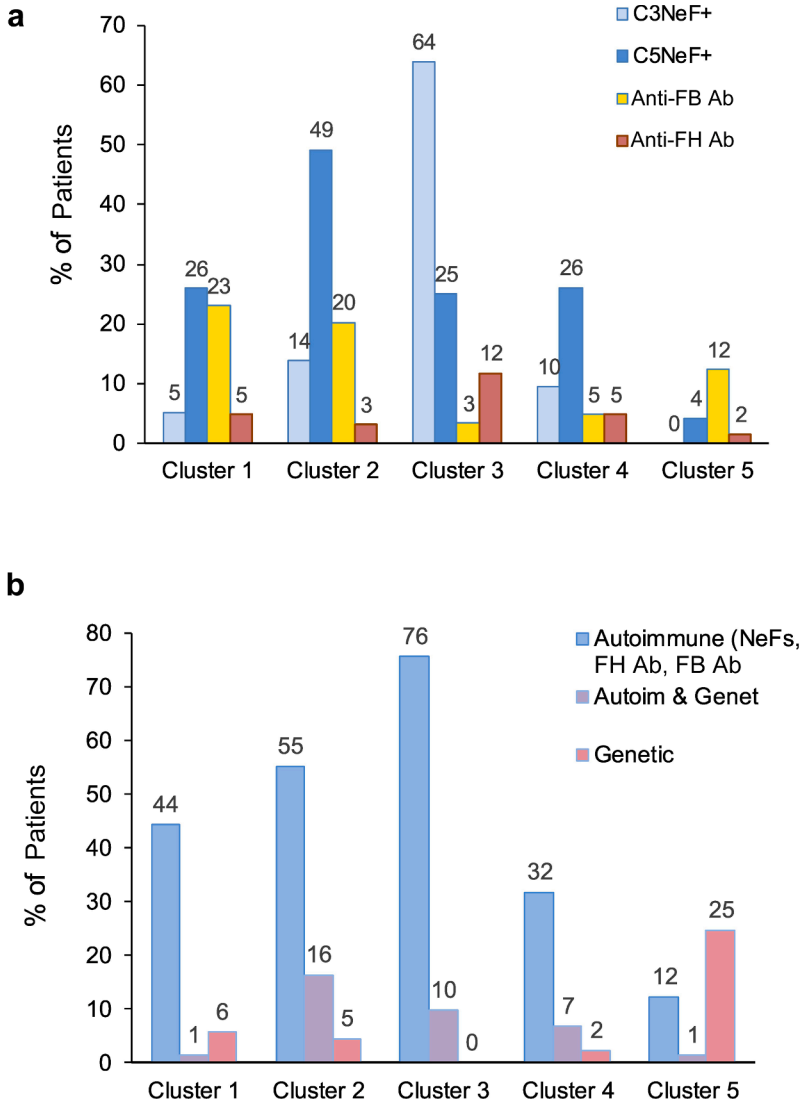
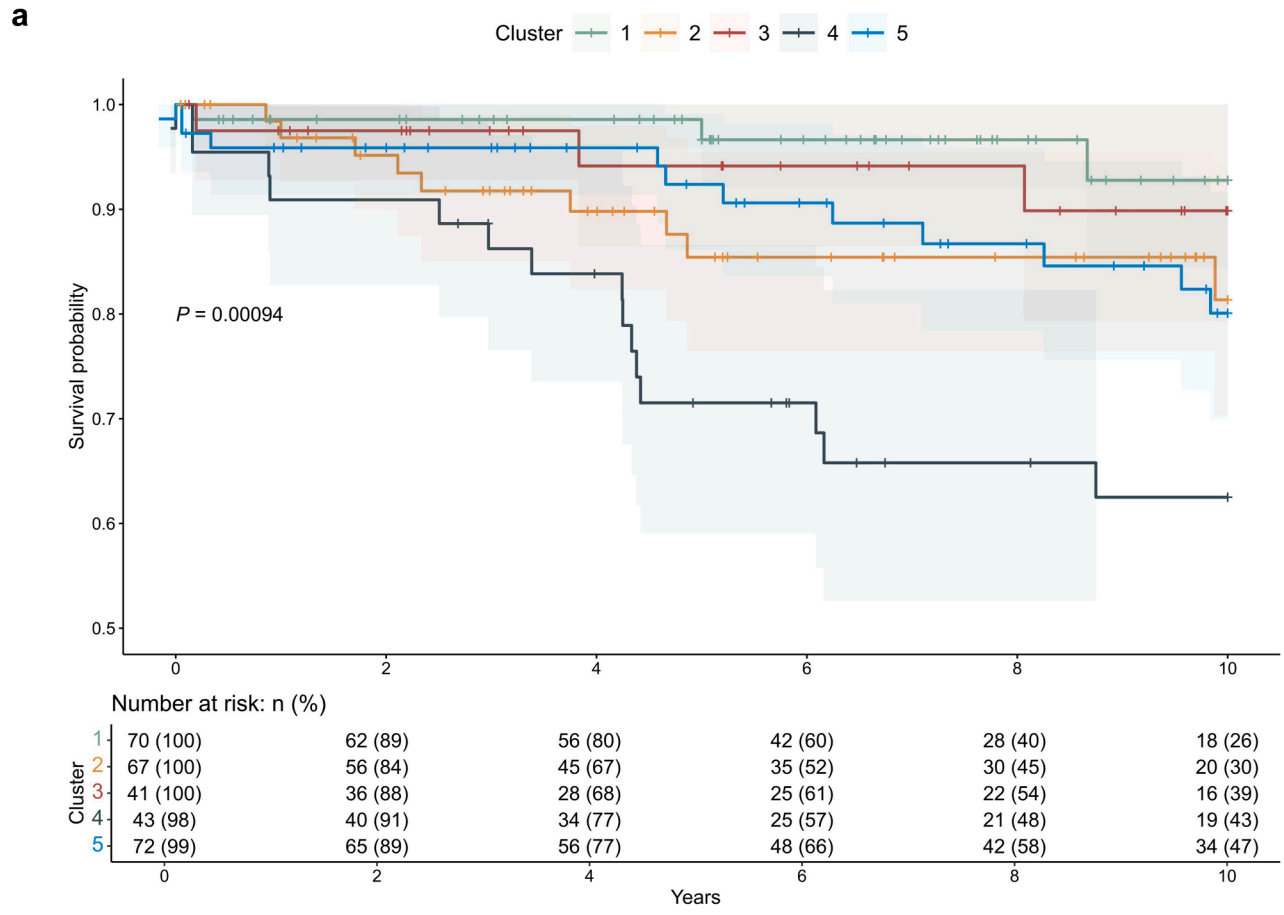


