C3 Glomerulopathy and related disorders in children: Etiology-Phenotype Correlation and Outcomes

Running title

Childhood C3G; etiology and outcome

Authors

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Abstract

Background and objectives: Membranoproliferative Glomerulonephritis (MPGN) and C3 Glomerulopathy are rare and overlapping disorders associated with dysregulation of the alternative complement pathway. Specific aetiological data for paediatric MPGN/C3 glomerulopathy are lacking, and outcome data are based upon retrospective studies without aetiological data.

Design, setting, participants, and measurements: Eighty prevalent pediatric patients with MPGN/C3 glomerulopathy underwent detailed phenotyping and long-term follow-up within the National Registry of Rare Kidney Diseases (RaDaR).

Results: Central histology review determined 39 C3 glomerulopathy, 31 immune-complex MPGN and 10 immune-complex glomerulonephritis (GN) cases. Patients were aged 2-15 (median 9) years. Median complement C3 and C4 levels were 0.31g/L and 0.14g/L respectively; acquired (anti-complement autoantibodies) or genetic alternative pathway abnormalities were detected in 46% and 9% patients respectively, across all groups including immune-complex GN. Median follow-up was 5.18 (IQR 2.13-8.08) years. Eleven patients (14%) progressed to kidney failure with 9 transplants performed in 8 patients, 2 of which failed due to recurrent disease. Presence of >50% crescents on initial biopsy was the sole variable associated with kidney failure in multivariable analysis (Hazard Ratio 6.2, $p = 0.045$). Three distinct C3 glomerulopathy prognostic groups were identified according to presenting eGFR and >50% crescents on initial biopsy.

Conclusions: Crescentic disease was a key risk factor associated with kidney failure in a national cohort of pediatric MPGN/C3 glomerulopathy and immune-complex GN. Presenting eGFR and crescentic disease help define prognostic groups in pediatric C3 glomerulopathy.

Acquired abnormalities of the alternative pathway were commonly identified but not a risk factor for kidney failure.

Introduction

Membranoproliferative glomerulonephritis (MPGN) is a pattern of glomerular injury characterised by increased mesangial matrix and cellularity and thickening of capillary walls[1]. MPGN classifies into immune-complex MPGN and C3 glomerulopathy based on relative complement and immunoglobulin staining on biopsy. C3 glomerulopathy sub-classifies into dense deposit disease (DDD) with characteristic dense osmophilic intramembranous deposits and C3 glomerulonephritis (C3GN) where other patterns of electron dense deposition are seen[2].

Immune-complex-MPGN and C3 glomerulopathy are rare, with estimated incidence of 1-4 cases per million population[3,4]. Acquired and genetic abnormalities associated with fluid phase dysregulation of the alternative pathway of complement have been identified in immune-complex MPGN and C3 glomerulopathy[5-16].

Immune-complex MPGN and C3 glomerulopathy carry a poor kidney prognosis, with median time to kidney failure around 10 years from diagnosis[10,17-21]. Following kidney transplantation, disease recurrence occurs in the majority of grafts and is the predominant cause of graft failure in 50%-90% of transplant recipients[10,19, 22-27]

A diagnosis of MPGN/C3 glomerulopathy in childhood has lifelong consequences for children and their families. Pertinent questions focus on aetiology, treatment and prognosis. Until recently, most information to address these questions is extrapolated from cohort analyses, comprising mixed groups of adults and children[10, 11] or small paediatric cohorts[28, 29].

Our aim was to build a cohort of children with MPGN/C3 glomerulopathy in order to describe the spectrum of histological disease, investigate the frequency of acquired and genetic alternative pathway defects and define clear prognostic groups to facilitate counselling and stratify emerging therapeutic options in children. We extended the cohort to include patients with immune-complex glomerulonephritis without MPGN pattern, who did not fulfil

diagnostic criteria for IgA nephropathy or systemic lupus erythematosus, whom we hypothesised may also have underlying alternative pathway dysregulation.

Here we report our findings from the National Study of MPGN, dense deposit disease and C3 glomerulopathy, which recruited children from all paediatric nephrology centres in Great Britain, using the infrastructure of the National Registry of Rare Kidney Diseases (RaDaR; https://rarerenal.org/radar-registry/).

Materials and Methods

Study Design

Patients were recruited into a multicenter observational cohort study from all pediatric kidney units in Great Britain. Prevalent patients with a diagnosis of MPGN, dense deposit disease, C3 glomerulonephritis, or immune-complex glomerulonephritis were identified by local clinicians and were eligible to be invited for recruitment into the study. Patients were recruited between 2011 and 2015.

Histopathologic data

Expert central pathology review included the original light microscopy, the original biopsy report and where available, immunostaining and electron microscopy. Kidney biopsies were classified according to the C3 glomerulopathy consensus report into 4 different sub-groups – i) C3 glomerulonephritis and ii) dense deposit disease (together comprising C3 glomerulopathy) and iii) immune-complex MPGN (immune-complex MPGN) and iv) immune-complex GN (together comprising immune-complex disease (IC-disease)) (Figure 1a).

Clinical and laboratory information

Clinical data was entered into clinical record forms into the RaDaR database and included height, serum creatinine, albumin and urinary P:Cr or A:Cr, C3, C4 and C3 nephritic factor to collect baseline (the time of initial diagnostic biopsy). Estimated glomerular filtration rate (eGFR) was calculated using the modified Schwartz formula[30].

UK kidney units routinely report clinical data to the Renal Registry via RaDaR – this data was extracted to provide prospective longitudinal data and determine outcomes.

Treatment information

Details of any use of angiotensin-converting enzyme inhibitors and angiotensin receptor blocker (ACE/ARB) or immunosuppression during the clinical course were extracted from RaDaR. Treatments were used at clinician's discretion. In general, patients received 1. no immunosuppression at any time - angiotensin-converting enzyme inhibitors and angiotensin receptor blocker (ACE/ARB) use; 2. corticosteroids and no other immunosuppression; 3 corticosteroids and mycophenolate mofetil (MMF). We identified a further group of patients receiving other non-specific immunosuppression that we further sub-divided into those using any of azathioprine, calcineurin inhibitor and an intense group for those who received any of cyclophosphamide, rituximab, eculizumab or plasma exchange at any time.

Complement testing, autoantibodies and genetics

Complement and autoantibody testing

At recruitment, blood samples were collected for further complement studies. Serum C3 and C4 were measured by immunoturbidimetric assays (Roche Cobas Analyser).

Screening for C3 nephritic factor was performed by immunofixation[1].

