Serum C3 levels in acute glomerulonephritis and postnephritic children

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Popović-Rolović, M. (1973). *Archives of Disease in Childhood*, 48, 622. **Serum C3 levels in acute glomerulonephritis and postnephritic children.** Measurements of β_1 C-globulin (C3) by the single radial immunodiffusion method were done in 128 patients with acute glomerulonephritis. In 88 patients the analyses were made during the first 3 weeks of the disease, and sequential determination was done thereafter until the end of the sixth month. During the first 4 weeks of the disease 90% of the patients had reduced C3 levels and the remaining 10% had normal levels. In the group of hypocomplementaemic patients only 80% achieved normal values after 6 months. The initial reduction of C3 concentration did not correlate with the severity of the acute phase of the disease or with the duration of haematuria and proteinuria. In 40 patients the analyses were done 2 to 4 years after the acute phase of the disease (the postnephritic group). 15% of these patients had a reduced concentration of C3. The mean values of C3 concentration in this group as a whole was significantly lower than in the control group of 30 healthy children ($P < 0.001$).

Since it was isolated as a purified protein (Müller-Eberhard, Nilsson, and Aronsson, 1960; Müller-Eberhard and Nilsson, 1960) β_1 C-globulin (C3) has been measured in a number of patients with various kidney diseases (Borgeaud, Paunier, and Humair, 1970; Gotoff *et al.*, 1965, 1969; Humair, 1968; Morse, Müller-Eberhard, and Kunkel, 1962; Northway *et al.*, 1969; Ogg, Cameron, and White, 1968; Tina *et al.*, 1968; West, Northway, and Davis, 1964; West *et al.*, 1965). Data so far reported indicate that in patients with acute glomerulonephritis C3 levels are reduced in the first few weeks of the disease (Borgeaud *et al.*, 1970; Gotoff *et al.*, 1965, 1969; Humair, 1968; West *et al.*, 1964). However, sequential determination has not been done extensively except by Humair (1968), and no studies have reported on C3 levels in the postnephritic period. The significance of normal C3 levels found in some patients with acute glomerulonephritis has not been elucidated (Gotoff *et al.*, 1969; Tina *et al.*, 1968; West *et al.*, 1964) and, moreover, the problem of correlation of the clinical course of the disease with C3 concentration is still a matter of controversy (Borgeaud *et al.*, 1970; West *et al.*, 1964).

The purpose of this study was to measure the initial C3 levels in a large group of patients and make sequential determinations during various phases of the disease and in the postnephritic period. It was felt that such investigations would show what proportion, if any, of patients had normal C3 levels and show a possible correlation between the clinical course of the disease and C3 concentration.

Material and methods

Measurements of C3 levels were performed in 128 patients with acute glomerulonephritis aged 2½ to 14 years.

In 88 patients analyses were done during the first 3 weeks of the disease and then they were followed until the end of a 6-month period. These patients were sporadic cases occurring mainly during autumn and spring in 1969 and 1970, and referred to the Children's University Hospital of Belgrade, where they were hospitalized regardless of the severity of the disease. None was from parts of the country with endemic Balkan nephropathy.

Clinical evaluation included complete history and physical examination; culture of the pharynx (or skin) and urine, chest x-ray, ECG, hippuran renogram, and laboratory assessment including serum creatinine, urea, and electrolytes, antistreptolysin O titre (ASO), and total haemolytic complement titre, daily urine analysis, and quantitative 24-hour urine protein determination.

The minimal criteria for diagnosis of acute glomerulonephritis and acceptance into the study were acute onset of the disease without a history of previous renal disease, macroscopical and/or microscopical haematuria with RBC casts and epithelial casts in the urinary sediment, oedema, significantly raised ASO titre (>200 Todd units) and, in the absence of the latter, a positive throat or skin culture for group A β -haemolytic streptococci. In addition, raised blood pressure and signs of reduced glomerular filtration were found in most cases.