Figure 4 | Distribution of complement abnormalities across the 5 clusters. The percentage of patients positive for each parameter is given for each cluster. (**a**) Autoimmune abnormalities. C3NeF+: positive in the C3NeF assay. C5NeF+: positive in the C5NeF assay. Anti-FB Ab: positive in the enzyme-linked immunosorbent assay (ELISA) for anti-FB antibodies. Anti-FH Ab: positive in the ELISA for anti-FH antibodies. C3NeF and C5NeF analyses were performed in 109 of 116 NeF+ patients. Anti-FH and anti-FB antibodies were assayed in 268 and 262 patients, respectively. The χ^2 test for distribution among clusters: C3NeF/C5NeF: $P < 0.0001$, anti-FH: $P = 0.21$, and anti-FB: $P = 0.019$. (**b**) Distribution of autoimmune (NeFs, anti-FB antibodies, and anti-FH antibodies) and genetic abnormalities alone and combined across the 5 clusters: Autoimmune: positive for autoimmune abnormalities alone. Genetic: carrier of genetic abnormalities alone. Autoim & Genet: double positive for autoimmune and genetic abnormalities. χ^2 test, overall $P < 0.0001$. FB Ab, factor B antibodies; FH Ab, factor H antibodies; NeF, nephritic factor.

(eculizumab [n = 37], iptacopan [n = 6], danicopan [n = 3], and avacopan and pegcetacoplan [n = 1]), the follow-up was discontinued at the last observation before the start of anti-complement therapy. The 5 clusters had distinct renal outcomes, with the best prognosis seen in cluster 1 and the worst prognosis in cluster 4 (Figure 5), over an average follow-up of 9.74 years from onset (cluster 1: 8.6 ± 7.2, cluster 2: 8.6 ± 7.6, cluster 3: 9.6 ± 7.6, cluster 4: 10.0 ± 9.4, and cluster 5: 12.2 ± 10.7 years).

Kaplan-Meier analysis showed that patients in cluster 4 had the lowest kidney survival probability at 10 years (Figure 5a). The hazard risk (with cluster 1 taken as the reference group) to develop end-stage kidney disease within 10 years in cluster 4 was 7.82 (95% confidence interval: 2.26–27.03, $P = 0.0012$).

At the last available follow-up, 52% of patients in cluster 4 had developed end-stage kidney disease, versus 13%, 23%, 17%, and 31% in clusters 1, 2, 3, and 5, respectively (Figure 5b and Supplementary Table S5, $P < 0.0001$). Conversely, in cluster 1, 74% of patients maintained a normal renal function, and approximately half (44%) had normal range proteinuria compared with 0% in cluster 4 (Figure 5b and Supplementary Table S5, $P < 0.0001$). The older age, higher prevalence of kidney