Screening for autoantibodies to FH using ELISA was performed as described previously, including epitope binding studies to short consensus repeats (SCR) 1-7 (N-terminus), 8-15, 16-18 and 19-20 (C-terminus)[2]. The ELISA was adapted to screen for autoantibodies to C3b and

FB using purified proteins (Comptech) and FHR proteins using recombinant FHR proteins generated in mammalian cell lines. Specificity of antibodies to FHR proteins was determined by western blotting. Screening for autoantibodies to CD35, CD46, CD55 was performed as described previously[3].

Control samples, as indicated, were randomly selected from a batch of 200 healthy blood donors (National Health Service Blood and Transplant) which were normally distributed ranging in age from 17 to 72 years of age, median age was 44, 56% female, 95% White-Caucasian). The 97.5 percentile was used to assign positive results.

Complement factor H-related protein 5 (CFHR5) was detected by western blotting using patient sera under non-reducing conditions. Plasma soluble C5b-9 (sC5b-9) levels were measured as described[4].

Genetic screening

Genetic screening of all exons and flanking regions of *C3*[5], *CFB*[6], *CFH*[7], *CFI* [8], *CD46*[9] and *DGKE*[10] was performed and rare genetic variants and common polymorphisms were identified following targeted next generation sequencing and confirmatory Sanger sequencing. Rare genetic variants were defined as minor allele frequency <0.01 in the exome variant server database (evs.gs.washington.edu). Screening for genomic disorders affecting *CFH*, *CFHR1*, *CFHR2*, *CFHR3* and *CFHR5* was undertaken using multiplex-ligation probe amplification[11].

Definitions and Outcomes

Duration of follow up was from baseline until latest available eGFR or kidney failure (eGFR<15ml/min/1.73m^2, onset of maintenance dialysis or pre-emptive transplantation). Patients with eGFR >90ml/min/1.73m^2 at latest follow up or if <90ml/min 1.73m^2, at latest follow up, within 15 ml/min/1.73m^2 of baseline eGFR kidney function were classified as

having either 1. Complete remission if latest urinary P:Cr <50mg/mmol or equivalent or 2. partial remission if latest urinary P:Cr between 50 and 300 mg/mmol or equivalent. Crescentic glomerulonephritis was defined as having crescents within >50% of viable glomeruli.

Statistical Analysis

Statistical analysis was performed using SPSS software (IBM). Baseline clinical and histological characteristics were expressed as median (interquartile range) for continuous variables and percentage for categorical variables. These were compared using Kruskal-Wallis (continuous variables) and Fishers exact (categorical variables). A Bonferroni correction was used for multiple comparisons. In order to determine risk factors for kidney survival, Cox proportional hazards models were used. We assessed baseline clinical, histological and complement risk factors, including complement levels at baseline and follow-up, presence of complement antibodies and rare genetic variants. Significant risk factors for kidney survival identified by unadjusted analysis were subjected to multivariable analysis. Kidney and transplant graft survival was determined using Kaplan-Meier method and group comparisons were performed using the log-rank test.

The MPGN/dense deposit disease/C3 glomerulopathy Rare Disease Group (RDG) of the Renal Association acted as a steering committee for the study.

Ethics statement

Ethical approval for this study was granted by North Somerset and South Bristol Research Ethics Committee (Ref 09/H0106/72, 12-11-09). Patients were included following informed consent / assent in accordance with the Declaration of Helsinki.

Results

Study Cohort

Eighty patients were recruited into the study, median 1.95 years (IQR 0.25 – 4.13) from baseline and followed up for median 5.18 (IQR 2.13-8.08) years. Following central histopathologic review, thirty-nine patients were classified as C3 glomerulopathy, including 14 patients with dense deposit disease and 25 with C3GN. The other 41 patients with IC-disease were classified as immune-complex MPGN (31 patients) and immune-complex GN (10 patients) (Figure 1a). Fifty-one of the 80 patients in this study were included in the recent NIHR BioResource Rare Diseases study which reported the results of whole genome sequencing and a genome wide association in 165 adult and paediatric patients with primary MPGN and C3 glomerulopathy[31].

Clinical Characteristics.

Patients were aged 2 to 15 (median 9) years at diagnosis (Figure 1b) and 45 (56.3%) patients were female. Patients typically presented with nephrotic-range proteinuria (68%), hypoalbuminaemia (76%) and hematuria (91%). Low eGFR (<90ml/min/1.73m^2) was a feature at presentation in 44% of patients. Patients with C3 glomerulopathy were the only patients to present with severe kidney dysfunction (eGFR <30 ml/min/1.73m^2) (Table 1).

Pathological features.

The most common pattern of glomerular injury was MPGN (55 patients; 69%), observed in 41 patients (100%) with immune-complex MPGN, 5 patients (36%) with dense deposit disease and 19 (76%) patients with C3 glomerulonephritis (Figure 1c). Other pathological features are summarised in table 1, notably crescentic glomerulonephritis was observed in 4 patients (5%), all dense deposit disease. Most patients displayed no evidence of chronic damage.

Complement abnormalities

C3 levels ranged from median 0.16 g/L in patients with DDD to 0.50g/L in patients with immune-complex GN. C4 levels were significantly lower in patients with immune-complex MPGN (median 0.12g/L) and immune-complex GN (median 0.13g/L) compared to patients with dense deposit disease (0.26g/L) (p=0.022) (Table 2).

Autoantibodies

Autoantibodies were identified in 37 patients (46%) (Table 2); C3 nephritic factor in 22 patients (39%), autoantibodies to FH (anti-FH) in 13 patients (17%) (Figure 2a), autoantibodies to FB (anti-FB) in 7 patients (9.1%) and autoantibodies to C3b (anti-C3b) in 5 patients (7%) (Figure 3). Eight patients had more than one autoantibody detected. (Table 3). There were no differences in serum C3 or C4 concentration at baseline regardless of whether an autoantibody was detected (Table 3).

C3 Nephritic factor was most likely to be detected in patients with dense deposit disease (62%; p = 0.042) (Table 2). Anti-FH bound predominantly to the N-terminus of FH in 10 of 13 patients (Figure 2b) and were not associated with the *CFHR*3/1 deletion in homozygosity (Table S1). The age of onset of disease in this group of patients was median 8 (range 3-11) years (Figure 2c).

C3 levels during follow up were lower (p=0.013) in patients who had detectable C3 nephritic factor. C4 levels during follow up were lower (p=0.027) in patients who had a detectable anti-FH Ab (Figure 4).

