

Complement analysis in children with idiopathic membranoproliferative glomerulonephritis: A long-term follow-up

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Fifty children with idiopathic membranoproliferative glomerulonephritis (MPGN), aged 2–14 years at apparent onset, were monitored for the presence of C3 nephritic factor (C3 NeF) and signs of complement activation in serum. In addition, C3 allotyping was performed in 32 patients. Observation time ranged from 2 to 20 (median 11) years. C3 NeF activity was detected at least once in 60% of the patients (in 11 of 26 with type I, in 15 of 17 with type II, and in four of seven with type III). C3 NeF-positive patients had significantly reduced levels of CH50 and C3 and elevated levels of C3dg/C3d. During follow-up, C3 levels were persistently normal in 62% of the patients with MPGN type I and in 43% with type III but in only 18% with type II. C3 allotype frequencies differed from those found in healthy controls with a significant shift to the C3F/C3FS variants in C3 NeF-positive patients. C3b(Bb)P as a marker for alternative pathway activation was not increased in C3 NeF-positive patients. Despite the presence of C3 NeF activity, C3 levels remained normal in six patients throughout the observation period. C3 NeF became undetectable in six patients, whereas seven developed C3 NeF activity during follow-up. There was no significant difference in renal survival probability in patients with or without C3 NeF activity. Neither C3 variants nor continuous low C3 or low CH50 levels had any prognostic value for the clinical outcome. No factor H deficiency was detected.

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Membranoproliferative glomerulonephritis (MPGN) is a progressive glomerular disease characterized by diffuse proliferative lesions and widening of the capillary loops (1,2). Based on the pathomorphological pattern, three types of MPGN have been described (3–6). Clinically, MPGN is characterized by severe proteinuria, usually of nephrotic degree, and a progressive course to end-stage renal disease (ESRD) in most patients (7–11).

Hypocomplementemia has been attributed to activation of complement (C) (Fig. 1) by circulating or tissue-deposited immune complexes or to hypercatabolism of activated complement com-

ponents (1,12,13), specifically of C3, the central component of the C system. In MPGN, consumption of C3 is mainly a result of the presence of C3 nephritic factor (C3 NeF) (14), which is predominantly found in patients suffering from the pathomorphological type II. This factor has also been observed in patients with partial lipodystrophy (15), meningococcal meningitis (16), and even in healthy individuals (17).

C3 NeF is an immunoglobulin G (IgG) or immunoglobulin M (IgM) auto-antibody, which seems to recognize neoantigenic structures on the alternative pathway convertase, C3bBb. It stabilizes C3bBb, prevents its physiological regulation by factors H and I, and increases the half-life of its spontaneous decay (18).

C3, the most abundant protein of the C system, is expressed in a number of different allotypic variants. The allele frequencies for the most common allotypes, C3S (slow running in agarose-gel electrophoresis) and C3F (fast running), are 0.80 and 0.19, respectively, in caucasoid populations (19,20). The molecular basis of the C3 polymorphism is a single base change in the DNA (21).

To further investigate the role of C in MPGN, we have collected data obtained within a longitudinal multi-center study. Clinical and pathomorphological manifestations in these patients, as well as a possible prognostic value of C3 NeF, have been reported previously (9).

In the current study we analyzed the influence of C3 NeF and distinct C3 allotypes on the long-term outcome of MPGN. Special emphasis was attributed to the possible influence of continuous C activation. In a later survey, a restricted number of patients were tested for complement regulatory proteins, factors H and I, where deficiencies have previously been associated with increased C consumption and renal pathology (22,23).

Materials and methods

Fifty children (27 girls, 23 boys) with idiopathic MPGN, who were aged 2.2–13.7 (median 9.8) years at apparent onset, were recruited in 17 pediatric centers from 1978 to 1982. Twenty-nine patients had initial symptoms before 1978 for a median time of 3.0 years. The diagnosis of MPGN was based on the clinical presentation and histopathology, as assessed using light and electron microscopy and immunofluorescence analysis. Other systemic diseases, especially lupus erythematosus, were excluded by clinical presentation, histopathology of renal biopsy, and specific serological tests. For differential diagnosis, all patients were screened for circulating anti-

nuclear antibodies (ANA) and elevated antibody titer to the streptococcal antigen, streptolysin O. During follow-up, two patients (one with lupus erythematosus and one with Wegener granulomatosis) were excluded from the study. The treatment protocol was decided by each center. Informed consent for participation in this study was obtained from the patients or their parents. The patients were followed-up regularly over a period of 1.9–20.5 (median 11.4) years. The median age at last observation or at ESRD (n=30) was 21.2 (range 5.4–29.1) years.