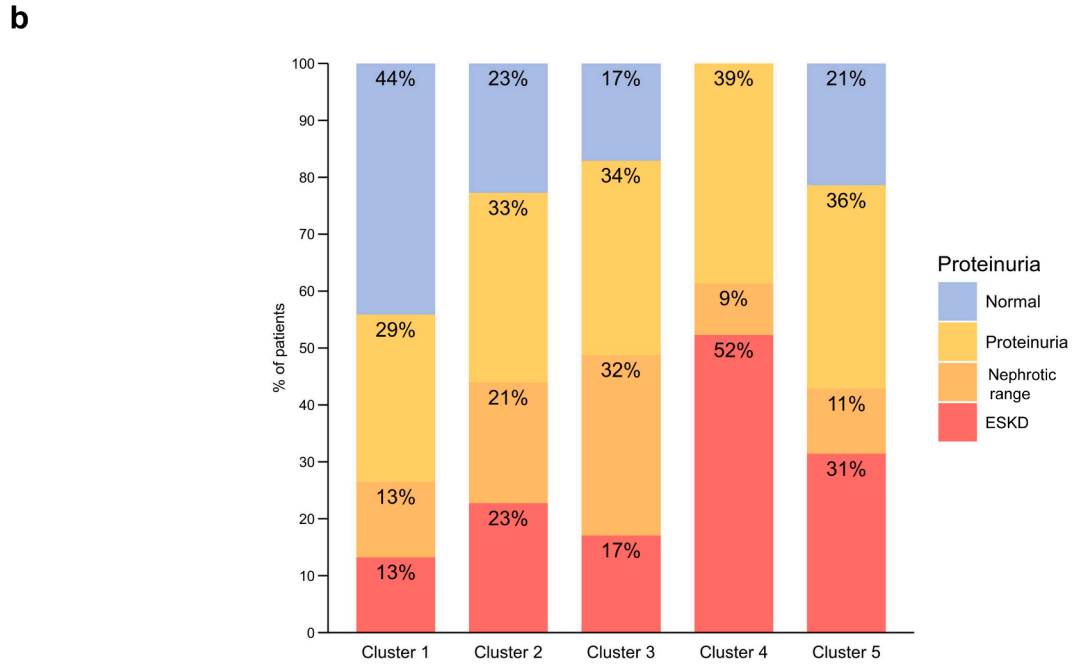


Figure 5 | Patients in cluster 4 have poor renal outcomes. (**a**) Kaplan-Meier analysis for survival from end-stage kidney disease (ESKD) according to the clusters. Patients were censored at last available follow-up with clinical information or at the event (need of dialysis or transplantation). For patients who received complement inhibitors, the follow-up was discontinued at the last observation with clinical data before the start of anticomplement therapy. (**b**) Histograms showing the distribution of patients with normal range proteinuria (normal, <0.3 g/24 h), with non-nephrotic proteinuria (proteinuria, 0.3–3.49 g/24 h), with nephrotic range proteinuria (nephrotic range, ≥3.5 g/24 h), or requiring renal replacement therapies (ESKD), at last follow-up. $P < 0.0001$; χ^2 test.

impairment at onset, and the higher percentage of sclerotic glomeruli of cluster 4 patients could contribute to worse outcomes because these parameters were independently associated with end-stage kidney disease in the whole cohort (Supplementary Table S6).

The percentage of patients with normal range proteinuria at follow-up did not differ between clusters 2, 3, and 5; however, nephrotic syndrome was more prevalent in cluster 3 (32%, Figure 5b and Supplementary Table S5).

At variance with clusters, histologic classification into C3GN, DDD, and IC-MPGN did not show a prognostic significance for long-term kidney survival (Supplementary Figure S5).

Among the 58 patients receiving a first kidney transplant, 24 (41%) had a biopsy-proven recurrence and 16 of them lost the graft for recurrence. Kaplan-Meier analysis showed a trend for higher risk of recurrence in the first kidney transplant in clusters 1 and 2, which however did not reach statistical significance (Figure 6a, $P = 0.09$). At the last follow-up after the first kidney transplant, we observed the highest prevalence of patients experiencing disease recurrence in cluster 1 (80%) and the lowest in cluster 5 (21%) (Figure 6b, $P = 0.018$ and Supplementary Table S7). There were no major differences in donor type or immunosuppression across clusters. Cluster 4 had the oldest recipients, consistent with the later disease onset in this group (Supplementary Table S7). Five additional patients received prophylactic anticomplement therapy (all with eculizumab) after transplantation, of whom one had a recurrence.

Evaluation of the relevance of the clinical, histologic, genetic, and biochemical variables for patient classification

The 29 different variables are expected to have a varying impact on cluster assignment of a new patient. We repeated leave-one-out bootstrapping using only 28 of the 29 variables and calculated the "loss-of-recall" for the corresponding left-out variable as the difference of the obtained recall to the original recall. We found that highly electron-dense deposits and percentage of crescents, IgA and fibrinogen staining, and presence of a trigger event were the most important variables. Missing one of these variables led to a loss of recall between 4% and 6% (details are given in Supplementary Figure S6 and Supplementary Table S8). Missing all immunofluorescenc variables decreased cluster recall by almost 16% (Supplementary Table S9). In contrast, percentage of sclerotic glomeruli, subendothelial deposits, and presence of renal impairment did not substantially improve classification (loss of recall $\leq$0.5%). The other variables showed an intermediate impact.