Autoantibodies to other complement regulatory proteins (FI, CD46, CD35, CD55 and CD59) (Figure S1) and FHR proteins (Figure S2) were not identified. Soluble C5b-9 levels at recruitment were elevated (median 223.3 ng/ml, (IQR 110.0-429.2), normal range <200ng/ml) (Table S2) though no trends associated with presence of complement autoantibodies or rare genetic variants

Genetic Analysis

Rare genetic variants in the complement genes examined were identified in six patients (8.6%) (Table 2). Of these, two patients had two rare genetic variants (Table S3). Most variants have previously been categorised as "likely benign" or of "uncertain significance"[32,33]. Three patients with rare genetic variants (50%) also had a complement autoantibody (Table S3).

The *C3* pR102G; c304C>G SNP was associated with a higher risk of dense deposit disease (Odds Ratio 3.14, p = 0.004; Table S4). None of the other SNPs were associated with a higher risk of disease (Table S4). No patients had the *CFHR*3/1 deletion in homozygosity (Table S1). There was no evidence of other copy number variation in this cohort (data not shown) and no genomic or proteomic evidence (Figure S4) of CFHR5 nephropathy. One previously reported patient with immune-complex GN had a likely pathogenic variant in *DGKE* found in homozygosity (c.323G>A; p.C108Y)[34] (Table 2).

Treatments

Treatments used in the cohort are summarised in Table 4 and S5. Overall, 16 patients (20%) received treatment with ACE/ARB only. The remainder all received immunosuppression with at least one agent, most commonly prednisolone (22 patients) or prednisolone in combination with MMF (17 patients). Fourteen patients received a more intense regimen of that included at least one of the following: rituximab, cyclophosphamide, plasma exchange or eculizumab.

Patients receiving ACE/ARB only were less likely to have eGFR <90ml/min/1.73m^2 (p=0.002) or albumin < 35g/l (p=0.006) at baseline. Patients receiving a more intense regime of immunosuppression were more likely to have C3 glomerulopathy (p = 0.001), eGFR < 90ml/min1.73m^2 (p < 0.001) or crescentic glomerulonephritis (p = 0.001).

Outcomes: disease remission

Complete or partial remission was observed in 28 patients (71%) with C3 glomerulopathy and 36 patients (88%) with immune-complex-disease. Amongst patients with C3 glomerulopathy, complete or partial remission was less likely amongst patients presenting with low albumin (p=0.013) or abnormal eGFR (p=0.012) and those receiving intense immunosuppression (p = 0.008) (Table S6). No clinical features were associated with a lower likelihood of remission in patients with immune-complex-disease (Table S7). The presence of C3 nephritic factor or anti-FH antibodies were not associated with remission in patients with either C3 glomerulopathy (Table S6) or those with immune-complex disease (Table S7).

Outcomes: kidney survival

During the follow-up period, 11 (14%) patients had progressed to kidney failure. In a multivariable analysis that included C3 glomerulopathy, crescentic GN, glomerulosclerosis, eGFR<90 at presentation and intense immunosuppression, only crescentic GN remained significantly associated with kidney failure (p=0.045, HR 6.2). The finding of rare complement gene variants or autoantibodies to complement components or complement levels at baseline or at follow-up did not associate with progression to kidney failure.

Kidney survival according to histological sub-group is shown in Figure S4a. We stratified patients with C3 glomerulopathy into three groups with significantly different short- and medium-term outcomes (Figure S4b). Of the patients with C3 glomerulopathy, all 14 patients with eGFR >90ml/min/1.73m^2 at baseline did not progress to kidney failure during the course of this study. All 8 patients with C3 glomerulopathy that progressed to kidney failure had eGFR <90ml/min/1.73m^2 at baseline. Amongst these, a pattern of crescentic GN identified patients with the shortest kidney survival, (mean = 1.7 years) compared with those that did not have crescentic GN (mean 8.3 years; p = 0.009). Three patients with IC-disease reached kidney failure, including two patients with immune-complex MPGN that did not progress to kidney failure until after 10 years.

Outcomes: kidney transplant

Of 11 patients that progressed to kidney failure, 8 have undergone kidney transplantation. Out of 9 transplant grafts, there were 4 cases of recurrent disease (all C3 glomerulopathy) of which 2 were lost due to recurrent disease (Figure S5).

Discussion

We report comprehensive etiological and outcome data from a national pediatric cohort of MPGN/C3 glomerulopathy.

Cohorts comprising immune-complex MPGN, dense deposit disease and C3 glomerulonephritis are well described (Table S8) and the distribution of these within our cohort is comparable, suggesting individual phenotypes are not seen more commonly in children. The predominant age for children to present (between 7 and 11 years) is in keeping with previous studies[28].

We identified acquired alternative pathway abnormalities in approximately half of patients, including in patients with immune-complex GN, suggesting a role of complement dysregulation in immune-complex GN and further studies are required.

C3 nephritic factor was the most commonly detected autoantibody in our cohort, though detected in a lower proportion than in previously reported mixed age-group cohorts[10, 11]. Our lower rate of C3 nephritic factor may be due to our wide ascertainment of cases or could reflect a lower prevalence of C3 nephritic factor in children.

Anti-FH in our cohort were identified in a comparable proportion of patients to previous reports[15, 16, 35] and we confirm specificity of anti-FH in MPGN/C3 glomerulopathy to the N-terminal regulatory domain of FH and the lack of association with *CFHR3/CFHR1* homozygous deletion in our cohort, in keeping with previous studies[15, 16]. We identified patients with anti-FB and anti-C3b, both previously reported in cohorts of immune-complex

MPGN and C3 glomerulopathy [36]. The proportion with anti-FB is in keeping with the recent study showing anti-FB in 14% of C3 glomerulopathy patients in contrast to 91% of patients with post infectious glomerulonephritis [37].

The rate of rare genetic variation in our cohort was low in comparison to larger cohorts[10, 11] and could be due our wide ascertainment of cases or a lower rate in children compared to adults. However, the predominance of acquired abnormalities compared to genetic is comparable to previous cohorts[10, 11]. A possible explanation for an autoimmune basis of MPGN/C3 glomerulopathy has been postulated in a recent study (to which 51 of our 80 patients contributed data), which showed an association of HLA type with MPGN/C3 glomerulopathy [31].

C3 levels were comparable, regardless of whether we identified an alternative pathway abnormality. It is possible that patients with no alternative pathway abnormality detected in our cohort have an acquired alternative pathway abnormality that we have yet to identify (e.g. C5 nephritic factor) and further work is being undertaken to assess this.

We also found that C4 levels were lower at presentation compared to at follow up in patients with immune-complex disease. In previous mixed age-group cohorts, low presenting C4 was reported in up to 15%, (Table S8) and in 25% of the previous largest paediatric cohort[28]. The finding of lower C4 may indicate a transient response to a triggering infection in paediatric MPGN/C3 glomerulopathy. In keeping with this is the observation C4 levels were higher at recruitment to the study. C4 levels were lower at follow up in those with anti-FH antibodies, implying ongoing classical pathway activation, possibly triggered by deposited antibody/complement immune complexes[38].