Preceding infection was recorded as upper respiratory infection in 82%, skin infection in 10%, and unknown in 8%. The latent period ranged from 4 to 30 days and significantly raised ASO titre was found in 98%. Though throat or skin culture was obtained routinely, data are not presented since almost all the children had received antibiotic therapy before entering hospital. Oedema was shown by subsequent loss of body weight ranging from 5 to 15% in 96% of cases, and as a positive history in the remaining 4%. Blood pressure was raised transiently in 90% of cases. Serum creatinine and urea were raised slightly or moderately (twice normal values) in 37%, and higher levels were recorded in only 11%.

In 40 children C3 levels were measured 2 to 4 years after the onset of the disease (postnephritic group). In the acute phase of the disease (sporadic cases from 1967-1968) these children were also in hospital for at least 8 weeks and the same criteria for acceptance into the study as was used in the group of 88 patients were applied. None had a history of previous renal disease; in 93% an earlier upper respiratory infection had been recorded and the ASO titre was significantly raised in 97%. The latent period ranged from 4 to 30 days. Blood pressure was raised in 77% and oedema was found in 87%. Serum creatinine and urea were slightly or moderately raised in 31% and higher levels were recorded only in 3%. All the patients had low levels of haemolytic complement titre.

During routine check-ups of the postnephritic group, in addition to physical examination and blood pressure, the urinary sediment was examined and 24-hour proteinuria determined. When the findings were not within normal limits, endogenous creatinine clearance was determined. In cases in which reduced C3 levels were found, the children were tested again.

C3 concentration was also measured in a control group of 32 healthy children from 3 to 13 years of age.

β_1C_{1A} globulin (C3) was determined according to the method of Mancini, Carbonara, and Heremans (1965) by the single radial immunodiffusion technique using commercial Behringwerke partigen plates.

The sera of both patients and healthy children were frozen and analyses were done every 2 to 3 weeks. After thawing, various serum dilutions were put into the wells on the plates and after exposure to room temperature for 48 hours the precipitin diameter was measured. The concentration of C3 was estimated from the standard curve. Standard sera were also obtained from Behringwerke.

Results

Concentration of C3 in the control group (32 healthy children) was 109.5 ± 16.3 mg/100 ml (mean $\pm$ SD).

In 88 patients with acute glomerulonephritis studied during the acute phase of the disease, C3 levels were measured in the course of the first 3 weeks of the disease. In the first week levels were measured in 35 patients, in the second week in 31 patients, and the third week in 22 patients.

The results of these investigations show that in 80 patients (90%) C3 levels were reduced during the first 3 weeks of the disease and were normal in 8 patients (10%).

Results of sequential determination of serum C3 levels in these patients are shown in Table I.

Table II indicates the time required for C3 concentration to become normal.

TABLE I

Sequential determination of serum C3 levels (mg/100 ml) in patients with acute glomerulonephritis

Time period	No. of patients	Mean $\pm$ SD
1st wk	34	26.7 ± 27.5
2nd wk	32	35.9 ± 33.9
3rd wk	38	40.2 ± 34.5
4th wk	17	43.8 ± 25.0
5th-6th wk	36	60.5 ± 26.5
7th-8th wk	26	69.2 ± 23.6
3rd mth	24	80.8 ± 18.2
4th mth	14	89.9 ± 27.4
5th-6th mth	20	99.8 ± 27.1
Control group	32	109.5 ± 16.3

TABLE II

Time taken for serum C3 (mg/100 ml) to become normal in patients with acute glomerulonephritis

Time period	Patients with normal C3 levels (%)
1st, 2nd, 3rd, and 4th wk	10
5th-6th wk	35
7th-8th wk	38
3rd mth	62
4th mth	71
5th-6th mth	80

Despite a marked tendency toward increased concentration and the fact that with the passage of time concentrations increased and became normal in a growing number of patients, not even after 6 months did all the patients show normal C3 levels.