A user-friendly, interactive web app for cluster assignment

We implemented a computational tool that allows assignment of patients to a cluster using a set of genetic, laboratory, and clinical parameters. The tool provided essential data portraying analysis results of the cohort of 295 patients including, among others, the patients' variable landscapes and 2-dimensional patient maps (Figure 7a and b, respectively; details in Supplementary Material, section "A user-friendly classification platform for MPGN patients"). It allows mapping a new patient into these analyses and predicting a personalized variable portrait (Figure 8). Specifically, the tool complements the support vector machine, 3-NN, and 9-NN classification probabilities by localizing the new patient in the 295-patient map and by providing a visual identity to compare with other patient and cluster portraits. The application of the tool was iteratively improved with focus on the impact of missing parameters and handling incomplete data as discussed above, and used to classify 14 patients newly recruited in the study (Supplementary Figure S7). The DECODE clustering web application is publicly available online (http://www.izbi.uni-leipzig.de/lha/interactive-mpgn-classification-tool/).

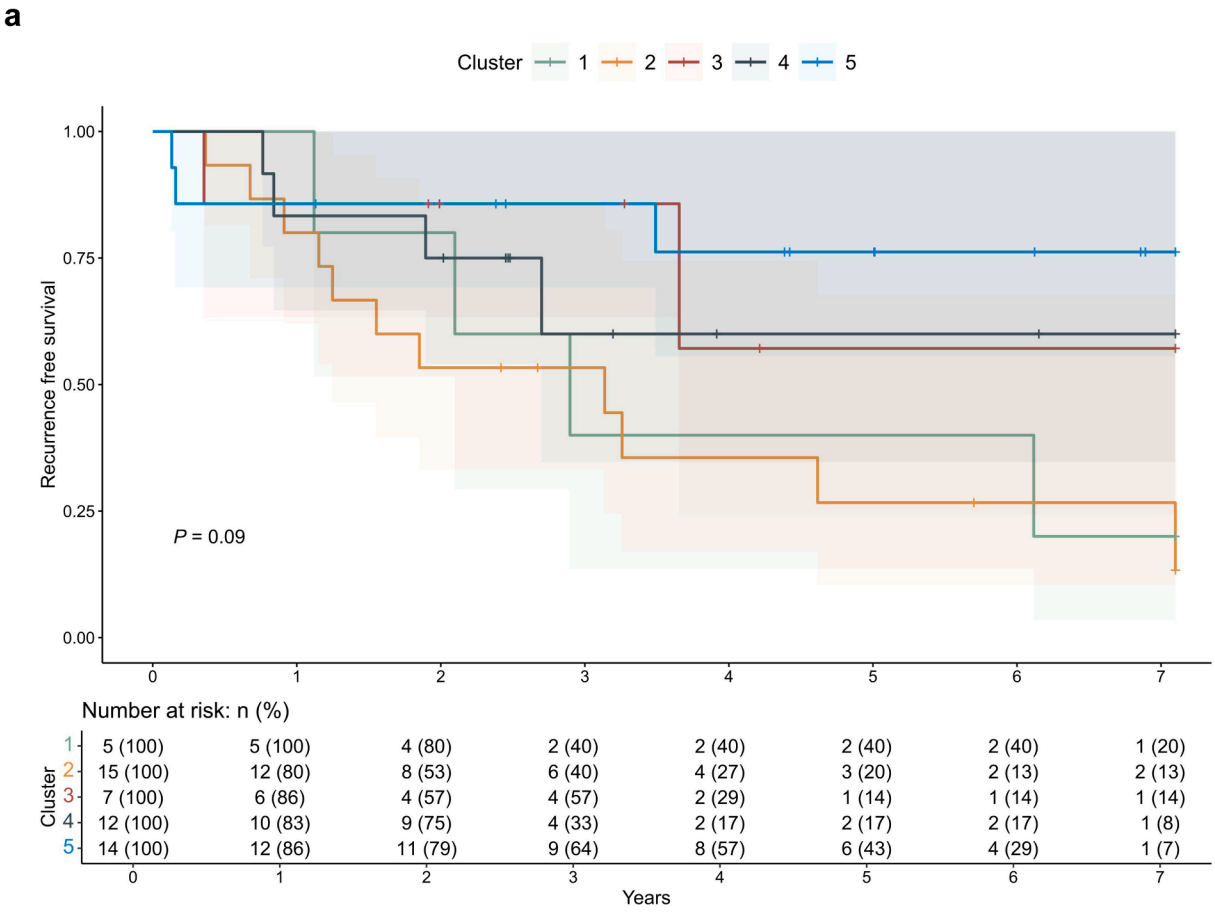
DISCUSSION

In a large cohort of 295 patients with C3G or idiopathic IC-MPGN, we demonstrate that a hierarchical clustering into 5 distinct groups outperformed the histology-based classification[4–6] in identifying underlying disease mechanisms. Unlike histology-based groups, the clusters demonstrated a prognostic value for long-term kidney outcomes and recurrence in the graft. To support clinical use, we developed a publicly available web application that estimates cluster assignment for new patients by comparing their profile with the 295 patients of the reference cohort.