Meanwhile, antibodies to other complement proteins were not detected (FI, CD35, CD46, CD55 and FHR proteins) and the finding of only one patient with a variant in *DGKE*

(previously described in MPGN[39]) suggesting that these do not play a major role in the aetiology of MPGN/C3 glomerulopathy.

This study did not set out to determine treatment efficacy, our data is limited to which treatments were used and are unable to take into account their timing in relation to disease onset and relationship to complement biomarkers that were performed upon recruitment to our study. However, our data helps provide an overview of treatments used in children with MPGN/C3 glomerulopathy. We report favourable outcomes in a majority of patients receiving either ACE/ARB only, or moderate immunosuppression, including either prednisolone only or prednisolone and MMF. The favourable outcomes of those receiving moderate immunosuppression are comparable with those in previous observational studies of children receiving prednisolone only[40] or in mixed adult and paediatric cohorts receiving a combination of MMF and prednisolone[41-44]. Otherwise, controlled trials in immune-complex MPGN and C3 glomerulopathy are lacking, with only a randomised-controlled trial in children with MPGN (before the classification of immune-complex MGPN and C3 glomerulopathy) reporting a benefit in kidney survival of long term treatment with high-dose corticosteroids to placebo[45]; however such doses are associated with adverse effects. In contrast, a final group (14/80; 18%) in our cohort received intense immunosuppression. These patients were predominantly C3 glomerulopathy and characterised by low eGFR, or >50% crescents at presentation and suggest that at least in some patients, these clinical characteristics prompted clinicians to offer more intense immunosuppression. Despite these treatments, these patients had the poorest outcomes highlighting that currently available treatments are likely to be ineffective in some patients with MPGN and C3 glomerulopathy and the unmet need for novel therapies.

We found that patients with C3 glomerulopathy and normal kidney function at presentation had a low risk of progression to kidney failure during follow-up. These patients and those with

immune-complex MPGN appear to have a more favourable outcome than previous large cohorts[10, 11], and are comparable to a recently published paediatric cohort of a similar size[40]. This could reflect the wide ascertainment of our cohort and ongoing follow up is required to determine their longer-term risk of kidney failure. Nonetheless, these data help the clinician offer more bespoke counselling on prognosis, possibly distinguishing patients with potentially more favourable longer-term outcomes from those with the worst short-term outcomes.

The question as to whether some children in our cohort actually had post-infectious GN, contributing to a more favourable outcome is important. However the vast majority had evidence of ongoing kidney disease at recruitment many months after onset and those recruited <6 months after diagnosis did not have better outcome than those recruited > 6 months after diagnosis, which points away from inadvertent inclusion of post-infectious GN patients. Transplant recurrence rate was comparable to previously described cohorts[25, 27].

Our study has a number of strengths. The multi-center recruitment encouraged wide ascertainment, regardless of disease severity and minimising the bias of reporting from cases referred to a single specialist centre. We were able to conduct central pathology review, which ensured consistent classification across our multiple centers and we followed patients longitudinally through the RaDaR database. However the study also has specific limitations not already discussed. We cannot rule out the possibility that some patients eligible for recruitment in our study were not included. Complement biomarkers from disease onset were limited to serum C3, C4 and C3 nephritic factor and are reliant upon local assays and data for sC5b-9 and other complement antibodies could only be measured from samples taken at recruitment to study, a distinct time point from baseline. Finally, our cohort had relatively few patients progressing to kidney failure, which could explain why we did not find significant associations between the complement profile of our patients and outcomes.

In summary, we propose that in children diagnosed with MPGN/C3 glomerulopathy and immune-complex GN, a cause of alternative pathway dysregulation should be considered and that priority should be given to screening for acquired abnormalities. We would start with C3 nephritic factor and anti-FH autoantibodies, though screening for anti-FB, C3b or rare complement genetic variants could be considered if initial screening does not identify an abnormality.

Currently available treatment strategies including immunosuppression with a combination of MMF and corticosteroids may have a role in management in addition to supportive treatments with ACE/ARB, but that children with abnormal kidney function at presentation, especially those with crescentic disease should be considered a priority for studies of novel treatments including those targeting the alternative pathway.

Disclosures

S.J. has received honoraria from and attended advisory boards for Alexion Pharmaceuticals and has attended advisory boards for Novartis Pharmaceuticals.

D.K has received honoraria from and attended advisory boards for Alexion Pharmaceuticals, has attended advisory boards for Novartis Pharmaceuticals and has received research income from Ra Pharmaceuticals.

E.W has received honoraria from and attended advisory boards for Alexion Pharmaceuticals, has attended advisory boards for Novartis Pharmaceuticals and has attended advisory boards for BioCryst Pharmaceuticals.

D.P.G. has received honoraria from and attended advisory boards for Alexion Pharmaceuticals.

T.H.J.G. has received honoraria from and/or attended advisory boards for Alexion Pharmaceuticals.

KJM has conflicts/disclosures from Gemini Therapeutics Ltd (C, F), Freeline Therapeutics (C), MPM Capital (C, R), Catalyst Biosciences (C, F); where C is consultancy, R is renumeration and F is funded lab work and has received research income from Ra Pharmaceuticals

CLH has received consultancy income from, or has attended scientific advisory boards for GlaxoSmithKline, Roche, Q32 Bio, Freeline Therapeutics, Biocryst Pharmaceuticals; all income is donated to the University and has have received research income from Ra Pharmaceuticals.

The remaining authors have nothing to disclose.

Author Contributions:

S.A.J., T.H.C. and T.H.J.G. designed the study. S.A.J. was chief investigator. E.K.S.W. carried out experiments, analysed the data, made the figures, drafted and revised the manuscript. H.T.C

and R.M. carried out central pathology review with support from H.L-B. K. J. M., C.L.H., I.P., H.D.,K.C. G.R. B.P.M., S.H., and V.W. carried out experiments. M.P. and D.K. supervised experiments. P.McA. was study co-ordinator. M.C. and H.M. recruited the most patients to the cohort. S.D.M. recruited patients and was chair of the RDG. S.A.J., T.H.C, E.K.S.W., H.L-B., K.J.M., C.L.H., M.P., D.K., D.P.G. and H.M., are members of the RDG

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Supplemental material Table of Contents

Figure S2 Screening serum from patients with C3 glomerulonephritis, dense deposit disease, immune-complex MPGN and immune-complex GN for auto-antibodies against complement factor H related proteins 1-5 (FHR1-5)

Figure S3 Western blot to detect complement factor H related protein 5

Figure S4 Kaplan-Meier analysis of kidney survival a) whole cohort by pathology and b) C3 glomerulopathy by stratified sub-groups.