Serum and EDTA-plasma were obtained from the peripheral blood and stored at –70°C until use. According to the original protocol, C analysis was scheduled to take place at 6-month intervals in the first 2 years and subsequently once per year. The first C analysis was usually performed 2 months after apparent onset of the disease. The median number of C measurements actually performed per patient was six (range four to 11). C3 NeF activity was measured at the same time. Activation-specific protein–protein complexes were analyzed only in the first plasma specimen obtained for analysis. No clinically apparent intercurrent infections that might have contributed to changes in the complement profiles were documented while samples were collected. In 32 patients additional phenotyping of C3 was carried out. Control data for C profiles were obtained from healthy blood donors (n=20). C3 phenotypes were compared with those of the healthy individuals reported by Rittner & Rittner (19). Total hemolytic complement activity (CH50) was determined according to Mayer (24). Functional activity of the alternative pathway was measured as described by Joiner et al. (25).

The C3 activation product C3dg/C3d was quantified by using double-decker rocket immunoelectrophoresis, according to Brandslund et al. (26). C3, C4, and Factor B were measured by radial immunodiffusion (Centeon Pharma/Chiron Behring, Marburg, Germany). In 19 patients, additional measurement of the regulatory proteins factors I and H were performed by using rocket immunoelectrophoresis, as described previously (27).

Activation-specific protein–protein complexes of the classical pathway – C1rsC1-Inhibitor – as well as the alternative pathway – C3b(Bb)P – were determined by enzyme-linked immunosorbent assay (ELISA), as described previously (28).

C3 NeF activity was assessed by using a hemolytic assay described previously by Rother (29). A patient was regarded as C3 NeF positive if the auto-antibody was detected at least once during the course of the disease. C3 phenotyping

Table 1. Long-term changes of C3 nephritic factor (NeF) in serum of patients with idiopathic membranoproliferative glomerulonephritis (MPGN)

MPGN	Type I	Type II	Type III	Total
No. of patients	26	17	7	50
C3 NeF positive at any time	11	15	4	30
C3 NeF persistently positive	7	9	1	17
C3 NeF initially positive then negative	2	1	3	6
C3 NeF initially negative then positive	2	5	0	7
C3 NeF persistently negative	15	2	3	20

was carried out by using high-voltage agarose-gel electrophoresis, according to Teisberg (30).

For data evaluation the statistical analysis system (SAS) (SAS, Cary, NC, USA) was used. The following procedures were applied: proc freq (Fisher's exact test for 2×2 or larger tables) (31), proc plot (for graphical presentation of percentages) (32) and proc lifetest (for survival analysis) (33).

Results

According to biopsy analysis, 26 patients showed MPGN type I, 17 type II, and seven type III. In 30 of the 50 patients C3 NeF activity was detected at least once during follow-up (Table 1). C3 NeF activity was more frequent in MPGN type II (15 of 17) than in type I (11 of 26) or type III (four of seven) ($p = 0.0083$, Fisher's exact test). C3 NeF was persistent in 17 patients, whilst it was present initially in only six; seven initially negative patients later became C3 NeF positive (Table 1).

The complement status according to the presence of C3 NeF at first measurement is given in Table 2. Significantly lower levels of CH50 and C3 and higher levels of C3dg/C3d were found in C3 NeF-positive patients, but not in persistently negative patients, as compared to normals ($p < 0.0004$). In contrast, levels of C3b(Bb)P were elevated in C3 NeF-negative patients compared to controls ($p = \text{NS}$), whilst C3 NeF-positive patients

had normal levels of this activation product; this may be explained by either a reduced ability to assemble the alternative pathway convertase or by competition of C3 NeF with properdin in stabilizing C3Bb, where C3BbC3NeF escapes recognition by the assay used for detection. Levels of factors H and I (regulatory proteins) in C3 NeF-positive and C3 NeF-negative patients were within the normal ranges.