This study, along with our previous publication in a smaller C3G/IC-MPGN cohort,[22] and other independent studies[17,32–34] challenge the notion of C3 and immunoglobulin glomerular staining as disease dichotomous discriminators.[5,35] Our findings align with earlier observations of complement activity and disease phenotype patterns among clusters.[22] Low C3 and high sC5b-9 levels indicating fluid-phase alternative pathway activation till the terminal pathway were observed in clusters 1 and 2, with classical pathway activation in the latter. Fluid-phase alternative pathway activation mainly restricted at the C3 level and intramembranous highly electron-dense deposits characterized cluster 3. Cluster 4 had normal C3 and sC5b-9 levels but bright glomerular C3 staining, indicating local complement activation. We also identified a new cluster 5, resembling cluster 4 in solid-phase complement activation but differing by younger onset and less biopsy evidence of sclerosis, fibrosis, and inflammation.

The expanded cohort improved clustering power, while separating NeFs and genetic abnormalities likely contributed to reveal cluster 5. The rarity of genetic abnormalities in cluster 4, contrasted with their enrichment in cluster 5, supports the above hypothesis.

Another novelty of this study in respect to our previous one[22] arises from in-depth characterization of autoimmune and genetic defects, which identified distinct pathogenetic mechanisms among clusters. In clusters 1 and 2, C5NeFs[13] and anti-FB antibodies[32,36] were prevalent. This finding is consistent with the *in vivo* complement profile of low C3 and



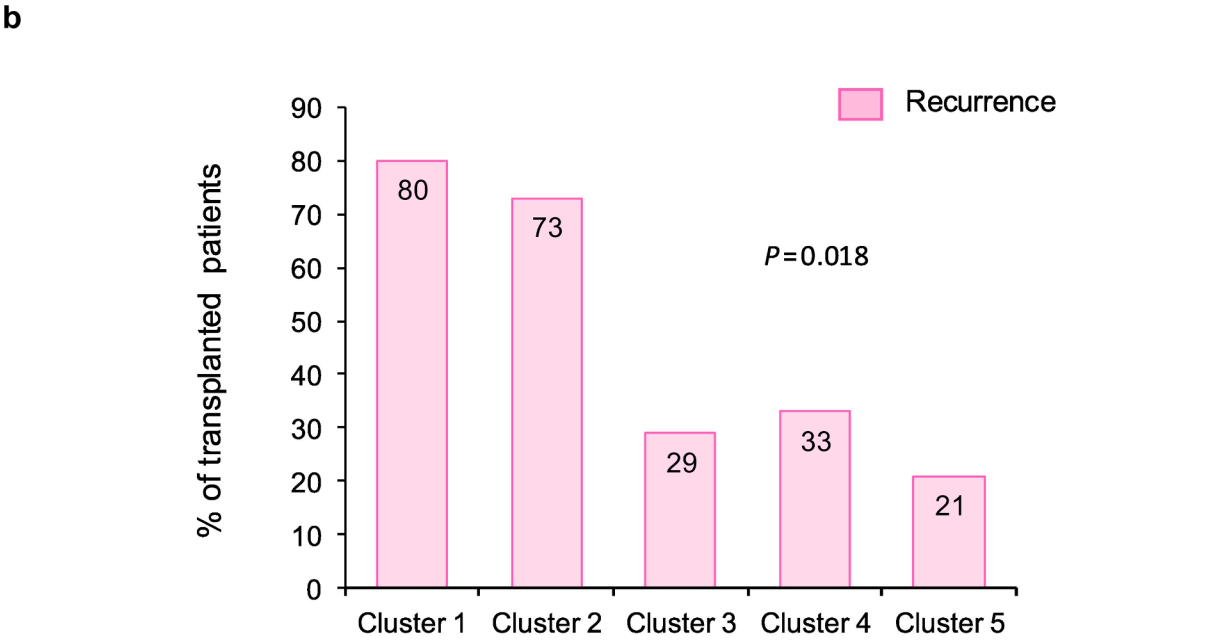


Figure 6 | Patients in clusters 1 and 2 have poor kidney transplant outcomes. (**a**) Kaplan-Meier analysis for survival from biopsy-proven recurrence according to the clusters. Patients were censored at last available follow-up with clinical information or at the event (need of dialysis or re-transplantation or death). The vast majority of post-transplant recurrences (>80%) were diagnosed based on *for-cause* allograft biopsies performed in patients with clinical suspicion of recurrence. (**b**) Histograms showing the distribution of kidney transplant patients with no recurrence and with post-transplant recurrence ($P = 0.018$; χ^2 test) at last follow-up.