Figure S5 Kaplan-Meier analysis of transplant graft survival in patients with C3 glomerulopathy

Table 1 Clinical and pathological characteristics at presentation in paediatric C3 glomerulopathy, immune-complex MPGN and immune-complex GN.

		Number of patients with available data	C3 glomerulopathy		Immune-complex disease	
			C3 glomerulonephritis	dense deposit disease	immune-complex MPGN	immune-complex GN
Number of patients		80	25	14	31	10
Median (IQR) age in years		80	9 (7-12)	9.5 (6-11)	9 (6-11)	8 (3-8)
Number (%) of male patients		80	8 (32)	9 (64.3)	14 (45.2)	4 (40)
Number (%) of patients with nephrotic range proteinuria (P:Cr >300mg/mmol, A:Cr>250mg/mmol or 4+ on dipstick)		73	13 (61.9)	7 (53.8)	22 (73.3)	8 (88.9)
Number (%) of patients with serum albumin ≤ 35g/l		75	16 (72.7)	10 (71.4)	26 (83.9)	5 (62.5)
Number (%) of patients with haematuria		60	16 (88.9)	12 (100.0)	19 (86.4)	8 (100.0)
Number (%) of patients with eGFR <90		75	10 (43.5)	12 (85.7)	7 (23.3)	4 (50.0)
Number (%) of patients with eGFR < 30 (including patients requiring temporary KRT)		75	4[a] (17.4)	4[a](28.6)	0 (0.0)	0 (0.0)
Number (% of histological sub-group) of patients with specified pattern of glomerular injury	Mesangial Proliferative GN	80	4 (16.0)	5 (35.7)	0 (0.0)	7 (70.0)
	Diffuse endocapillary proliferative GN		2 (8.0)	0 (0.0)	0 (0.0)	2 (20.0)
	Crescentic GN		0 (0.0)	4 (28.6)	0 (0.0)	0 (00.0)
	Membranoproliferative GN		19 (76.0)	5 (35.7)	31 (100.0)	0 (0.0)
	Other		0 (0.0)	0 (0.0)	0 (0.0)	1(10)[b]
Number (% of histological sub-group) of patients with specified amount of glomerulosclerosis	None	74	18 (78.3)	12 (92.3)	23 (82.1)	9 (90.0)
	1-25		3 (13.0)	1 (7.7)	5 (17.9)	1 (10.0)
	26-50%		2 (8.7)	0 (0.0)	0 (0.0)	0 (0.0)
Number (% of histological sub-group) of patients with specified amount of crescents	None	74	18 (78.3)	3 (23.1)	26 (92.9)	7 (70.0)
	1-50%		5 (21.7)	6 (46.2)	2 (7.1)	3 (30.0)
	>50%		0 (0.0)	4 (30.7)	0 (0.0)	(0.0)
Number (% of histological sub-group) of patients with specified amount of interstitial fibrosis/tubular atrophy	None	69	15 (71.4)	10 (76.9)	18 (69.2)	8 (88.9)
	1-25%		5 (23.8)	3 (23.1)	6 (23.1)	1 (11.1)
	26-50%		1 (4.8)	0 (0.0)	2 (7.7)	0 (0.0)

eGFR, estimated glomerular filtration rate calculated by modified Schwartz formula and expressed in ml/min/1.73m^2; P:Cr, urinary protein:creatinine ratio; A:Cr, urinary albumin:creatinine ratio

[a] includes 1 patient with C3 glomerulonephritis and 3 patients with dense deposit disease requiring kidney replacement therapy (KRT). b One patient had focal and segmental necrotising GN

Table 2 Prevalence of complement abnormalities in C3 glomerulopathy, immune-complex MPGN and immune-complex GN.

	Number of patients with available data		C3 glomerulopathy		Immune-complex	
		All	C3 glomerulonephritis	dense deposit disease	immune-complex MPGN	immune-complex GN
Median (IQR) serum C3 at presentation g/L, n	57	0.31 (0.14-0.50) 57	0.39 (0.16-0.44) 18	0.15 (0.09-0.45) 11	0.23 (0.15-0.65) 21	0.50 (0.29-0.80) 7
Median (IQR) serum C4 at presentation g/L, n	57	0.14 (0.07-0.26) 55	0.19 (0.08-0.26) 16	0.26 (0.15-0.31) 11	0.12 (0.06-0.14 21	0.13 (0.07-0.18) 7
Number (%) of patients with C3 nephritic factor[a]	80	22 (28.9)	6 (26.1)	8 (61.5)	7 (23.3)	1 (10.0)
Number (%) of patients with anti-FH Ab	78	13 (16.7)	3 (12.5)	3 (21.4)	4 (13.3)	3 (30.0)
Number (%) of patients with anti-FB Ab	77	7 (9.1)	2 (8.3)	1 (7.1)	4 (13.8)	0 (0)
Number (%) of patients with anti-c3b Ab	77	5 (6.7)	0 (0)	2 (15.4)	3 (10.7)	0 (0)
Number (%) of patients with any complement autoantibody	80	37 (46.3)	10 (40.0)	9 (64.3)	14 (45.2)	4 (40.0)
Median (IQR) sC5b-9ng/L, n	72	223.3 (110.0-429.2) 72	401.0 (591.8) 21	324.8 (251.8) 12	378.6 (493.9) 30	346.1 (224.6) 9
Number (%) of patients with Rare Genetic Variant in complement gene[b]	70	6 (8.6)	1 (4.5)	1 (8.3)	4 (14.8)	0 (0)
Number (%) of patients with Rare Genetic Variant in *DGKE*	70	1 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (12.5)

Anti-FH Ab = anti-factor H autoantibodies, Anti-FB Ab = anti-factor B autoantibodies, Anti-c3b Ab = anti-c3b autoantibodies, sC5b-9 = soluble C5b-9, *DGKE* = Diacyl Glycerol Kinase Epsilon

n, number of patients with data available; IQR, inter-quartile range; %, percentages expressed as number of patients tested. [a] C3 nephritic factor detected at any point during presentation or follow up; [b] complement genes tested were *C3*, *CFB*, *CFH*, *CFI* and *CD46.* Comparisons made in shaded rows are between C3 glomerulopathy and immune-complex disease.