Long-term changes of C3 concentrations are summarized in Table 3. Thirty-five per cent of patients with MPGN type II showed persistently very low C3 levels ($< 300 \mu\text{g/ml}$) at all times during the observation period, whilst this applied to $< 20\%$ of those with types I and III combined.

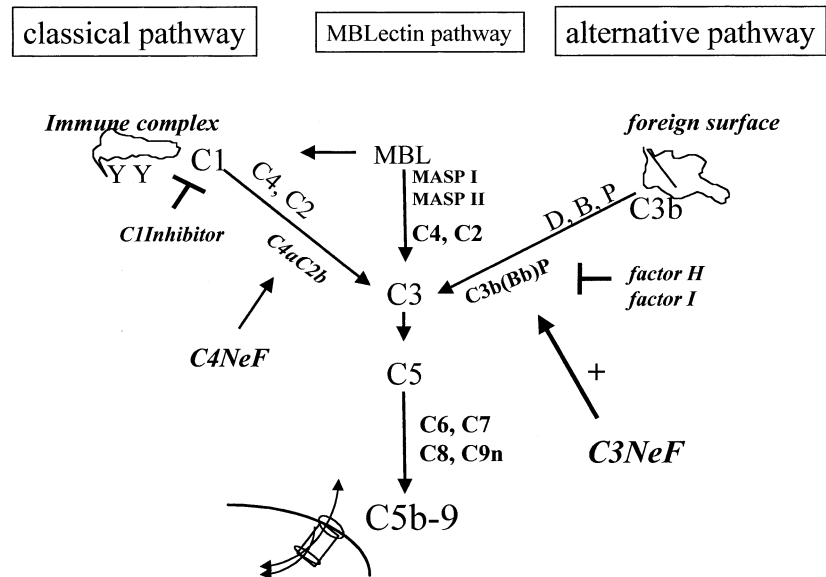
Renal survival analysis showed no significant difference between patients who were C3 NeF positive at any time and those who were negative. The median time to reach ESRD was 13.6 and 14.2 years for patients who were C3 NeF positive at any time and negative, respectively ($p = \text{NS}$) (Fig. 2). However, of the 17 patients with persistent C3 NeF activity, only nine reached ESRD during follow-up whereas all seven patients with initially negative but subsequently positive C3 NeF activity reached ESRD ($p = 0.031$, Fisher's exact test) (Table 4). Low C3 levels did not contribute to the occurrence of ESRD in C3 NeF-positive or C3 NeF-negative patients (Table 4).

Table 2. Initial complement profiles in patients with idiopathic membranoproliferative glomerulonephritis (MPGN) (in brackets number of patients tested)

	Normal (mean $\pm$ 2SD)	C3 NeF positive at any time during follow-up (n=30)			C3 NeF persistently negative during follow-up (n=20)		
		Mean	SD	p	Mean	SD	p
CH 50 (% of standard)	100.0 $\pm$ 20	43.2	38.2	< 0.0004	88.7	29.8	NS
C3 ($\mu\text{g/ml}$)	825.0 $\pm$ 275	286.0	229.0	< 0.0004	690.0	226.0	NS
C4 ($\mu\text{g/ml}$)	300.0 $\pm$ 200	279.0	85.0	NS	275.0	121.0	NS
B ($\mu\text{g/ml}$)	300.0 $\pm$ 200	143.0	45.0	NS	154.0	35.0	NS
C3dg,d (mU/l)	55.0 $\pm$ 20	174.0	87.0	< 0.0004	57.0	23.0	NS
C3b(Bb)P (U/ml)	5.3 $\pm$ 2.5	3.6	3.1 (15)	NS	24.0	33.3 (9)	NS
C1rs-C1INH (U/ml)	8.7 $\pm$ 2.6	7.3	5.1 (15)	NS	8.9	2.0 (9)	NS
Factor H ($\mu\text{g/ml}$)	520.0 $\pm$ 160	557.0	192.0 (15)	NS	544.0	118.0 (4)	NS
Factor I ($\mu\text{g/ml}$)	75.0 $\pm$ 15	84.0	49.0 (15)	NS	101.0	22.0 (4)	NS

p was calculated by using the Student's *t*-test. The respective p-value were adjusted according to Bonferroni.