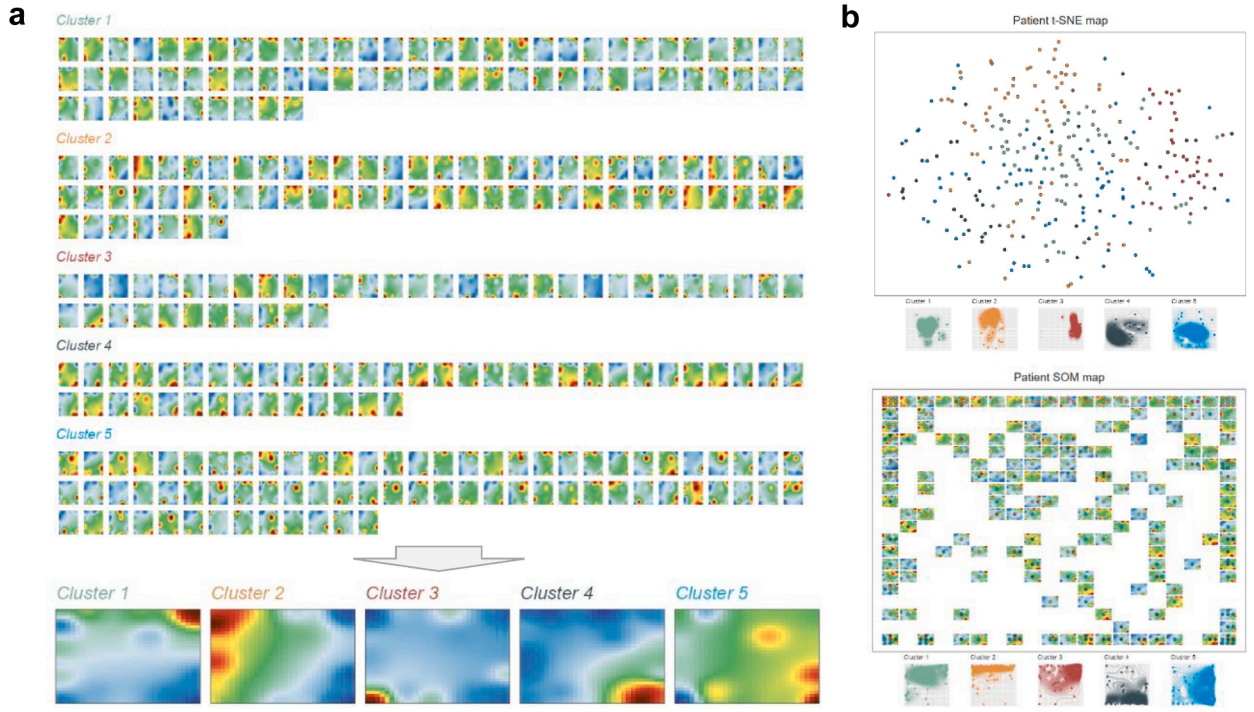


Figure 7| Features' and patients' landscapes of the 295 patients with C3 glomerulopathy (C3G)/immune complex–mediated membranoproliferative glomerulonephritis (IC-MPGN). (**a**) Single patients' variable landscapes are averaged into cluster-specific landscape portraits. (**b**) Patient landscapes of the 295 patients with C3G/IC-MPGN are derived from t-SNE (top panel) and patient SOM (bottom panel) approaches. SOM, self-organizing maps; t-SNE, t-distributed stochastic neighbor embedding.