Table 3 – Complement profile of patients with anti-complement autoantibodies

	Complement Autoantibody						
	C3 Nephritic Factor	Anti-complement Factor H autoantibodies	Anti-complement Factor B autoantibodies	Anti-C3b autoantibodies	Any Antibody	No Detectable Antibody	P value
Number of patients testing positive (% of cohort)	22 (28.9)	13 (16.7)	7 (9.2)	5 (6.7)	37* (46.3)	43 (53.7)	
Serum C3 at presentation (g/l, median (IQR))	0.0.17 (0.0.09-0.50)	0.23 (0.14-0.52)	0.41 (0.09-1.15)	0.17 (0.15-0.17)	0.23 (0.13-0.69)	0.31 (0.14-0.49)	0.83
Serum C4 at presentation (g/l, median (IQR))	0.21 (0.11-0.26)	0.120 (0.09-0.26)	0.11 (0.0.08-0.26)	0.14 (0.14-0.15)	0.15 (0.06-0.26)	0.14 (0.10-0.26)	0.77
Plasma C5b9 at recruitment to study (ug/ml, median (IQR)	192.5 (95.21-360.10)	209.50 (121.15-998.3)	110.94 (48.89-339.40)	328.86 (130.81-415.64)	189.80 (103.12-342.24)	247.63 (146.47-466.02)	0.29
	Eight patients had more than one anti-complement autoantibody. All of these had a C3 nephritic factor plus additional autoantibodies as follows; 1 with anti-factor B, 3 with anti-factor H, 1 with anti-C3b, 1 with anti-factor H and anti-factor B, 2 with anti-factor H and anti-C3b autoantibodies						

P-values comparing patients with any antibody and those with no detectable antibodies (shaded)

IQR – interquartile range

Table 4 Treatments received in paediatric C3 glomerulopathy, immune-complex MPGN and immune-complex GN

		Number of patients	Treatment					
			ACE / ARB	Pred	Pred/MMF	Pred/+	Intense	
All Patients (n, %)		80	16 (20.0)	22 (27.5)	17 (21.3)	11 (13.8)	14 (17.5)	
Pathological sub-group Number (% of sub-group) receiving each treatment	C3 glomerulonephritis	25	7 (28.0)	4 (16.0)	3 (12.0)	4 (16.0)	7 (28.0)	
	dense deposit disease	14	2 (14.3)	2 (14.3)	3 (21.4)	1 (7.1)	6 (42.9)	
	immune-complex MPGN	31	4 (12.9)	12 (38.7)	8 (25.8)	6 (19.4)	1 (3.2)	
	immune-complex GN	10	3 (30.0)	4 (40.0)	3 (30.0)	0 (0)	0 (0)	
Number (%) of patients with nephrotic range proteinuria *		50	8 (16.0)	16 (32.0)	13 (26.0)	6 (12.0)	7 (14.0)	
Number (%) of patients with non-nephrotic range proteinuria		23	7 (30.4)	5 (21.7)	3 (13.0)	4 (17.4)	4 (17.4)	
Number (%) of patients with eGFR <90		33	1 (3.0)	9 (27.3)	7 (21.2)	4 (12.1)	12 (36.4)	
Number (%) of patients with eGFR >90		42	13 (31.0)	12 (28.6)	9 (21.4)	6 (14.3)	2 (4.8)	
Number (%) of patients with serum albumin <35g/l		57	7 (12.3)	15 (26.3)	13 (22.8)	9 (15.8)	13 (22.8)	
Number (%) of patients with serum albumin >35g/l		18	8 (44.4)	5 (27.8)	3 (16.7)	1 (5.6)	1 (5.6)	
Number (%) of patients with Histology showing >50% crescents		4	0	0	0	0	4 (100.0)	
Number (%) of patients with Histology showing <50% crescents		76	16 (21.1)	22 (28.5)	17 (22.4)	11 (14.5)	10 (13.2)	

ACE/ARB, Angiotensin Converting Enzyme inhibitor or Angiotensin Receptor Blocker; Pred, Prednisolone; MMF, mycophenolate mofetil; Pred/+, includes patients receiving Pred in combination with Azathioprine or tacrolimus; intense, includes patients that received any of rituximab, cyclophosphamide, plasma exchange or eculizumab.

* nephrotic range proteinuria defined as P:Cr >300mg/mmol, A:Cr>250mg/mmol or 4+ on dipstick P:Cr = urinary protein:creatinine ratio, A:Cr = urinary albumin:creatinine ratio

eGFR = estimated glomerular filtration rate calculated by modified Schwartz formula and expressed in ml/min/1.73m^2

n, number of patients; %, percentages expressed as number of patients tested.

[a] P-values comparing patients receiving intense immunosuppression and patients receiving any other treatments

[b] P-values comparing patients receiving ACE/ARB and patients receiving any other treatments