Since the initial reduction of C3 concentration in the first 3 weeks of the disease varied from 5 to 53

mg/100 ml, the patients were divided into two groups: one with initially markedly reduced levels—below 15 mg/100 ml—and the other with initially slightly reduced levels—from 30 to 53 mg/100 ml.

Further investigations were done to ascertain (1) whether the initial reduction of concentration was related to the severity of the acute phase of the disease, (2) whether the initial reduction correlated with the duration of proteinuria and haematuria, and (3) whether there was any difference in the outcome of the disease between the two groups.

To estimate the severity of the acute phase of the disease the following clinical and laboratory findings were studied: oedema (estimated as loss of body weight), mean blood pressure, sedimentation rate (Westergren, first 2 hours), serum urea and creatinine levels, haematuria (RBC/hpf), and 24-hour proteinuria. A comparison of these parameters was made between the two groups. The mean values were calculated and the difference was estimated according to Student's 't' test. No statistically significant difference between any of the tested parameters was found (from $P > 0.10$ to $P > 0.90$).

The duration of haematuria and proteinuria in these two groups was followed for 1 year and by the end of that time 80% of the patients in each group showed normal values. A comparison of the duration of haematuria and proteinuria was made between the two groups according to the Wilcoxon, Mann and Whitney U-test and no statistically significant difference was found ($P > 0.36$ and $P > 0.42$, respectively).

Since it was established that in 20% of patients the serum C3 concentration had not become normal 6 months after the onset of the acute phase of the disease, a group of 40 children who had recovered from the acute phase of the disease 2 to 4 years

earlier were investigated (the postnephritic group). The results of the measurements of C3 concentration in these children showed that 6 out of 40 (15%) had not achieved normal values within this period either.

In the 6 children with reduced C3 levels the reduction was not pronounced except in one case, the values being 73, 70, 64, 67, 64, and 46 mg/100 ml.

The mean value of C3 concentration in this group as a whole was lower (86.5 ± 14.8) than in the control group, and the difference was statistically significant ($P < 0.001$).

During a control examination of the postnephritic children it was found that in 30 all the findings were within normal limits, while in the remaining 10 certain abnormalities were observed, as shown in Table III. All these children had minimal proteinuria and haematuria, while 5 also had reduced C3 levels. Of the total of 6 patients with reduced levels, 5 were in the group 'with sequelae' and only 1 had all findings within normal limits and belonged to the group of 30 children 'with no sequelae'. This child was subjected to analysis twice more within 3 months and C3 values obtained were 41, 46, and 51 mg/100 ml. Since he showed no other abnormalities, C3 concentration was measured in his parents and one brother and normal values were obtained in all three.

Discussion

Before individual components of complement were isolated as purified proteins (Müller-Eberhard *et al.*, 1960; Müller-Eberhard and Nilsson, 1960), occasional findings of normal values of haemolytic complement titre in patients with acute glomerulonephritis were not given special attention and were

TABLE E
Postnephritic group: clinical and laboratory findings

Case no.	Age at subsequent testing (yr)	Blood pressure (mmHg)	Proteinuria analysis (mg/24 hour)	
			1st	2nd
1	4	85/55	37	160
2	8	90/60	77	431
3	9	70/55	140	248
4	10	95/75	60	192
5	11	90/60	40	60
6	15	115/60	133	360
7	15	90/60	40	214
8	15	120/70	190	215
		100/60		
9	15	100/70	203	100
10	15	105/75	161	295

usually attributed to delay in performing the analysis (Fischel and Gajdusek, 1952; Kellett and Thompson, 1939; Lange, Wasserman, and Slobody, 1960; Reader, 1948; Wasserman *et al.*, 1965).