Fig. 1. Complement activation pathways. During the course of the reaction cascade mediators of inflammation are generated and the membrane attack complex is formed. The reaction sequence is controlled by fluid phase and membrane associated regulatory proteins. B, factor B; C5b-9, membrane attack complex; D, factor D; MASP, mannan-binding ligand-associated serine protease; MBL, mannan-binding ligand; P, properdin.



The allele frequencies of the major C3 variants C3S, C3FS, and C3F in our MPGN patients differed from those reported for the normal Caucasian population ($p = 0.0029$). A significant shift in allele distribution to the C3FS or C3F phenotype was found in C3 NeF-positive patients ($p < 0.0001$) (Table 5). However, the C3FS/F phenotype did not predict ESRD (data not shown).

Discussion

This study comprises, to date, the largest group of European children with idiopathic MPGN who have undergone regular monitoring for the presence of C3 NeF and signs of C activation in serum over an extended time-period.

C3 NeF activity was observed in 87% of patients with MPGN type II but in <50% of patients with types I and III. This result is in line with the findings of Schena et al. (34) that were obtained in a smaller series of adult patients with MPGN types I and II. In >50% (17 of 30) of our C3 NeF-positive patients, especially in those with type II, C3 NeF was present persistently. Independently of the histological type of MPGN, the presence of C3 NeF activity was associated with

signs of C activation, as demonstrated by reduced serum levels of CH50 and C3 and increased concentrations of C3dg/C3d.

To our surprise, C3(Bb)P levels were normal in C3 NeF-positive patients, despite ongoing C activation. This may be explained by a low concentration of C3 NeF-stabilized C3 convertase leading to permanent depletion of C3. In addition, C3 NeF might compete with properdin for the same binding site on the C3 convertase, C3bBb. A lack of C3 hypercatabolism in six C3 NeF-positive patients with C3 levels persistently within the normal range may indicate the occurrence of different kinds of C3 NeF, as proposed by Ohi et al. (35). In six of 104 patients with MPGN, these authors detected C3 NeF activity in the absence of hypocomplementemia. It may be speculated that C3 NeF associated with non-hypocomplementemia does not interfere with factors I and H in dissociating the alternative pathway convertase, C3bBb. Recently, Spitzer et al. (36) reported that a reduction in C3 NeF activity may be explained by a structural alteration of factor B serving as an auto-antigen for C3 NeF.

Analysis of factors I and H in our study did not reveal any deficiency, which also may have accounted for C3 hypercatabolism by excessive

Table 3. Long-term changes of serum C3 concentrations in 50 patients with idiopathic membranoproliferative glomerulonephritis (MPGN) (levels below 550 $\mu\text{g/ml}$ are regarded as subnormal)

MPGN	Type I	Type II	Type III	Total
No. of patients	26	17	7	50
C3 persistently <300 $\mu\text{g/ml}$	5	6	1	12
C3 moderately changed (300 $\mu\text{g/ml}$ to >550 $\mu\text{g/ml}$)	5	8	3	16
C3 persistently >550 $\mu\text{g/ml}$	16	3	3	22

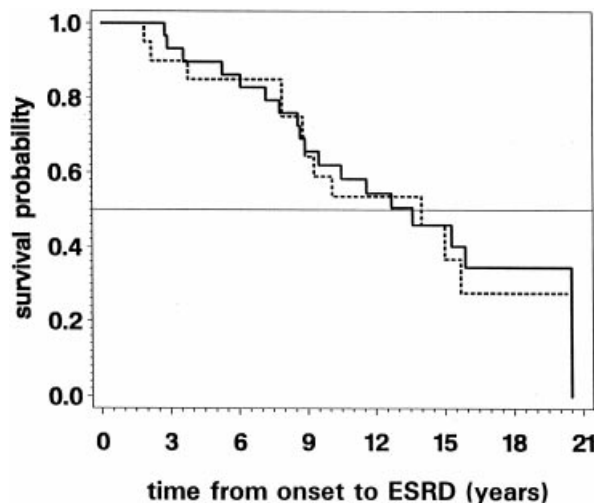


Fig. 2. Renal survival probability from apparent onset of idiopathic membranoproliferative glomerulonephritis (MPGN) to end-stage renal disease (ESRD). Straight line: patients with C3 nephritic factor (NeF) activity. Dotted line: patients without C3 NeF activity.

circulating C3bBb, as outlined by West & McAdams (37).