Figure 1

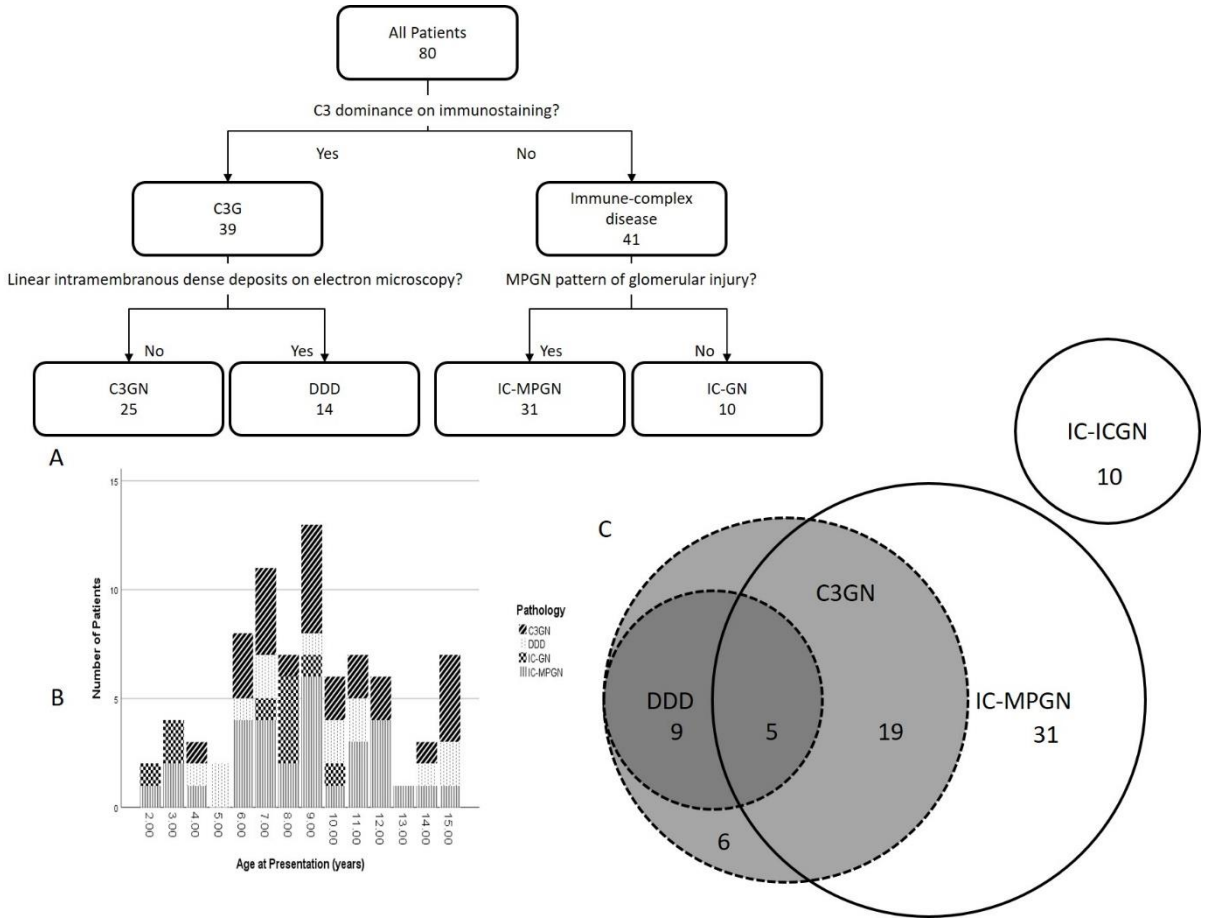

Figure 1a. Classification of pathology following central review. Patients with C3 glomerulopathy (C3G) were sub-classified into C3 glomerulonephritis (C3GN), dense deposit disease (DDD). Patients with non-C3 glomerulopathy had immune-complex forms of glomerulonephritis and were sub-classified into immune-complex membranoproliferative glomerulonephritis (IC-MPGN) and immune-complex glomerulonephritis (IC-GN) . 1b. Age distribution of patients. Age of presentation ranged from 2 years to 15 years, categorised by pathology classification. 1c. Overlap of C3 glomerulopathy (dashed-circles) and MPGN (dotted-circle). In total, 55 patient (shown in bold circle) in this cohort were noted to have an MPGN pattern of glomerular injury. In dotted circle are 39 patients with C3 glomerulopathy, sub-classified into C3 glomerulonephritis (outer area) and dense deposit disease (darkest grey, inner area). 19 patients (76.0%) with C3 glomerulonephritis and 5 patients (35.7%) with dense deposit disease have an MPGN pattern of injury. A further 41 patients included in this cohort do not have C3G and sub-classify into the 31 patients with an MPGN pattern of glomerular injury (immune-complex MPGN) and 10 patients with immune-complex GN (IC-GN). Of 25 patients now classified as C3 glomerulonephritis, prior to the availability of the C3 glomerulopathy consensus report, 19 patients would have been classified as MPGN and 6 patients would not have been classified at all.

Figure 2

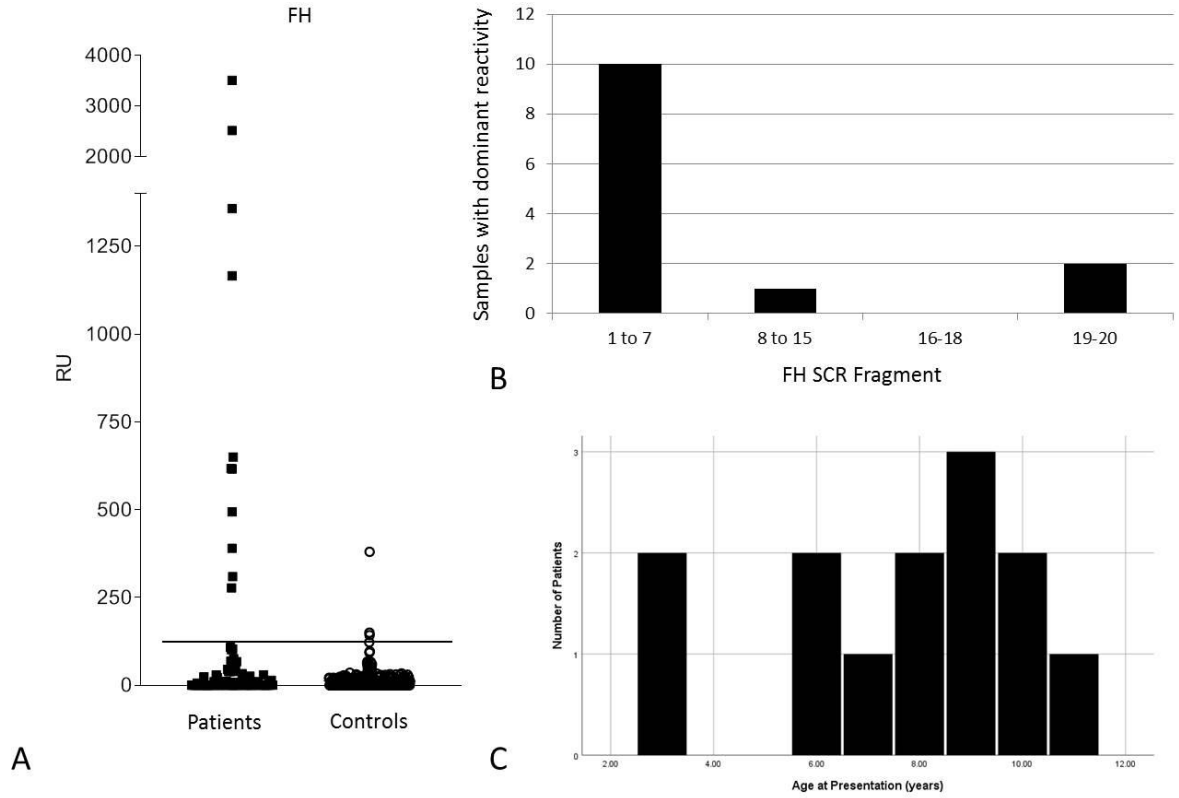

Figure 2 A. Screening serum from 78 patients with C3 glomerulonephritis, dense deposit disease, immune-complex MPGN and immune-complex GN for auto-antibodies against complement factor H (FH). Line indicates 97.5th percentile of samples from blood donor controls, the minimum threshold for identifying an autoantibody. RU = response units titrated to standard published in (Goodship et al., 2012). B. Epitope binding in patients with autoantibodies to FH was tested against short short consensus repeat (SCR) fragments (1-7, 8-15, 16-18 and 19-20). The predominant reactivity was to SCR1-7 in 10 cases out of 13. C. Age of onset of patients with auto-antibodies against complement factor H.