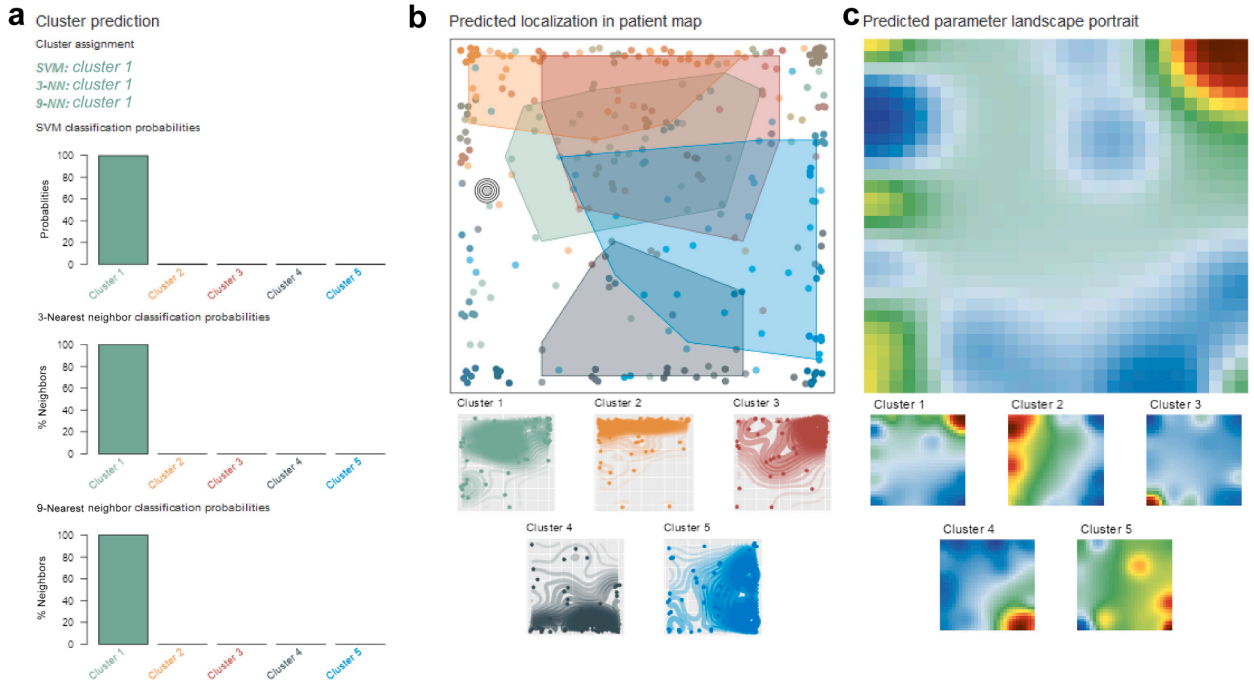


Figure 8| The interface for clinicians allows assignment of new patients to a cluster and provides a visual control of the results. (**a**) Classification of a patient is performed by different algorithms (SVM and kNN) using the provided parameters. (**b**) The patient is also mapped into the patient map (the round target, located close to the cluster 1 core region), allowing to compare its position with the specific regions of the clusters. (**c**) A personalized clinical portrait of the patient is generated for direct comparison with the cluster-specific portraits shown below. Details of data portraying and the color codes are given in Supplementary Material (section "A user-friendly classification platform for MPGN patients"). k-NN, k-nearest neighbors; SVM, support vector machine.

high sC5b-9 in these patients because C5NeFs stabilize the alternative pathway C5 convertase[11,13] and anti-FB antibodies overactivate the C3 convertase.[32,37,38] In clusters 1 and 2, fluid-phase activation products of C3 and C5 accumulate in the glomerulus, promoting electron-dense deposit formation and recruiting inflammatory cells, as reflected by marked endocapillary proliferation. The 2 clusters differ in that cluster 2 also showed strong glomerular C1q and immunoglobulin staining. We propose that in cluster 1, disease was primarily driven by autoimmune abnormalities of the alternative pathway, whereas in cluster 2, complement activation likely began via the classical pathway—triggered by immunoglobulin-antigen complexes—and was sustained by autoimmune and genetic factors promoting chronic alternative pathway overactivation.

One could argue that clusters 1 and 2 were incidentally enriched with postinfectious GN cases because anti-FB antibodies were found frequently in acute postinfectious GN.[39] We discard this possibility because we excluded patients with typical postinfectious GN from our cohort. In addition, in 84% of anti-FB^{+} patients, the antibodies were detected at >6 months from onset, unlike their rapid disappearance in postinfectious GN.[39]

Regarding cluster 3, the high prevalence of C3NeFs, which specifically stabilize the C3 convertase,[13] fits with *in vivo* complement profile of low C3 and mostly normal sC5b-9 levels. How this abnormality translated into the formation of intramembranous ribbon-like highly electron-dense deposits is still ill defined, although evidence is available that accumulation of apolipoprotein E along with complement proteins may contribute.[40]

The peculiar features of cluster 5, namely, the paucity of autoimmune abnormalities and a relatively high prevalence of gene abnormalities mainly affecting *CFH*, could explain the local kidney complement activation seen in this cluster, as factor H is a crucial regulator of alternative pathway on surfaces such as the fenestrae of glomerular endothelium and the glomerular basement membrane[41–43] lacking other complement regulators.[44–46]

A strength of our stratification model is its prognostic value, whereby cluster 4 had the highest incidence of end-stage kidney disease during follow-up, whereas most patients in cluster 1 retained normal kidney function long-term. Although clusters 4 and 5 share a relatively normal circulating complement profile, cluster 5 captures a subset of patients with earlier disease onset and better kidney outcome than cluster 4. This division indicates that the 5-cluster classification adds clinical and biological resolution as compared with the previous 4-cluster solution.[22] The finding that patients in cluster 4 had later onset and more severe chronic lesions[47] might suggest that this cluster was enriched with patients receiving a delayed diagnosis. However, time from disease onset to biopsy did not differ from cluster 5, which had fewer sclerotic lesions. Thus, we cannot definitively determine whether the findings in cluster 4 reflect delayed detection of subclinical disease or a more aggressive disease course.

In line with published data,[8,48] post-transplant recurrence was frequent across the C3G/IC-MPGN cohort and often led to graft loss. Recurrence was particularly frequent in clusters 1 and 2, possibly reflecting ongoing fluid-phase complement activation driven by autoimmune features.