In most of our patients with MPGN type II, C3 levels were persistently low throughout the observation period. This was in contrast to patients with MPGN types I and III where C3 levels were found to be decreased in only $\approx 50\%$. Similar results in children and adult patients studied over a shorter duration have been reported by others (7).

In our population, the presence of C3 NeF activity was not associated with a more rapid progression to ESRD (Fig. 2). This finding is in contrast to that of other groups who found an accelerated deterioration of renal function in C3 NeF-positive vs. C3 NeF-negative adult patients

(34,38). It also contradicts the observations of Seino et al. (39), suggesting a more rapidly progressive course of MPGN if C3 NeF activity is present. West (40) hypothesized that C3 NeF is only a predisposing factor for development of MPGN.

No differences were detected in the severity of initial proteinuria in C3 NeF-positive and C3 NeF-negative patients; however, in our previous report (9) a significant association of renal survival probability and initial nephrotic syndrome was demonstrated. There was also no correlation of severity of proteinuria at onset and the degree of complement activation (data not shown).

Analysis of the influence of therapeutic options on the clinical outcome was not part of the initial study design, as the treatment was left to the discretion of each center. We did not detect any drug-related effect on outcome in any group of patients, which also included all seven patients who were initially C3 NeF negative but then developed C3 NeF activity and subsequently ESRD. In these patients too, no precipitating event was identified that might have been associated with the appearance of C3 NeF activity.

An increased frequency of the C3F allotype has been described in various inflammatory diseases affecting the kidney, including immunoglobulin A (IgA) nephropathy and systemic vasculitis (41,42). In our patients with MPGN, C3 allotype distribution differed similarly although the increased proportion of patients with the C3FS/C3F phenotype was restricted to C3 NeF-positive patients ($p < 0.0001$). Our findings confirm the preferential association of C3 NeF with the C3F variant, as proposed by Finn & Mathieson (43). It

Table 4. Outcome of patients suffering from membranoproliferative glomerulonephritis (MPGN) according to the presence of C3 nephritic factor (NeF) activity and persistently low C3 levels

	Patients without ESRD	Patients with ESRD	n
C3 NeF			
Persistently positive	8	9	17
Initially positive then negative	4	2	6
Initially negative then positive	0	7	7
Persistently negative	8	12	20
Total	20	30	50
C3 in C3 NeF-positive patients			
Persistently $<300 \mu\text{g/ml}$	4	7	11
Moderately changed ($300 \mu\text{g/ml}$ to $>550 \mu\text{g/ml}$)	5	8	13
Persistently $>550 \mu\text{g/ml}$	3	3	6
Total	12	18	30
C3 in C3 NeF-negative patients			
Persistently $<300 \mu\text{g/ml}$	1	1	2
Moderately changed ($300 \mu\text{g/ml}$ to $>550 \mu\text{g/ml}$)	2	0	2
Persistently $>550 \mu\text{g/ml}$	5	11	16
Total	8	12	20

Table 5. C3 phenotypes in 32 patients with idiopathic membranoproliferative glomerulonephritis (MPGN)

	C3 phenotype	Total number	Incidence (%)	p
Control population (ref. 19, Rittner 1974) (n=2316)	C3F	95	4.1	
	C3FS	700	29.9	
	C3S	1521	65.0	
MPGN patients (n=32)	C3F	6	18.8	0.0029
	C3FS	10	31.2	
	C3S	16	50.0	
C3 NeF positive at any time (n=19)	C3F	6	31.6	<0.0001
	C3FS	7	36.8	
	C3S	6	31.6	
C3 NeF negative (n=13)	C3F	0	0.0	NS
	C3FS	3	23.1	
	C3S	10	76.9	

p-values were calculated by Fisher's exact test.

appears that the C3F/C3FS phenotype facilitates the formation of C3 NeF as an auto-antibody.

In conclusion, complement activation in children with idiopathic MPGN is related to the presence of C3 NeF in serum and predominates in the pathomorphological type II. The presence of C3 NeF, a certain C3 allotype, or low C3 levels, are of no prognostic value for the clinical outcome.

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