Figure 3

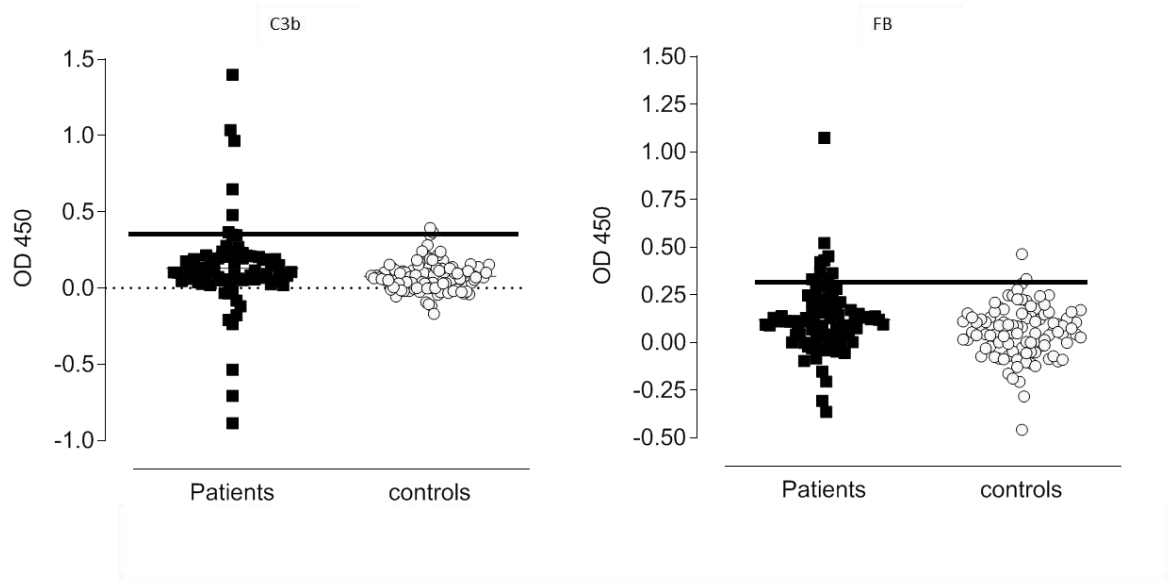

Figure 3. Screening serum from 77 patients with C3 glomerulonephritis, dense deposit disease, immune-complex MPGN and immune-complex GN for auto-antibodies against C3b and complement factor B (FB). Line indicates 97.5th percentile, the minimum threshold for identifying an autoantibody. Autoantibodies were identified against C3b (5 patients) and FB (7 patients.)

Figure 4

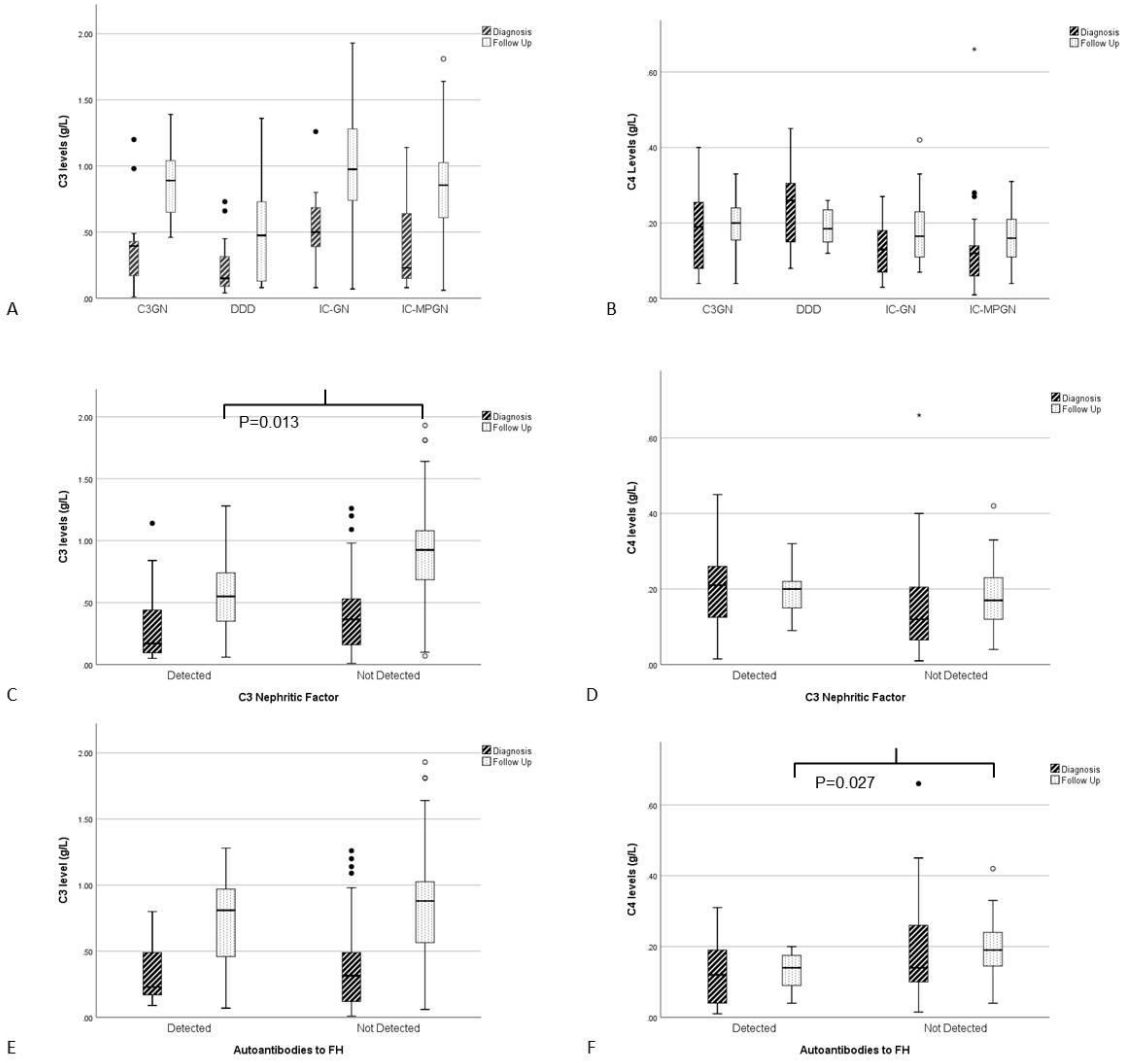

Figure 4 Box and whisker plot showing C3 and C4 levels at diagnosis and at follow up depending on (A+B) the 4 pathological sub-groups, and whether or not patients had (C+D) detectable C3 nephritic factor or (E+F) anti-FH autoantibody