The results of this work generated the hypothesis that cluster-based classification could aid decision-making in selecting the most suitable anticomplement therapy for individual patients. This approach may be particularly relevant, as a growing number of anticomplement drugs[21,49–55] are in clinical development for C3G/IC-MPGN. Given the complexity of the disease, it is unlikely that a single agent will be effective for all patients. On the basis of current insights, we propose that cluster 1 patients might benefit from FB inhibitors[21,49,50] and potentially from the anti-inflammatory effects of C5 inhibitors;[51–53] cluster 2 patients, who also show signs of classical pathway activation, may require broader complement inhibition at the C3 level;[50] cluster 3 patients could be more responsive to alternative pathway inhibitors[54] than to C5 blockade. For clusters 4 and 5, therapies targeting complement activation on cell surfaces[50,55] may offer advantages, while minimizing the side effects associated with systemic complement inhibition. These associations remain speculative, and additional studies will be important to assess the prognostic relevance and potential therapeutic implications of clusters.

Other groups have previously used either histologic or serologic parameters to identify subgroups of patients with C3G/IC-MPGN and prognostic markers.[56,57] Our study integrates all available information with additional clinical and genetic parameters through the clustering approach.

The assembly of a sufficiently large cohort of patients required recruitment from several centers over more than 15 years. This was done by necessity due to the rarity of the disease. We overcame this limitation by the rigorous data collection structure of our registry and careful revision of histologic diagnoses. In addition, biochemical data at onset and follow-up were verified and updated for this study, whereas complement genetic and autoimmune analyses were centralized in our laboratories. Nonetheless, because of missing data, renal function and proteinuria were categorized as ordinal instead of continuous variables, which may have limited the level of detail captured in the analysis.

Given the complexity of our clustering model, its direct use for disease classification in clinical practice is currently impractical. To address this, we developed a publicly available web application that, on input of data at diagnosis, provides clinicians with the probability of assigning new patients to a specific cluster. This tool may support the integration of clustering approaches into prospective datasets. Future studies should also investigate whether cluster membership may change over time, which could further refine its clinical utility. However, validation by independent

groups remains a prerequisite before clinical implementation of the application.

In conclusion, this study proposes a classification of primary C3G/IC-MPGN into 5 clusters based on clinical, histologic, and complement profiling data at disease onset, which promises to be instrumental to diagnose, predict the prognosis, and support clinical management by tailoring therapeutic strategies, toward a precision medicine. This model is extendable to other complex complement-related conditions.

APPENDIX

Contributors to the Italian Registry of MPGN

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DISCLOSURE

MN has received honoraria from Alexion Pharmaceuticals for giving lectures and for participating in advisory boards, and she has received research grants from Omeros, Gemini, Novartis, and BioCryst Pharmaceuticals. AB has received honoraria from Alexion Pharmaceuticals and BioCryst Pharmaceuticals. ED has received honoraria for lectures and advisory boards from Novartis and Apellis-Sobi. GR has consultancy agreements with AbbVie, Alexion Pharmaceuticals, Novartis Pharma, and BioCryst Pharmaceuticals. MV has consultancy agreement with Novartis, Apellis, and SOBI; has received honoraria for lectures and advisory boards from Roche, Travere, BioCryst, Chemocentrix, Alexion, Glaxo, Santhera, PureSpring, Bayer, and WebMD; and participates as a principal investigator in clinical trials sponsored by Alexion, Novartis, Apellis, Bayer, Travere, Chinook, and Roche. FA has received honoraria from CSL-Vifor, AstraZeneca, and Bayer for giving lectures, and for participating in advisory boards. All the other authors declared no competing interests.

DATA STATEMENT

Sharing individual participant data with third parties was not specifically included in the informed consent form for the study, and unrestricted sharing of these data may pose the threat of revealing participants' identities, as permanent data anonymization was not carried out. To minimize this risk, individual participant data underlying the results reported in this article will be available 3 months after publication and for 5 years after publication. Researchers shall submit a methodologically sound proposal to raredis@marionegri.it. To gain access, data requestors will need to sign a data access agreement and obtain the approval of the local ethics committee.

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AUTHOR CONTRIBUTIONS

MN, AB, MB, and GR conceived the study. MN drafted the manuscript. MB conducted hierarchical clustering and performed other statistical analyses. RP, HL-W, MS, RD, ZI, CMe, MA, and LQ carried out the experiments. ED, MR, MM, CMa, EB, and GN retrieved, updated, and reviewed clinical and biochemical data from the MPGN Registry. ED, MM, and CC reviewed biopsy reports and diagnoses. SG and EB coordinated sample collection. MV, FE, GLM, EVi, AP, AR, GD, EVe, AA, GB, MG, FA, UM, and GM made substantial contributions to patient recruitment and clinical data collection. MN and AB were responsible for funding acquisition and study supervision. MN, AB, MB, ED, MM, and GR reviewed and edited the manuscript. All authors approved the final version of the manuscript.

Supplementary material is available online at www.kidney-international.org.

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