The incidence of normal C3 levels found in patients with acute glomerulonephritis has differed considerably in the various studies so far reported and a different aetiology was suggested as a possible explanation (Tina *et al.*, 1968; West *et al.*, 1964). Gotoff *et al.* (1969) were the first to prove that two patients with normal C3 levels were typical cases of poststreptococcal glomerulonephritis. In our study it was found that the incidence of normocomplementaemia in a large number of patients with acute glomerulonephritis was 10%. These patients were the subject of a separate study (M. Popović-Rolović, unpublished data) in which it was found that their disease did not differ from typical 'hypocomplementaemic' poststreptococcal glomerulonephritis.

Sequential determination of C3 levels has been performed in relatively small groups of patients (Borgeaud *et al.*, 1970; Gotoff *et al.*, 1965, 1969) except in the study of Humair (1968); it was found that results became normal within 2 to 4 months. We found that only 80% of the patients achieved normal values by 6 months. The discrepancy between our findings and the data of Humair (1968) and others (Borgeaud *et al.*, 1970; Gotoff *et al.*, 1965, 1969) is so far unexplained.

The problem of correlation between complement concentration and the severity of the acute phase of the disease has not been systematically investigated before (Borgeaud *et al.*, 1970; Fischel and Gajdusek, 1952; Kellett and Thompson, 1939; Lange *et al.*, 1960; Reader, 1948; West *et al.*, 1964). Our study has proved, by comparing clearly defined parameters of the acute phase of the disease, that the initial reduction of C3 levels does not correlate with

the severity of the acute phase. Our findings also indicate that the initial reduction probably does not influence the final outcome of the disease.

We know of no previous investigations of C3 concentration in patients several years after the acute phase of their disease (postnephritic children). Our data indicate that not even after this period do all the patients have normal concentrations. The possibility that some of our patients with reduced C3 levels were in reality suffering from exacerbations of chronic glomerulonephritis from the beginning of the disease could not be ruled out completely without renal biopsy (Edelmann, Greifer, and Barnett, 1964). However, this possibility seems very unlikely because these patients were followed closely during the acute phase of the disease and thereafter, and the course of their disease did not differ from that of the others. The mean concentration of C3 in this group as a whole was significantly lower ($P < 0.001$) than in the control group of healthy children.

Reduced C3 levels in this group may be due to three causes. (1) That complement is still being consumed by the creation of immune complexes (Michael *et al.*, 1966); (2) the impaired relation between synthesis and catabolism which seems to exist in the acute phase of the disease (Alper and Rosen, 1967) may last considerably longer; and (3) it is possible that inhibitors of complement also continue to be produced for a long time or are periodically activated as a kind of protective mechanism (Pickering, Gewurz, and Good, 1968). Obviously this problem cannot be resolved without parallel investigation of the metabolism of C3 and analysis of the biopsy materials for ultrastructural and immunohistological changes.

From the practical point of view, one should not forget that children affected by acute glomerulo-

II

II control examination in 10 patients with sequelae

Urinary sediment analysis (RBC/hpf)		Creatinine clearance (ml/min per 1.73 m ²)	Serum C3 analysis (mg/100 ml)		Outcome
1st	2nd		1st	2nd	
0-1	0	105	64	70	Intermittent proteinuria
0-1	0	105	76	80	Intermittent proteinuria
0	0	102	72	75	Constant proteinuria
0-2	0	120	110		Intermittent proteinuria
4-6	0-2	168	100		Intermittent haematuria
0		130	70	69	Constant proteinuria
0-1	0	163	60	69	Intermittent proteinuria
0	0	115	56	72	Constant proteinuria
0-1	0	145	80		Intermittent proteinuria
0-1	0	135	100		Constant proteinuria

nephritis have a favourable prognosis (Čalić-Perišić *et al.*, 1968; Dodge *et al.*, 1962; Edelmann *et al.*, 1964; Perlman *et al.*, 1965). We consider that our results contribute towards the elucidation of the natural course of the disease in acute glomerulonephritis and that for the time being there is not enough evidence for interpreting a low serum C3 concentration as an unfavourable prognostic sign.

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